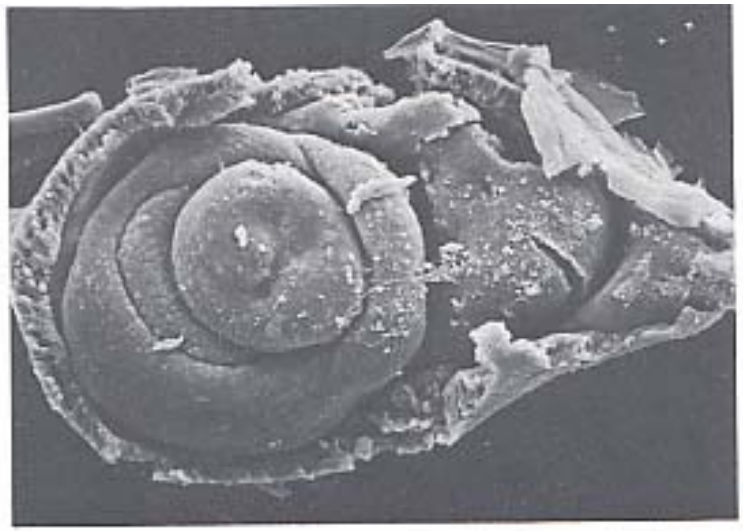
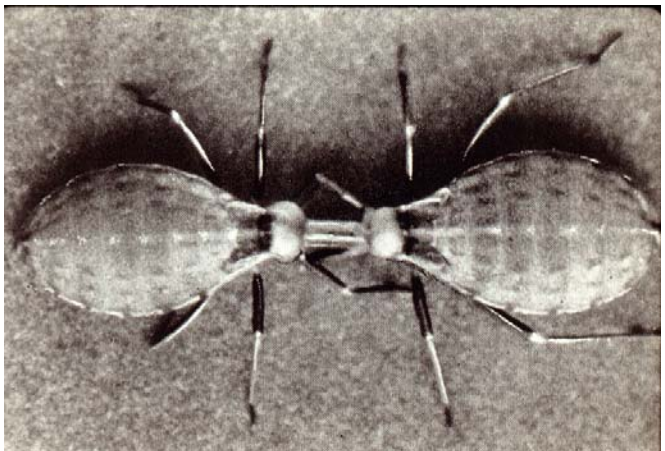
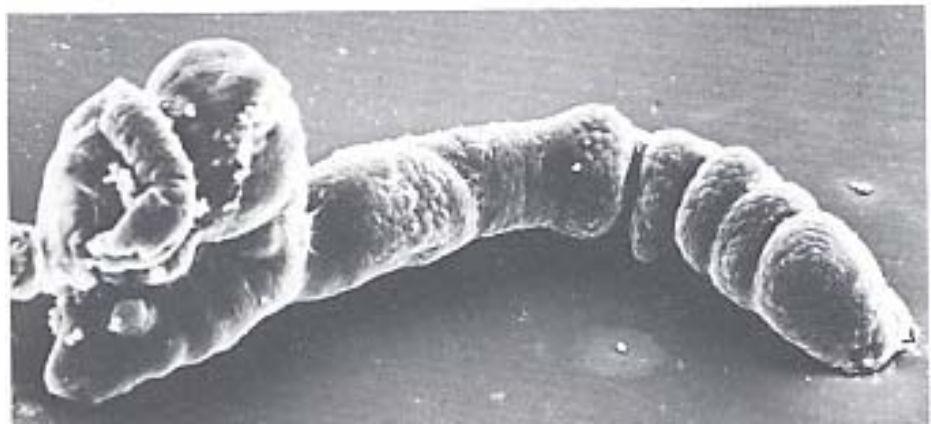
PHYSIOLOGY AND DEVELOPMENT

1. Pre-embryonic development
2. Embryonic development
3. Post-embryonic development
   a. Growth
   b. Molting
   c. Metamorphosis
   d. Aging







STUDIES TO ELUCIDATE CONTROL MECHANISMS REGARDING EGG AND EMBRYO DEVELOPMENT

A. Early studies involved removal, destruction, disruption or separation of various parts of the egg or embryo by:

1. Microsurgery
2. Centrifugation
3. Grafting
4. Ultraviolet light
5. X-rays
6. Cautery
7. Ligation

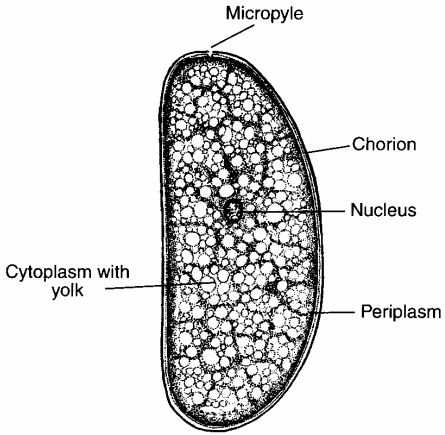
B. Modern studies involve:

1. Mutants
2. Microsurgical lasers
3. Various molecular techniques:
   a. Visual molecular probes such as specific antibodies
   b. Cloning
   c. Labeling various transcripts
   d. Nuclear transplantation techniques

Developmental studies involve 3 major processes:

How does a fertilized egg go from

one cell



to a multicellular adult

1. Differentiation: **The development of specialized cell types from the single fertilized egg. Example. The border cells in the egg assist in forming the micropyle and do nothing else while the pole cells differentiate into the germ cells of the adult.**

2. Determination: **When a cell or tissue becomes irreversibly committed to develop into its original fate or designation. Also, the commitment is irreversible. Example. Removal of a leg imaginal disc and put it into the abdomen of the adult and it always becomes a leg.**

3. Morphogenesis: **Includes**

a. Morphogenetic movements shaping the final embryo

b. Pattern formation

c. Creation of body shape and appendage formation

PRE-EMBRYONIC DEVELOPMENT

Because of our knowledge of its genetics, *Drosophila* is not only the "workhorse" of modern genetics but has also become the "workhorse" of modern developmental biology. Thus, this small fly with four pairs of polytene chromosomes that encode at least 5,000 genes has taken us to our current understanding of how genetics influences behavior, physiology, and most recently developmental biology.

CONTRIBUTIONS TO THE DEVELOPING EMBRYO

MATERNAL CONTRIBUTIONS to egg

A. Egg package covering

1. Egg shell and vitelline envelope

B. Stored reserves for embryo

1. Yolk (vitellin, Vt)

2. Lipids

C. Machinery for protein synthesis

1. Maternal ribosomes

D. Instructions prior to zygote formation

1. Transcription factors (mRNA) and proteins from the maternal effect genes in the nurse cells or trophocytes

E. Tranovarially transmitted pathogens, symbionts, etc.

F. Female oocyte nucleus

PATERNAL CONTRIBUTIONS to egg

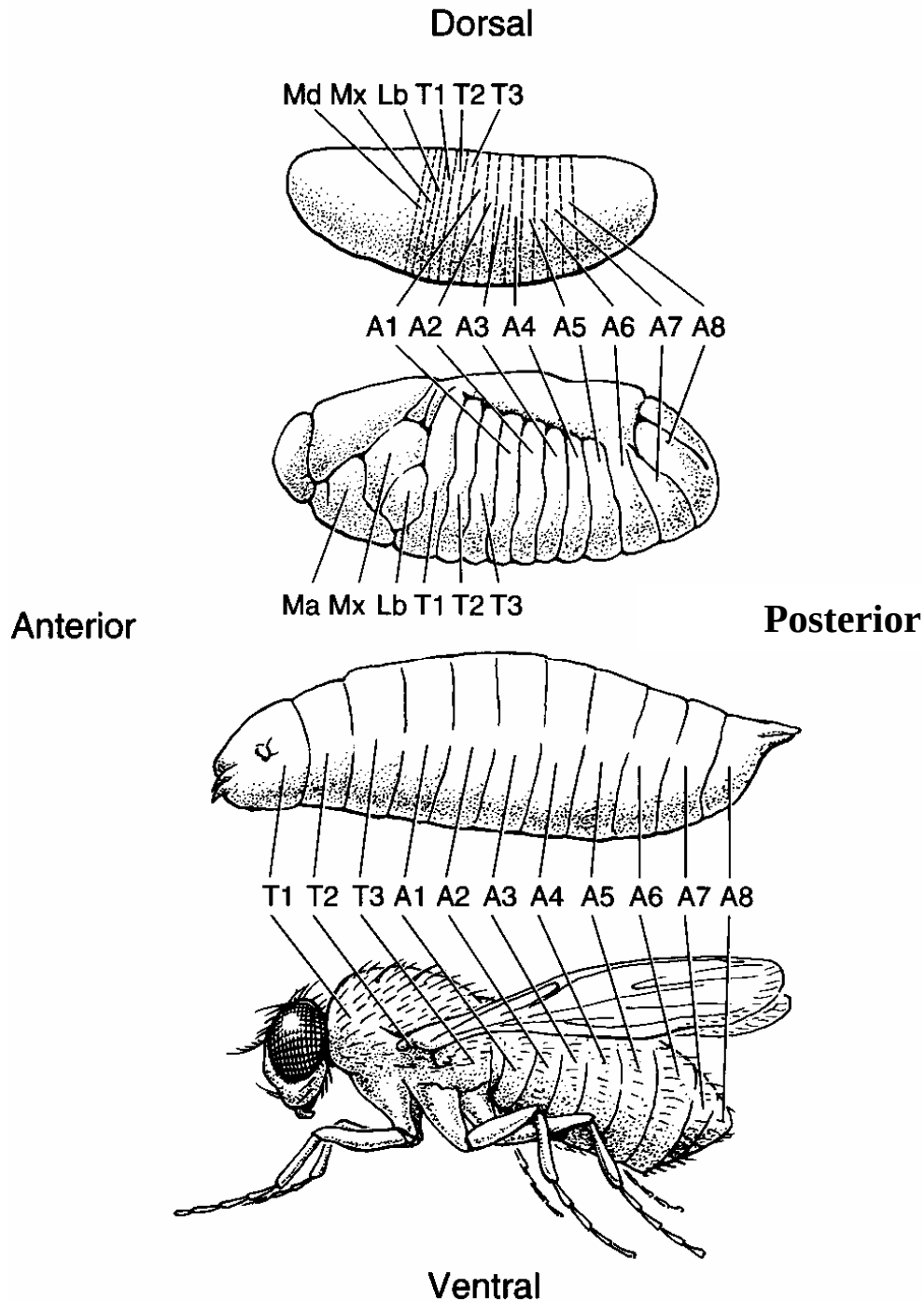
A. Sperm (sperm nucleus)

B. Sometimes uric acid, Pyrrolizidine alkaloids, etc.

How is the egg architecture controlled prior to zygote formation?

