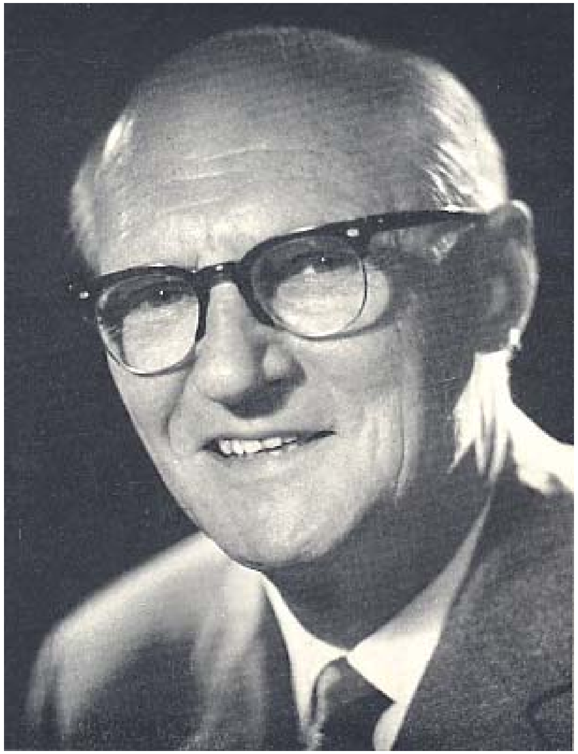
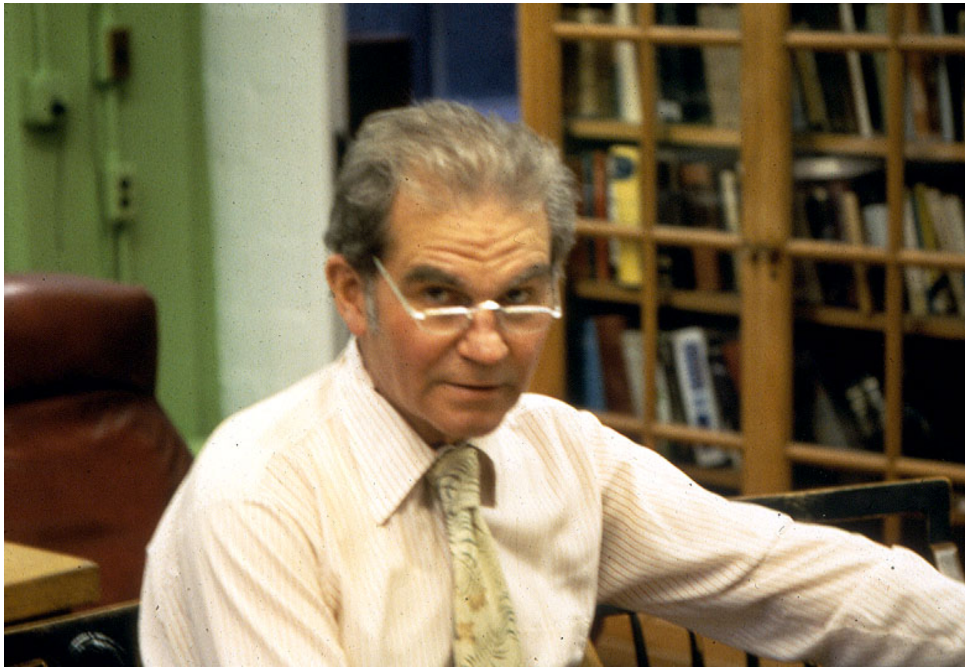
ENDOCRINE SYSTEM
OR
NEUROENDOCRINE SYSTEM

Sir V.B. Wigglesworth



Carroll Williams



FUNCTIONS OF THE NEUROENDOCRINE SYSTEM

Along with the nervous system, hormones provide the necessary communication between all the cells that constitute a multicellular animal

Nervous system-Is involved in rapid transfer of short-term events and coordination of short-term events. Electrochemical information involving neurons.

Neurosecretory cells-Neurons have electrical activity but involved in the production and release of neurosecretion that produces their effect as chemicals.

Endocrine system-Is involved in the integration and coordination of long-term events through chemicals called hormones.

TECHNIQUES FOR STUDYING THE INSECT ENDOCRINE SYSTEM

First generation insect endocrinologists (1920-1950's) used the following techniques:

Mainly identified the major endocrine glands and some of their functions

- Ligation
- Parabiosis
- Extirpation
- Transplantation of gland
- Reimplantation of gland
- Light microscopy

Second generation endocrinologists (1950-1960's) used the following techniques:

Hormones were chemically identified as to their structure and biosynthetic pathways

- SEM and TEM of glands
- Antibodies to the hormone
- Gas and liquid chromatography
- Mass spectrometry
- Nuclear magnetic resonance (NMR)
- High-performance liquid chromatography

Third generation endocrinologists (1970-1980's) used the following techniques:

Determined hormone titer

- Enzyme-linked immunoassay (ELISA) and RIA-radioimmunoassay)

Fourth generation endocrinologists (1980s until now) are using the following techniques:

Locate and determine the genes involved in hormone production, determining the molelcular struction and action. Identifying various receptors.

- PCR
- Other molecular techniques

Kopec's ligation experiment in 1917 with gypsy moth larva was the first to show that hormones were present in insects.

Partial purification of brain hormone or PTTH) from the head of *Bombyx mori*

96,000 Bombyx heads (fresh wt. 720 g)

↓

Acetone powder

↓ Washed with 80% EtOH; filtered

Residue

↓ Extracted with 2% aqueous NaCl,

Extract

↓ Heated in boiling water for 10 min
Cooled rapidly; filtered

Filtrate

↓ $(NH_4)_2SO_4$ added (80% saturation); filtered

Precipitate

↓ Dissolved in water
Acetone added; filtered
Acetone added to filtrate; filtered

Precipitate

↓ Dissolved in water
Saturated aqueous solution of picric acid added; filtered

Precipitate

↓ Dissolved in 0.1 M Tris buffer (pH 7.8)
Acetone added; filtered

Precipitate (''crude PTTH'')

↓ Gel filtration on Sephadex G-50 (fine)

Sephadex G-50 (fine) fraction

↓ Chromatography on DEAE-Sepharose Cl 6B

DEAE-Sepharose fraction

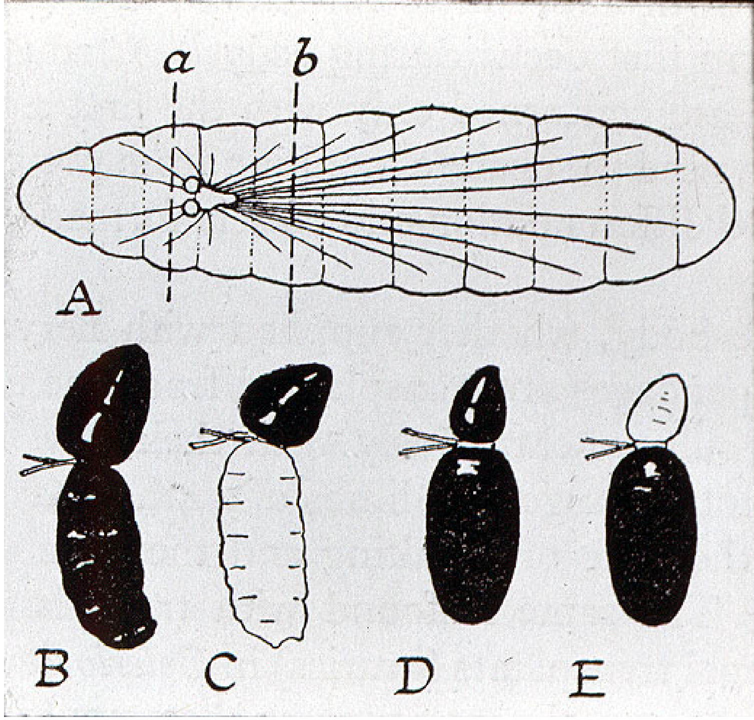
↓ Chromatography on SP-Sephadex C-25

SP-Sephadex fraction

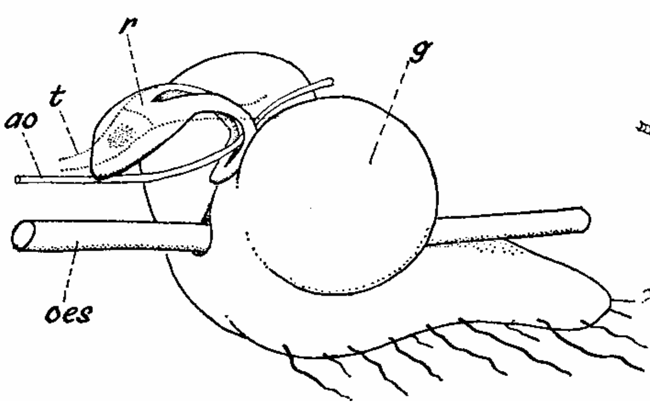
↓ Gel filtration on Sephadex G-50 (superfine)

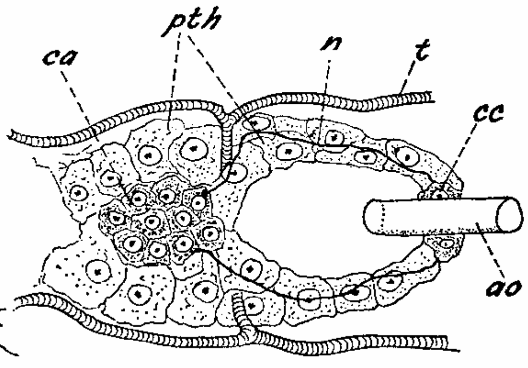
Highly purified brain hormone

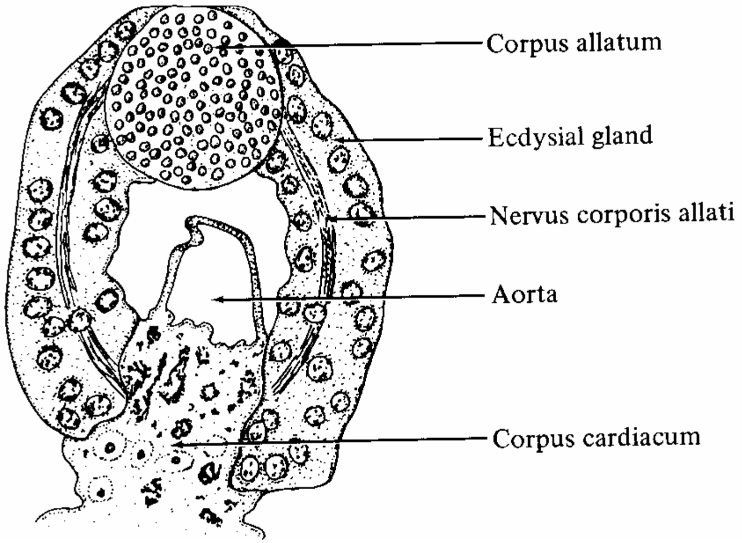
Ligation-Separating parts of the body by using human hair, silk, or fine string to tie off and separate the blood supply of one area from the other. Note in photo to the right that the ring gland (Weismann's ring) is found within the section between a and b.



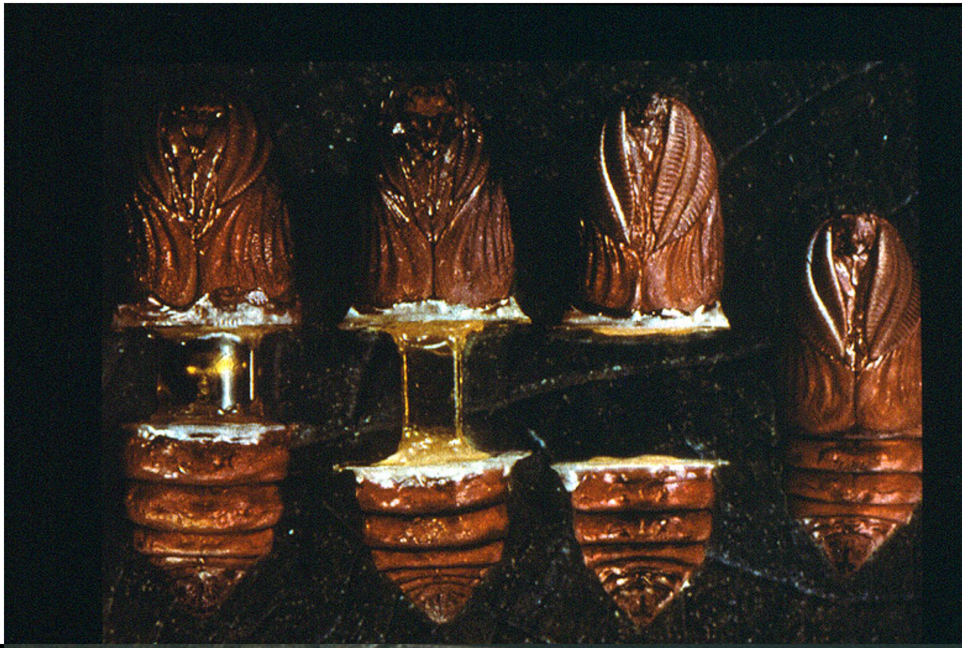
Weismann's ring or rind gland

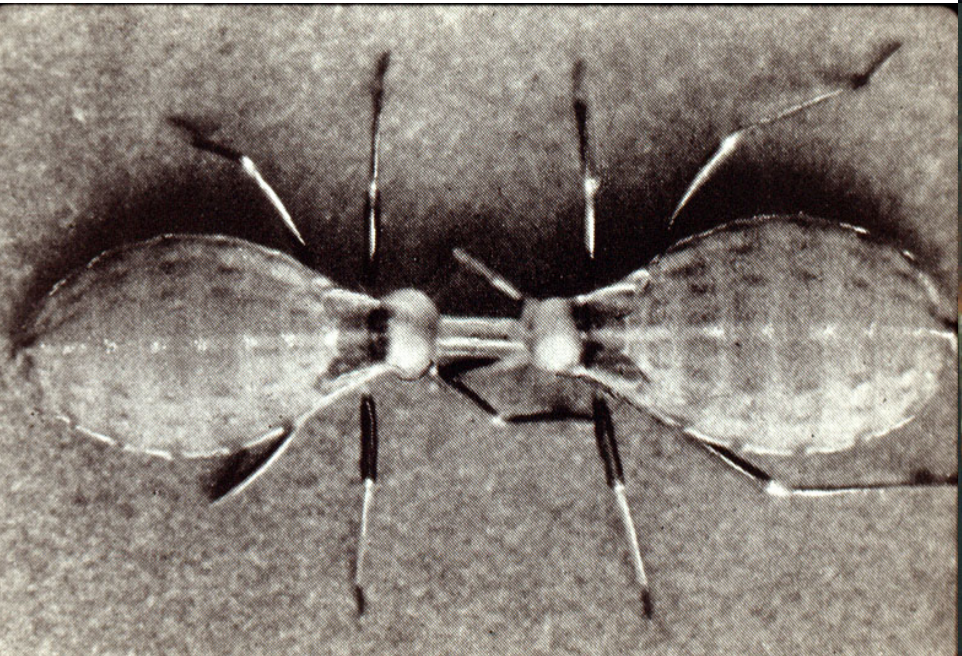


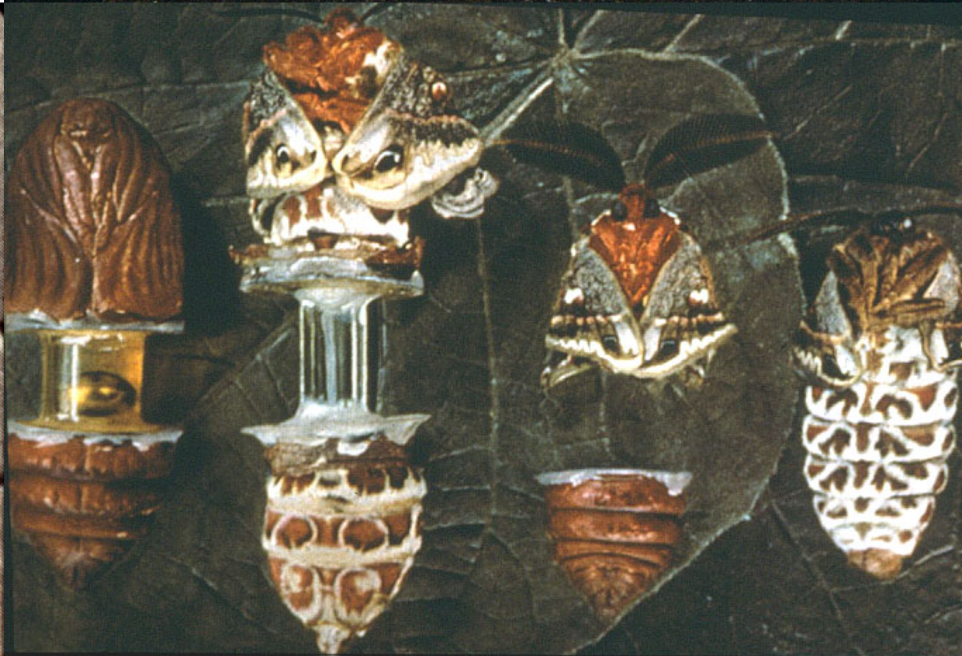




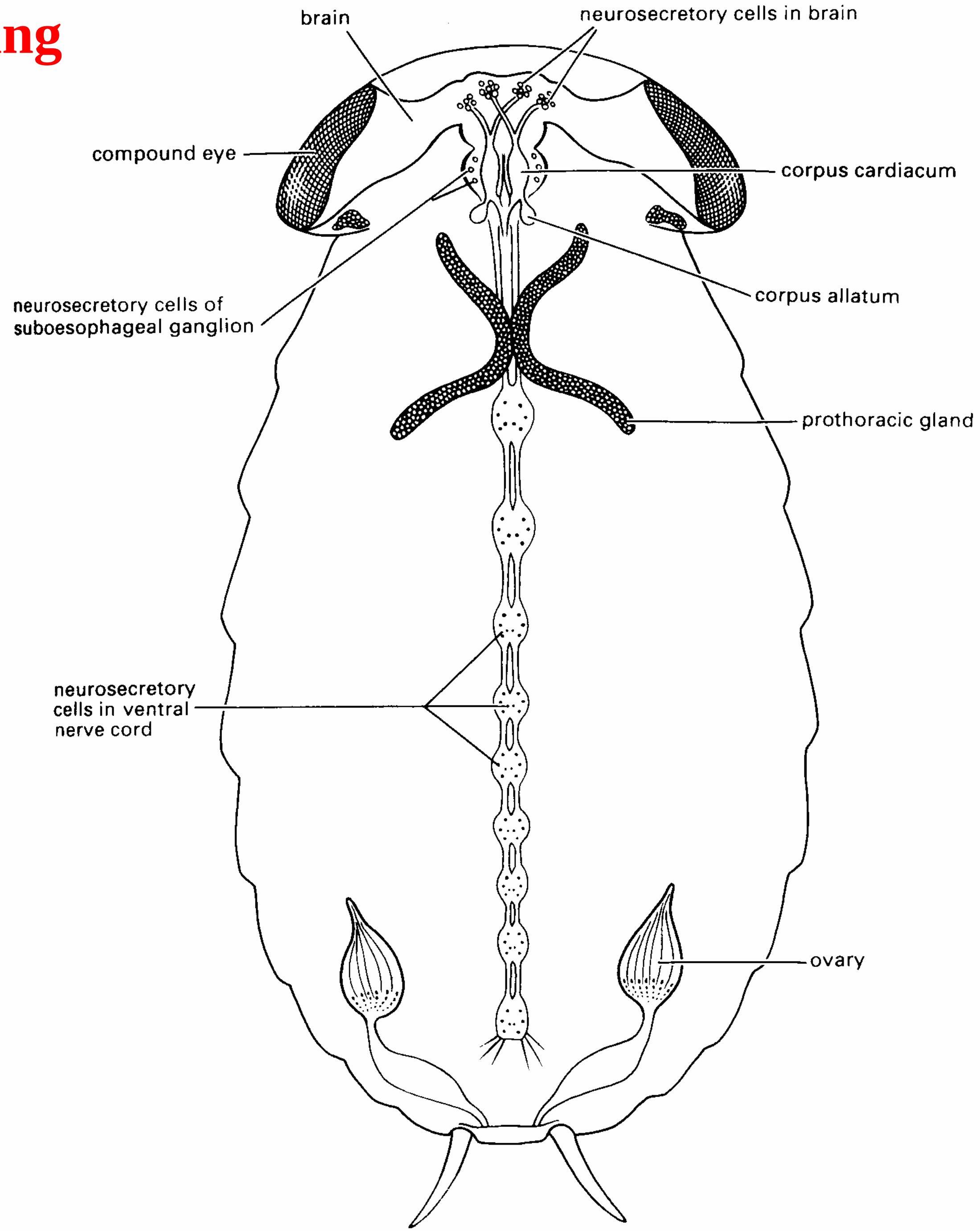
Parabiosis-Connecting the blood supply of two individuals by using various techniques.







