

**Hormones
II
Hormones in Moulting and
Metamorphosis**

Insect Structure Function & Physiology

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Topics

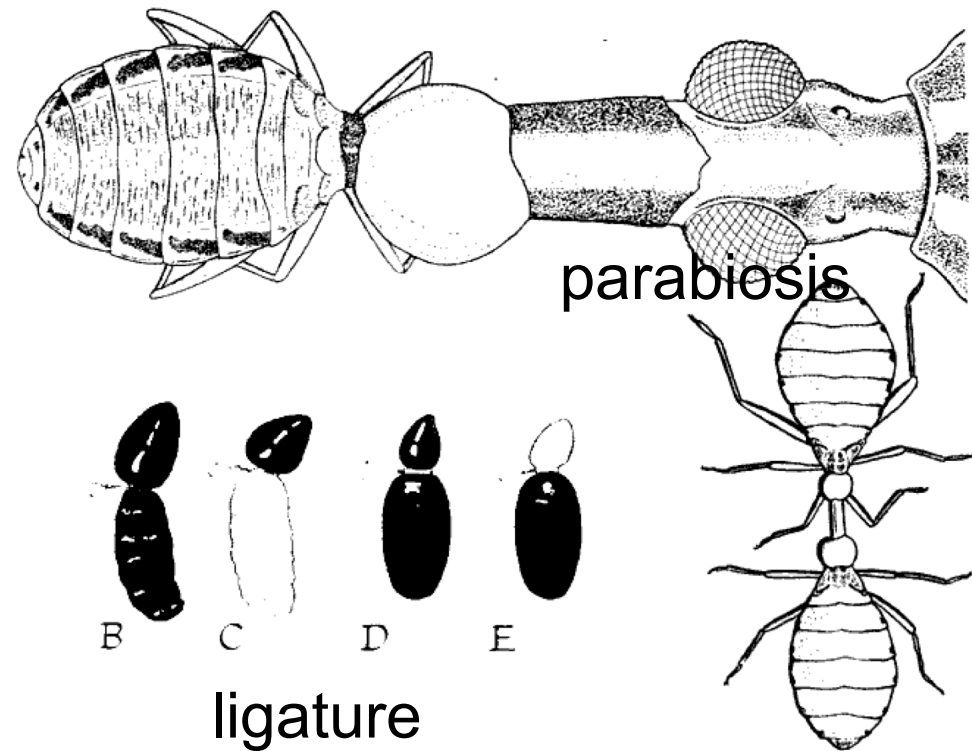
- A history of insect hormone research and the main model systems
- Prothoracicotropic hormone
- Ecdysone
- Juvenile hormone

Objectives

- Know the names and origins of the main hormones involved in moulting
- Be able to show how the different hormones are regulated to act in concert
- Be able to describe the main experimental approaches used in hormone research
- Be aware of the promise and application of hormone analogs or antagonists as insecticides
- Appreciate the difference between hormonal regulation of moulting in hemimetabolous and holometabolous insects

History of PTTH (brain hormone) and ecdysone (moulting hormone)

1. Ligature experiments. Kopec in 1910's and 1920's. If a gypsy moth (*Lepidoptera*) final instar larva is ligated and bisected after the final larval moult, the front half pupates while the posterior half does not, though it remains viable for weeks.
2. Kopec concluded that the brain is the source of a substance required for metamorphosis.



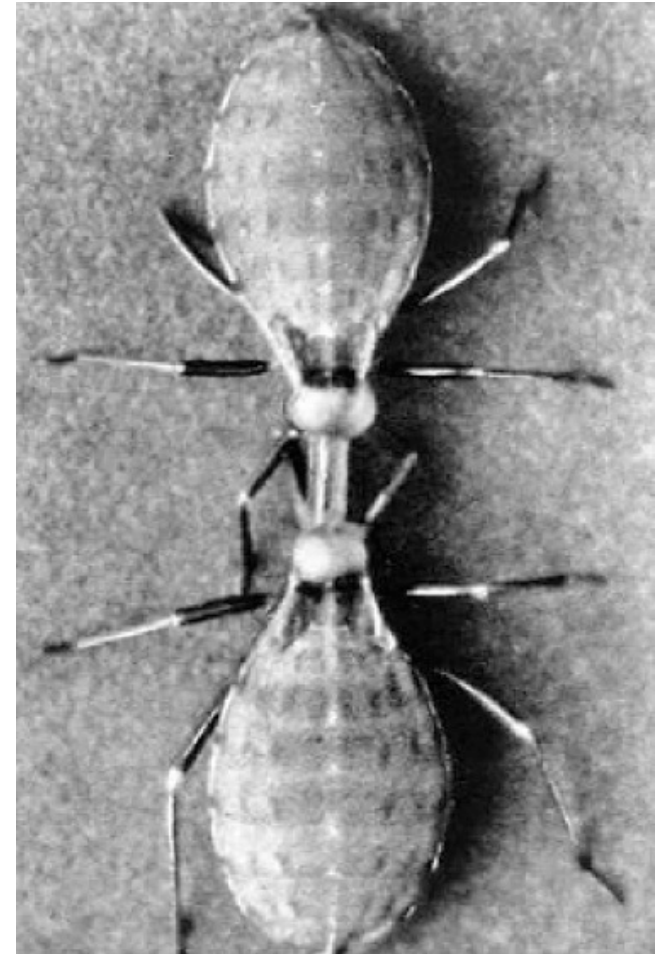
History of PTTH (brain hormone) and ecdysone (moulting hormone)

1. Wigglesworth experiments in the 1930's: the endocrine control of moulting using *Rhodnius prolixus*, a blood-sucking reduviid that transmits Chagas' disease (a trypanosomiasis) in Central and South America. The larvae require a large blood meal before a moult can occur.
2. Head removal resulted in a long-lived larva that did not moult. If removal of brain is delayed past a critical phase after the blood meal, the moult ensues anyway.
3. The "head critical period" was defined as the time at which the moult can proceed independent of the brain, as determined by ligature or removal (extirpation) (approximately 3 days in *Rhodnius*).



History of PTTH (brain hormone) and ecdysone (moulting hormone)