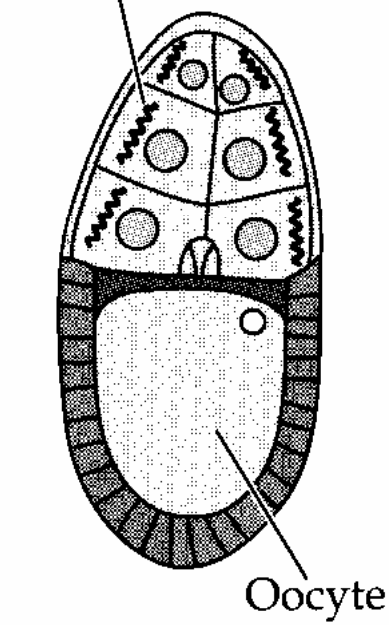
and

What kinds of polarity (anterior/posterior) and (directional dorsal/ventral) directions and regional specificity are needed prior to zygote formation and where do they come from?

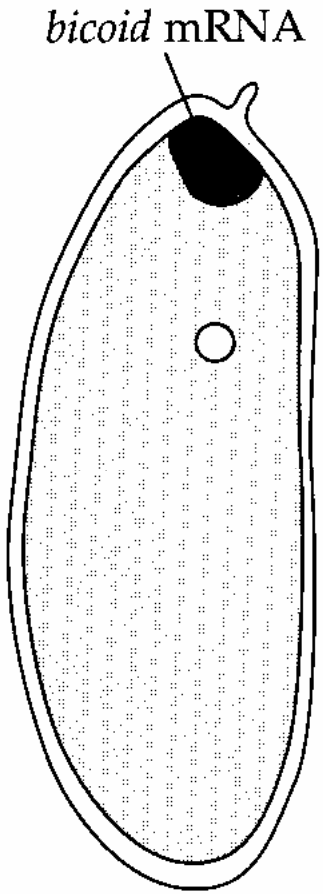


MATERNAL TRANSCRIPTS (mRNA)

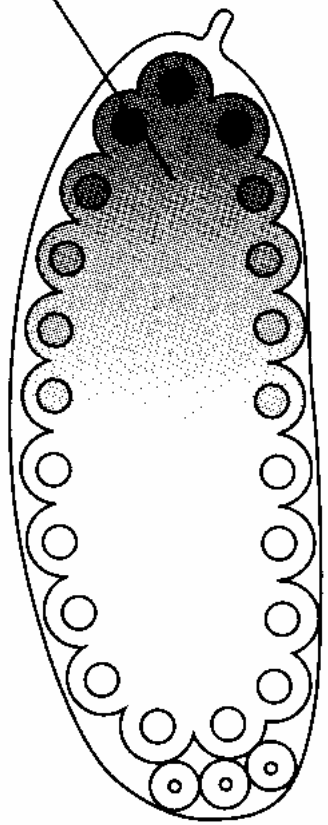
Mid-oogenesis	Completion of oogenesis	Syncytial blastoderm	Cellular blastoderm



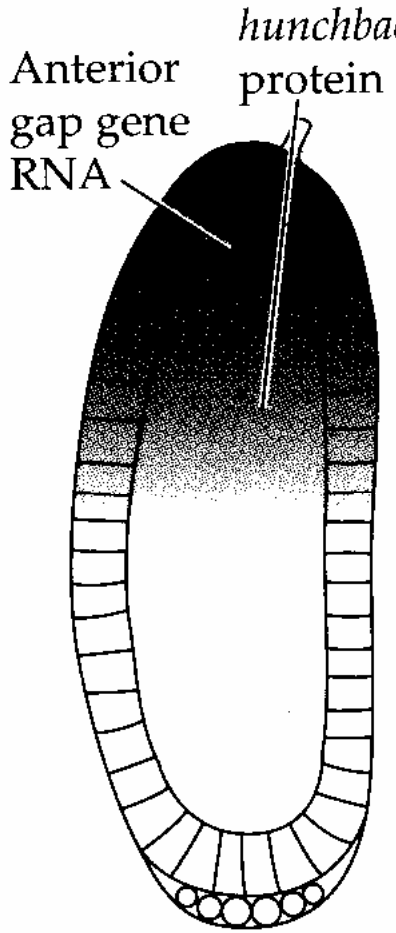
Ovarian nurse cells secrete *bicoid* mRNA into oocyte



bicoid mRNA localized in anterior by products of *exuparantia* and *swallow*

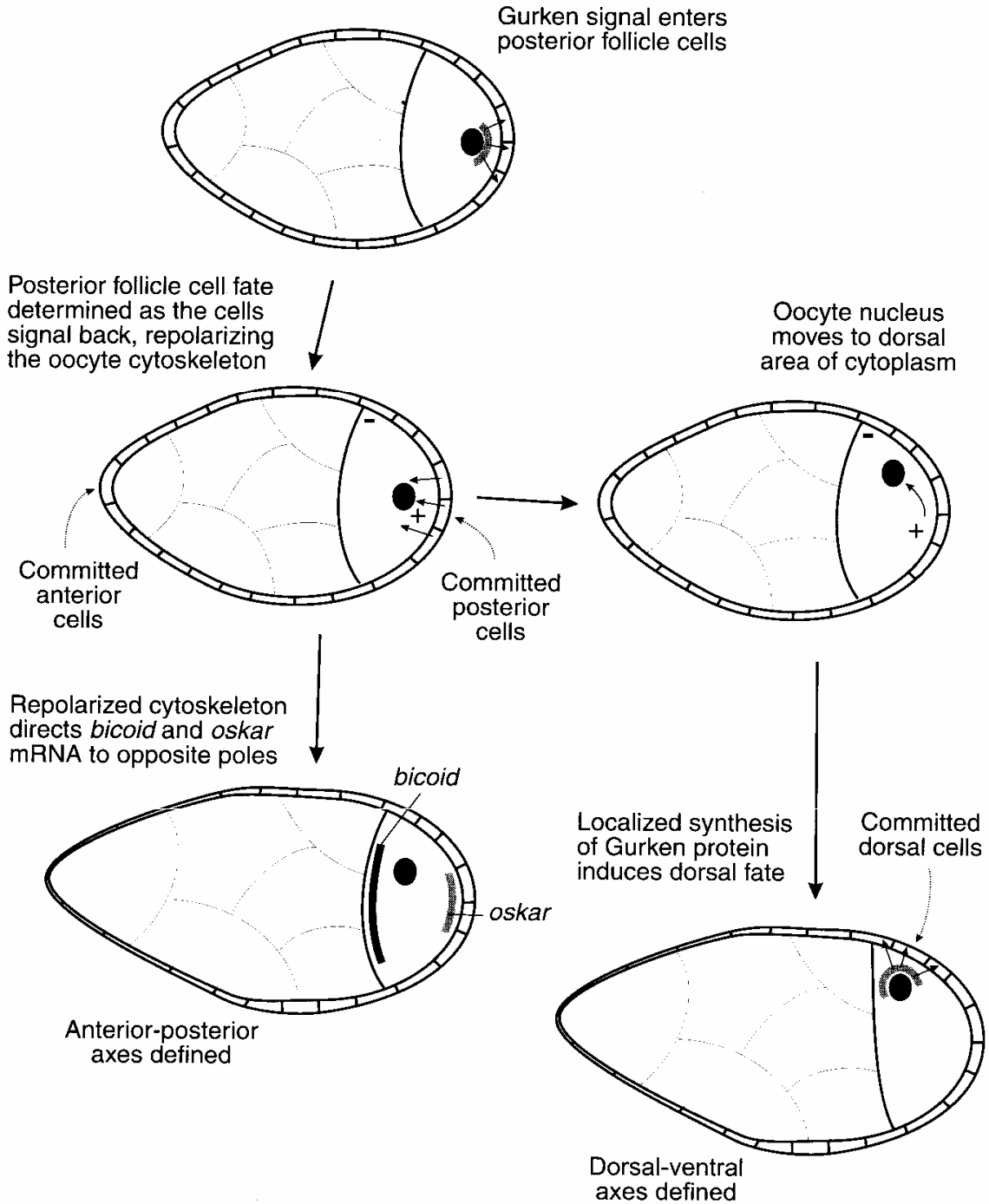


bicoid mRNA translated and forms protein gradient



bicoid protein activates anterior gap genes and the *hunchback* gene

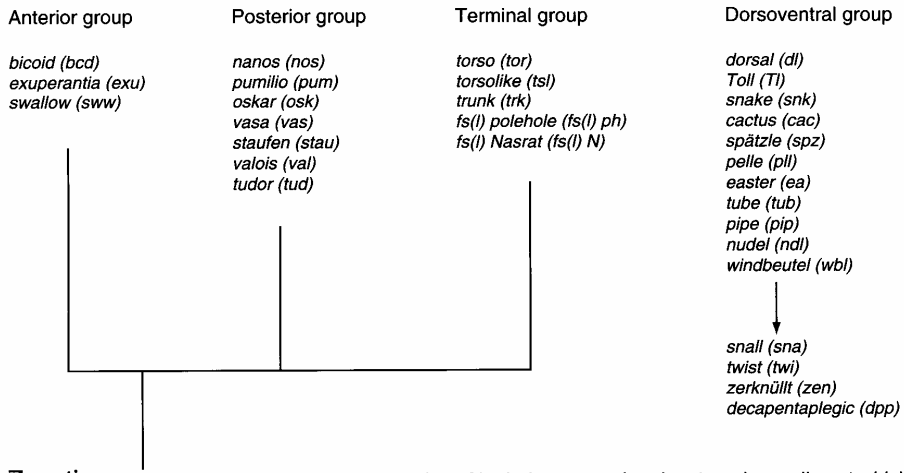
Genes have been identified, for Example gurken, that are required in the oocyte for the proper development of the surrounding follicle cells



GENETIC INSTRUCTIONS FOR EGG DEVELOPMENT

1. **MATERNAL GENOME**
   - **a. Maternal effect genes**
2. **ZYGOTE GENOME**
   - **a. Gap genes**
   - **b. Pair-rule genes**
   - **c. Segment polarity genes**
   - **d. Homeotic genes**

Maternal genes Genes from the mother that produce transcription products that have their regulating effect on establishing the pattern and polarity of the egg prior to blastoderm formation. The anterior, posterior, and terminal maternal gene groups have a regulatory effect on the zygotic genes referred to as gap genes. In general, they produce the address labels for the different regions of the egg.