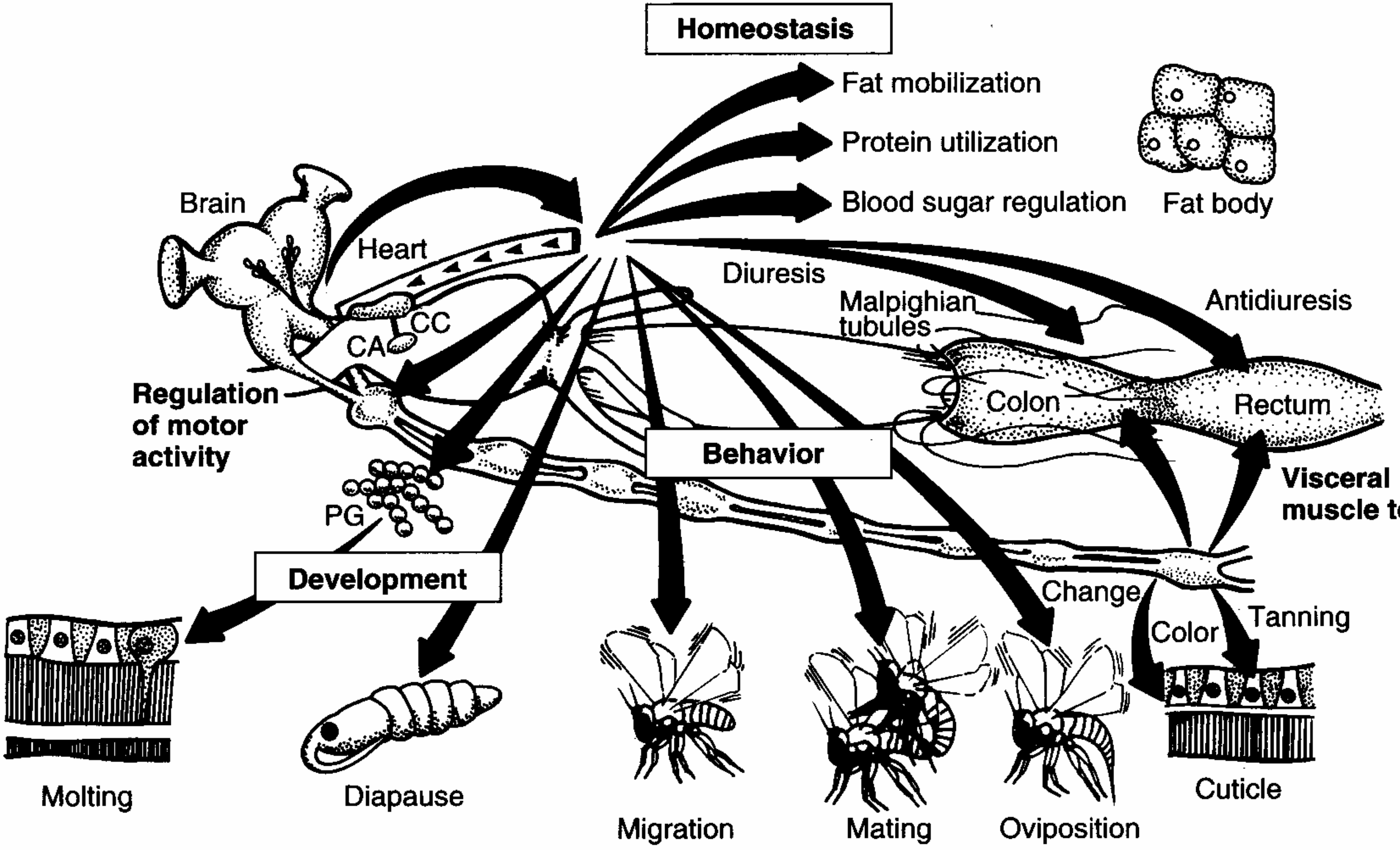
Generalized scheme showing the location of various endocrine glands and neurosecretory cells in an insect



Areas of insect biology that hormones play a major role

1. Regulation of molting
2. Determination of form at metamorphosis
3. Effects on polymorphism
4. Regulation of diapause
5. Involvement in reproduction
6. Regulation of metabolic activities and general body functions
7. Regulation of behavior
8. Regulation of preprogrammed cell death

Major physiological functions regulated by neurohormones



Insect endocrine glands & neurosecretory cells & location

Active Principle	Origin	Target	Role/function
I. Nonneural hormones			
A. Immature insects			
Ecdysone	ecdysial gland	epidermis	initiates molt
Juvenile hormone	corpora allata	epidermis	controls or directs fate of metamorphosis at molt
B. Adult insects			
Ovarian hormone (ecdysteroids)	ovarian tissue-follicle cells	fat body	initiates + regulates the production of vitello-genin (VG)
Juvenile hormone	corpora allata	fat body	primes fat body to become competent to produce vitellogenin

Insect endocrine glands & neurosecretory cells & location

Active Principle	Origin	Target	Role/function
Juvenile hormone	corpora allata	ARG's	affects development and production of glandular secretion
Juvenile hormone	corpora allata	follicle cells	activates patency and uptake of VG by the follicle cells

Insect endocrine glands & neurosecretory cells & location

Active Principle	Origin	Target	Role/function
II. Neural hormones and peptide hormones			
A. Ecdysiotropin (PTTH) (=prothoracicotropic hormone)	brain hormone protocerebrum	ecdysial glands	developmental-stimulates and regulates production and release of ecdysone
B. Bursicon	MNSC and thoracicoabd. ganglion of flies	epidermis	dev.-stimulates scleroti-zation and melanization of cuticle
C. Eclosion hormone	brain of pre-ecdysis moths	abdominal ganglion	behavior-synchron. of eclosion with photoperiod
D. Ecdysis-triggering	epitracheal glands (ventrolateral tracheal tube near each spiracle)	CNS (abdomin. ganglia)	Beh.-synchron. of eclosion
E. Allatostatins	Brain	corpora allata	dev./beh/homeostasis inhibits JH production
F. Allatotropin	Brain	corpora allata	dev./beh/homeostasis stimulates JH production
G. Diuretic hormone	brain/cc and thoracic ganglia	Malpig. Tubules	homeostasis-controls diuresis or fluid secretion

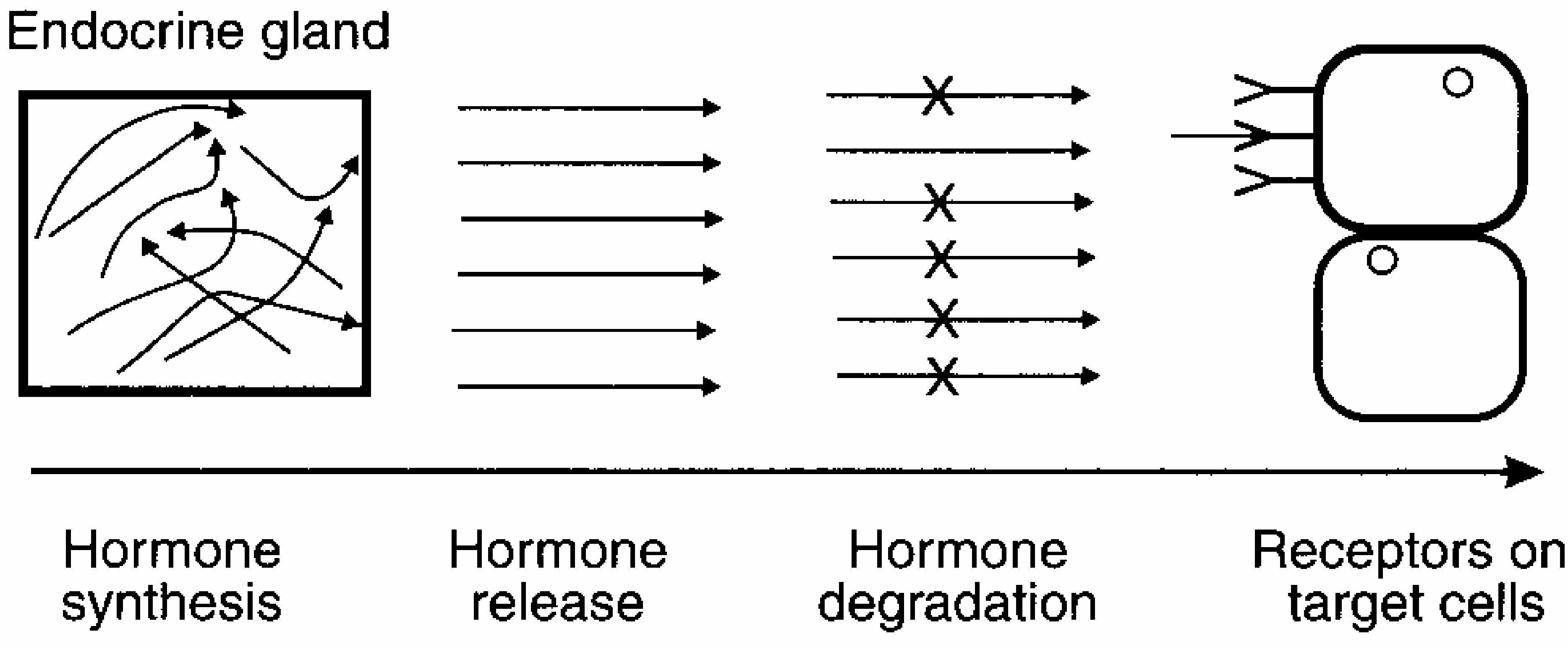
Insect endocrine glands & neurosecretory cells & location

Active Principle	Origin	Target	Role/function
H. Mating inhibition	ARG of male	female's brain	beh.-prevents remating
I. Oviposition initiation	ARG of male	oviduct?	beh.-initiations egg laying
J. Cardioacelerator	brain/CC	myocardium	Homeostatis-increase in freq. + amplitude of muscle contraction
K. Proctolin	brain/CC	hindgut and poss. Visceral muscle in general	homeo.-muscles contrac-tion, defecation, egg-lay-ing, + heartbeat
L. Dromyosuppresin	brain/CC	muscles of crop	inhibits muscle contract
M. Ovarian ecdysteroidogenic hormone (OEH) (also know as EDNH) (these may be similar to PTTH)	brain	ovaries	stimulate ovarian tissue to produce ecdysteroids

HORMONES-Chemicals produced in a gland that are released into the blood and have their effect somewhere else in the animal

Hormonal activity in the blood is influences by

1. **Hormone synthesis-The effective titer must be research to work**
2. **Hormone release**
3. **Hormone degradation**
4. **Receptors on the target cells-These change in number in**



LOCATION OF ENDOCRINE GLANDS IN DIFFERENT INSECTS

Not only does the location of the glands different in different insect orders but the hormones used for various functions may also vary.

Prothoracic glands------Produce ecdysone

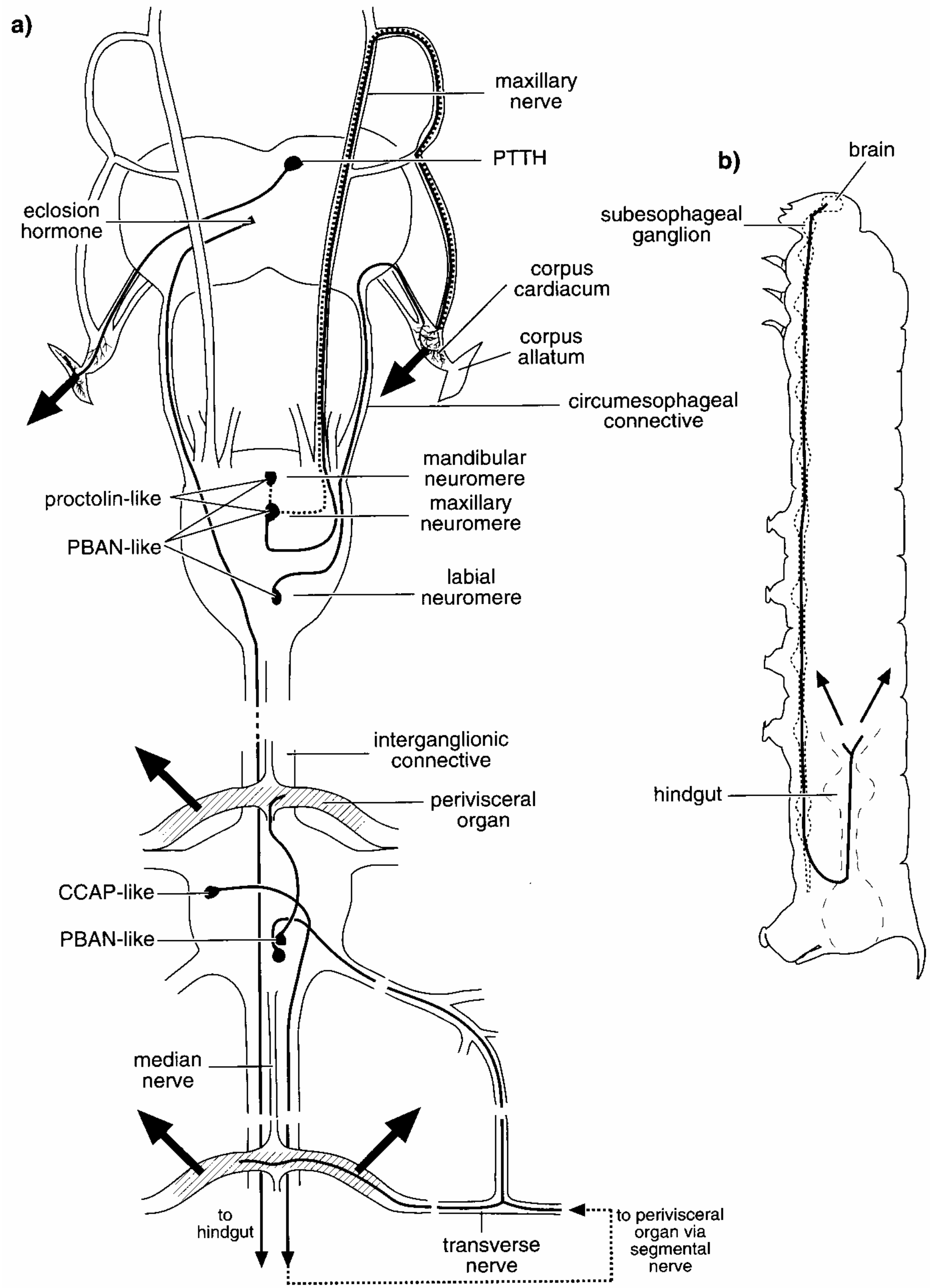
Corpora allata-----------Produces JH

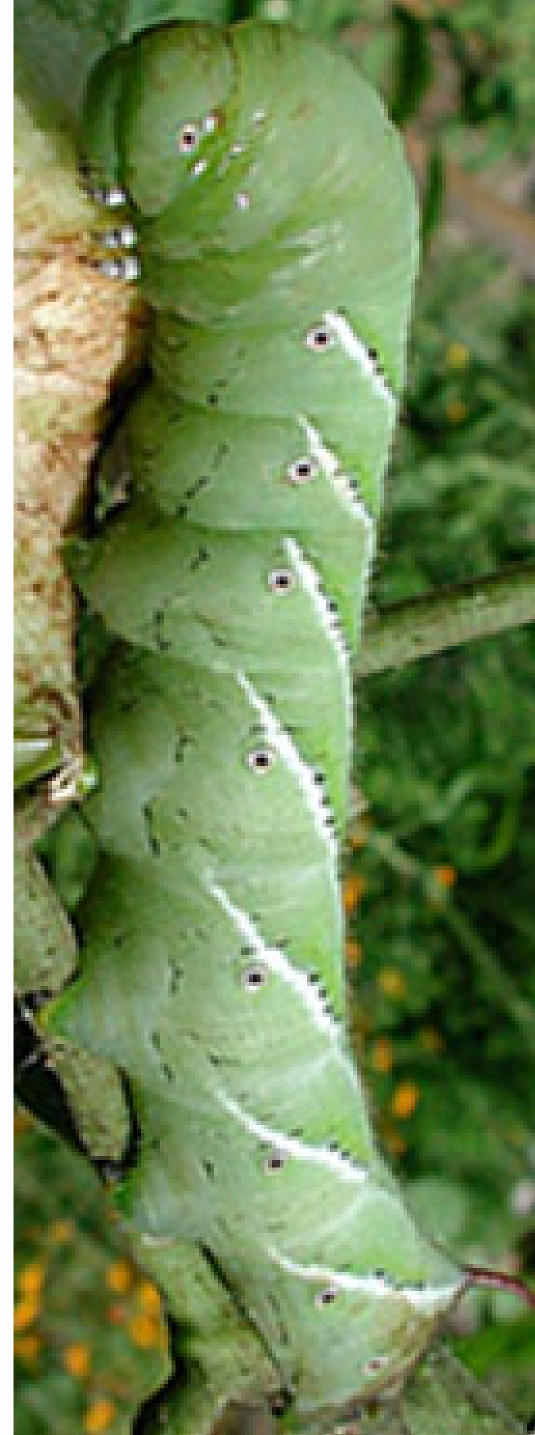
Corpora cardiaca-------Stores and releases brain hormones. Also produces and releases some peptides such as adipokinetic hormones

Midgut endocrine cells-Produce various peptides. Open + closed cell types.

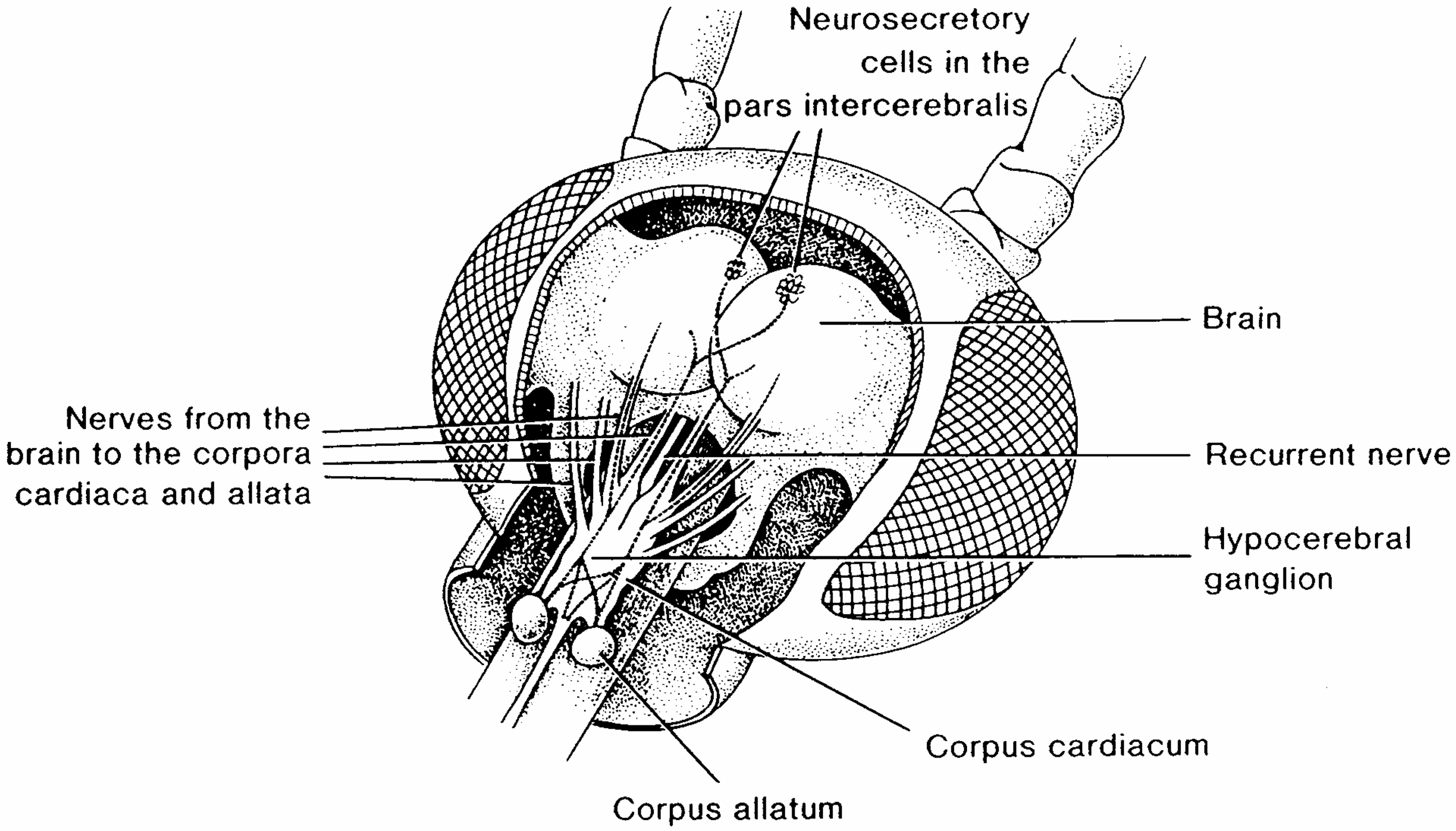
Epitracheal glands------Produce the ecdysis triggering hormone in Lepidoptera

Neurosecretory cells----Produce neurosecretion (peptides or biogenic amines); located in the various ganglia of CNS


a)
maxillary nerve
PTTH
eclosion hormone
corpus cardiacum
corpus allatum
circumesophageal connective
mandibular neuromere
proctolin-like
maxillary neuromere
PBAN-like
labial neuromere
interganglionic connective
perivisceral organ
CCAP-like
PBAN-like
median nerve
to hindgut
transverse nerve
to perivisceral organ via segmental nerve
b)
brain
subesophageal ganglion
hindgut