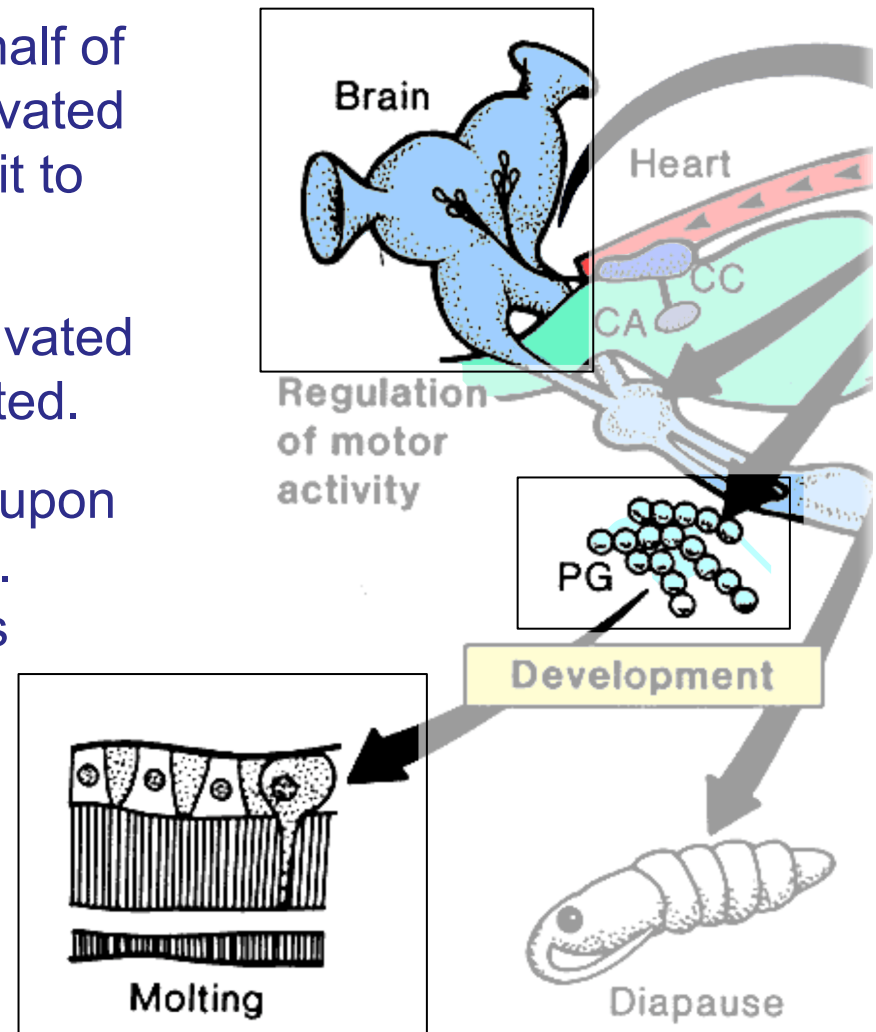
4. An activated larva (i.e. some days after feeding and past the head critical period), when parabiosed (joined) to a brain-removed larva could induce moulting in the brain-removed larva, therefore an endocrine factor is responsible because no neural or epidermal connectivity existed between the parabiosed larvae.
5. Implantations of parts of CNS allowed the hormone source to be localised to the anterior part of the brain:
 - Suboesophageal ganglion: no effect,
 - Posterior part of brain: no effect.
 - Anterior part of the brain: an effect.



History of PTTH (brain hormone) and ecdysone (moulting hormone)

Williams working on the silkmoth (1947, 1948):

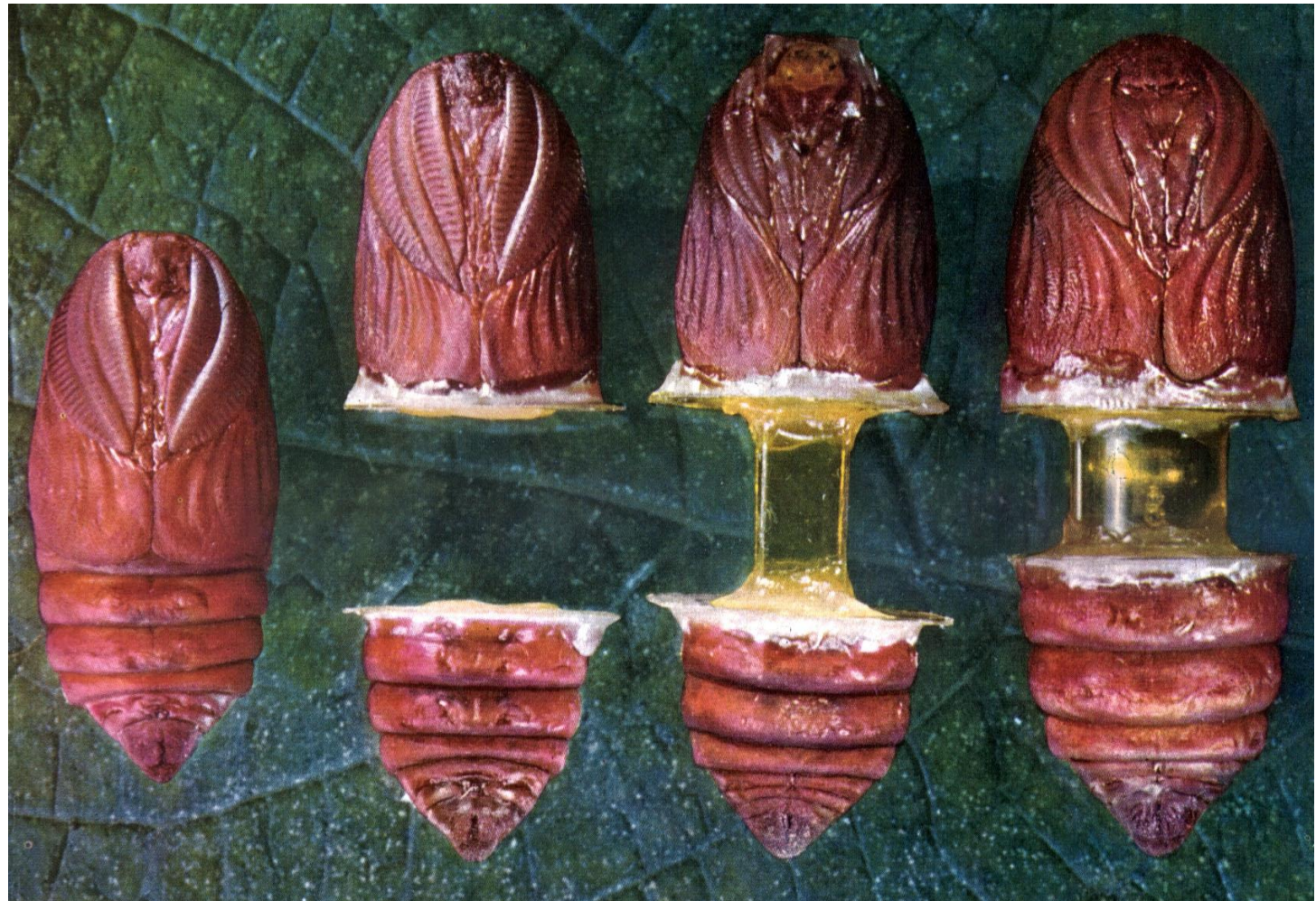
- an "activated" brain placed in anterior half of bisected pupa caused moult, while activated brain placed in posterior did not cause it to moult.
- the posterior half moults only when activated brain *and* prothoracic gland are implanted.
- Proved that the "brain hormone" acted upon the prothoracic gland to initiate a moult. Subsequently this "brain" hormone was renamed prothoracicotropic hormone (PTTH).