Zygotic genes Genes resulting from the union of both the paternal and maternal compliment which in one way or another are involved in segmentation of the developing embryo. These segmentation genes provide the segmentation identity which was already determined by the address labels provided by the maternal genes.

Gap genes: Genes that regulate large overlapping regions of the embryo and cover several parasegments; influenced by the maternal genes.

hunchback (hb)
Krüppel (Kr)
knirps (kni)
giant (gt)
tailless (tll)

→

Pair-rule genes: Genes that regulate and act in double segmental units or alternate parasegments; influenced by the gap genes.

even skipped (eve)
hairy (h)
runt (run)
fushi tarazu (ftz)
odd-paired (opa)
odd-skipped (odd)
sloppy-paired (slp)

→

Segment-polarity genes: Genes that regulate and affect every parasegment and usually do this by controlling cell-to-cell communication of signals from the different morphogens; influenced by the pair-rule genes.

engrailed (en)
wingless (wg)
patched (ptc)
gooseberry (gsb)
paired (prd)
armadillo (arm)
cubitus-interuptus (ci ^{D})
fused (fu)
naked (nkd)
hedgehog (hh)
dishevelled (l(l)dsh)

Homoeotic genes Genes that are active along the anterior-dorsal axis and give the parasegment its identity in expression; often influence appendage formation.

proboscipedia (pb)
Deformed (Dfd)
Sex combs reduced (Scr)
Antennapedia (Antp)
Ultrabithorax (Ubx)
Abdominal-A (abd-A)
Abdominal-B (abd-B)

Polarity and regional specificity are determine very early in the egg

Dorsoventral pattern-20 genes control this

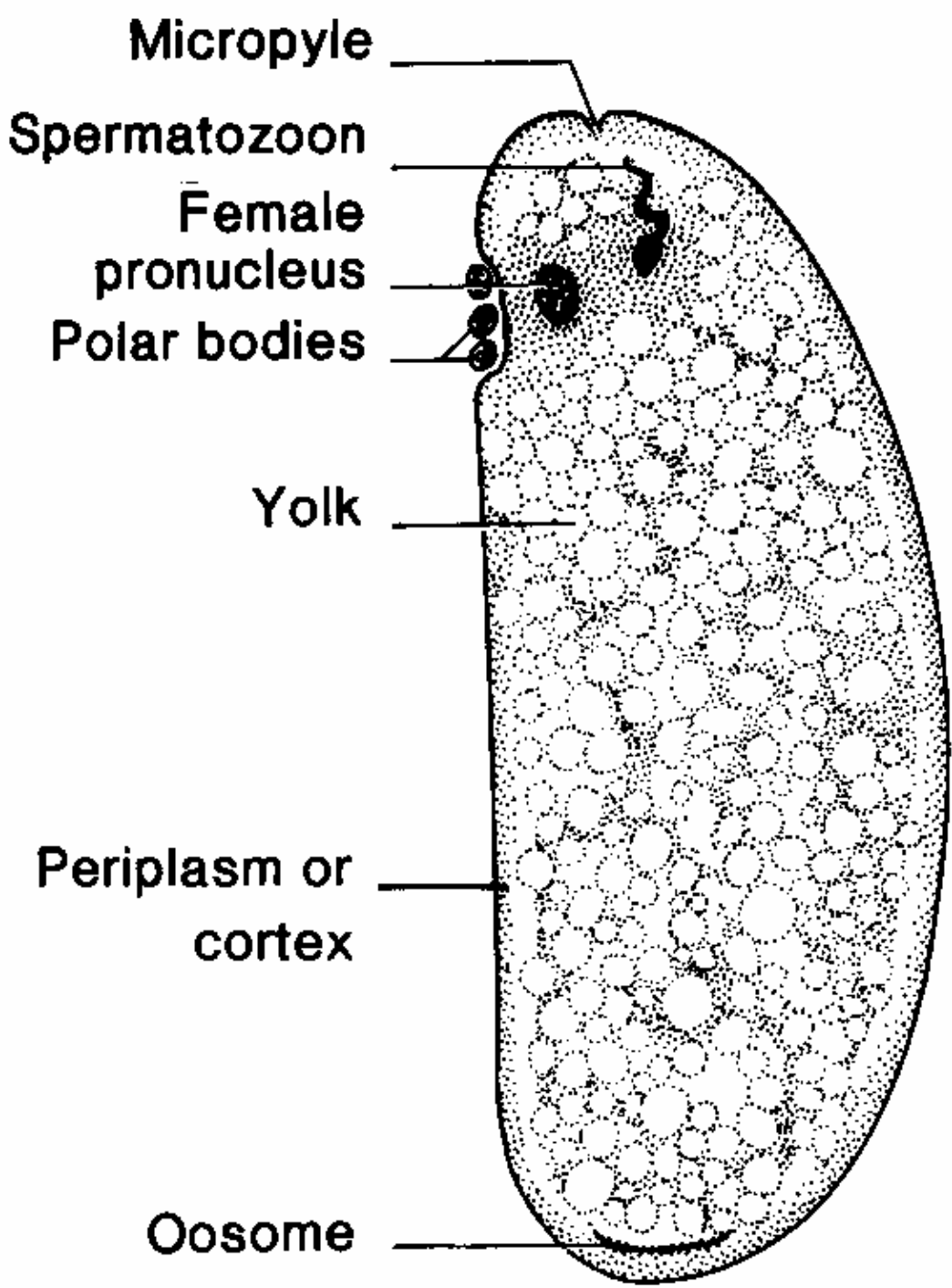
Antero-posterior pattern-50 genes control this

Regional specificity-comparable to the external segmentation but do not coincide exactly with the true segments. These regions are called parasegments. Thus, region T1 or the prothoracic area of the egg will become T1 of the adult.

The internal composition of the egg is not uniform

Immediately beneath the plasma membrane the cytoplasm, called periplasm, is very different than elsewhere.

Also, the egg has been divided into regions based on proteins that have been derived from the female genome or produced in situ that influence the sequential activation or repression of specific genes by these gradients. These proteins are called **morphogens**.



All of the events requiring movements or changes in shape usually involve various cytoskeletal filaments such as actin and microtubulin

Skeletal molecules must be in place to hold other molecules in place and to move molecules and the general shape of the embryo once it is formed. Some of the molecules involved are actin and microtubulin.

Stein, W.D. and F. Bronner. 1989. Cell shape-determinants, regulation, and regulatory role. Academic Press, N.Y.

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Anterior group

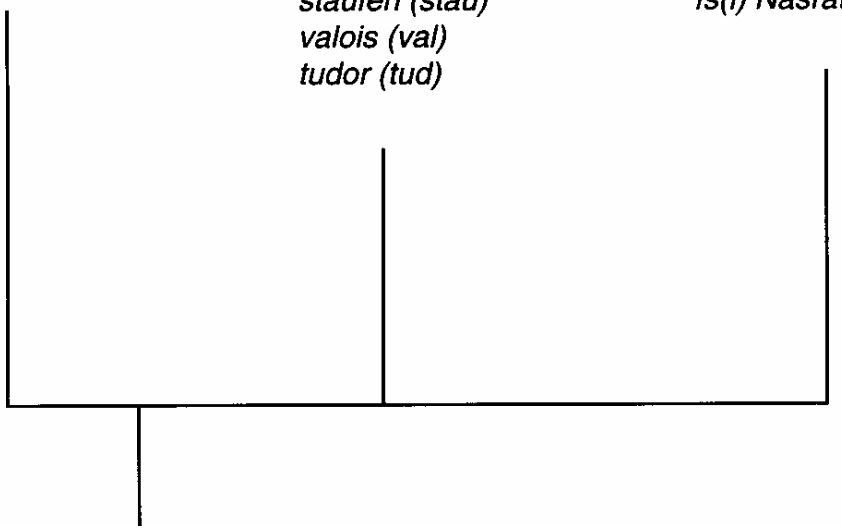
bicoid (bcd)
exuperantia (exu)
swallow (sww)

Posterior group

nanos (nos)
pumilio (pum)
oskar (osk)
vasa (vas)
staufen (stau)
valois (val)
tudor (tud)

Terminal group

torso (tor)
torsolike (tsl)
trunk (trk)
fs(l) polehole (fs(l) ph)
fs(l) Nasrat (fs(l) N)



Dorsoventral group

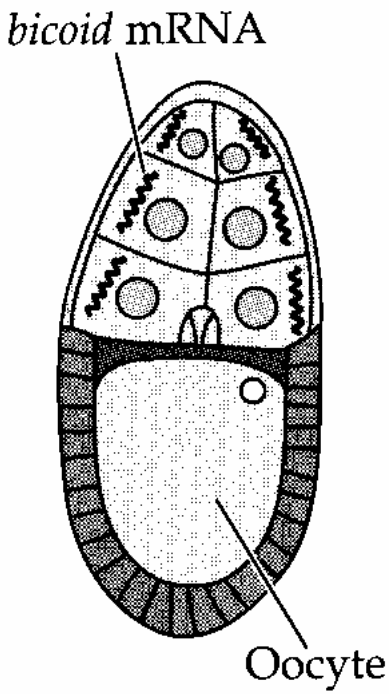
dorsal (dl)
Toll (Tl)
snake (snk)
cactus (cac)
spätzle (spz)
pelle (pll)
easter (ea)
tube (tub)
pipe (pip)
nudel (ndl)
windbeutel (wbl)

snail (sna)
twist (twi)
zerknüllt (zen)
decapentaplegic (dpp)