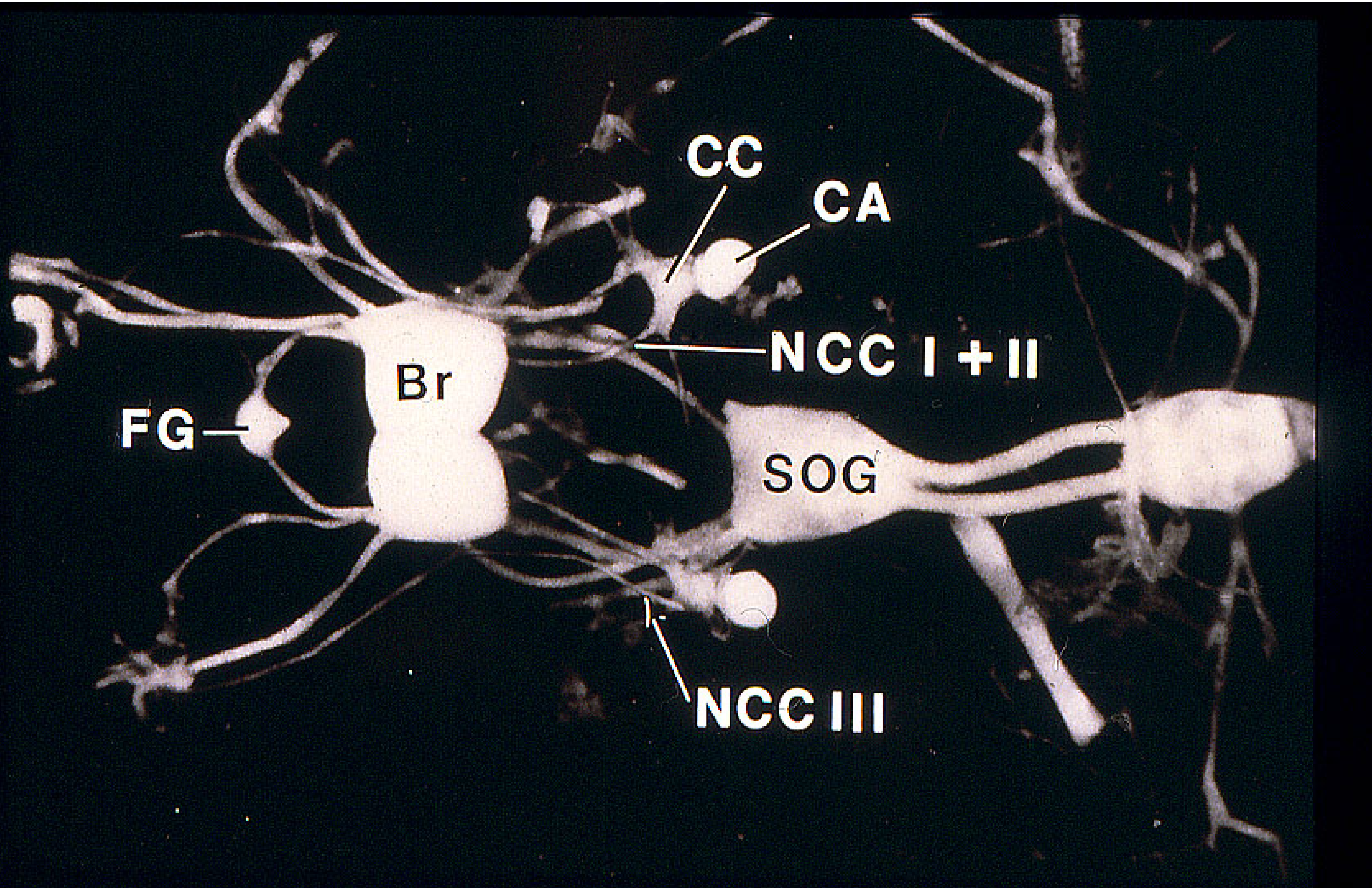


Orthoptera

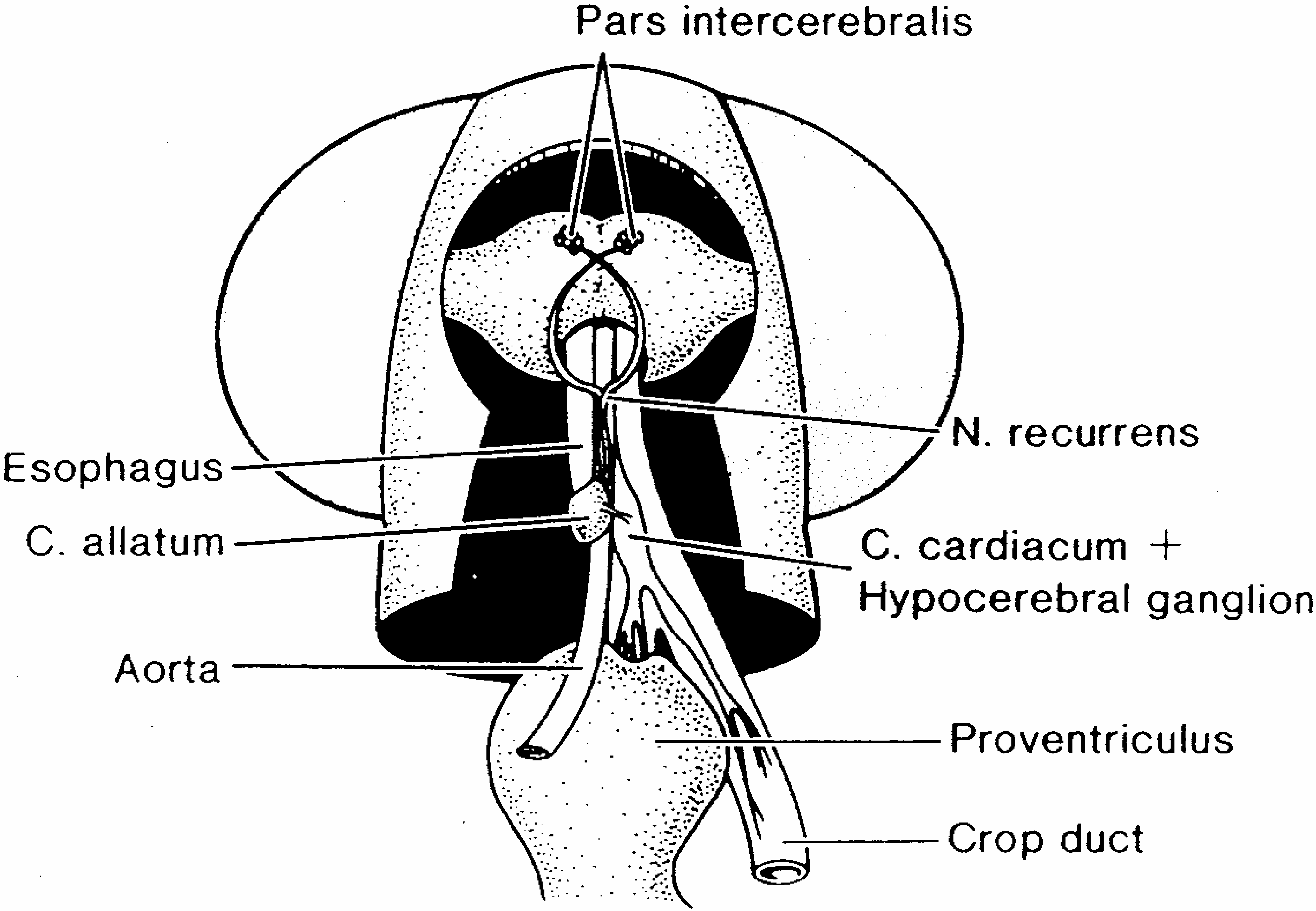


Neurosecretory cells in the pars intercerebralis
Brain
Nerves from the brain to the corpora cardiaca and allata
Recurrent nerve
Hypocerebral ganglion
Corpus cardiacum
Corpus allatum

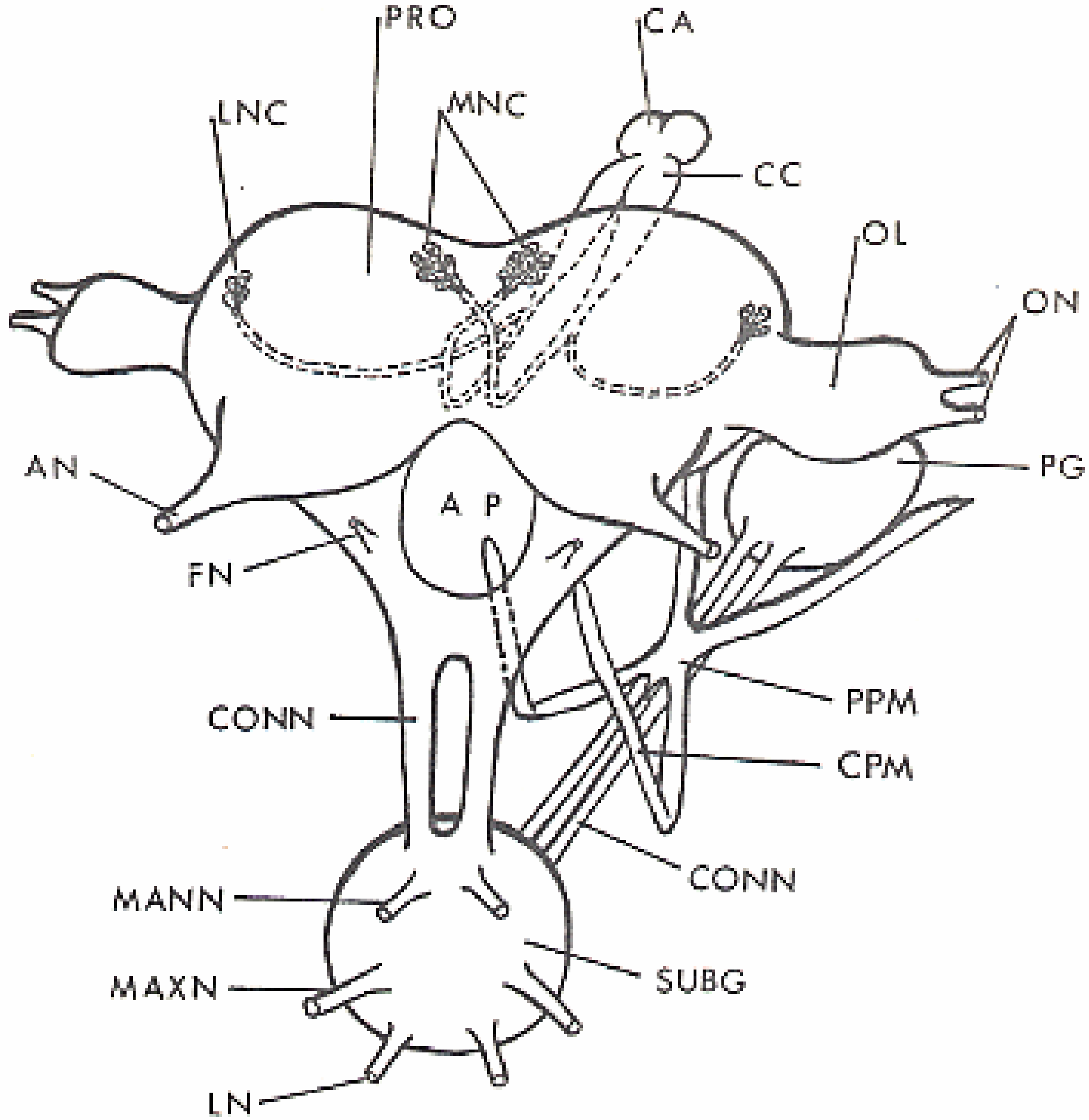
Whole mount of nervous/endocrine systems of gypsy moth larva



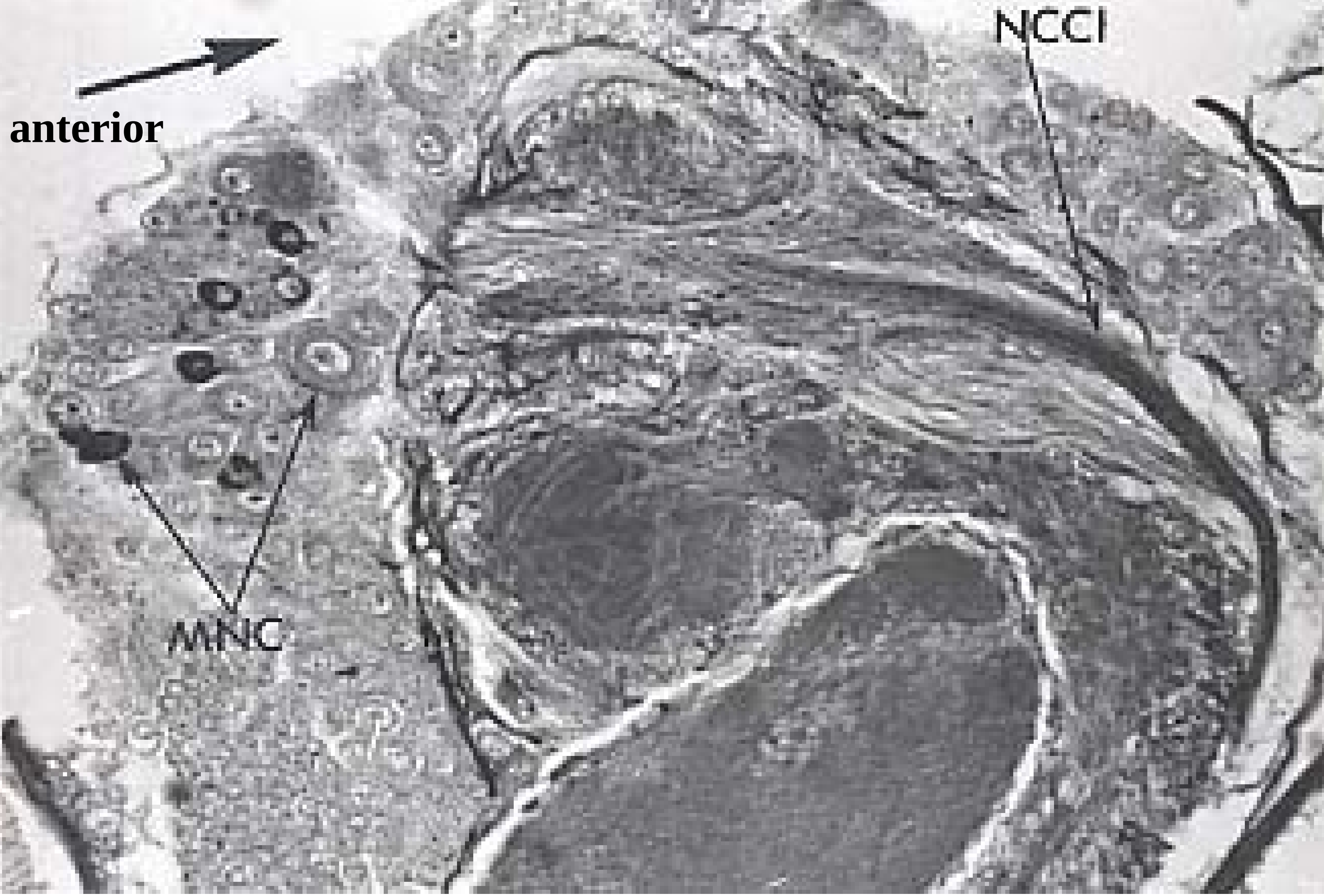
Diptera


Pars intercerebralis
N. recurrens
Esophagus
C. allatum
C. cardiacum +
Hypocerebral ganglion
Aorta
Proventriculus
Crop duct

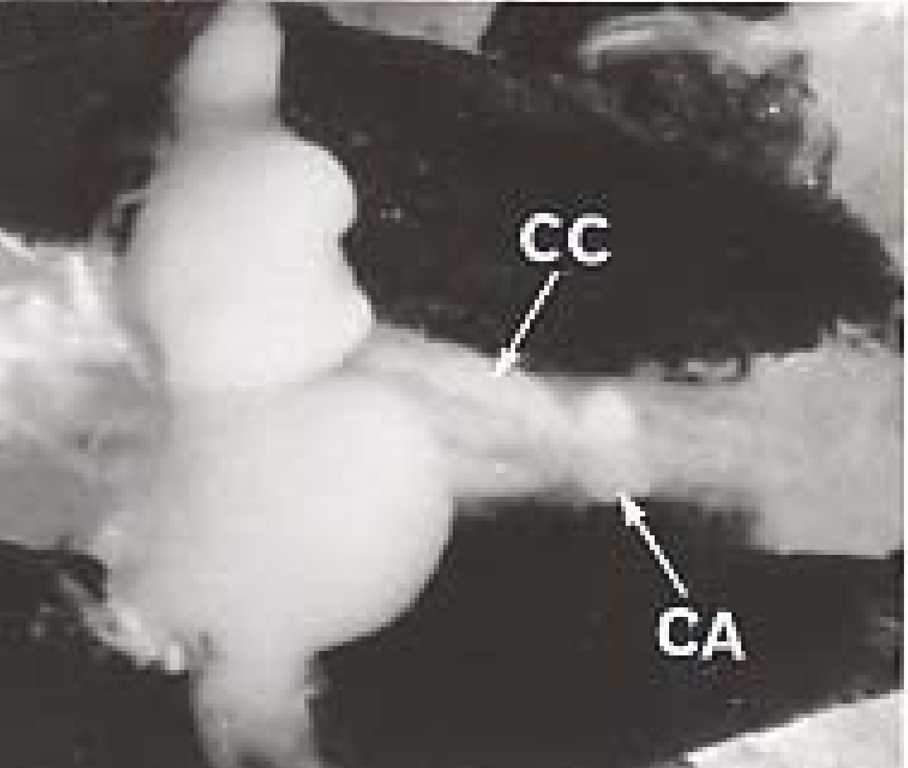
LNC=lateral neurosecretory cell
PRO=protocerebrum
MNC-median neurosecretory cell
CA=corpus allatum
OL=Optic lobe
ON=Optic nerve
PG=prothoracic gland
CPM=cephaic portion of the PG
CONN=Connectives
SUBG=suboesophageal gland
LN=labial nerve
MAXN=macillary



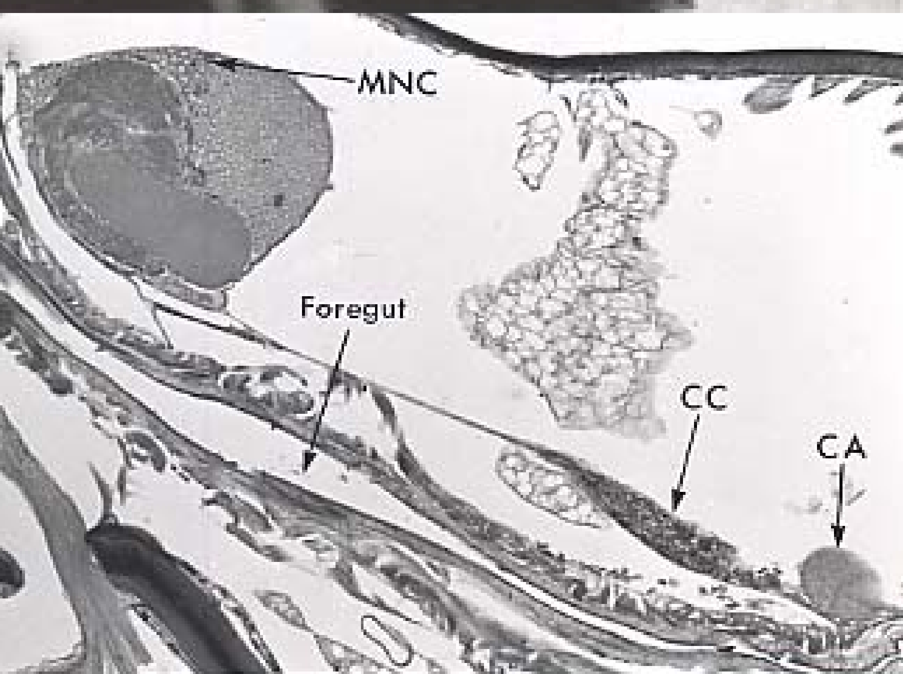
Endocrine glands in relation to head central nervous system in *Zootermopstis angusticollis*. From Yin. 1972. PhD. Disseration

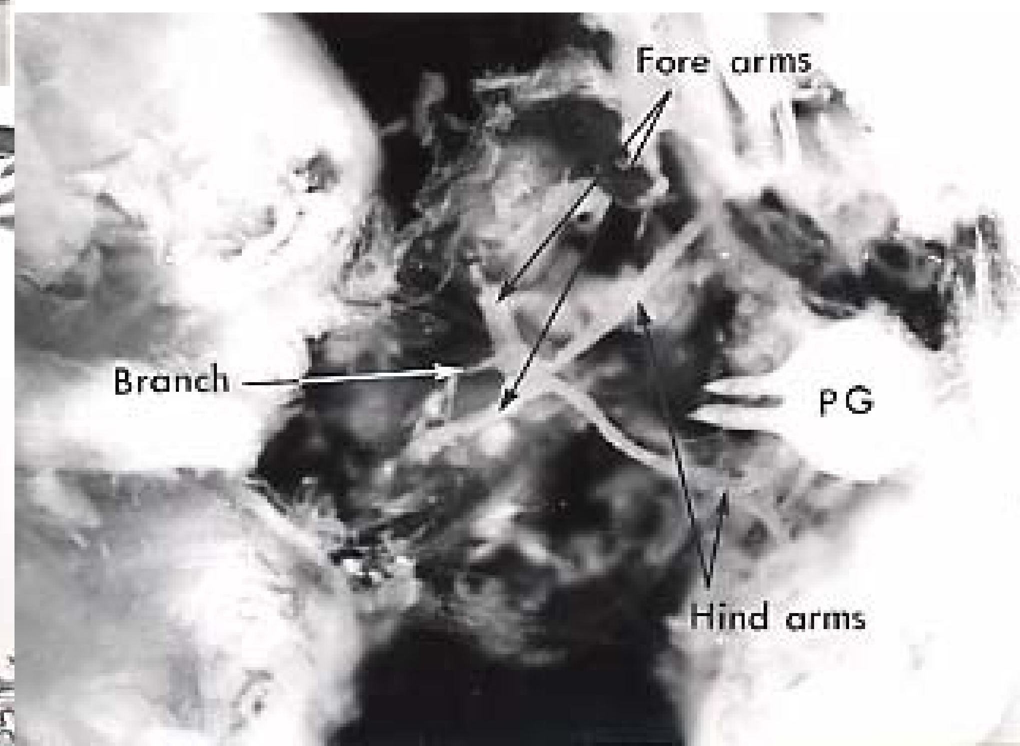


Longitudinal section through pars intercerebralis of 8th instar female termite larva showing the median neurosecretory cells and their axons using a PAF stain. NCCI=nervus corporis cardiacum interni