History of PTTH (brain hormone) and ecdysone (moulting hormone)

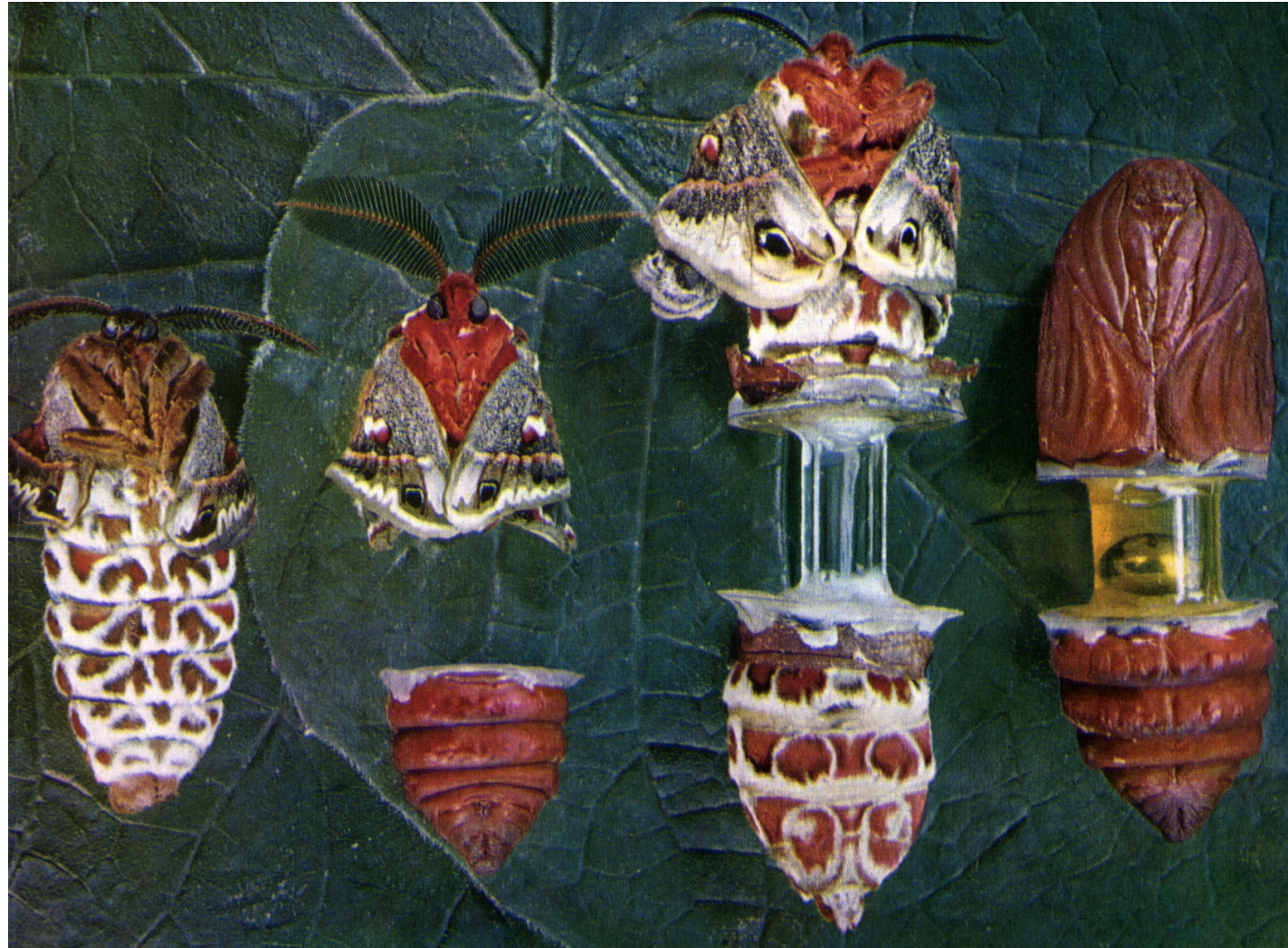
HALVED PUPAE test the effects of injury on metamorphosis. For purposes of comparison, Dr. Williams used four moth pupae of exactly the same age. The pupae are from left: a whole pupa; a halved pupa whose two raw ends are covered with a plastic coating; a halved pupa whose two ends are connected with a tiny plastic tube; and finally a halved pupa with the sections connected by a plastic tube but with a movable ball bearing in the tube to stop any tissues from extending between the segments.

From *Life* magazine 11 Feb 1952 about work of Carol Williams. Available free online.



History of PTTH (brain hormone) and ecdysone (moulting hormone)

A MONTH LATER the experiment is complete. Pupa Number 1 developed normally. In Number 2 the front end metamorphosed but the tail did not. In Number 3, the wound healed and a tissue thread grew through the tube, providing a bridge across which hormones could flow. Both ends developed. In 4, where the motion of the ball prevented tissue growth, no development took place. Dr Williams concluded from these experiments that injuries must heal before the insect metamorphoses



Hormones in Moulting and Metamorphosis

Prothoracicotropic hormone: Released from brain neurosecretory cells, accumulates in CA, prompts the prothoracic glands to release ecdysone

Ecdysone: Released from prothoracic gland targets the epidermis to initiate moulting

I. apolysis

II. enzyme secretion

III. digestion and reabsorption

Juvenile hormone: Secreted by endocrine cells of the corpora allata, widespread targets, maintains juvenile stage

Eclosion hormone: Released from brain (a neuropeptide) through corpora cardiaca (in Manduca through proctodeal nerve NH area from cells with somata in brain) and targets the CNS to produce eclosion (or ecdysis) behaviour.

Ecdysis triggering hormone (ETH): Released from peripheral nerve cells associated with the trachea near the spiracles. Trigger the onset of ecdysis behaviour

Bursicon: Released from neurosecretory cells of thoracic and abdominal neuromeres. Targets epidermis to produce sclerotization and melanization of the cuticle