MATERNAL TRANSCRIPTS (mRNA)

Mid-oogenesis	Completion of oogenesis	Syncytial blastoderm	Cellular blastoderm

Anterior bicoid



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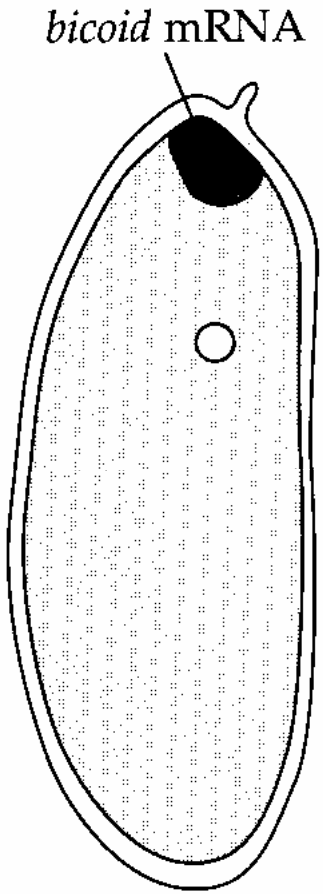

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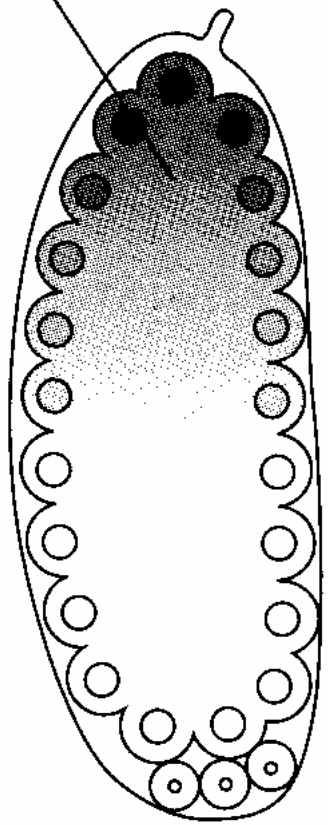

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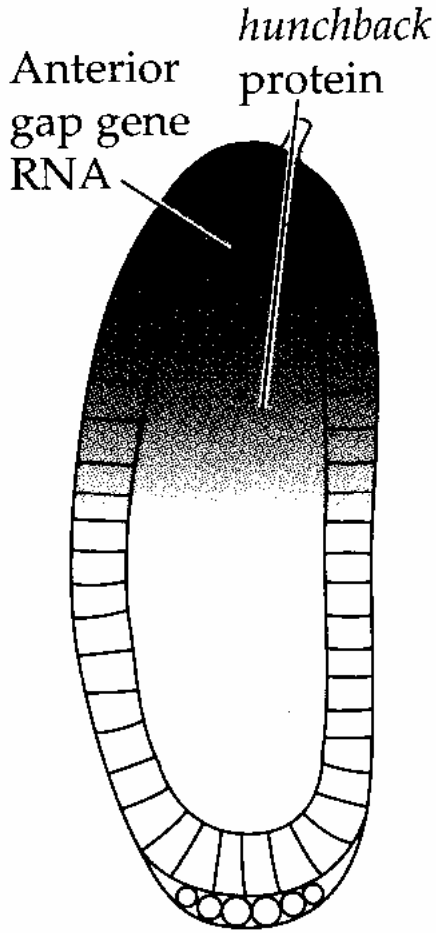


bicoid protein activates anterior gap genes and the *hunchback* gene

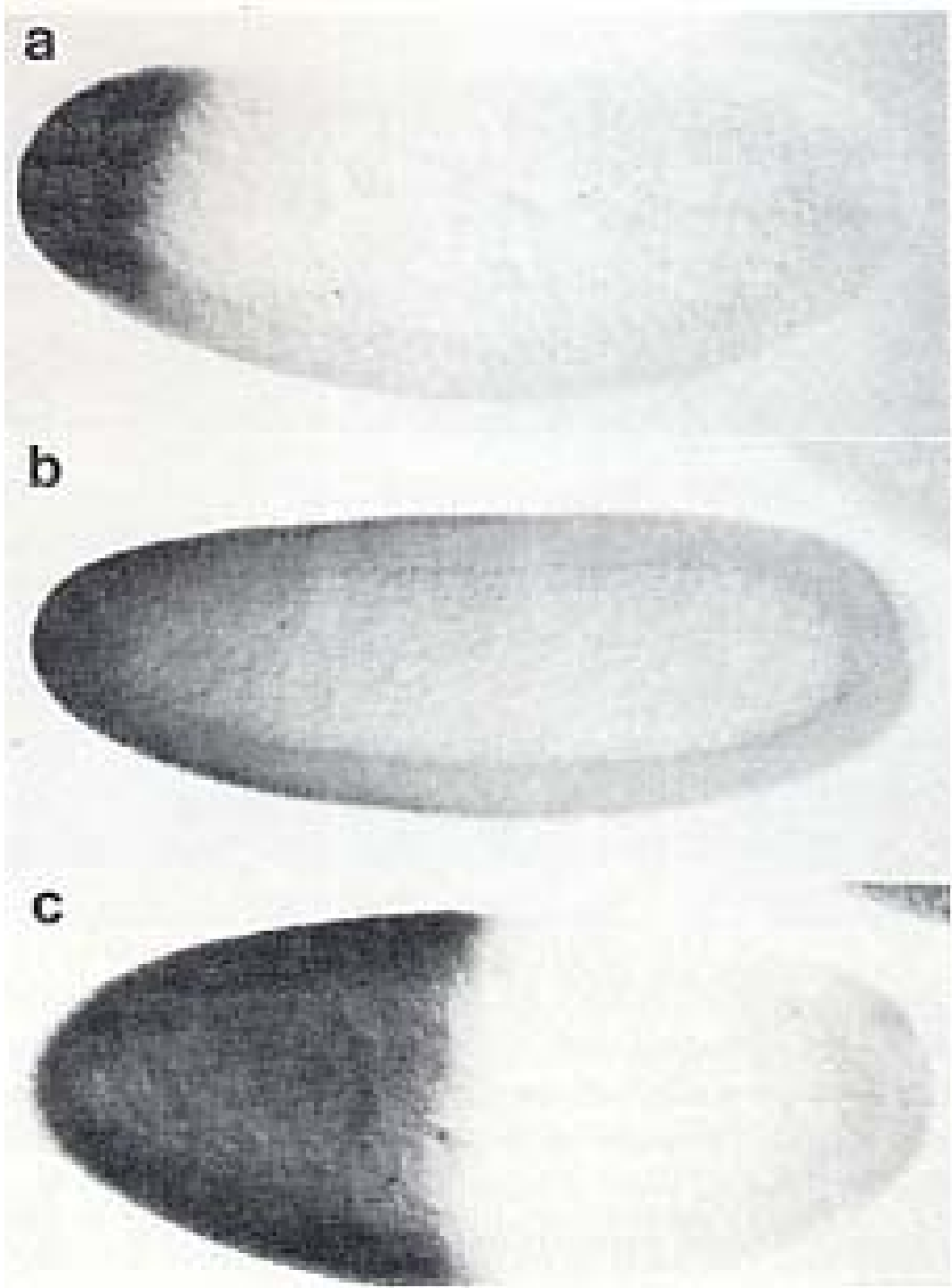
ANTERIOR DETERMINATION

a. Detection, using a probe for the bicoid mRNA

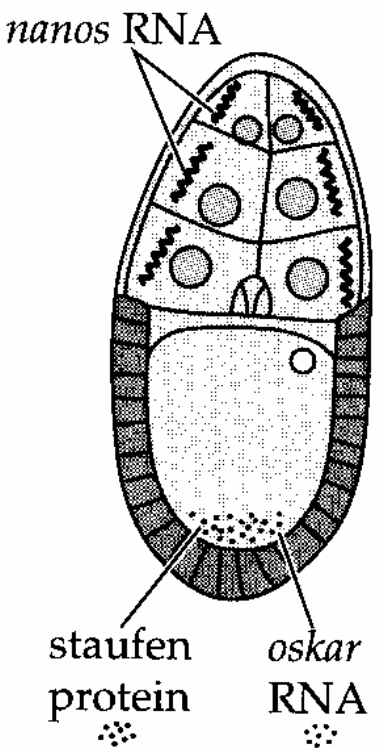
b. the bicoid protein

plus the

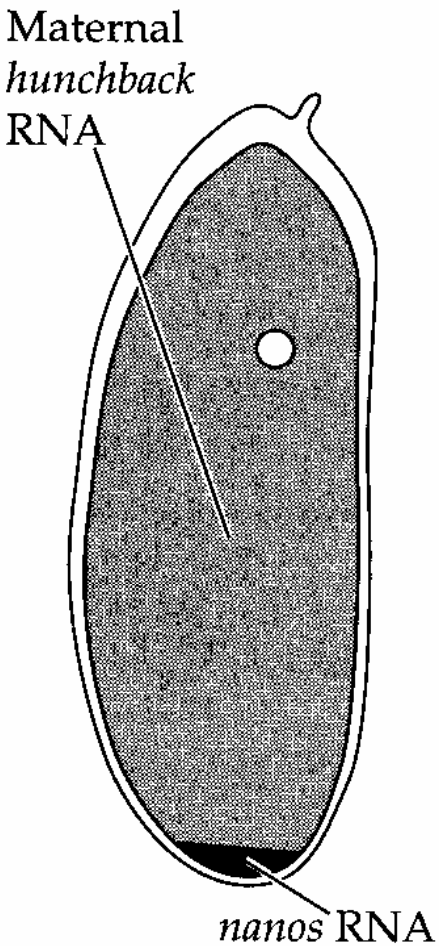
c. presence of the hunchback (hb mRNA) in the anterior of the eggs of *Drosophila*.