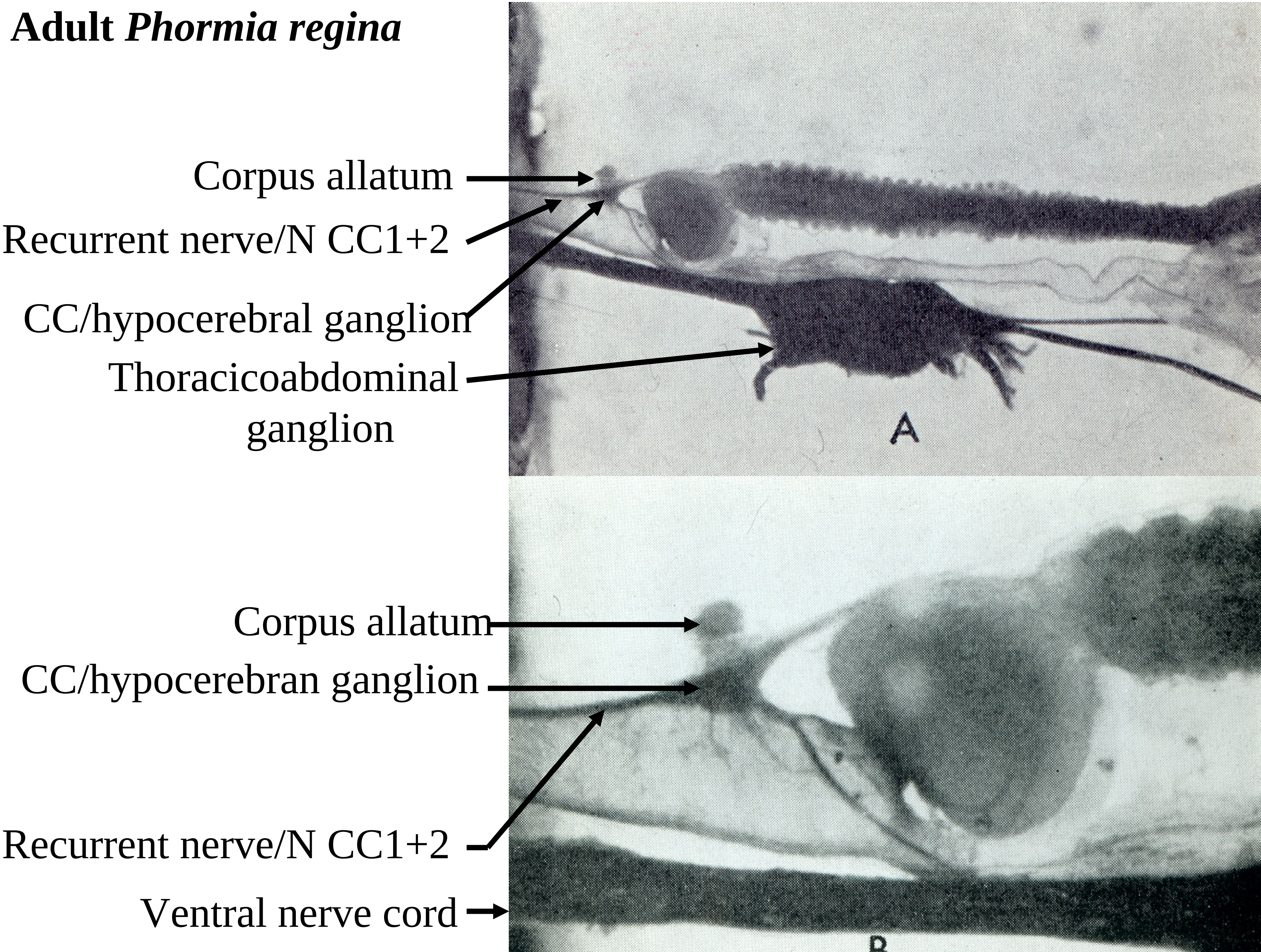


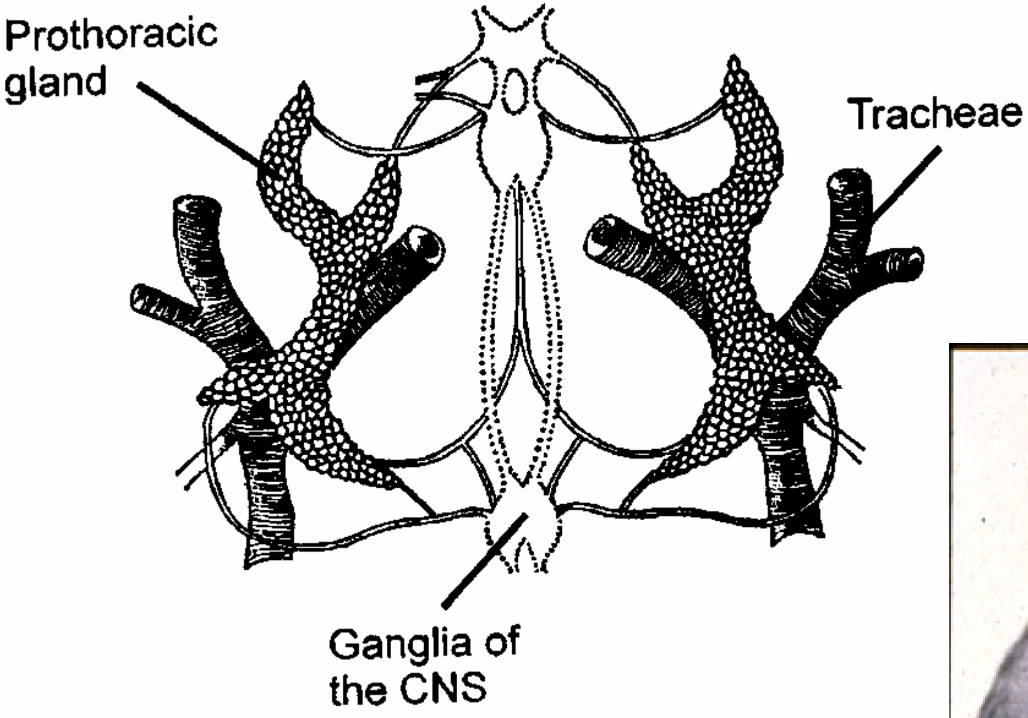
Whole mount on left and histological section showing the CA, CC, MNC and brain. Below is a whole mount of a 7th instar male *Zootermopsis* showing the arms and branches of the ecdysial gland, also known as the prothoracic gland. PG is the prothoracic ganglion.



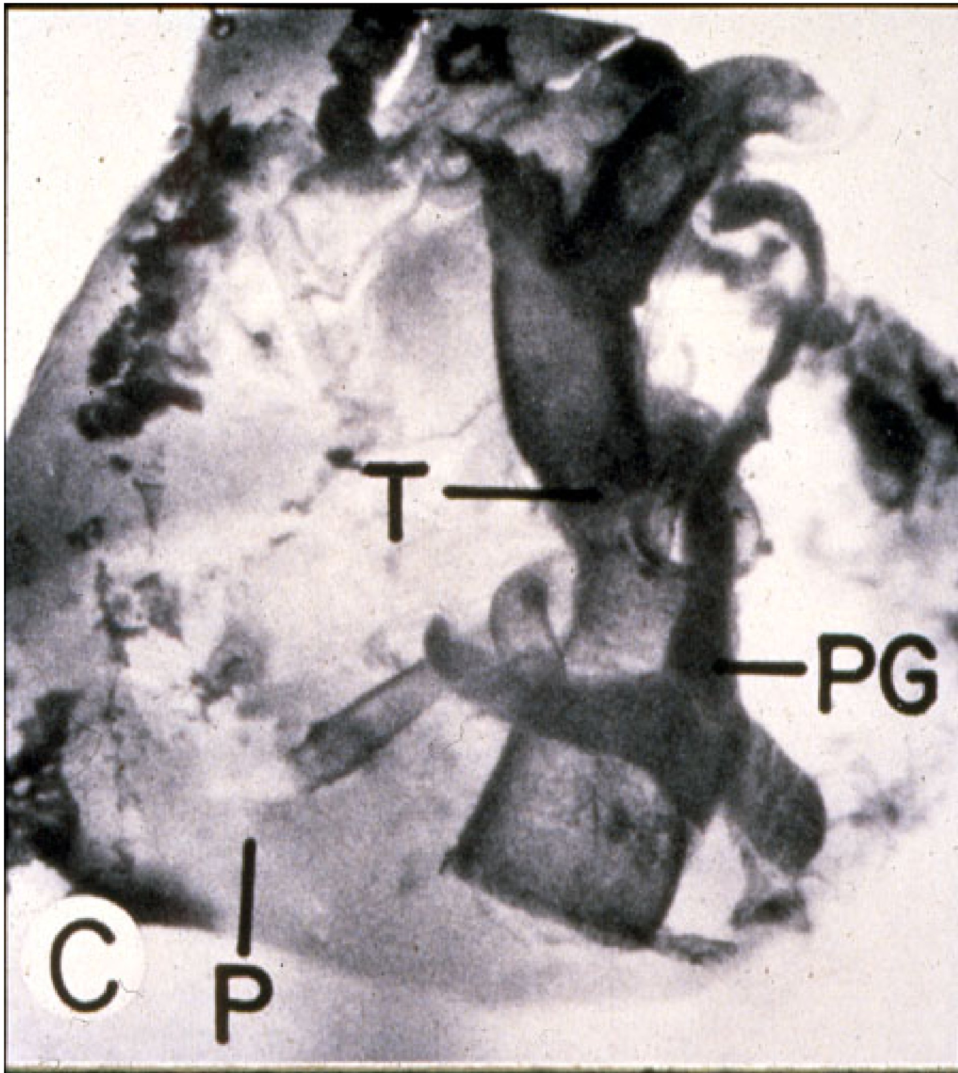


Adult *Phormia regina*





Prothoracic glands of Lepidoptera.
PG=prothoracic gland
T=trachea



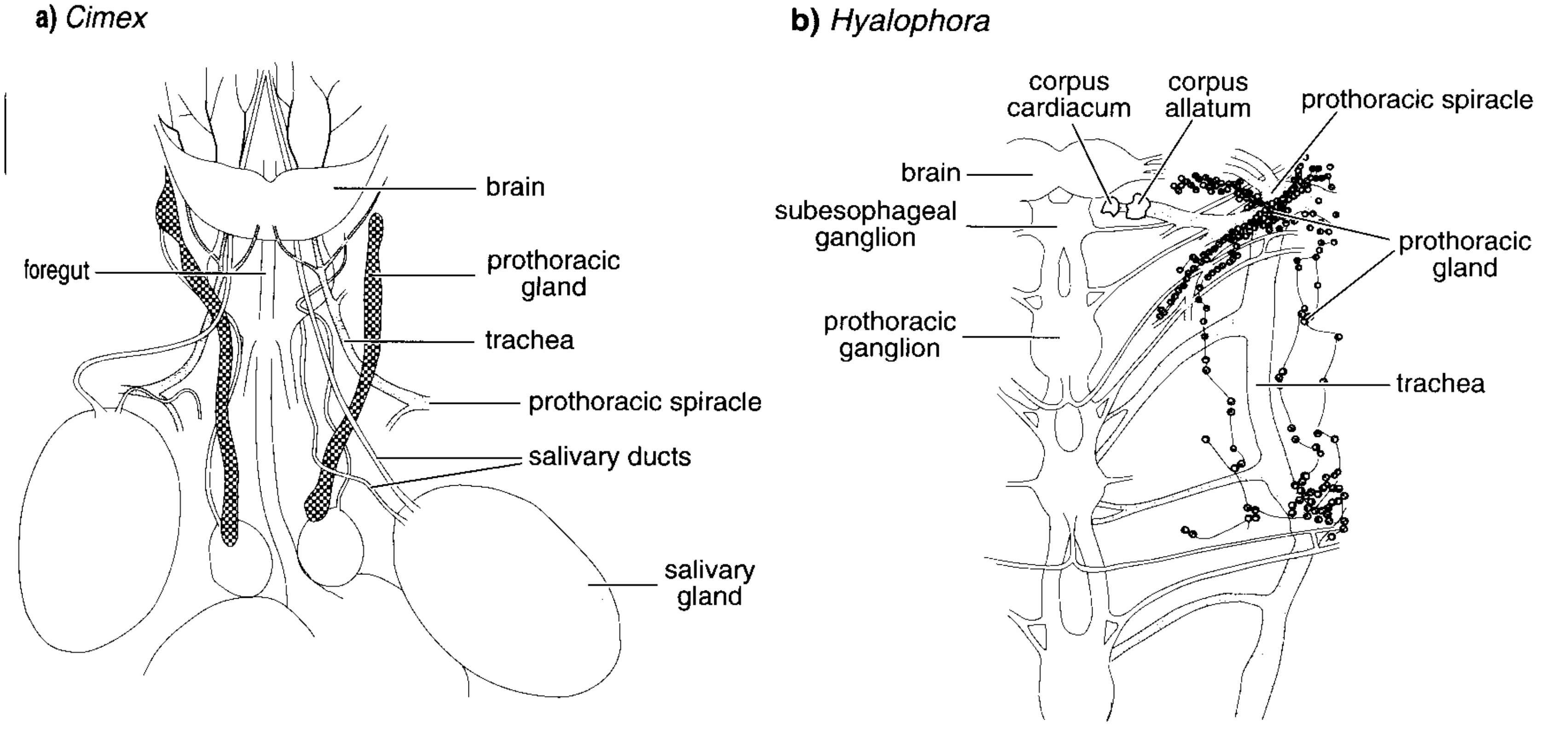
REMEMBER-What happens to the ecdysial or prothoracic glands in almost all adult insects?

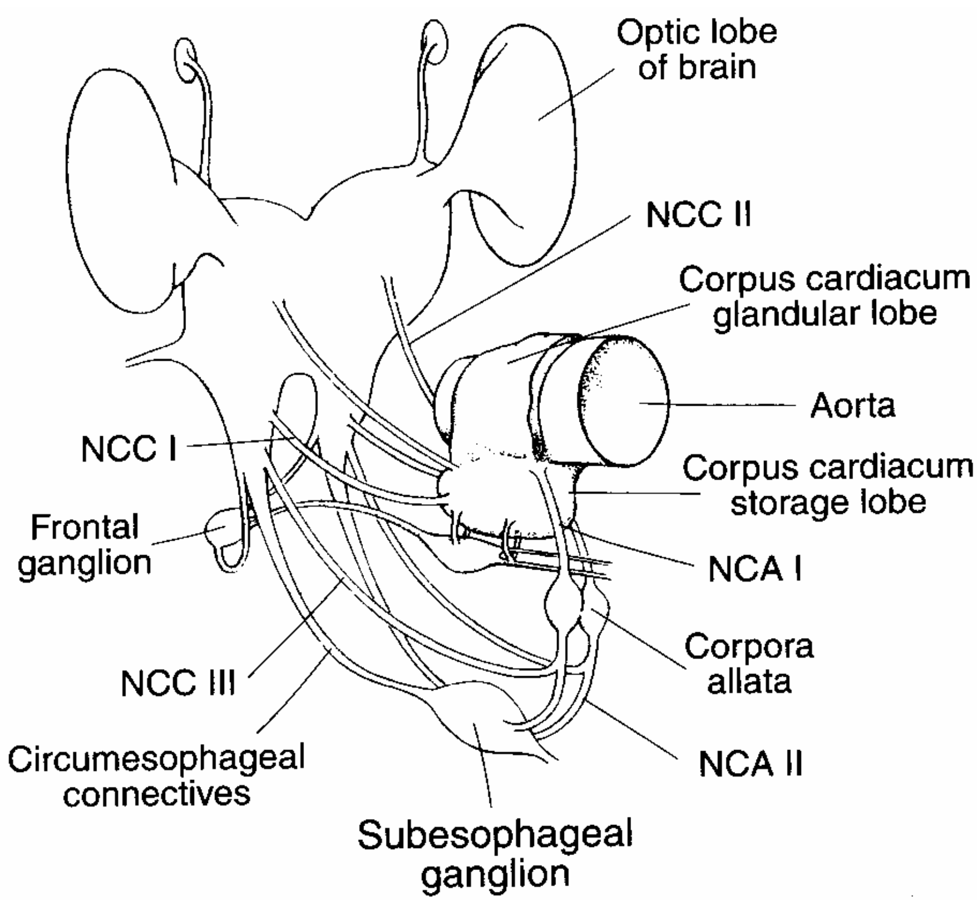
What group do they remain during adulthood and keep on molting?

The Apterygota-especially the Thysanura

What signal is essential for their destruction or histolysis?

The absence of JH hormone



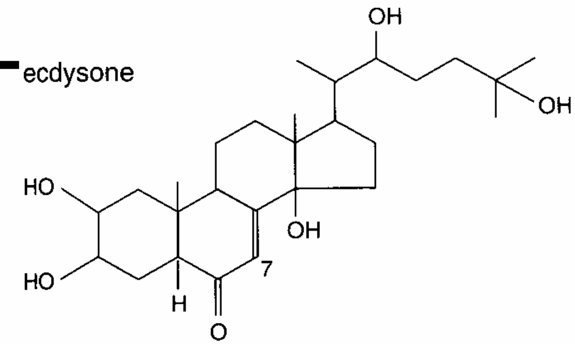

Optic lobe
of brain
NCC II
Corpus cardiacum
glandular lobe
Aorta
NCC I
Corpus cardiacum
storage lobe
Frontal
ganglion
NCA I
Corpora
allata
NCC III
Circumesophageal
connectives
NCA II
Subesophageal
ganglion

ECDYSTEROIDS-

1. Ecdysone is a steroid hormone
2. Insects cannot synthesize sterols, they must get them in their diet (cholesterol or phytosterol)

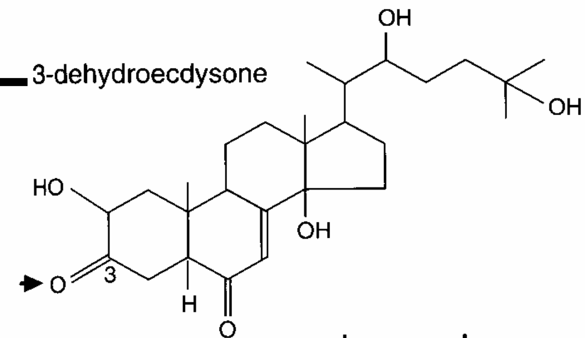
Ecdysone is the most common ecdysteroid produced in insects

ecdysone



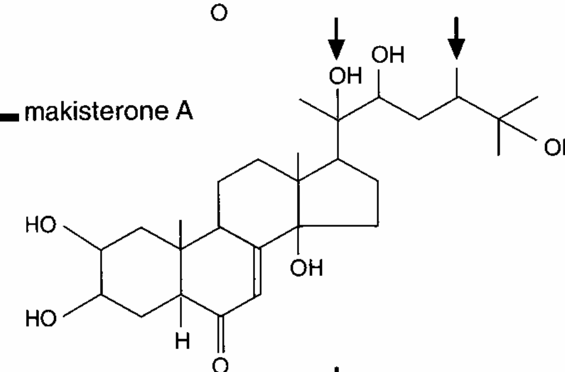
Some larval leps. Use 3-dehydroecdysone that is coverted to ecdysone by enzymes in the hemolymph

3-dehydroecdysone



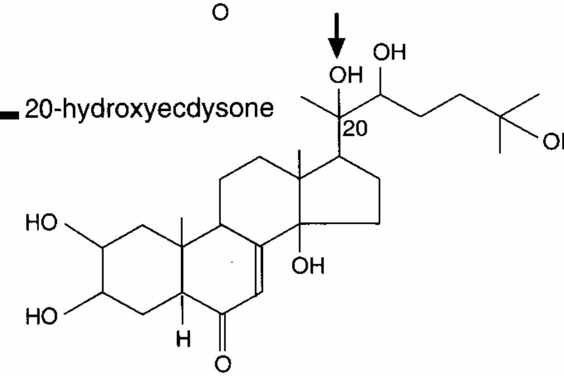
In the honeybee and Heteroptera, makisterone is the main ecdysteroid produced and used

makisterone A



In the Diptera is release from the ovaries and stimulates the fat body to produce vitellogenin. Ecdysone converted to 20-hydroxyecdysone in fat body.

20-hydroxyecdysone



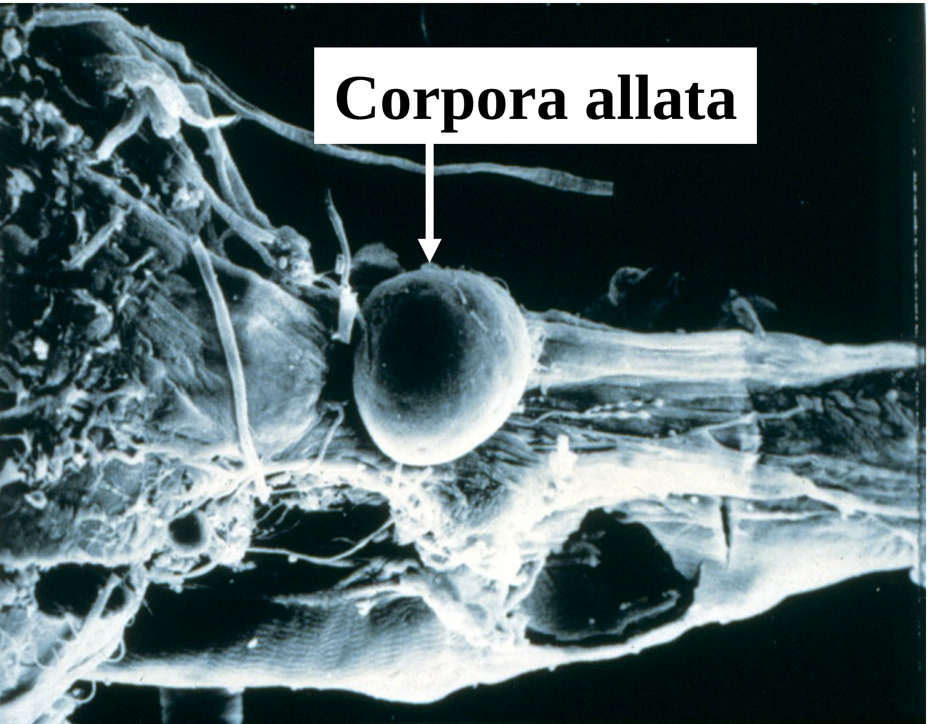
JUVENILE HORMONES

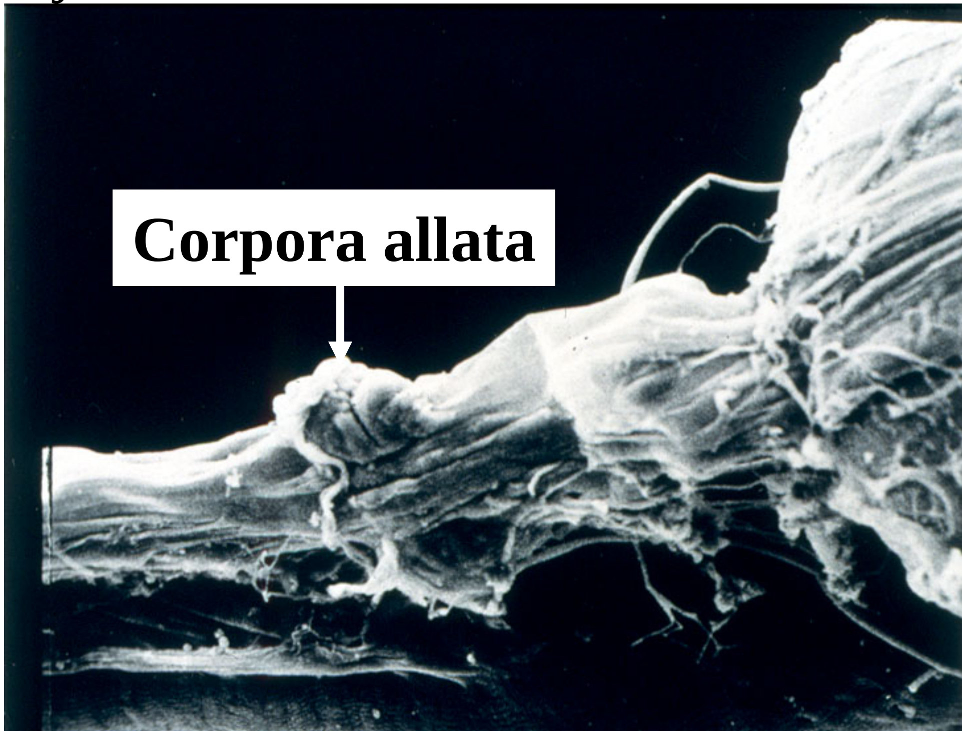
Are sesquiterpenes produced by the corpora allata. Several different forms have now been discovered (see next slide).