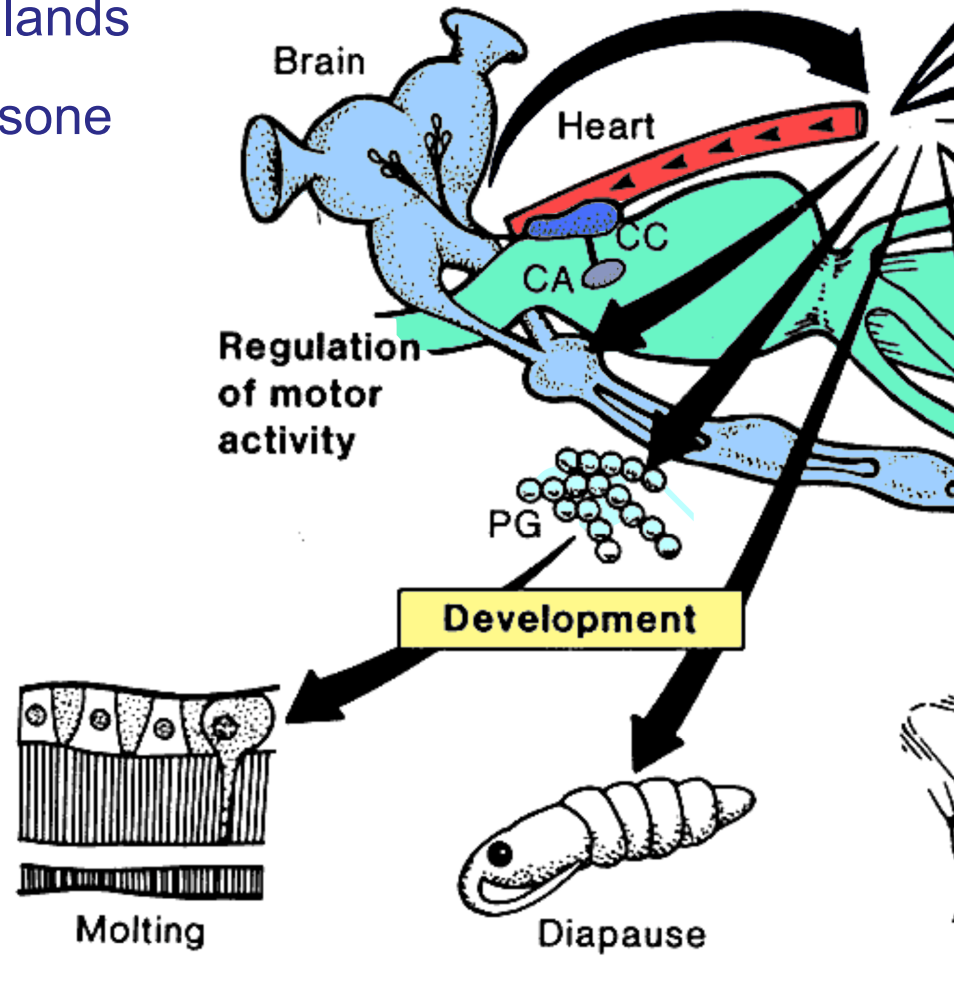
prothoracicotropic hormone

From: brain neurosecretory cells

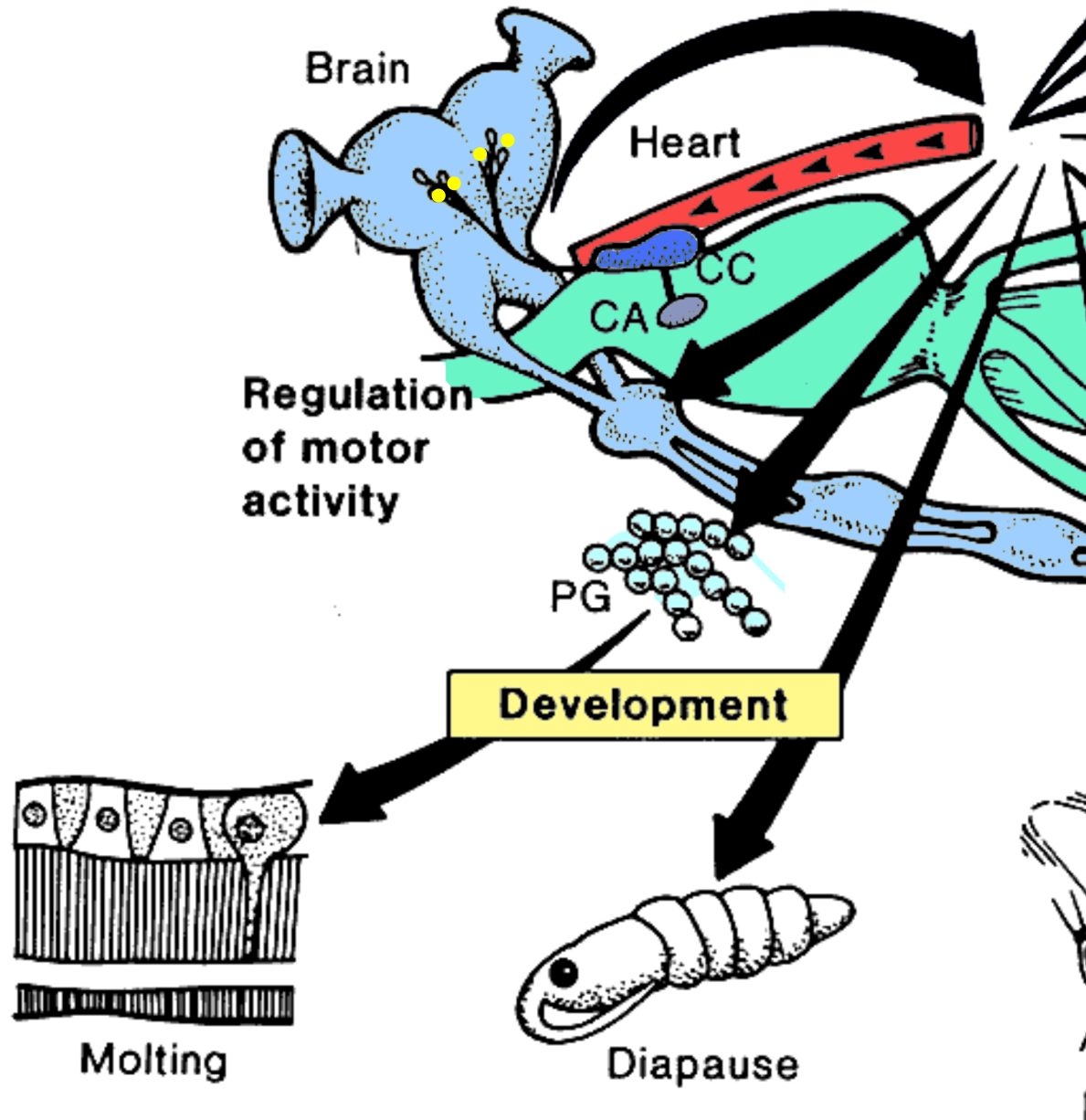
To: CA or CC

To: prothoracic glands

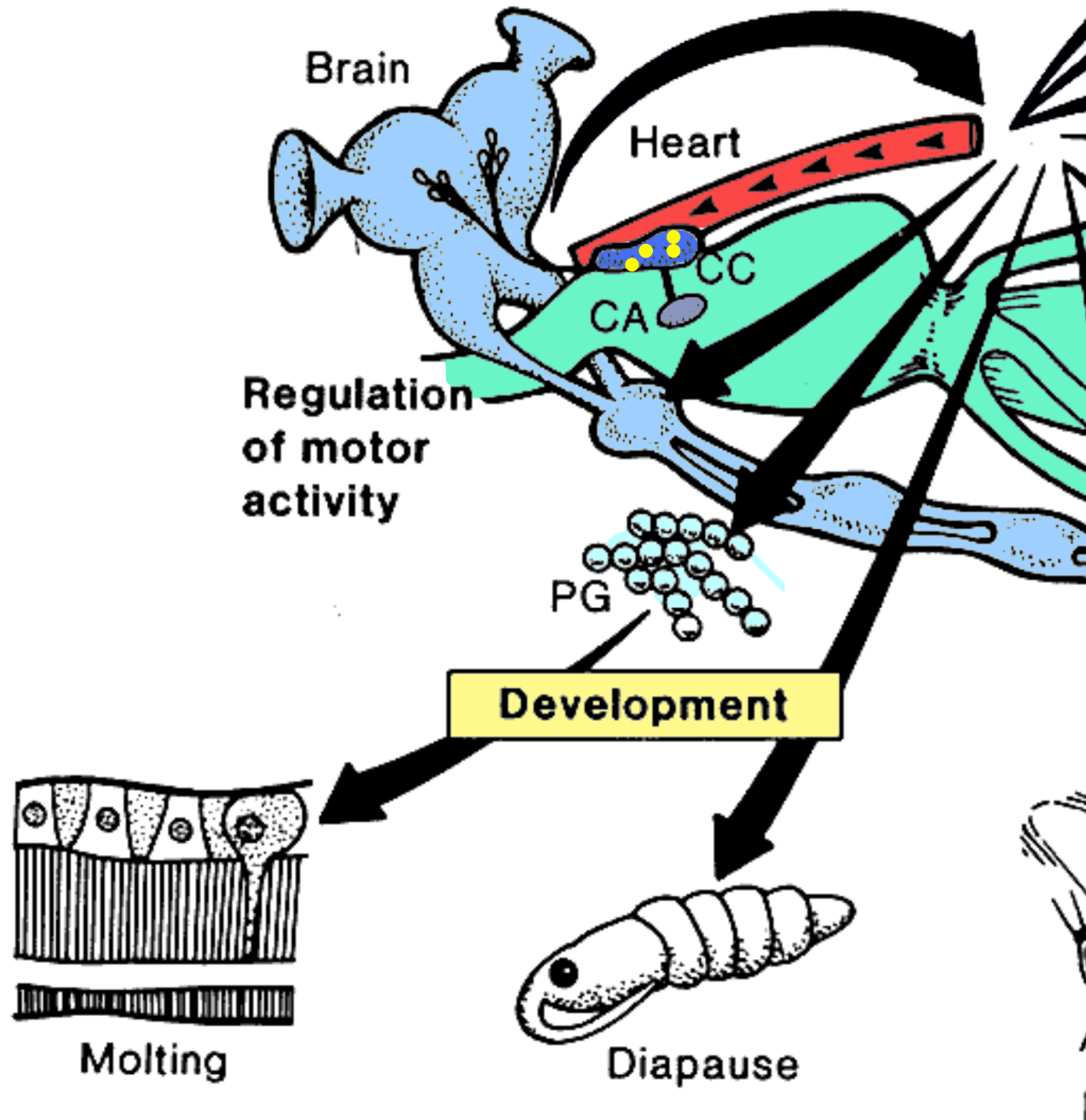
To: release ecdysone

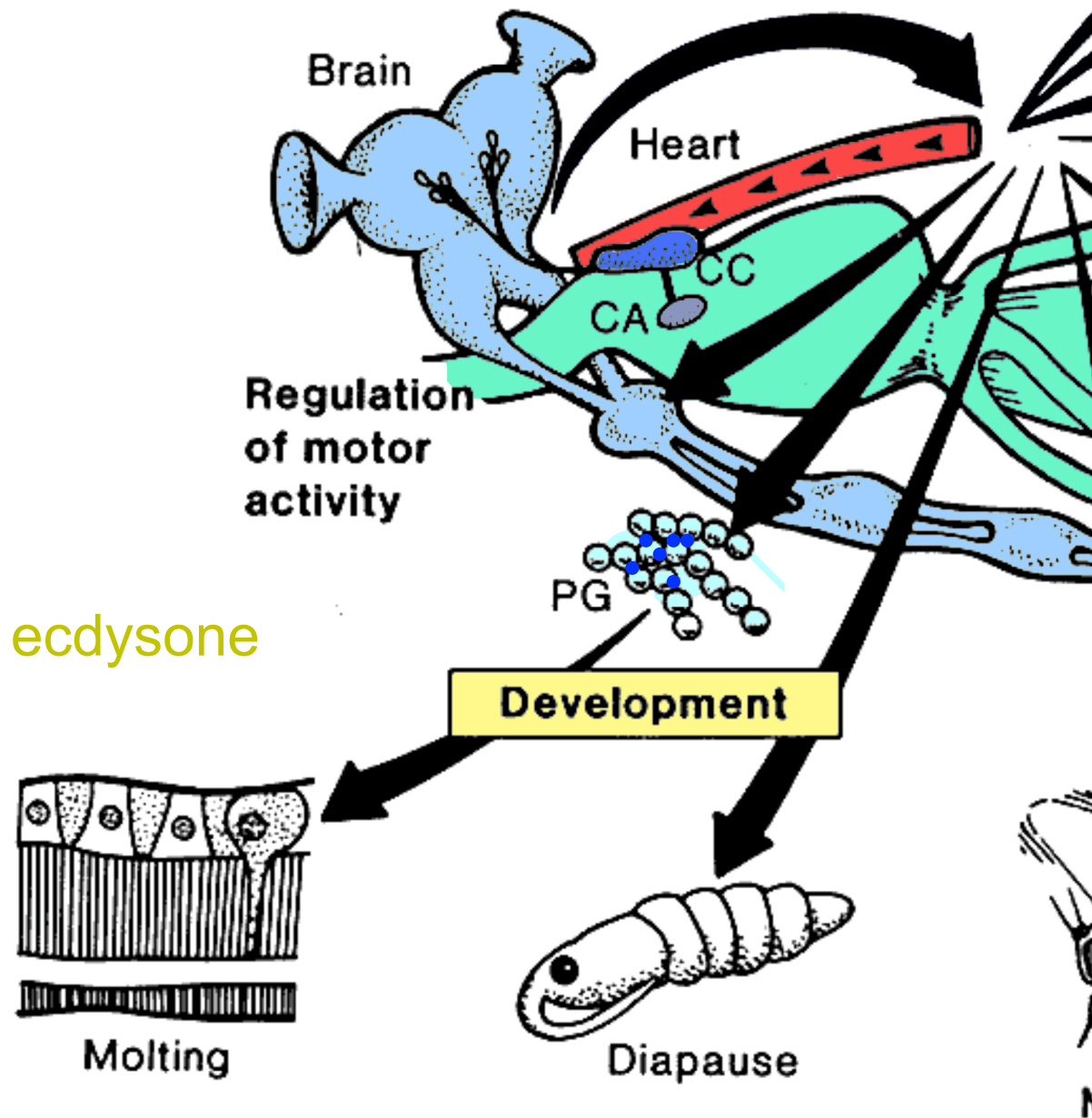


prothoracicotropic hormone



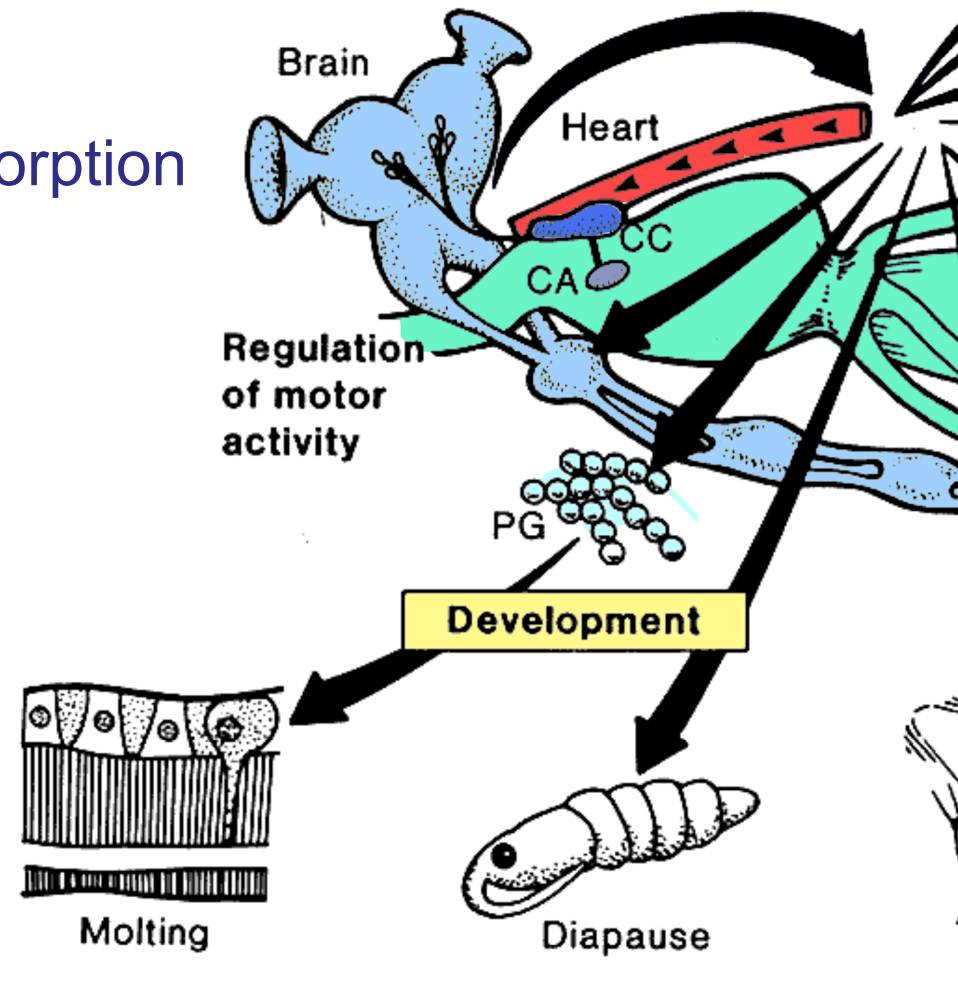
prothoracicotropic hormone





Ecdysone : Released from prothoracic gland targets the epidermis to initiate moulting:

- I. apolysis,
- II. enzyme secretion
- III. digestion and reabsorption



Secretion of PTTH from brain

- PTTH is a neurosecretory polypeptide produced by neurons in the brain.
- In most insects, the neurosecretory axons project to the **corpora cardiaca**.
- In lepidoptera, where the **corpora allata** are the neurohemal organs.
- PTTH is turned on for a short period to initiate the moult.
 - In *Rhodnius*, PTTH release is required for several days
 - In *Manduca* it is required for only a few hours. Acts as a trigger.