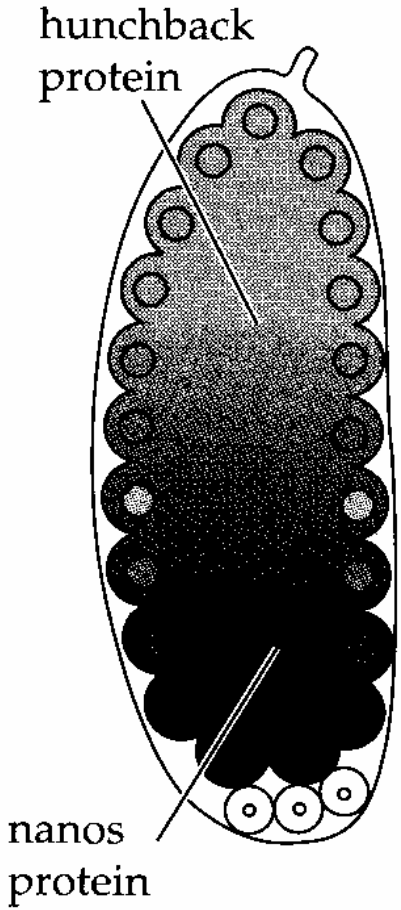
Posterior: nanos



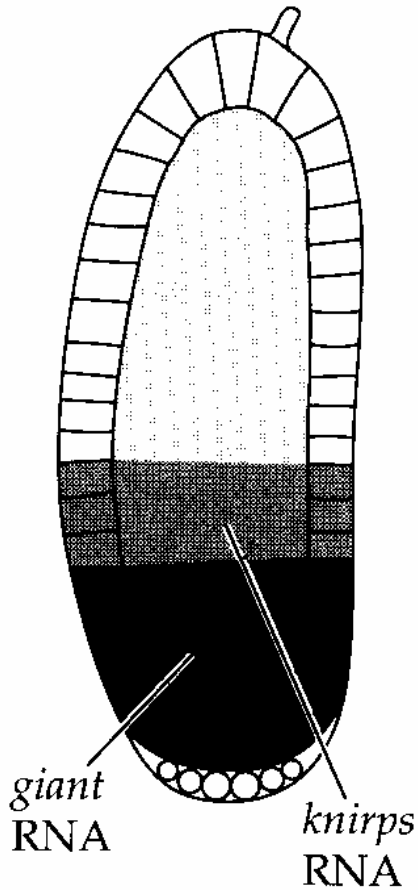
Ovarian nurse cells secrete posterior "scaffold" to bind *nanos* mRNA



nanos mRNA secreted by ovarian nurse cells localized to posterior pole

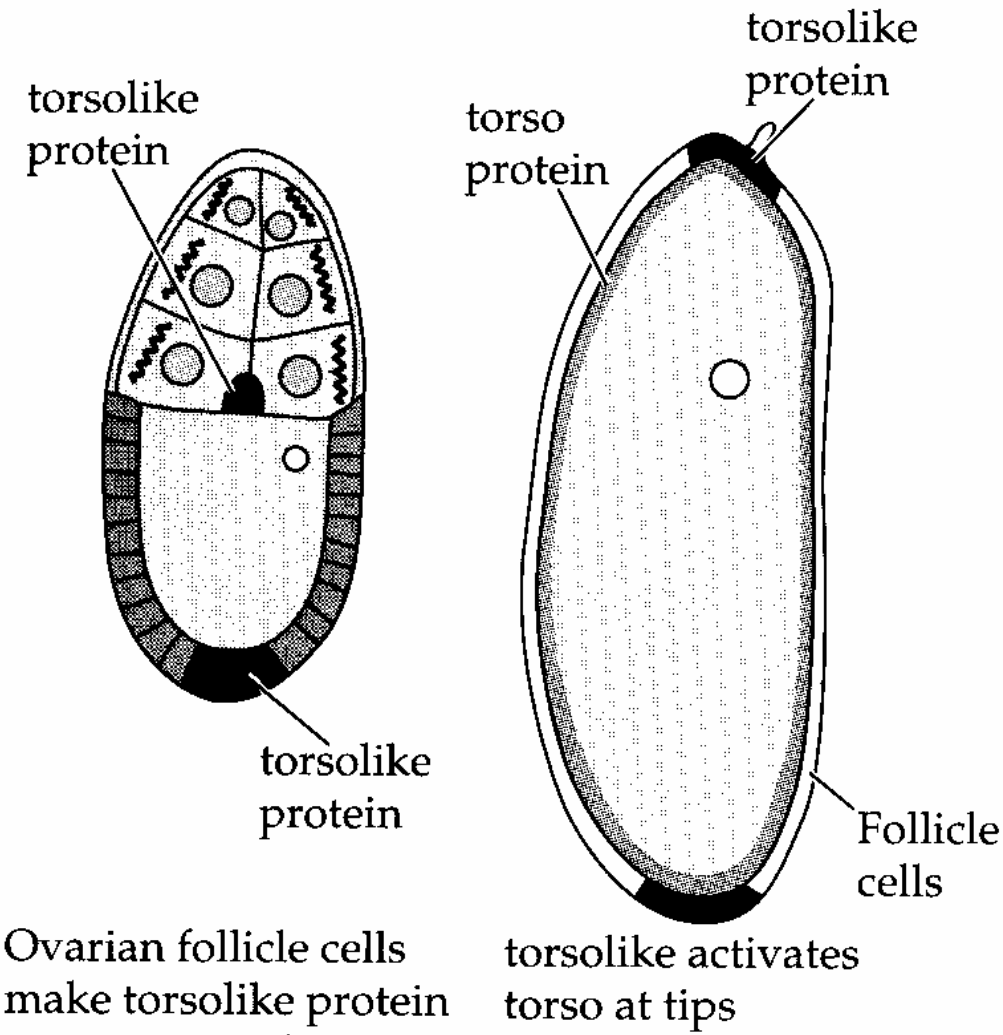


nanos mRNA translated and blocks translation of *hunchback* message in posterior of embryo



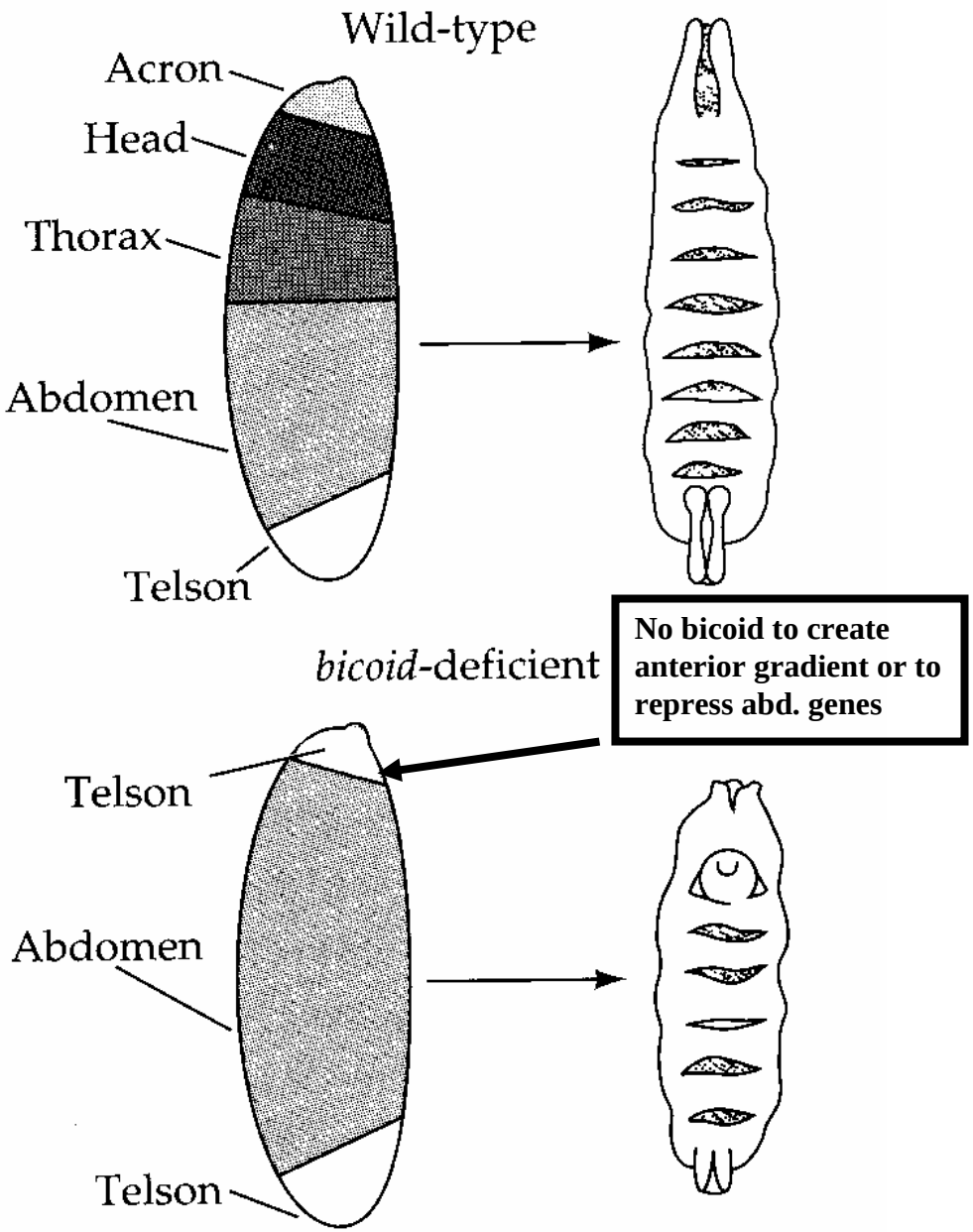
nanos activates *knirps* of posterior gap genes (such as *giant*)