Analogues of JH, especially methoprene have been successfully used in insect control. Used against mosquito larvae and fleas (Siphotrol).

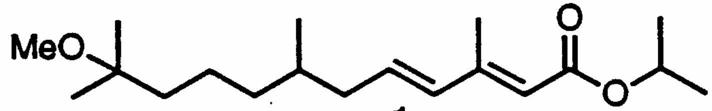
Precocene-from the common bedding plant, ***Ageratum houstonianum***

An extract of this plant produced a chemical that selectively kills the cells of the corpora allata, thus no JH is produced. Below on the left is a photo showing the healthy, normal CA in adult *Phormia regina* while on the right is the effect of precocene injection. Notice CA size.

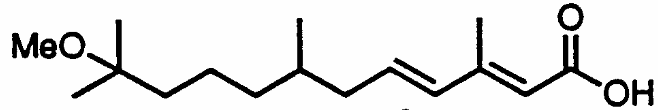




Methoprene is a JH analogue and is used in insect control and experiments

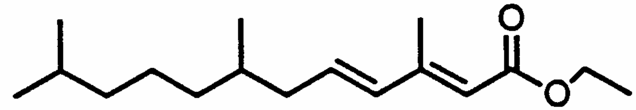


1 Methoprene

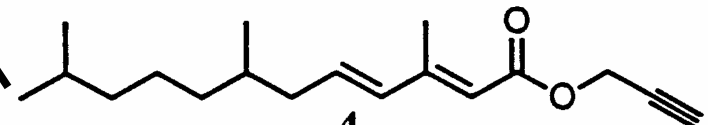


2 Methoprene acid

Hydroprene and kinoprene are JH analogues and are used in insect control

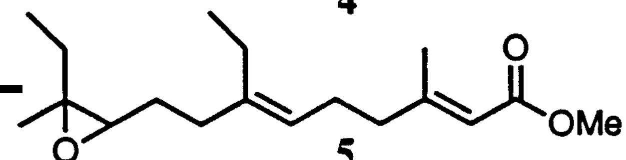


3 Hydroprene

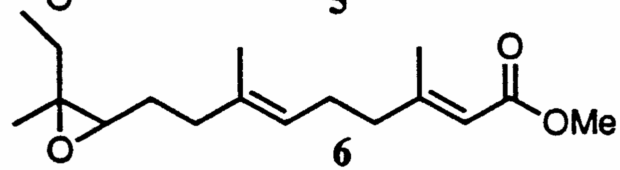


4 Kinoprene

JH I is found mainly in the Lepidoptera

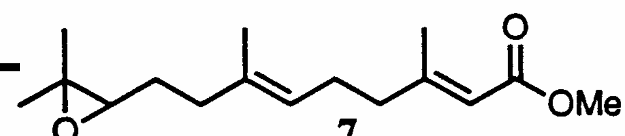


5 Juvenile Hormone I (JH I)

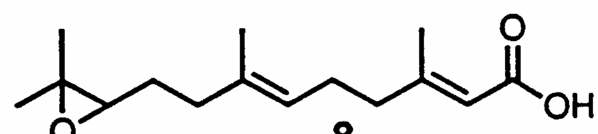


6 JH II

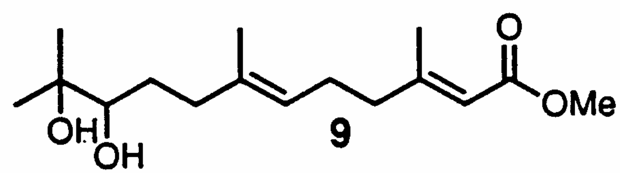
JH III was discovered by Dr. Yin (tell story about its discovery, etc.). Found mainly in the Cyclorrphaphous dipterans.



7 JH III



8 JH III acid



9 JH III diol

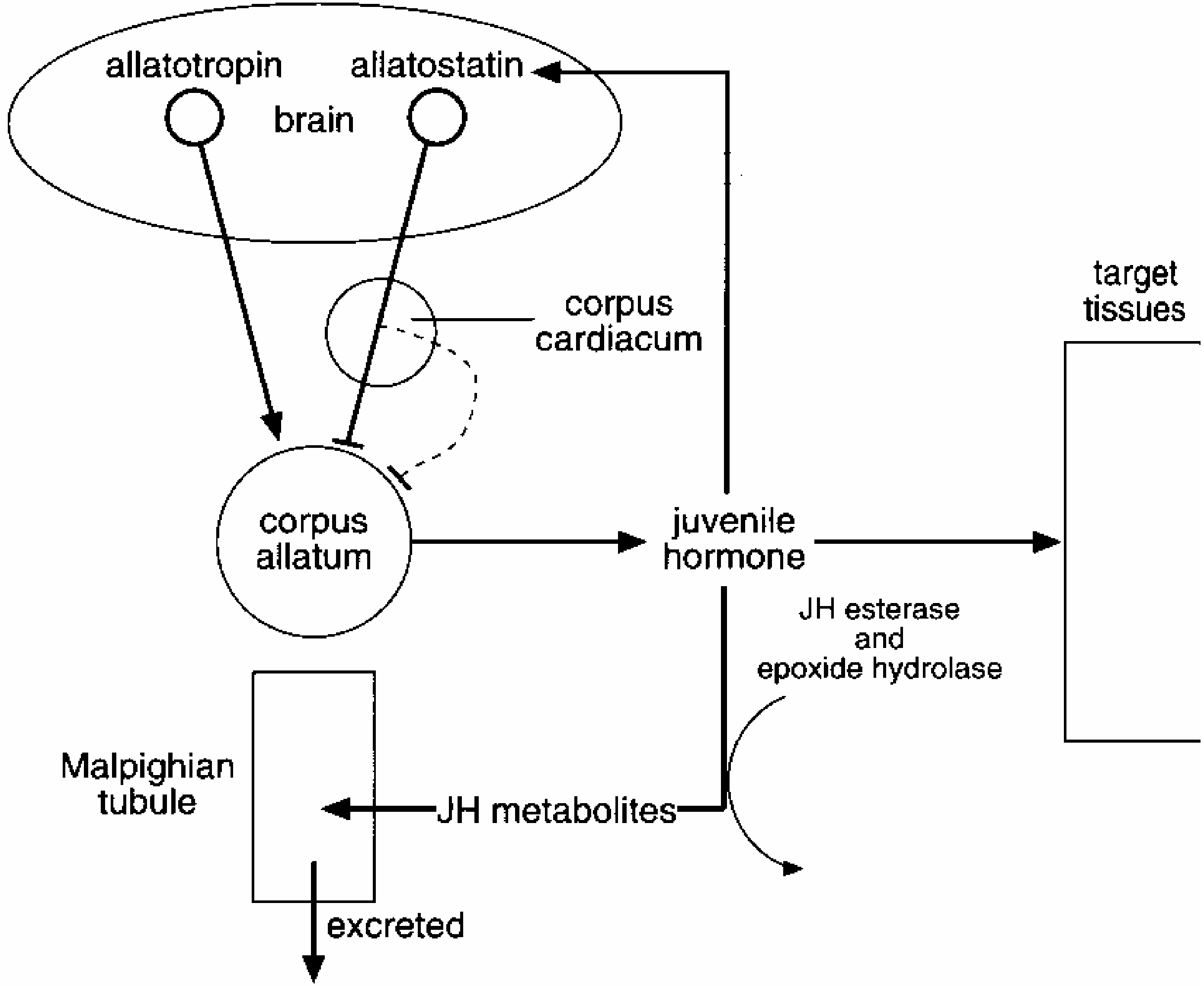
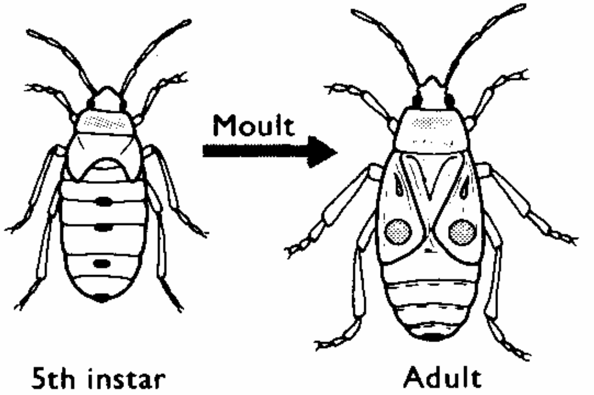
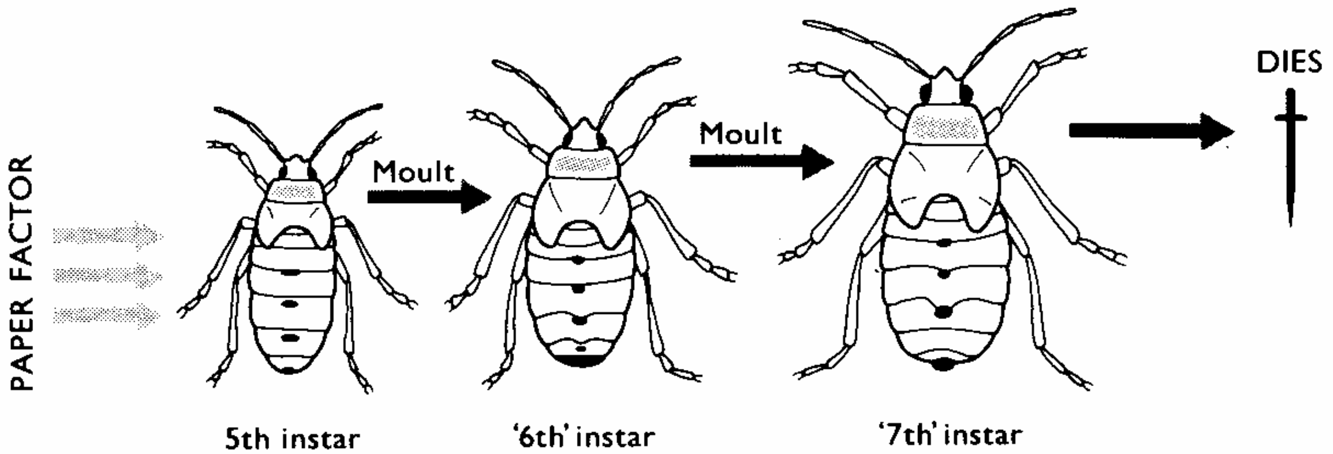


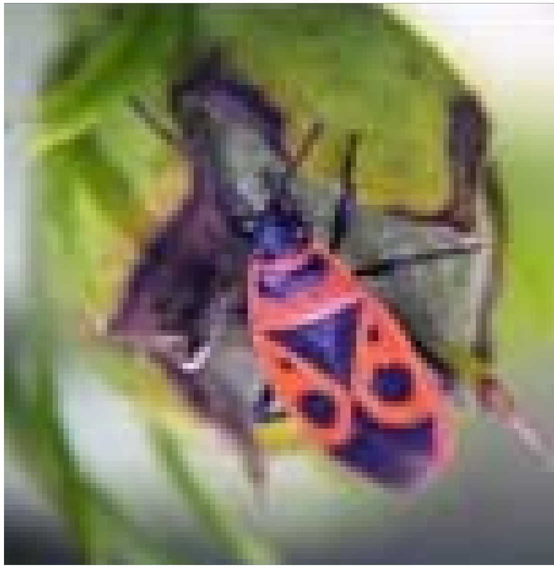
Fig. 21.11. Juvenile hormone. Regulation of hemolymph titer involves the balance between synthesis in the corpora allata and degradation and excretion by the Malpighian tubules.

PAPER FACTOR

1. **Beginning of idea for using hormone analogs to control insects-Zoecon started**



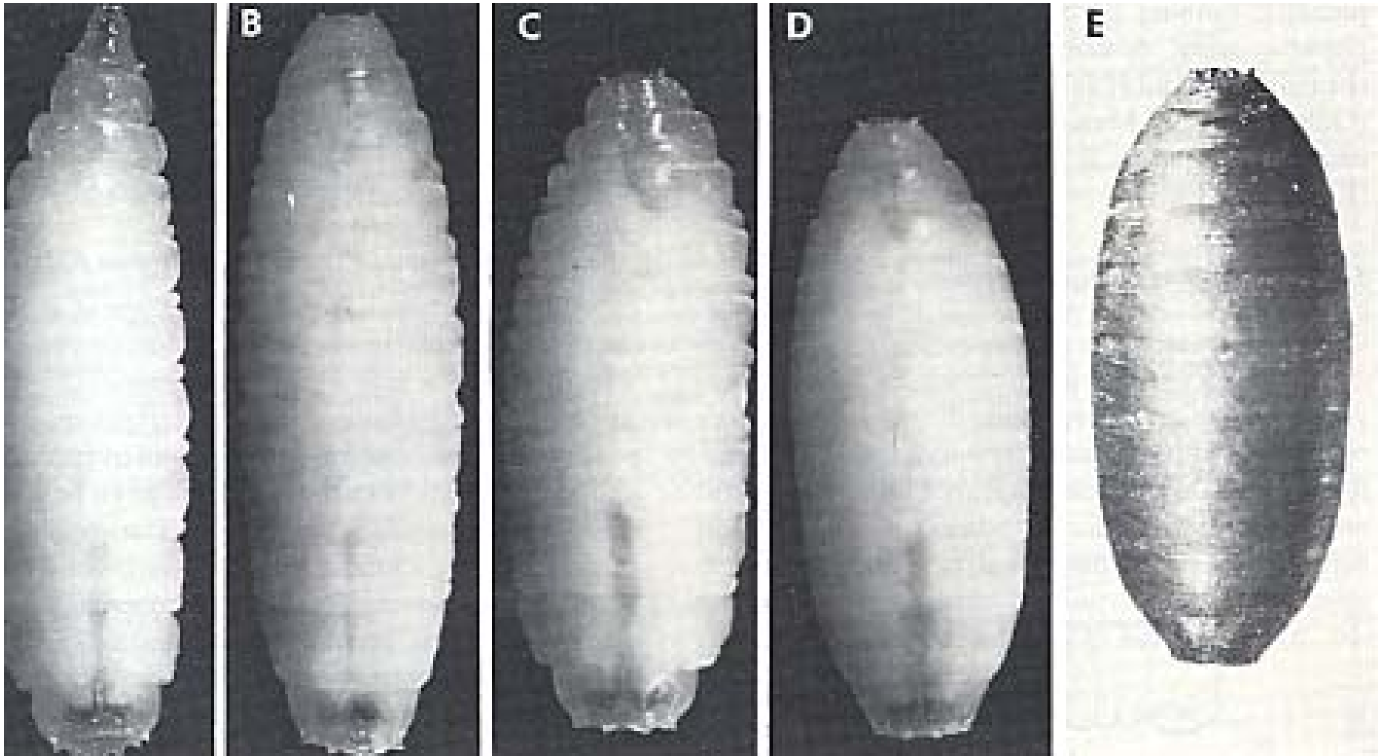




Carroll Williams and John Law getting the paper factor from brown paper towels

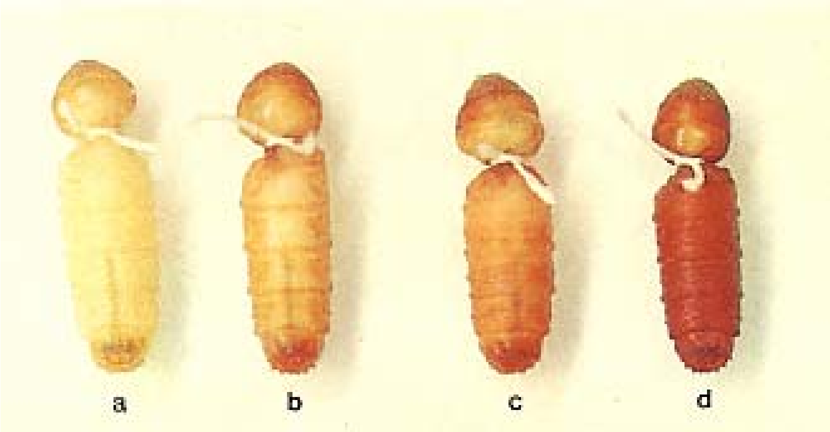
PUPARIATION IN THE CYCLORRHAPHOUS DIPTERA-

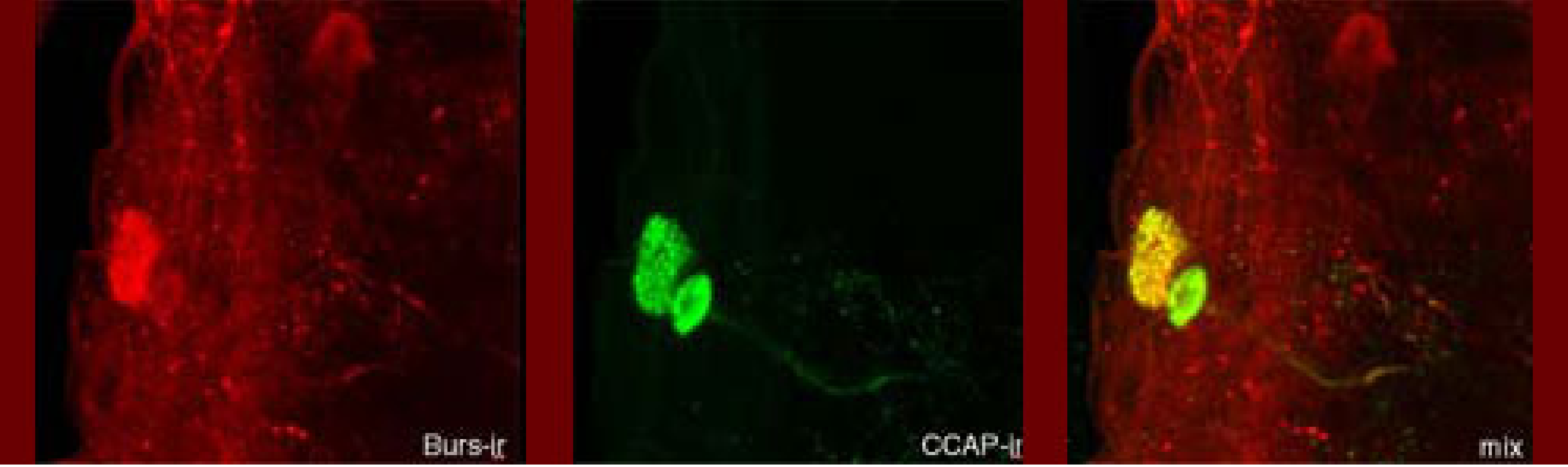
1. Cuticle of last larval instar becomes the pupal case or puparium.
2. Injecting JH into the last larva does not lead to a supernumary larva like in some other insects
3. What causes the tanning and darkening of the cuticle?



BURSICON-Neurosecretory hormone that controls tanning (sclerotization) and mechanical properties of the cuticle during and after a molt. Found in most ganglia of the CNS.

1. Cuticle of newly emerged adult fly is soft and plasticized.
2. Soon, however, it becomes smooth and rigid due to the tanning process and also it becomes darkened due to the melanization process.
3. Ecdysone or eclosion hormone causes the release of bursicon, which is the neurohormone that causes both plasticization and melanization.





Identification of the nerve cells in the abdominal ganglion of cockroaches using an antibody against bursicon. The nerve cells which contain bursicon also produce another hormone, called crustacean cardioactive peptide or CCAP. CCAP is involved in triggering the motor activity that allows the animal to crawl out of its old cuticle. Bursicon is labeled in red and CCAP is labeled in green. The two figures are overlaid to show that CCAP and bursicon are both in the same nerve cell.

Vertebrate hormones in insects-

Insulin-like peptide. In 1975 T. Normann suggested that an insulin-like activity in decapitated blowfly, *Calliphora*, was due to a lack of a hypotrehalosemic hormone of cephalic origin. He suggested it the neuroendocrine gland complex, the corpus cardiacum-corpus allatum was the likely site for its release. Chen and Friedman (1977) also made a similar assumption using *Phormia regina* and pointed to the CC-CA as the site of the compound.