- PTTH regulates the secretion of ecdysteroids by the prothoracic glands.



Prothoracic Glands

Found in prothorax, produce ecdysteroids

Gland cell structure: folded plasma membrane, typical of secretory cells

Ecdysteroid is not stored: it is released as it is synthesised

Prothoracic glands are not found in adults: programmed cell death during metamorphosis

“Ring gland” of Diptera is a fusion of CA, CC and PG

What is the moulting stimulus?

- In *Rhodnius*, stretch receptors in the abdomen monitor abdominal distension from blood meal
- Other hemiptera (bugs) use the same process: *Oncopeltus* injected with saline will moult to a small adult.
- In Lepidoptera the stimulus is unknown. In *Manduca* larvae stretch receptor input is *not* the important cue. In most Lepidoptera the stimulus is closely correlated with the insects' size, but how it is measured and triggers PTH release is unknown.



Courtesy TDR Image Library

Hormones in Moulting and Metamorphosis

Juvenile hormones

Play a role in many aspects of reproduction and development including

- metamorphosis,
- regulation of behaviour in honeybee colonies, polyphenisms of locusts and aphids,
- larval and adult diapause,
- ovarian development.

Juvenile hormones are terpenoids.

Five structurally related forms are known in insects.

JH is produced by intrinsic secretory cells of the CA. The rate of secretion is determined by the rate of production, i.e. it is not stored.

In most insects, the CA come under nervous control.

Name

JH got its name through conclusion from parabiosis experiments.

Last instar larvae, parabiosed to earlier instar larvae, moult into an intermediate adult/immature form.

Conclusion: a blood borne juvenilising factor is responsible.

Origin

CA implant -> moult into an intermediate adult/immature form.

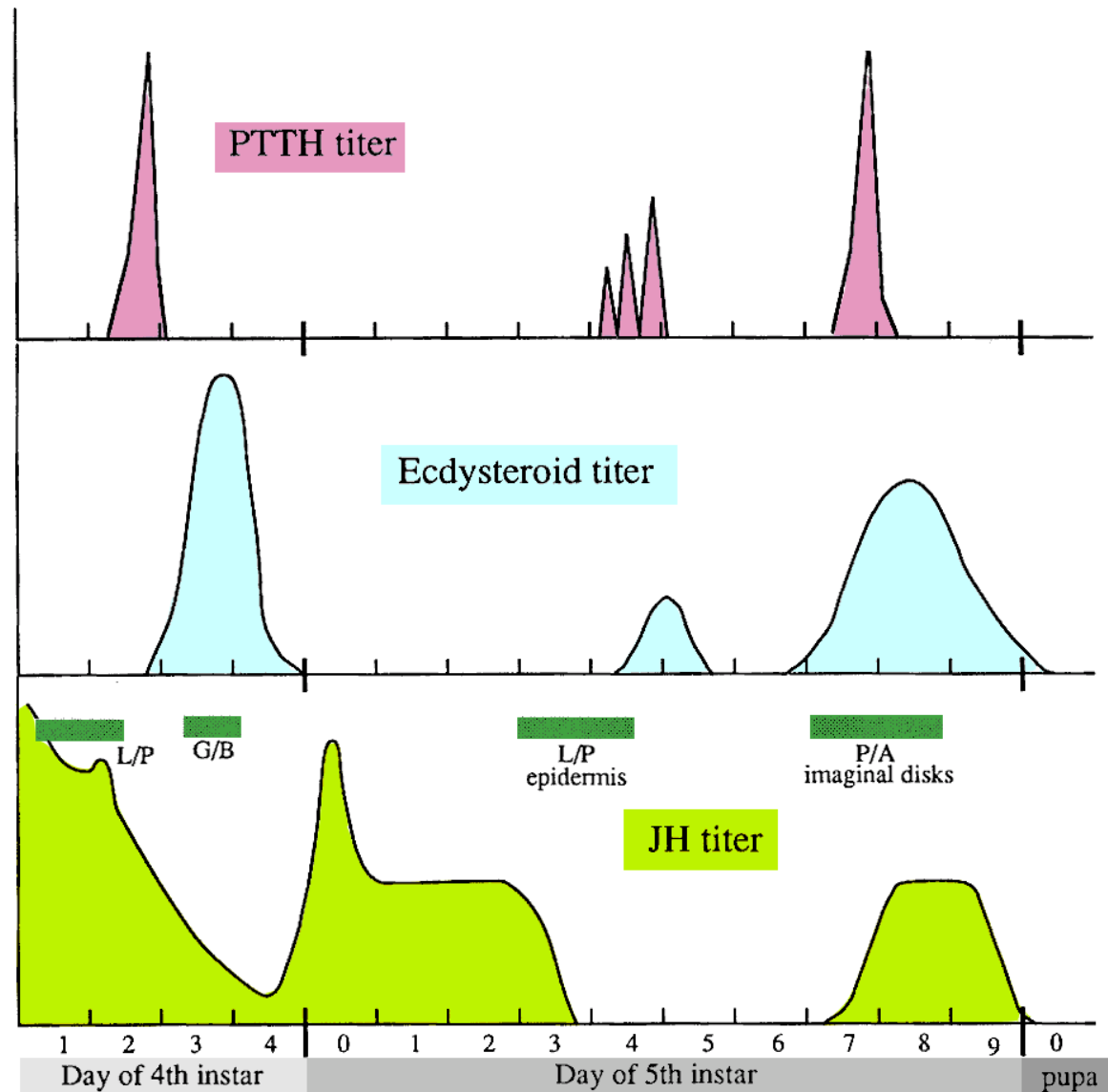
CA removal causes premature metamorphosis in many insects.