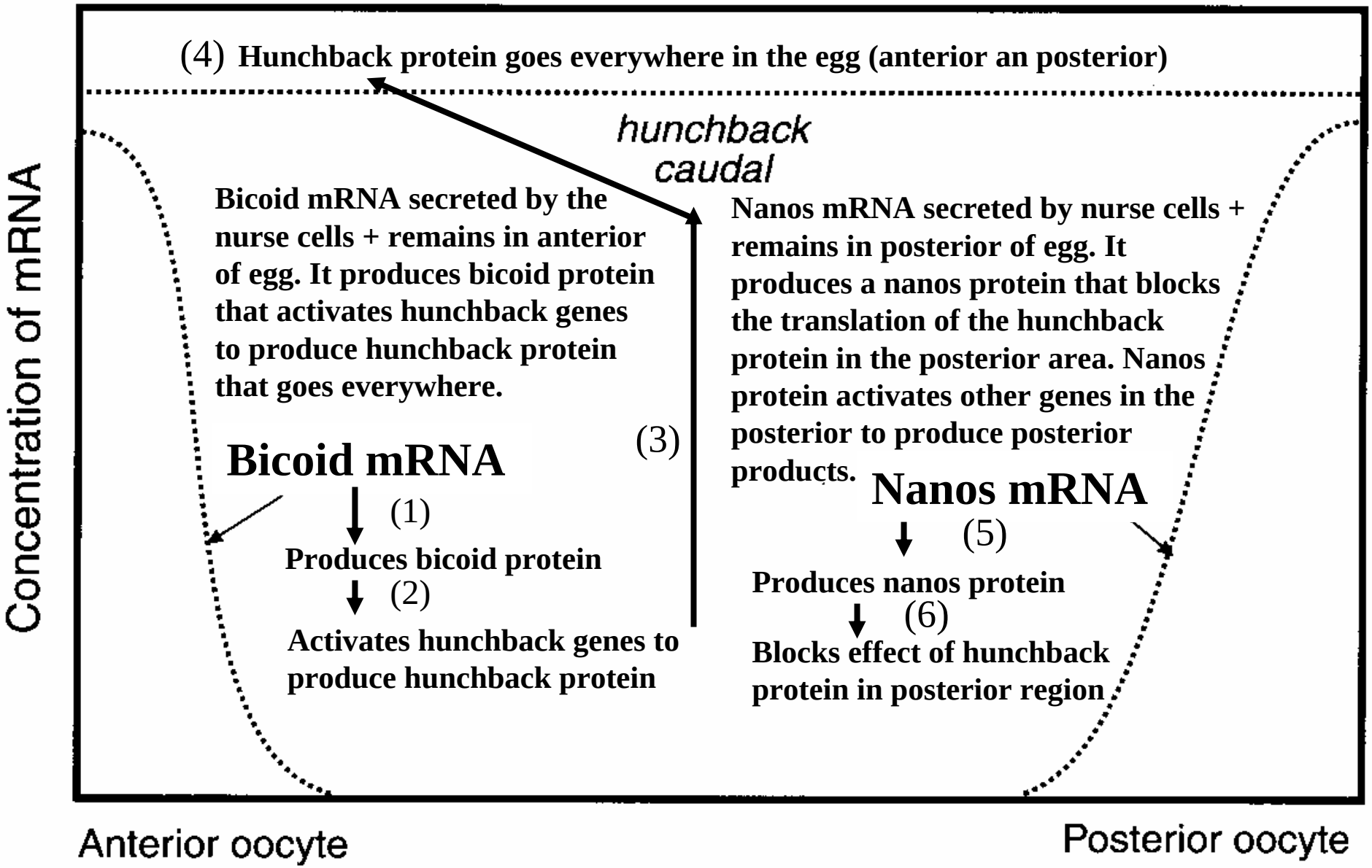
Terminal: torso



Ovarian follicle cells make torsolike protein at anterior and posterior tips

torsolike activates torso at tips


Wild-type
Acron
Head
Thorax
Abdomen
Telson
bicoid-deficient
No bicoid to create anterior gradient or to repress abd. genes
Telson
Abdomen
Telson
nanos-deficient
Acron
Head
Thorax
Telson
torso-deficient
Head
Thorax
Abdomen


(4) Hunchback protein goes everywhere in the egg (anterior an posterior)
hunchback
caudal
Concentration of mRNA
Bicoid mRNA secreted by the nurse cells + remains in anterior of egg. It produces bicoid protein that activates hunchback genes to produce hunchback protein that goes everywhere.
Bicoid mRNA
(1)
Produces bicoid protein
(2)
Activates hunchback genes to produce hunchback protein
(3)
Nanos mRNA secreted by nurse cells + remains in posterior of egg. It produces a nanos protein that blocks the translation of the hunchback protein in the posterior area. Nanos protein activates other genes in the posterior to produce posterior products.
Nanos mRNA
(5)
Produces nanos protein
(6)
Blocks effect of hunchback protein in posterior region
Anterior oocyte
Posterior oocyte

The expression and repression of these maternal genes finally results in the turning on of the zygotic genes in certain areas of the egg. The first of these is the **GAP GENES**. Once expressed, other genes activate the **PAIR-GENES** that create parasegments, which in turn activate the **SEGMENT-POLARITY GENES** that affect every parasegment

Zygotic genes Genes resulting from the union of both the paternal and maternal compliment which in one way or another are involved in segmentation of the developing embryo. These segmentation genes provide the segmentation identity which was already determined by the address labels provided by the maternal genes.

Gap genes: Genes that regulate large overlapping regions of the embryo and cover several parasegments; influenced by the maternal genes.

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Krüppel (Kr)
knirps (kni)
giant (gt)
tailless (tll)

Pair-rule genes: Genes that regulate and act in double segmental units or alternate parasegments; influenced by the gap genes.

even skipped (eve)
hairy (h)
runt (run)
fushi tarazu (ftz)
odd-paired (opa)
odd-skipped (odd)
sloppy-paired (slp)

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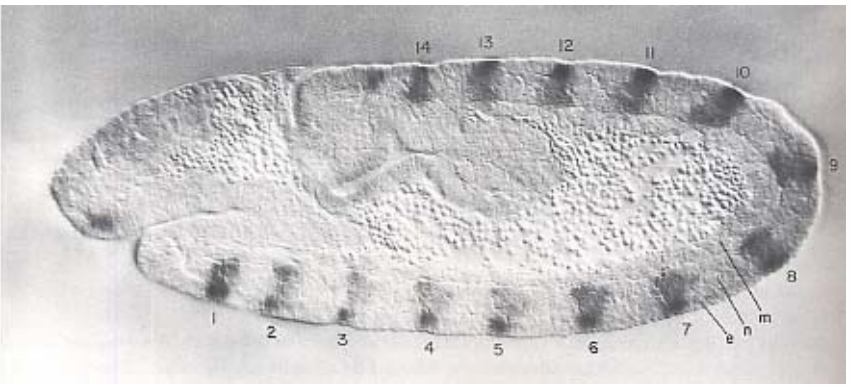
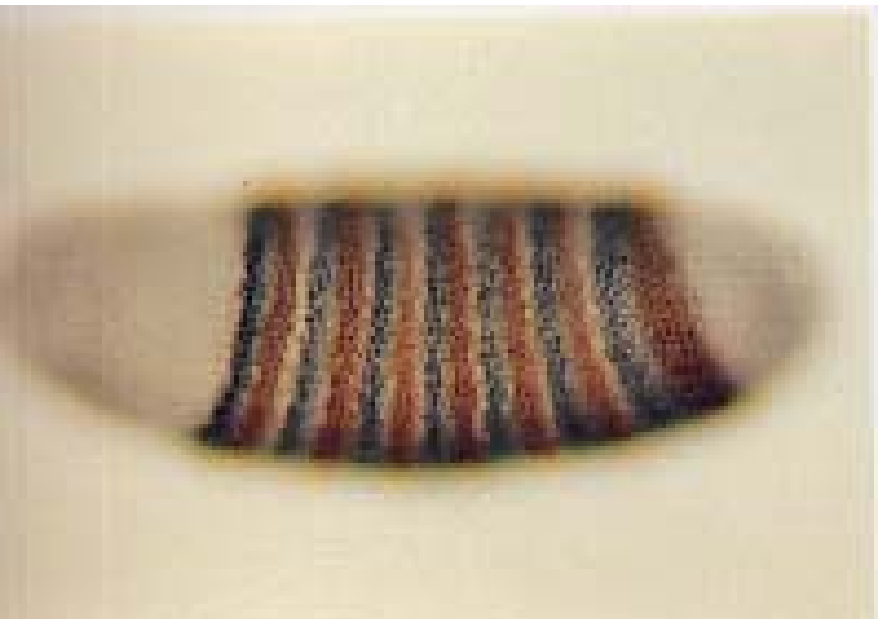
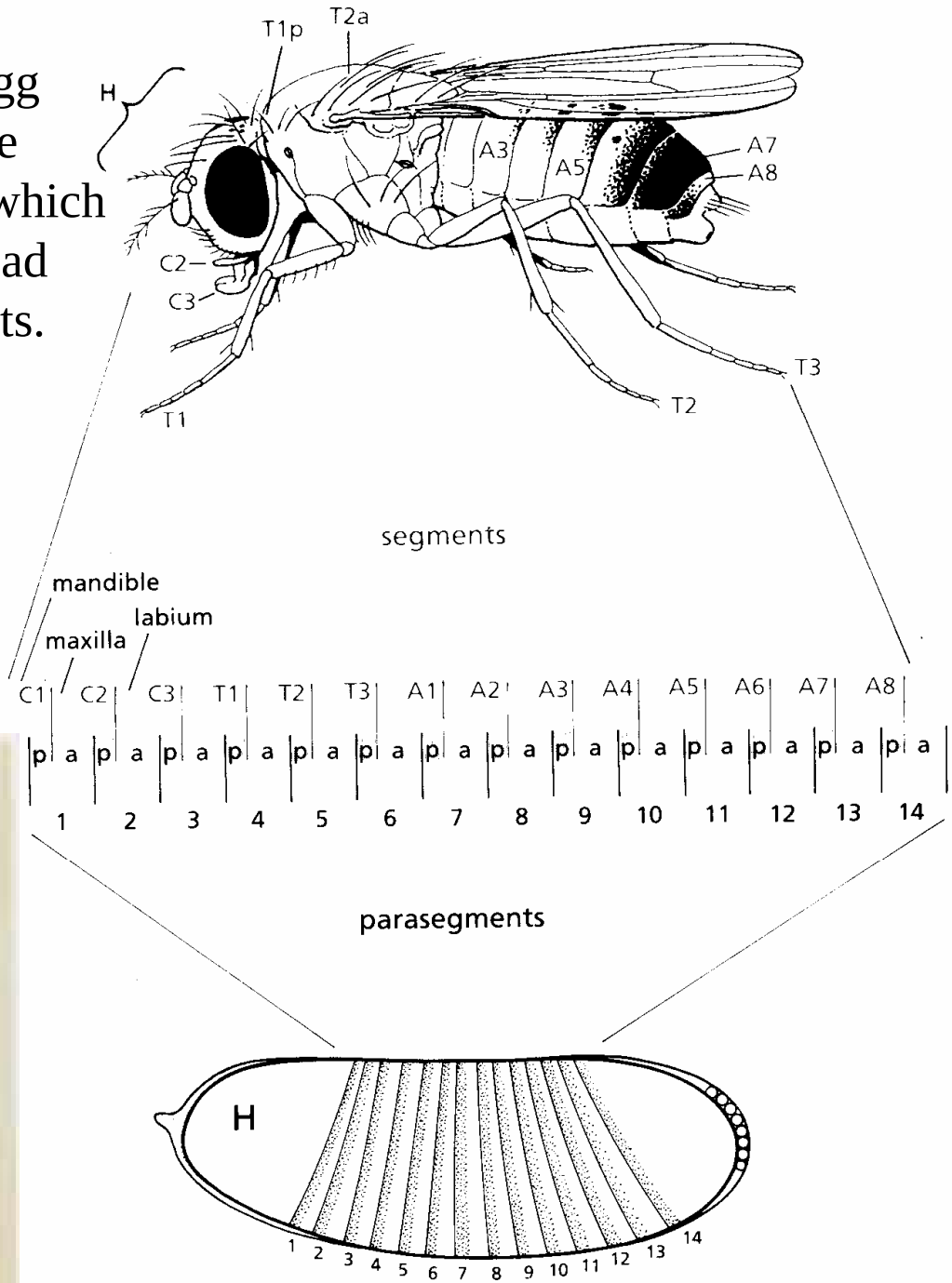
engrailed (en)
wingless (wg)
patched (ptc)
gooseberry (gsb)
paired (prd)
armadillo (arm)
cubitus-interuptus (ci ^{D})
fused (fu)
naked (nkd)
hedgehog (hh)
dishevelled (l(l)dsh)