OTHER VERTEBRATE PEPTIDES ARE BEING FOUND IN INSECTS

In Press. Neurons of the Adult Queen Blow Fly, *Phormia regina* (Meigen)(Diptera: Calliphoridae) Reacted Positively to Antisera Against the Mosquito Ovary Ecdysteroidogenic Hormone I and the Fruit Fly Insulin Receptor
HEPING LIN[1], CHIH-MING YIN[1], JOHN G. STOFFOLANO, JR.[1], AND ROBERT S. GAROFALO[2]

Cao, C.; Brown, M.R. 2001. Localization of an insulin-like peptide in brains of two flies. Cell Tissue Res. 304: 317-321.

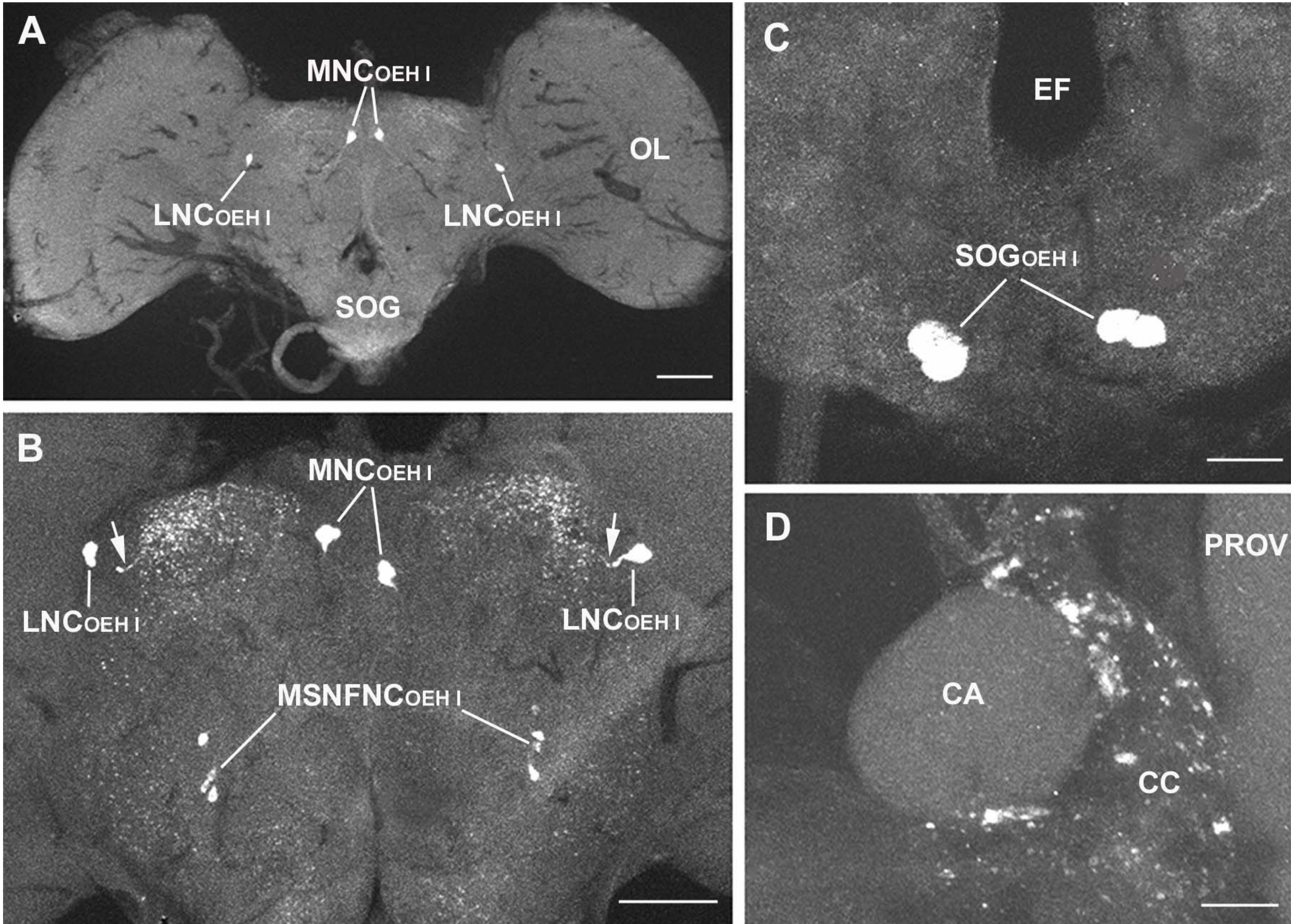
Chen, C.; Jack, J.; Garofalo, R.S. 1996. The *Drosophila* insulin receptor is required for normal growth. Endocrinology 137: 846-856.

Ruan, Y.; Chen, C.; Cao, Y.; Garofalo, R.S. 1995. The *Drosophila* insulin receptor contains a novel carboxyl-terminal extension likely to play an important role in signal transduction. J. Biol. Chem. 270: 4236-4243.

Rulifson, E.J.; Kim, S.K.; Nusse, R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296: 1118-1120.

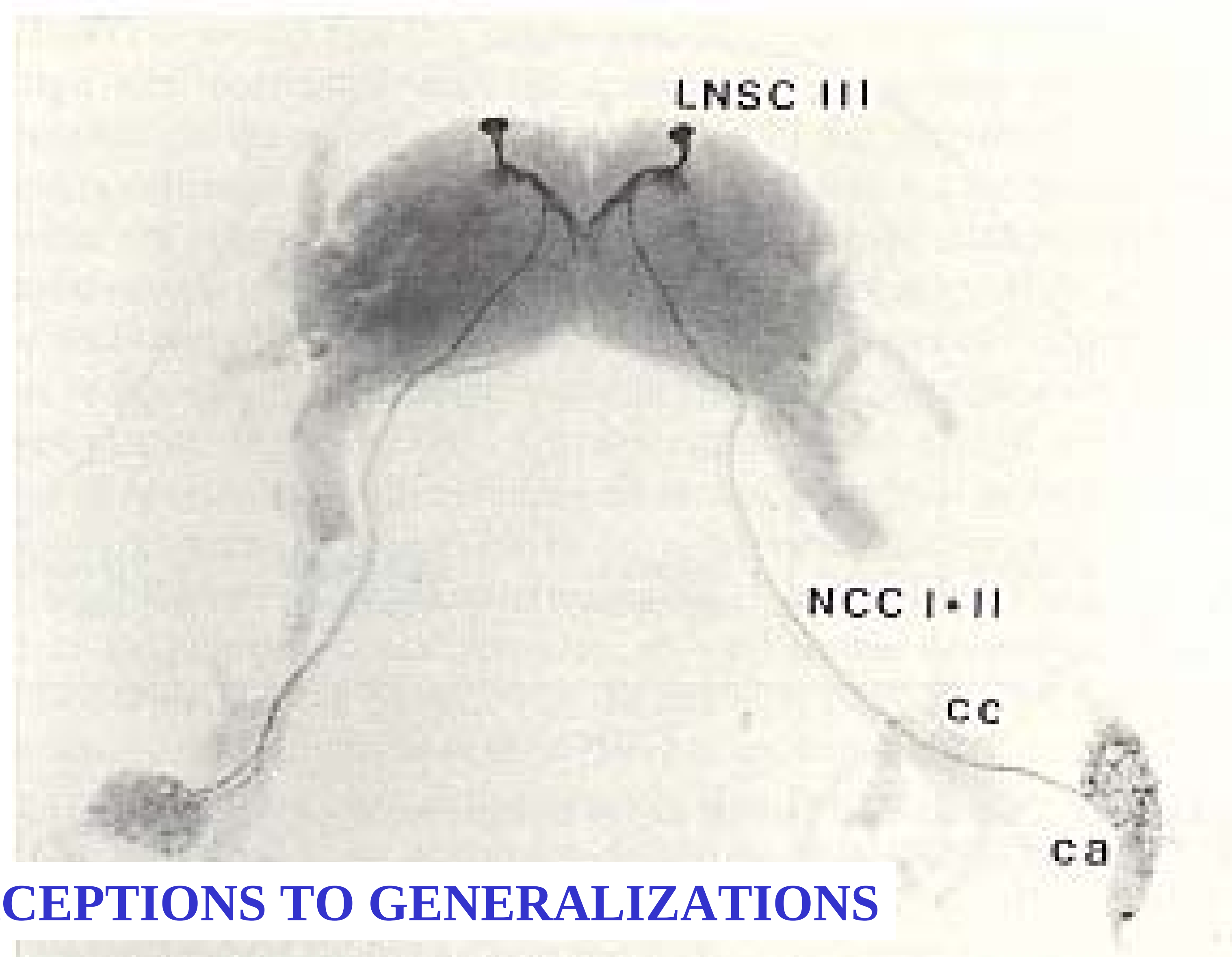
RULIFSON ET. AL. SUGGESTED THE RECEPTOR IS IN THE CC/CA COMPLEX BUT NOW SHOWN (SEE NEXT SLIDE)

Brain of *Phormia regina* showing the presence of *Aedes aegypti* ovary ecdysteroidogenic hormone I (OEH) in the brain of female (A), male (B), and (C) suboesophageal ganglion of the female. Fig. D shows the insulin-receptor immunopositive signalds in the CC-CA complex of female.



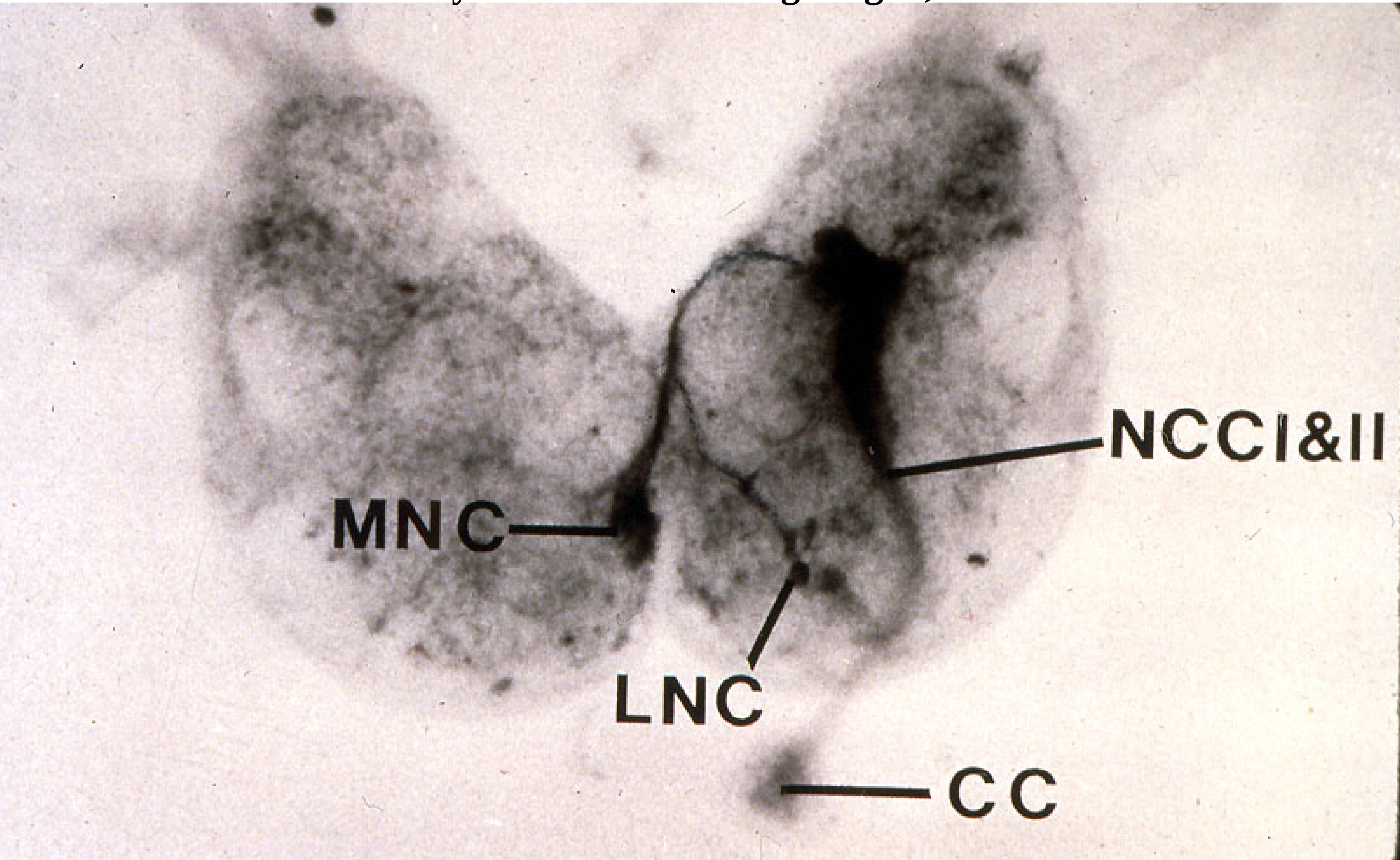
PTTH-Used an immunohistochemical stain with an antibody against PTTH. PTTH is produced in the LNSC III cells in the brain. Travels down the NCC I+II through the CC and is released from the CA

5th instar of *Manduca Sexta*. In most insects the CC is the release site of PTTH but, in Leps., it is the CA where release occurs.



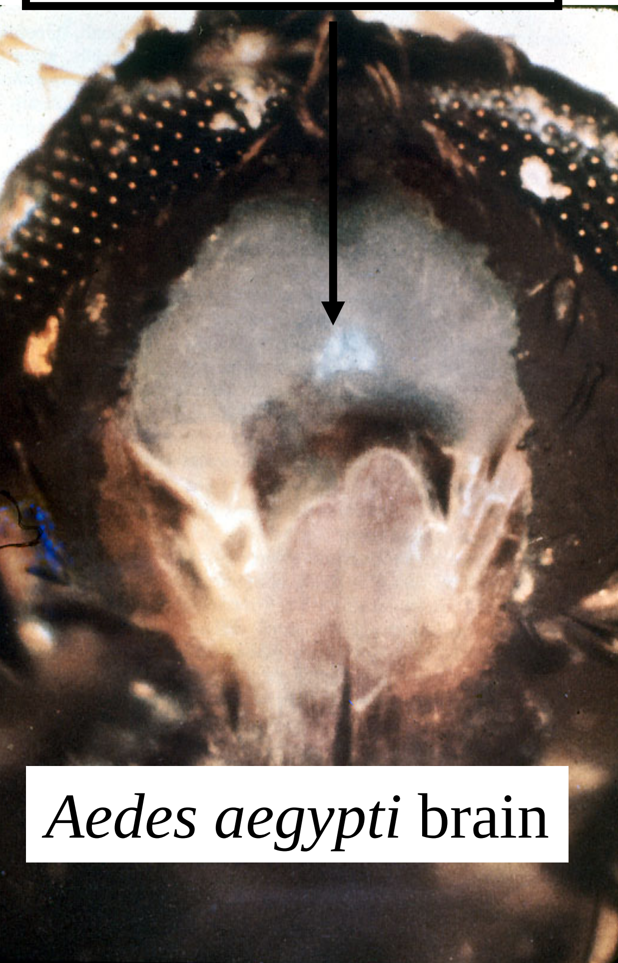
ALWAYS EXCEPTIONS TO GENERALIZATIONS

Cobalt backfilling of 6th instar larva of gypsy moth showing the median neurosecretory cells (MNC), lateral NSC (LNC), the nervi corpora cardiaca (NCCI&II) and the corpus cardiacum (CC). Note the passage of the dye between the neurosecretory cells and the storage organ, the CC>



Neurosecretory cells-Specialized cells that are both nervous and secretory. Identified basically by specific staining techniques and electron microscopy. In transmitted light they appear blue because of the Tyndall effect of light scattering due to the fine droplets of neurosecretion found in the cells. The droplets are electron dense (See below right photo)

Tyndall blue effect



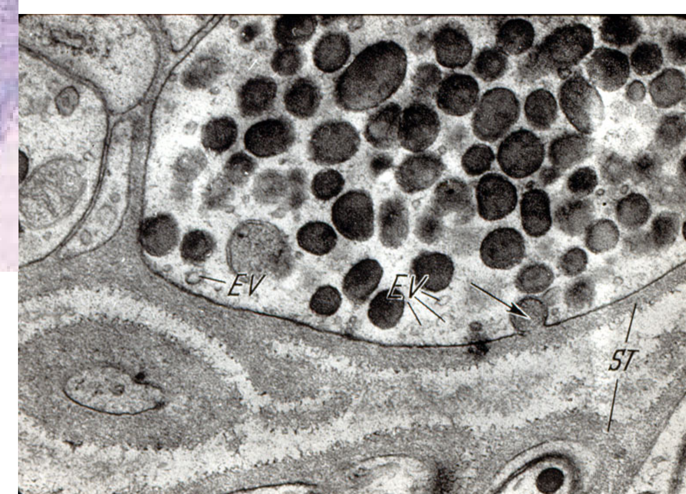
Aedes aegypti brain

Medial neurosceretory cells

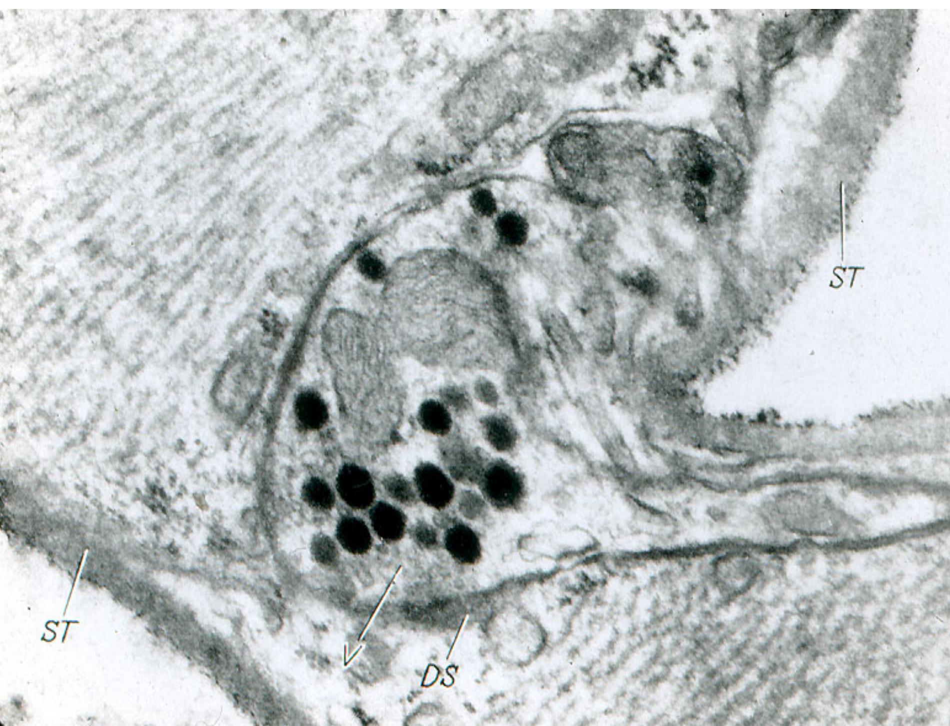
Paraldehyde fuschin

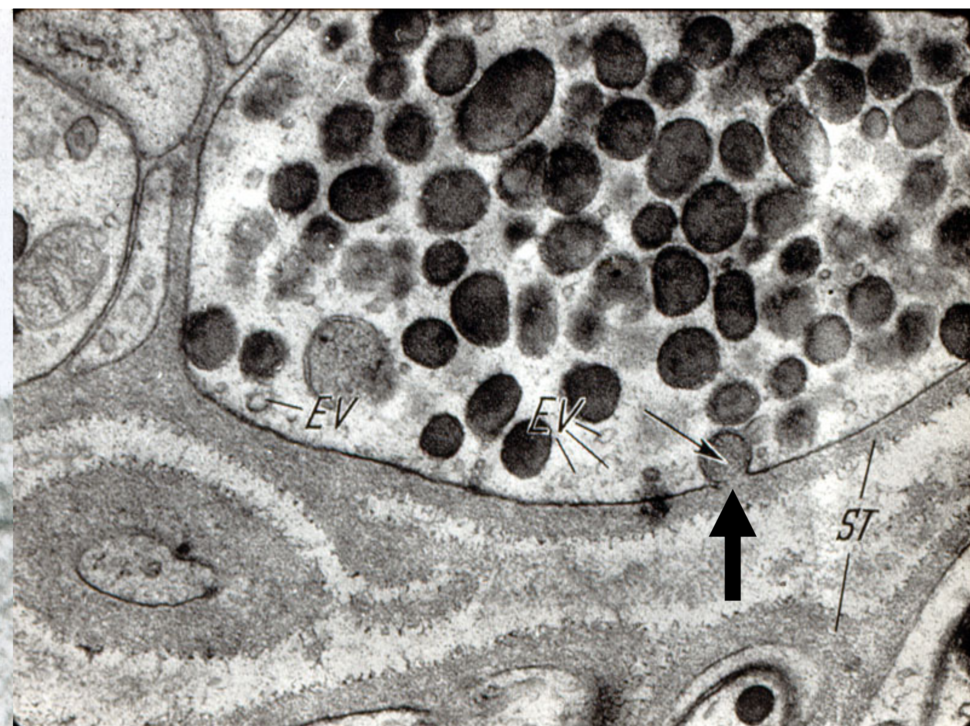
Brain of *Phormia regina*

Electron microscopy

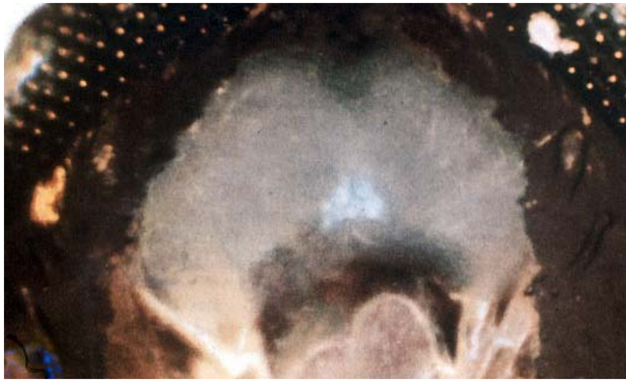


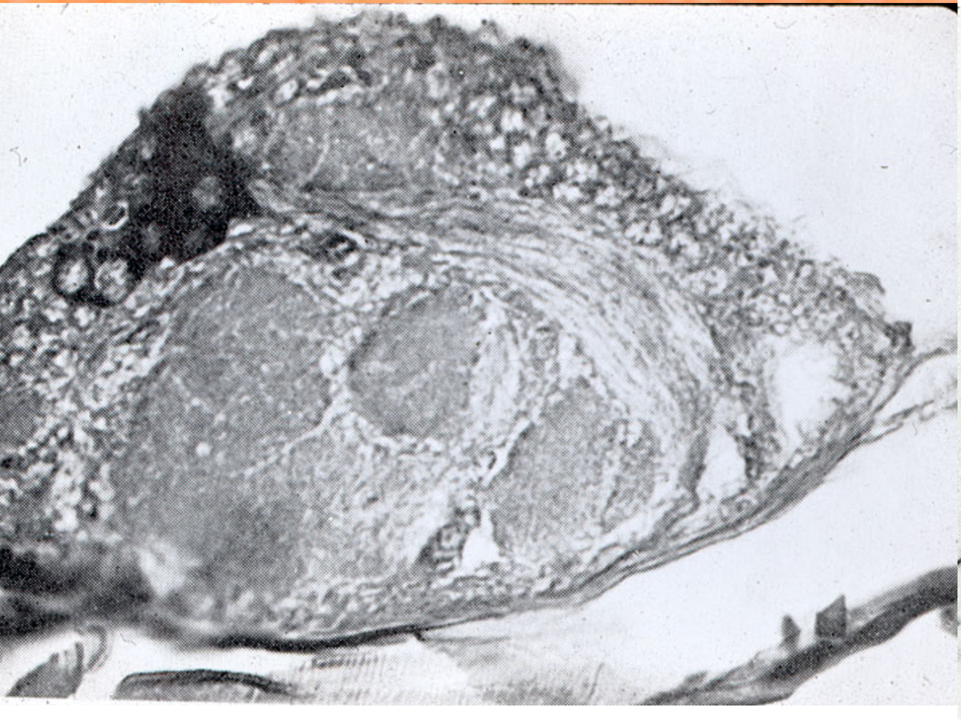
Neurosecretory cells can release their secretion directly onto tissue, as seen in bottom left with the material being released onto heart muscle. Release occurs as a result of fusion of the membrane of the vesicles of neurosecretion with the membrane of the cell through exocytosis (see bottom right bold arrow).