Conclusion: corpora allata are the origin of JH

Hormones in Moulting and Metamorphosis

Green bars indicate the approximate timing and duration of the JH-sensitive periods for larval versus pupal determination (L/P), for green versus black larval pigmentation (G/B), and for pupal versus adult determination (P/A).

The epidermis and imaginal disks have independent times of commitment to pupal and adult development. (From Nijhout, 1994.)

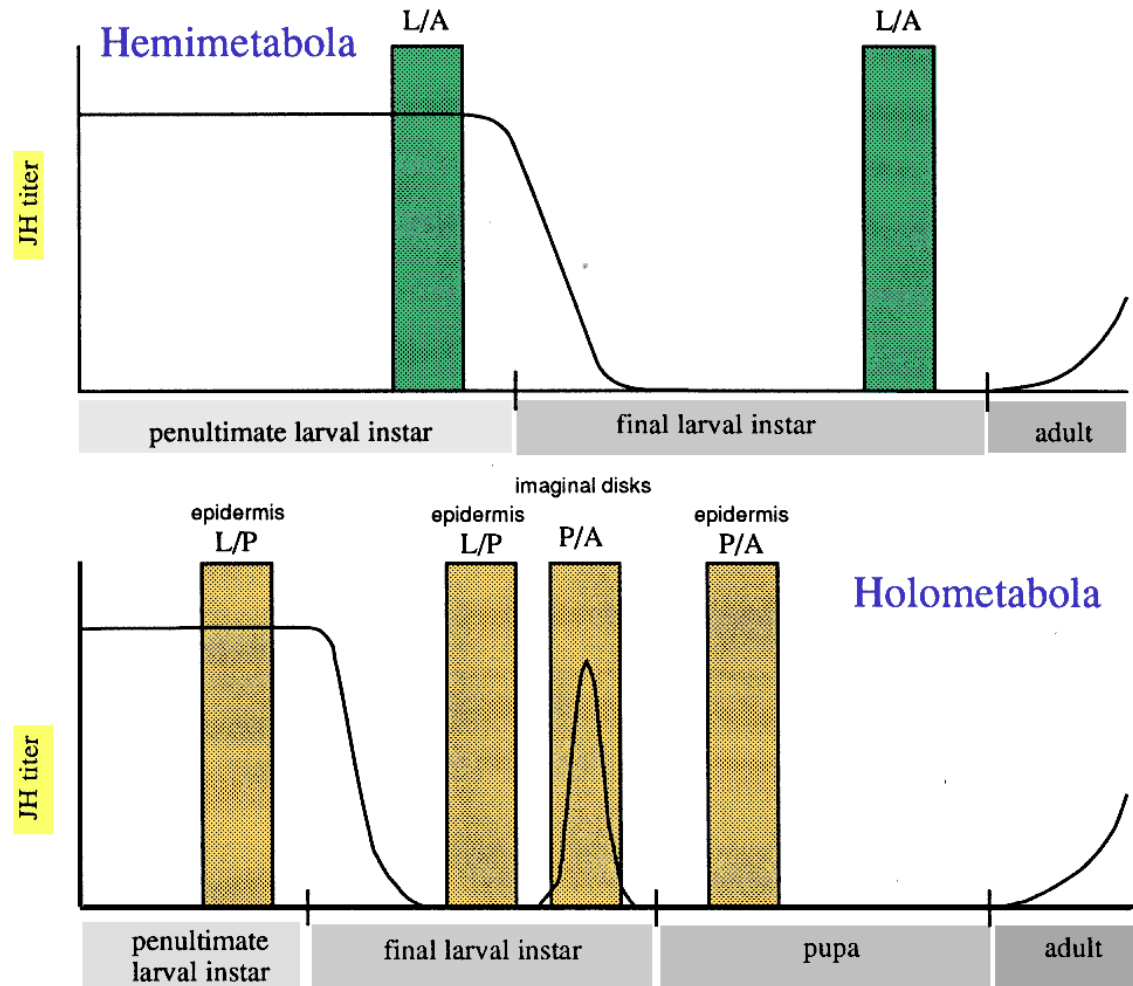


Hormone titers during the last two larval instars of *Manduca sexta*.