PARASEGMENTS

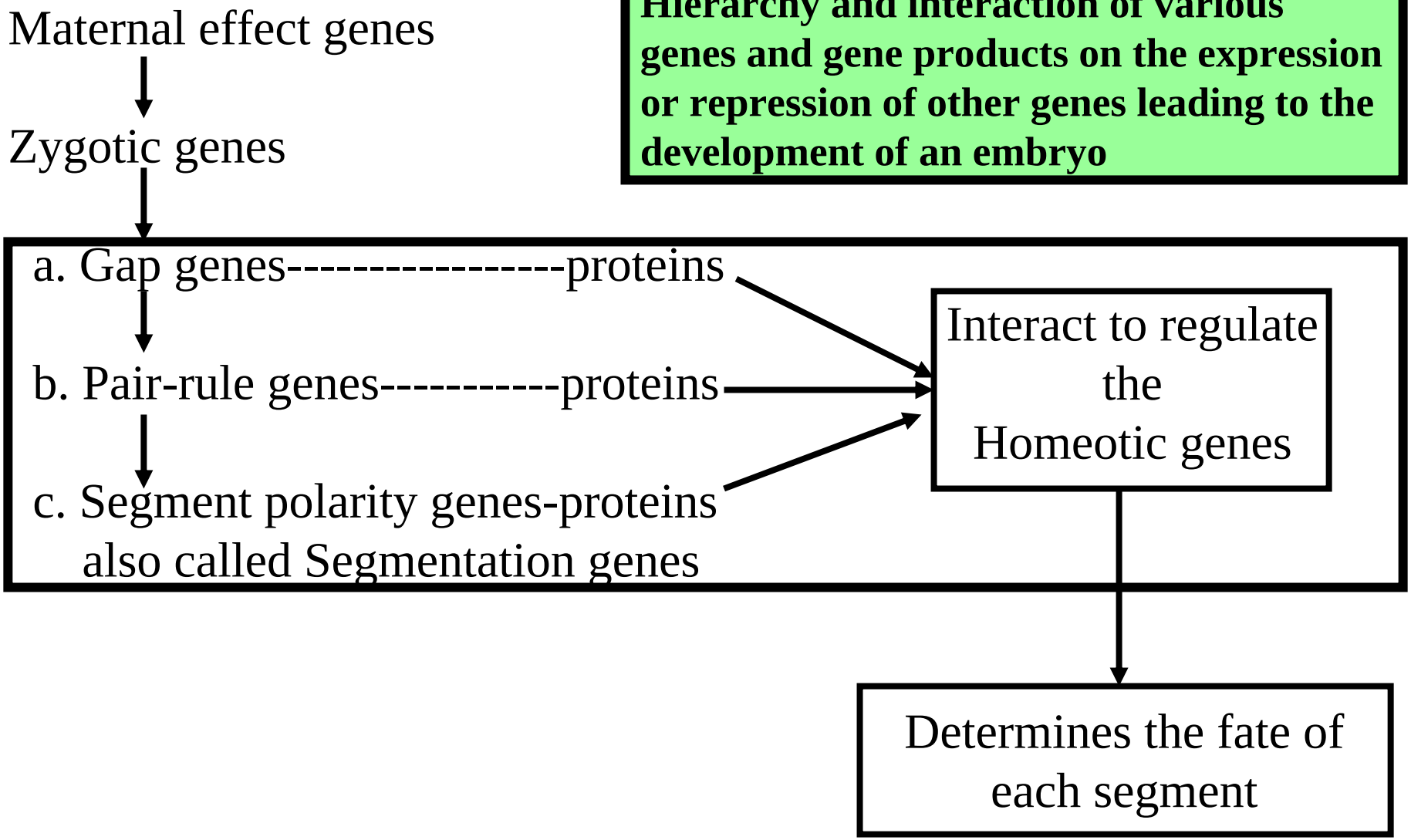
Maternal effect genes determine the egg pattern of the **gap genes**, which regulate the expression of the **pair-rule genes**, which in turn, when transcribed divide the broad gap regions into individual parasegments.







Hierarchy and interaction of various genes and gene products on the expression or repression of other genes leading to the development of an embryo