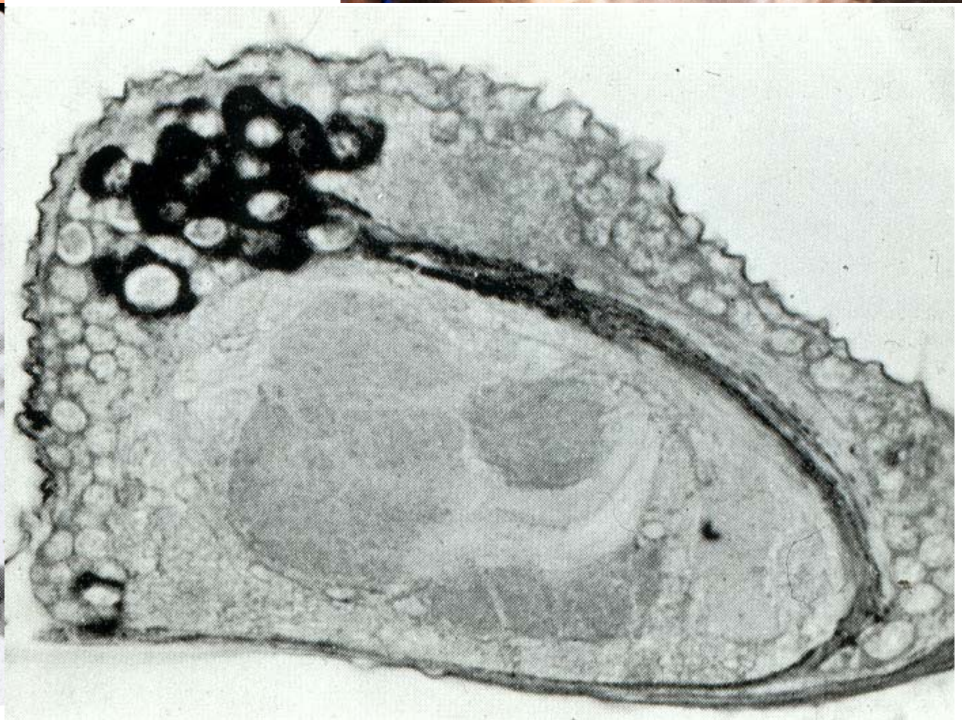




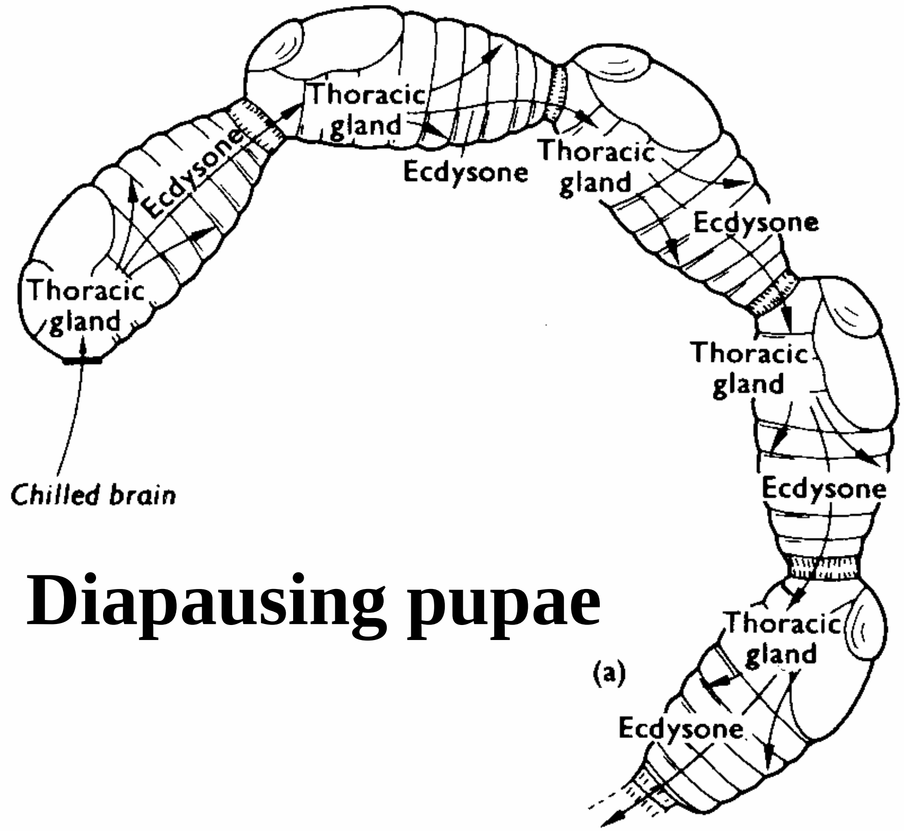
Brain neurosecretory cells in *Aedes aegypti*. Seen as blue cells because of Tyndall blue effect caused by droplets of neurosecretion. Note bottom left cells, using PAF to show no release but in bottom right you can see it in the axon.

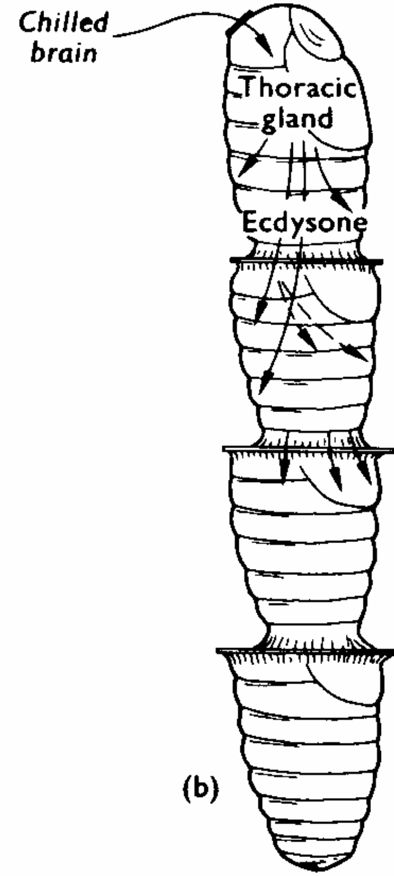




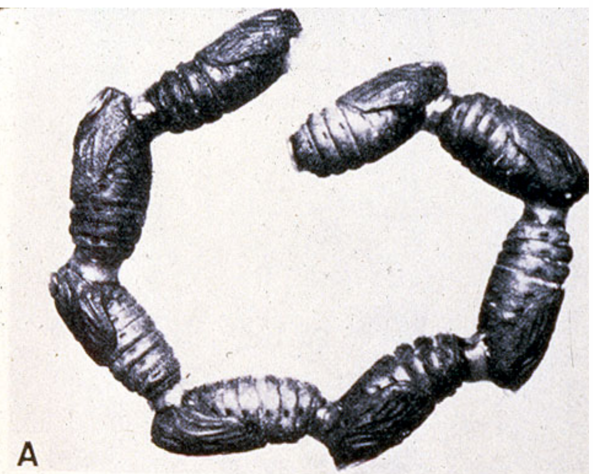


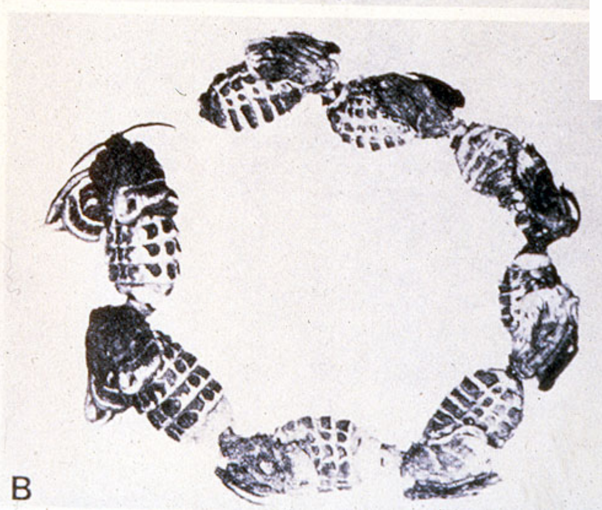
THE BRAIN HORMONE

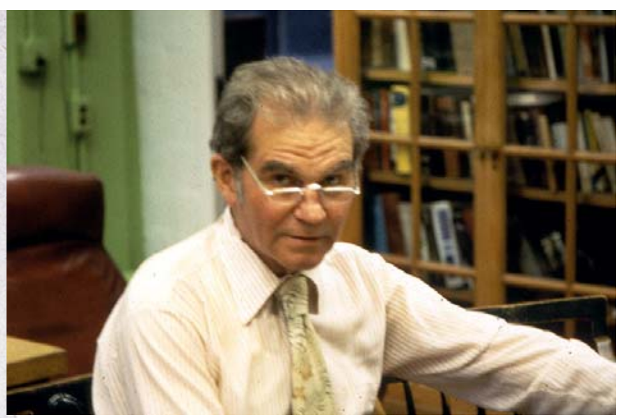


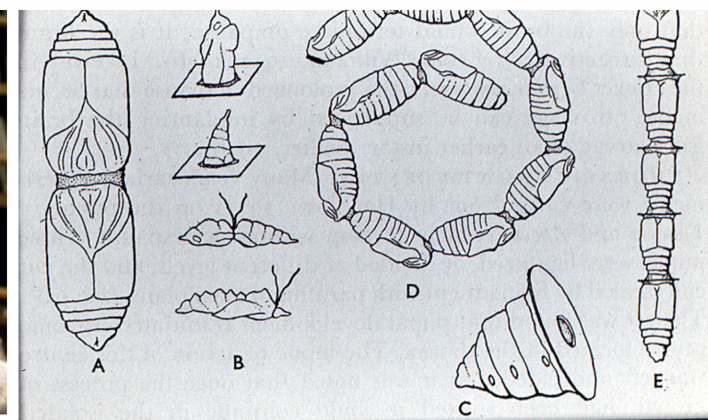


Diapausing pupae









Transport of hormones in hemolymph-

Ecdysteroids are relatively insoluble in water. Transported by binding proteins

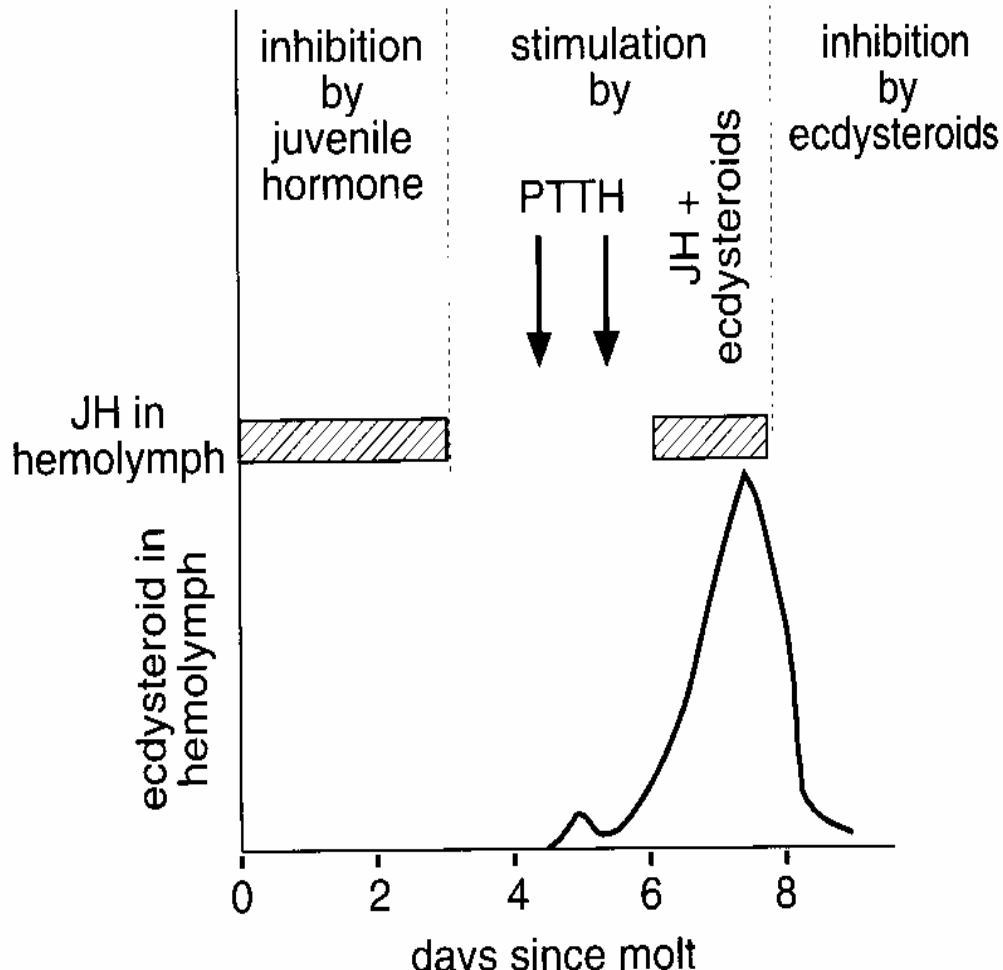
JH is slightly soluble in water + is also transported by binding proteins

Peptides are soluble in water and need no binding protein for transport

Control of titers in hemolymph-

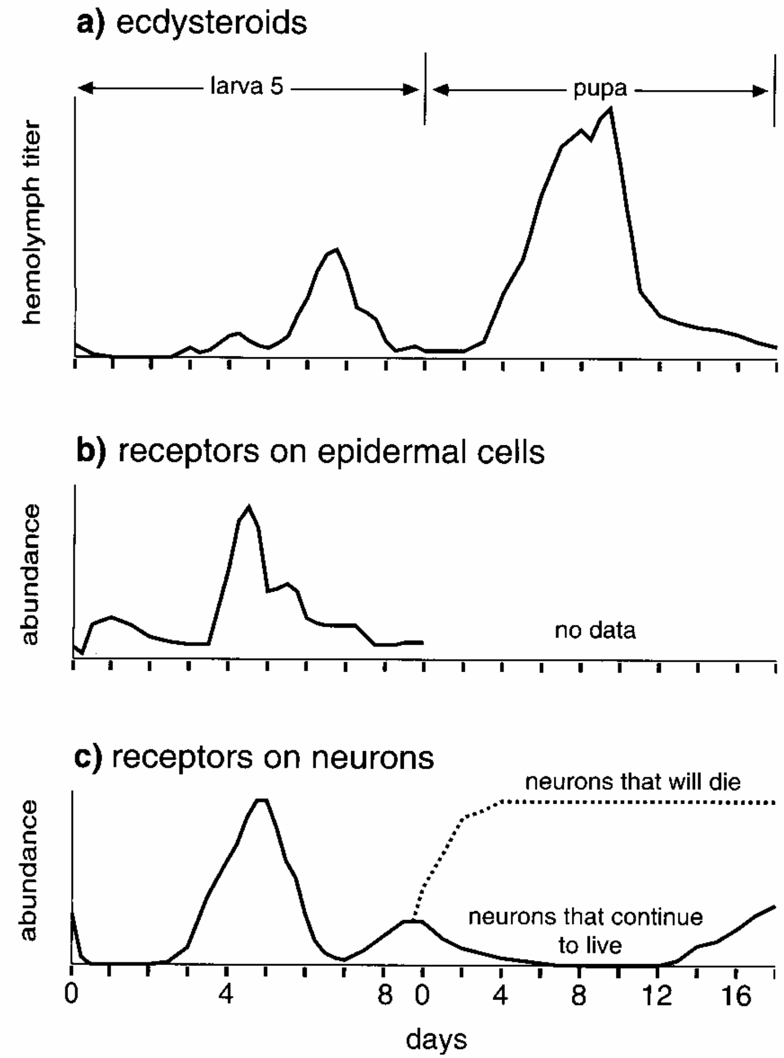
Critical titer-the concentration of the hormone in the hemolymph where it can produce an effect on Its target site.

Critical window-the time frame in which the hormone can actually have its effect on the target site or tissue. This probably depends on the presence of appropriate receptors.



Mode of action of hormones-

1. Activity within a cell depends on specific receptors for that hormone
2. The response of different tissues depends on the presence + number of receptors. This varies with development. Thus, different tissues will respond at different times
3. Receptors are in the cell membrane or within the cell
4. Both Ecdy. + JH are lipophilic so they pass through the cell membrane and have their effect within the cell
5. Cause inactive genes to become active or can inactivate other genes
6. In immature insects, JH has no effect by itself but it modifies the responses to ecdysteroids. In adult insects JH can produce an effect by itself.