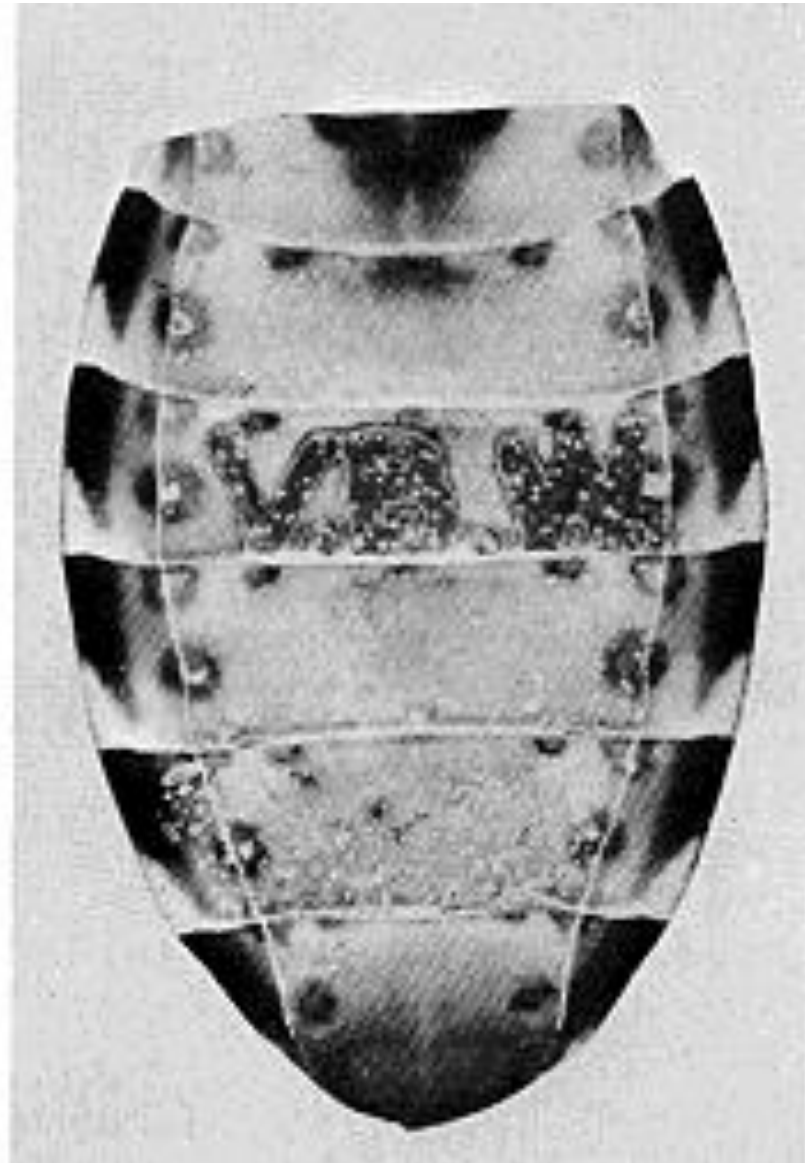
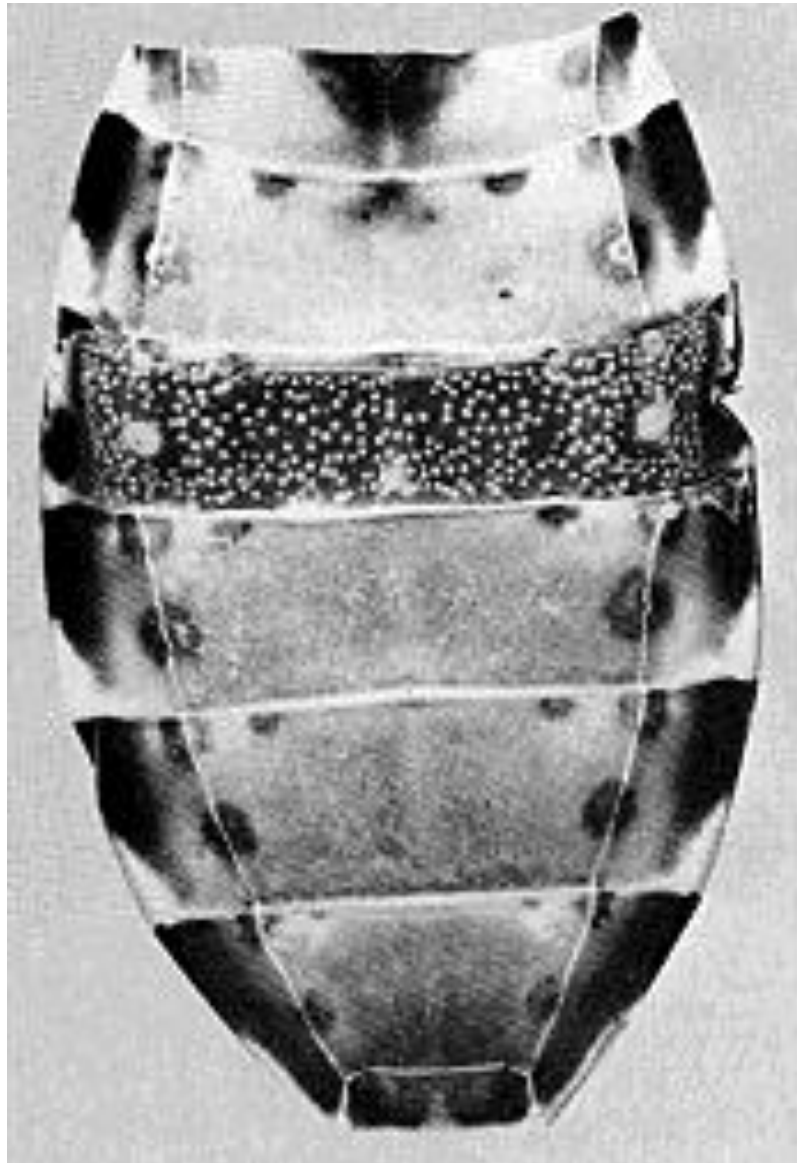
Hormones in Moulting and Metamorphosis

- Holometabola and hemimetabola have different JH profiles
- JH-sensitive periods determined by applying artificial JH analogs to larvae of different ages
- Defines the “window” when the cells (e.g. epidermis) have the appropriate receptors.

General Rule
 If JH is present, maintain the current state (hence its juvenilising effect).
 If JH is absent, switch developmental pathways.



Typical juvenile hormone titer profiles in hemimetabolous and holometabolous insects. Hypothetical JH-sensitive periods indicated by bars. In Hemimetabola JH-sensitive period near the end of each larval instar, during which the commitment to larval versus adult development is controlled. In the Holometabola there is a JH-sensitive period for larval versus pupal determination near the end of each larval instar, except in the final larval instar. The two JH-sensitive periods in the final larval instar are based on the findings in *Manduca sexta*. There is an additional JH-sensitive period for pupal versus adult determination early in the pupal stage, at the outset of adult development. (From Nijhout, 1994.)



Left: application of a band of juvenile hormone to the cuticle of an adult *Rhodnius* results in the formation of larval cuticle (speckled band) when the insect is forced to undergo an extra molt.

Right: Dr. Wigglesworth has printed his initials with juvenile hormone.

From: <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/I/InsectHormones.html>

Pest control

Moulting is an ideal target for insecticides because it is arthropod-specific. Genomics can lead to structure of specific receptors or signalling molecules that can be chemically emulated.

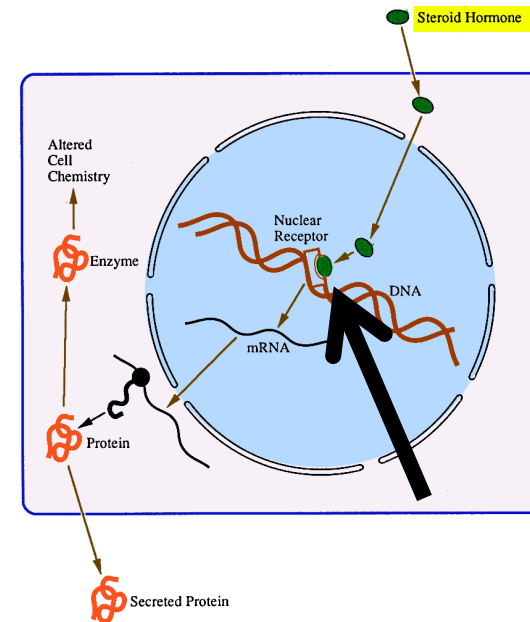
Ecdysone receptor-binding agonists are selective pesticides, called moulting-accelerating compounds

Juvenile hormone analogs are commonly used, e.g. methoprene. Known as insect growth regulators (IGRs). Not commonly used in field due to

- lack of outdoor stability
- limited insect control spectrum
- slow toxic action (extra JH is often not lethal until the adult moult).

Very effective in control of fleas on domestic animals and as the mosquito larvicide, Altosid

Cattle feed to control dung flies



Dhadialla, T. S., G. R. Carlson, et al. (1998). "New insecticides with ecdysteroidal and juvenile hormone activity." Annual Review of Entomology 43(1): 545-569.

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Nijhout, H. F. (1994). *Insect Hormones*. Princeton, Princeton University Press.