Mode of action of hormones-

7. Peptide hormones and biogenic amines are lipophobic, thus they will not pass through the cell membrane. Specific receptor proteins for these substances are present in the cell membranes. Activation of these receptors activates secondary messengers (e.g., cAMP + cGMP) with the cells

3 important aspects concerning gene regulation in eucaryotic cells

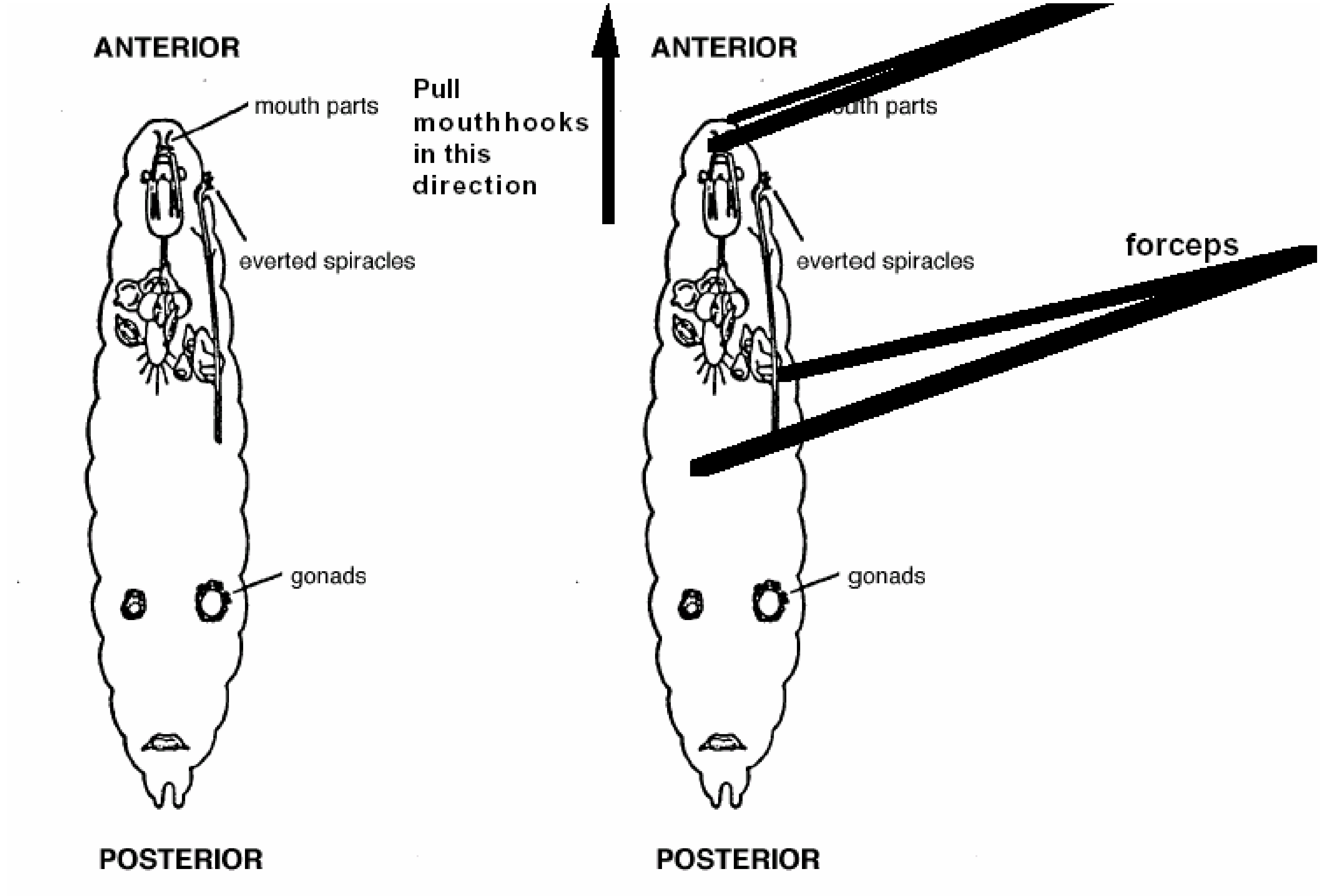
1. **There is a similarity of DNA in all nuclei of an organisms that is quantitative as well as qualitative. Each cell possesses a complete and identical set of genetic information. How do cells respond differently then?**

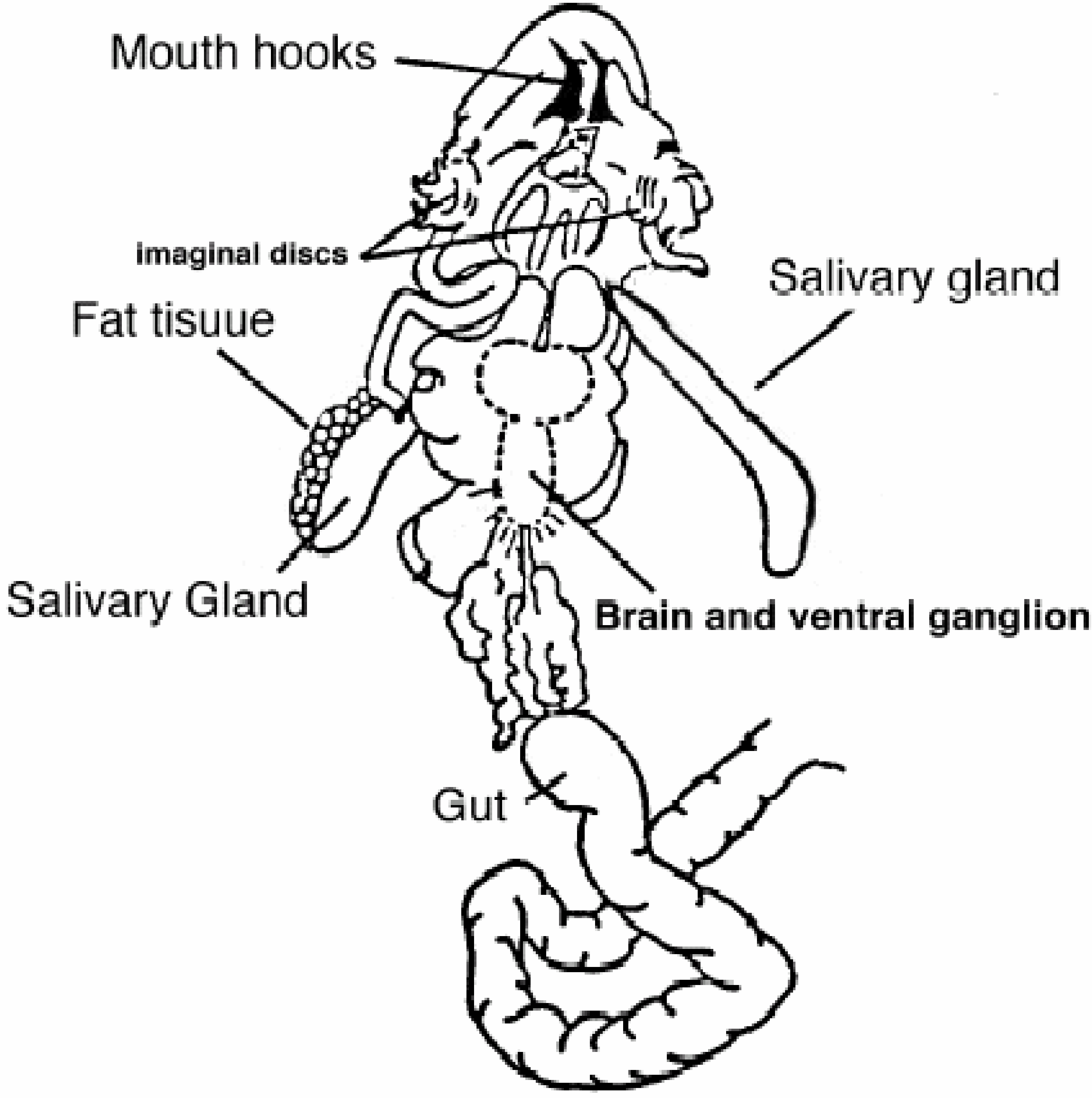
2. **The availability of genetic information for transcription is restricted: 5-20% of the genome is transcribable at at time and the speciic sequences expressed are different in each cell type, thus reflecting the metabolic requirements of the cell.**

3. **Cells are able to modulate gene expression in response to specific demands. Such modifications in gene readout occur during development and differentiation, during the cell cycle, and in response to hormones.**

a. **How are specific regions of the genome rendered transcribable?**
b. **How are genes ‘turned on’ or ‘turned off’**
c. **Specific repressor proteins called histones**

For a discussion of removing the salivary glands in *Drosophila* and looking at heat shock genes, see the following http://biosci191.bsd.uchicago.edu/labdocs/20191F02PolyteneLab.pdf




Mouth hooks
imaginal discs
Salivary gland
Fat tisuue
Salivary Gland
Brain and ventral ganglion
Gut

Temperature induced puffing of polytene chromosomes

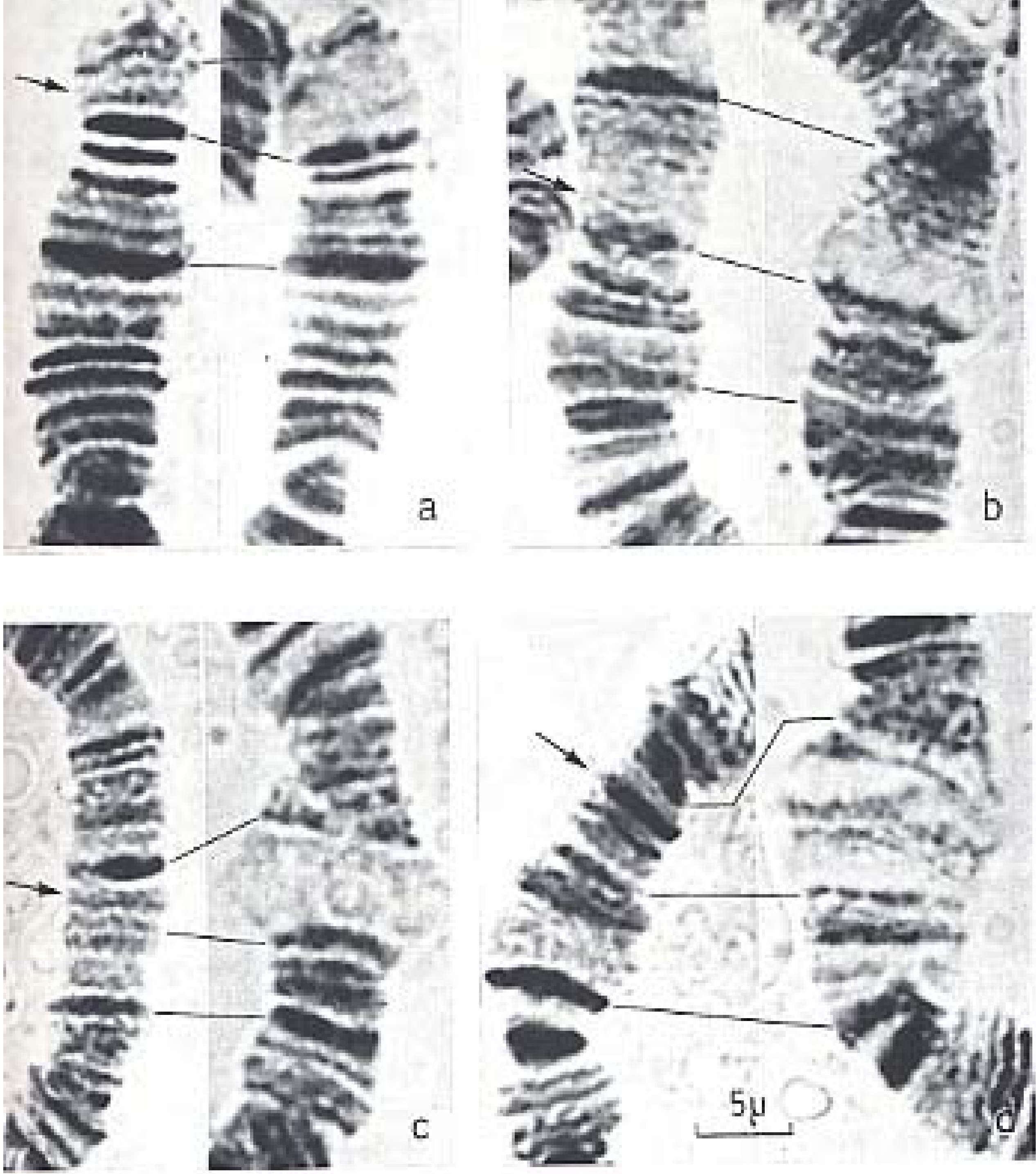
Chromosome puffing activated in *Drosophia* chromosomes due to different temperature treatments.

a. 48ºC

b. 36ºC

c. 81ºC

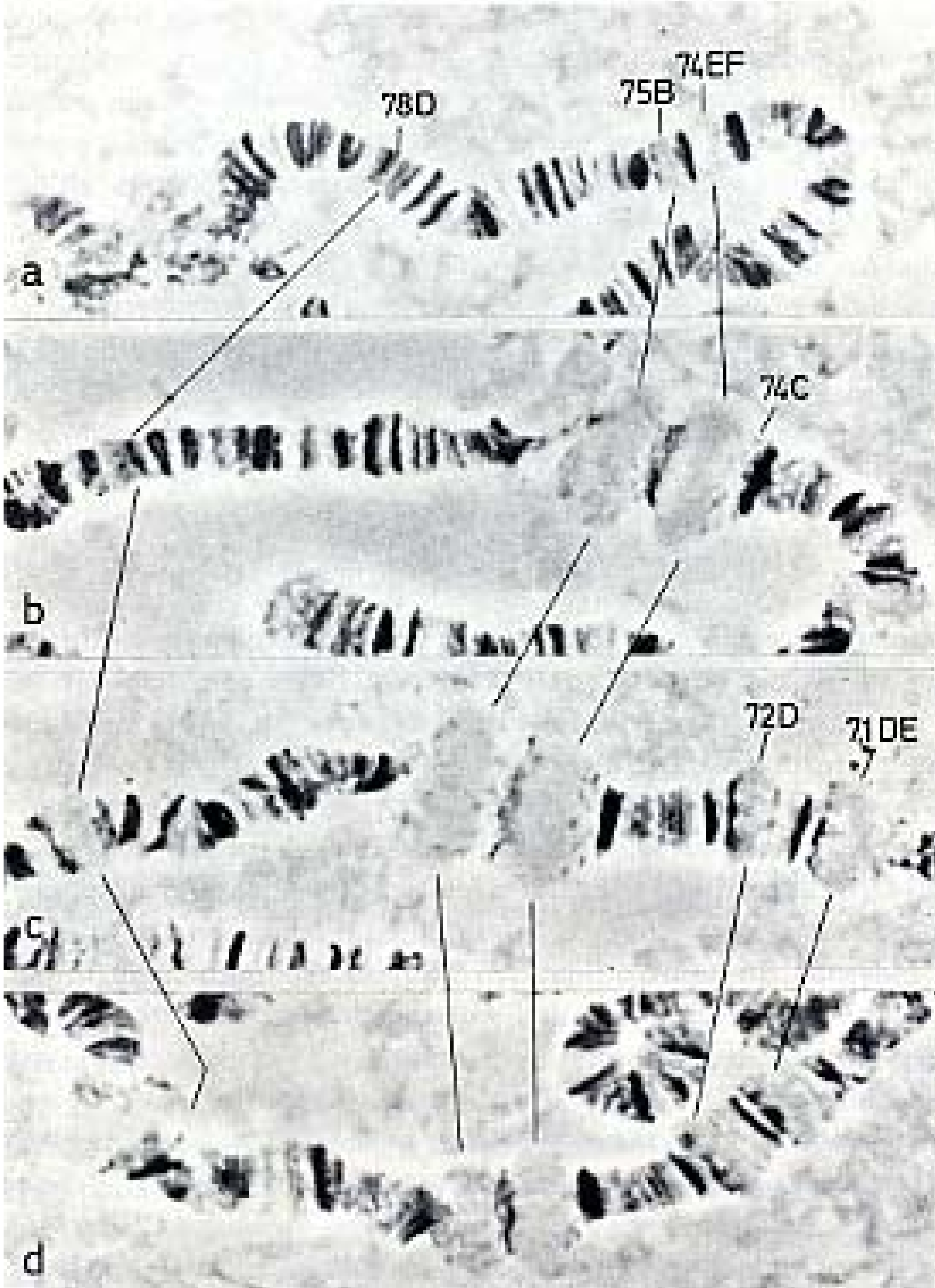
d. 32ºC



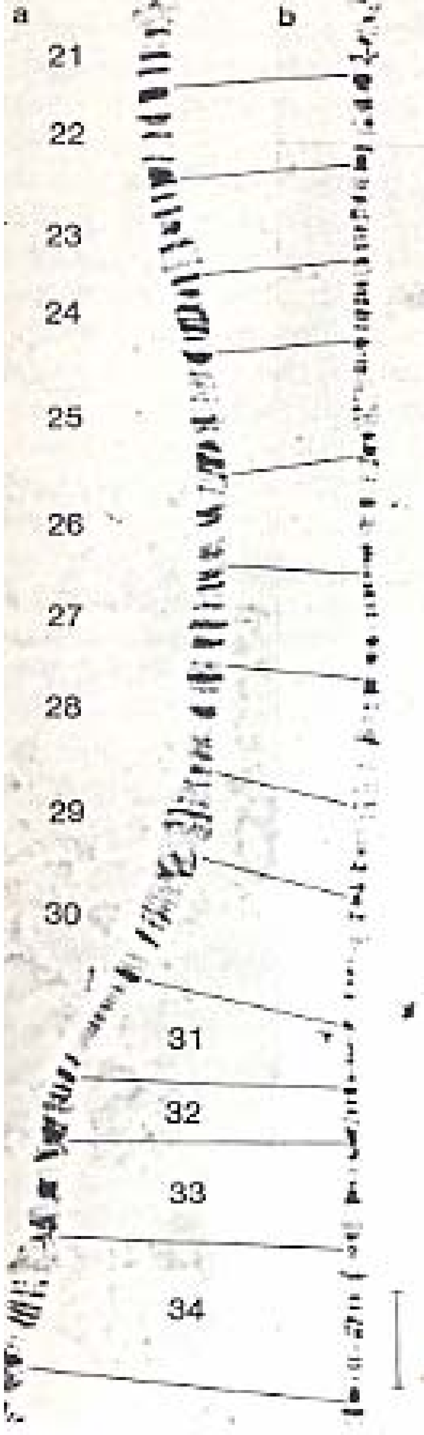
Ecdysteroid induction of puffing patterns in *Drosophila* polytene salivary gland chromosomes

In vitro induction of puffing pattern changes after incubation with 20-hydroxyecdysone at 1, 2, 4, and 6 hr. after incubation

Puffing patterns change with respect to tissue, age of insect and stage of insect



Banding pattern differences of chromsome 21 of *Drosophila melanogaster* showing the difference between the puffing of the same chromosome in the salivary gland (a) and fat body (b).



Baermann's (1961) evidence that puff sites are sites of active messenger RNA synthesis.

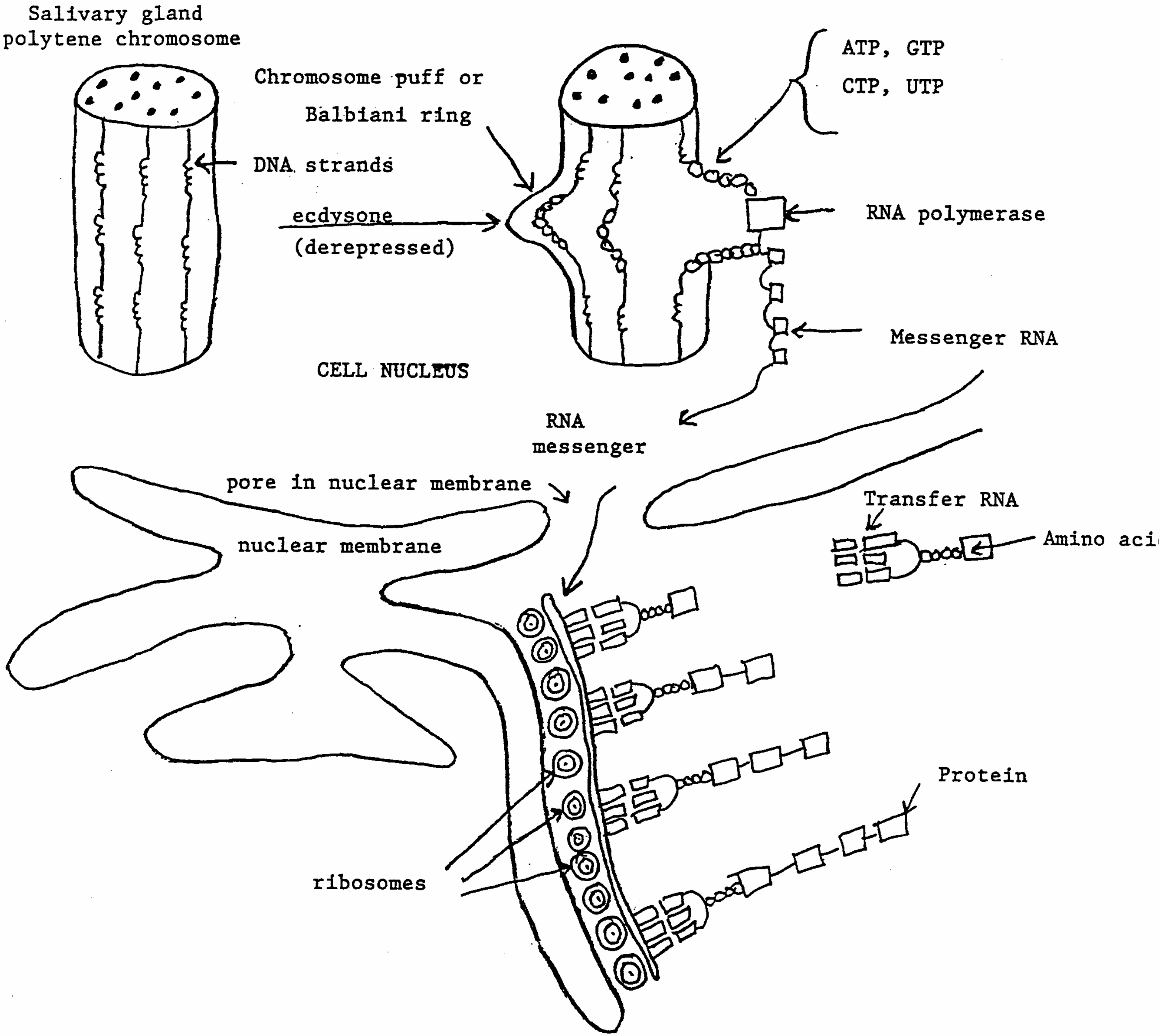
He took 2 interbreeding species of *Chironomus*

species	A	B
1. Produced major salivary protein	+/+	-/-
2. Large puff present	+/+	-/-

He crossed A with B (+/-) he got hybrid that produced intermediate amounts of protein. What he saw in the puffing patterns were:

(+/-) x (+/-) crossed he got	+/+	with 2 puffs at region
	+/-	with 1 puff at region
	-/-	with no puffs

Using Medelian genetics he demonstrated that the puff site correlates with production of salivary protein



Salivary gland
polytene chromosome
Chromosome puff or
Balbiani ring
ATP, GTP
CTP, UTP
DNA strands
ecdysone
(derepressed)
RNA polymerase
Messenger RNA
CELL NUCLEUS
RNA
messenger
pore in nuclear membrane
nuclear membrane
Transfer RNA
Amino acid
Protein
ribosomes

RESEARCH ON THE INSECT NEUROENDOCRINE SYSTEM

I. MODEL SYSTEMS

A. Role of hormones on behavior

B. Role of hormones on development

C. Effects of environmental factors (light and temp. and food) on physiological events acting via the endocrine system

II. APPLIED ASPECTS

A. 3rd generation pesticides (IGR's or insect growth regulators)

B. JH mimics or analogs-use as insect growth regulators (e.g., methoprene)

C. Harvesting nature's treasures-anti-corpora allata compound from the common bedding plant (see next slide)

http://www.altosid.com/

Methoprene is an Insect Growth Regulator (IGR), which is the active ingredient in the larvicide Altosid. A larvicide attacks mosquitoes in the larval stage, when they are waterborne and concentrated together, before they emerge as breeding, biting adults. Methoprene's disruption of the mosquito growth cycle allows it to be defined as a bio-rational agent, rather than a conventional pesticide. It specifically targets mosquito larvae, but does not kill them until they reach their next developmental stage, the pupae. This can be key to preserving the natural food chain, since mosquito larvae can be a minor food source for other organisms. In addition, extensive studies have shown that methoprene breaks down quickly in the environment, spares non-target organisms, and poses no hazard to humans.