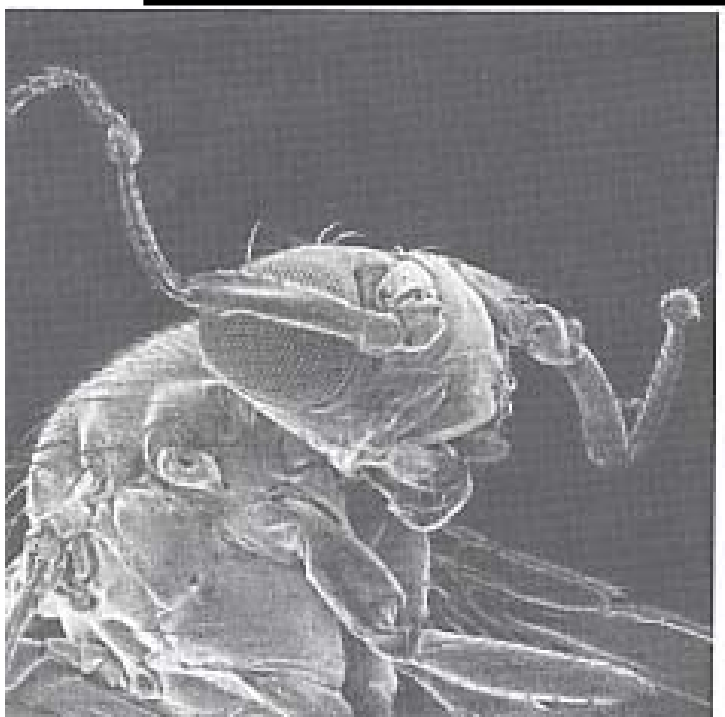


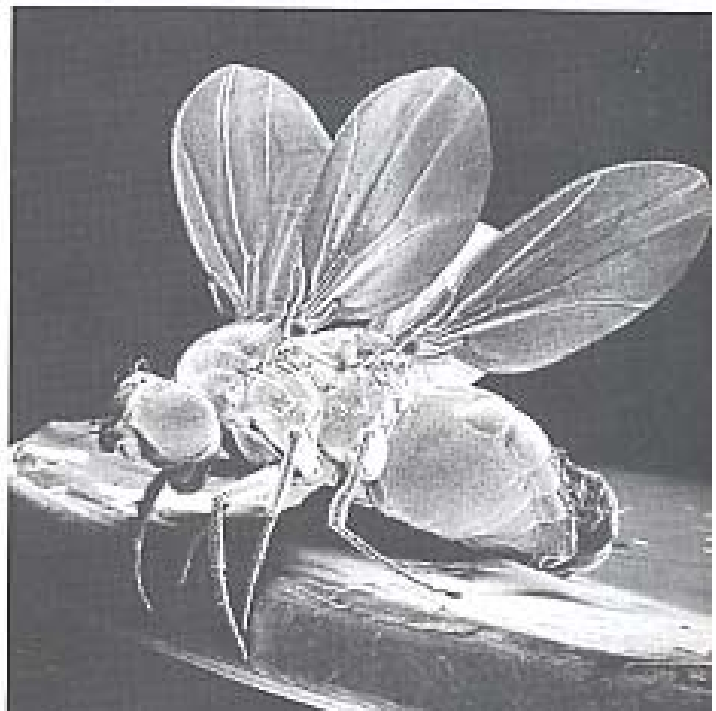
Originally it was thought that the ability of various master or regulatory genes to produce such major changes (two pairs wings in flies) via single-gene mutations could provide a simple mechanism to account for the evolution of major morphological changes. An example of this could be the loss of one pair of wings in primitive Diptera, as fossil evidence now reveals they were once four-winged (Riek, 1977). This original interpretation, however, was incorrect since the ultrabithorax (ubx) gene is also found in the Lepidoptera, Crustacea, and annelids; thus, it was prior to the origin of halteres and, in fact, wings. What occurred in the Diptera was a change in the way the ubx gene set was regulated. Even more exciting than demonstrating the presence of homeotic genes is the evidence that most homeotic genes studies to date-whether they are from earthworms, centipedes, insects, frogs, or humans-have a highly conserved (retained through evolution) region of the DNA that encodes a sequence of 180 nucleotide pairs. This is known as the **homeobox** and represents a highly conserved and uniform sequence of DNA. The reason this regions has been so resistant to evolutionary change remains speculative.

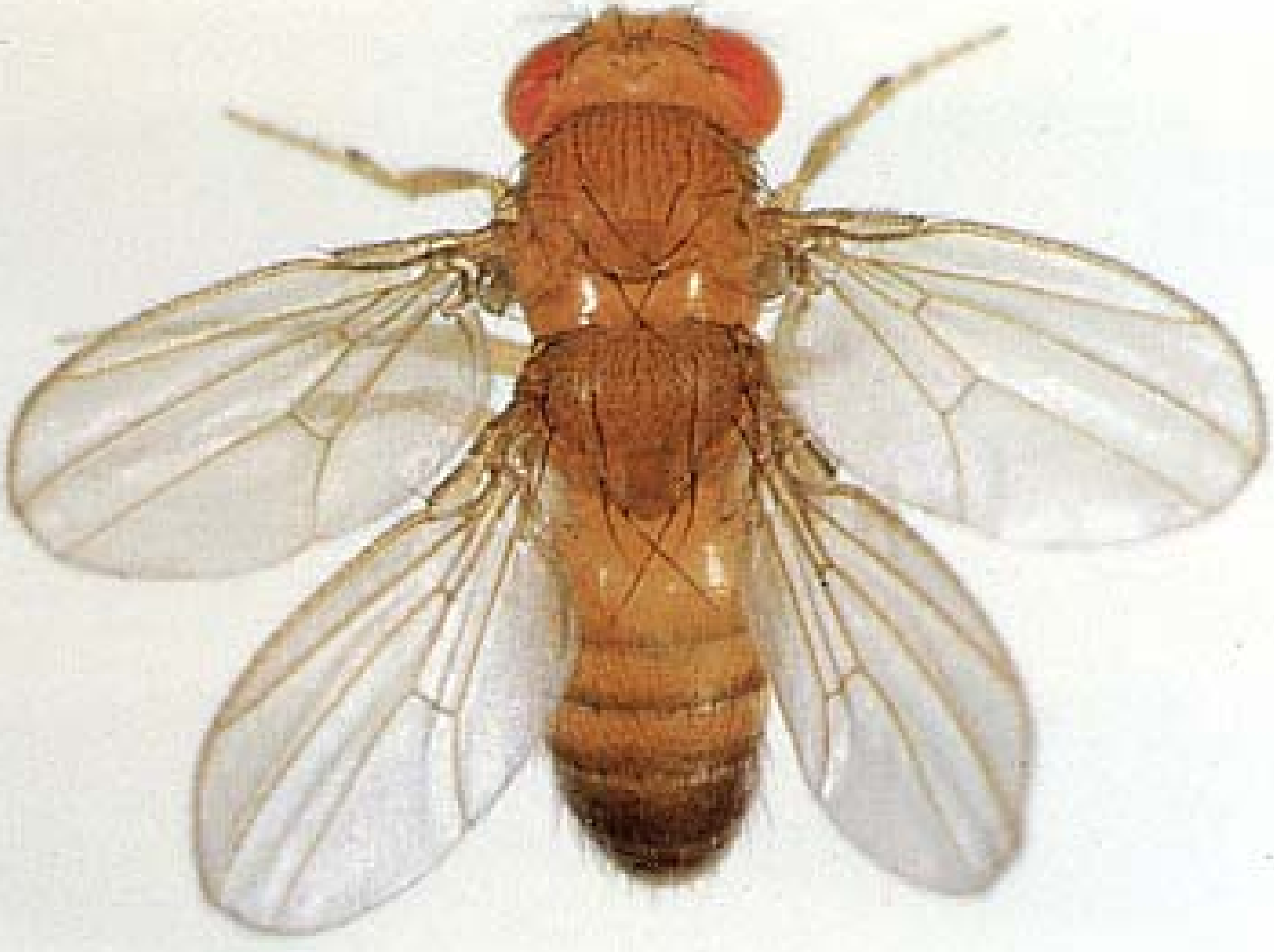
Homoeotic genes Genes that are active along the anterior-dorsal axis and give the parasegment its identity in expression; often influence appendage formation.

proboscipedia (pb)
Deformed (Dfd)
Sex combs reduced (Scr)
Antennapedia (Antp)
Ultrabithorax (Ubx)
Abdominal-A (abd-A)
Abdominal-B (abd-B)

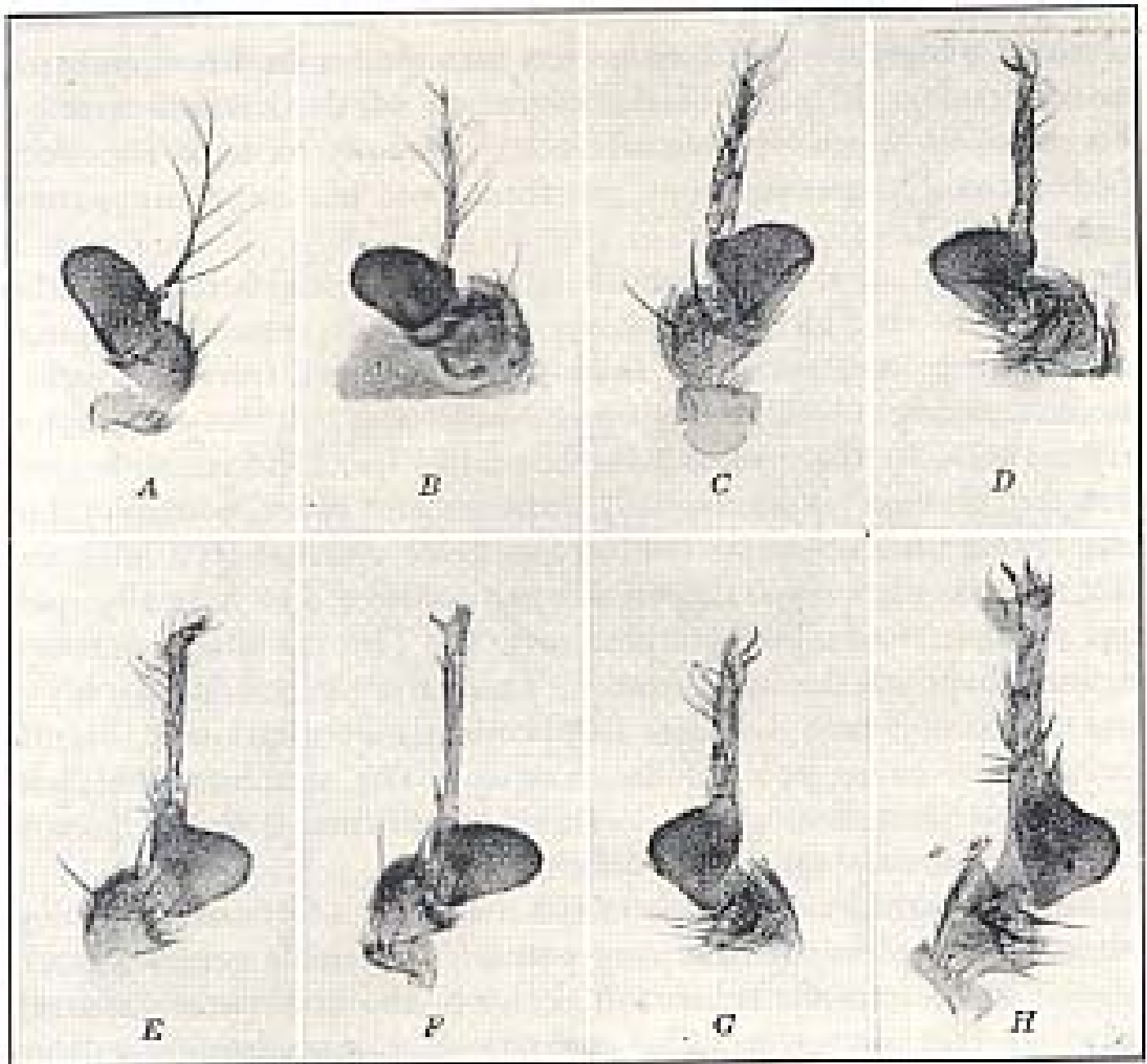
Homeotic selector genes encode a system of molecular address labels. These genes have been located in *Drosophila* and are found only in those areas showing abnormal development, either because a gene is absent or it has mutated. They are found in each parasegment of the blastoderm. Each parasegment has its own set. If the address labels are changed, that parasegment behaves as if it were somewhere else (i.e. another segment). Activation of these genes is controlled by segmentation genes.

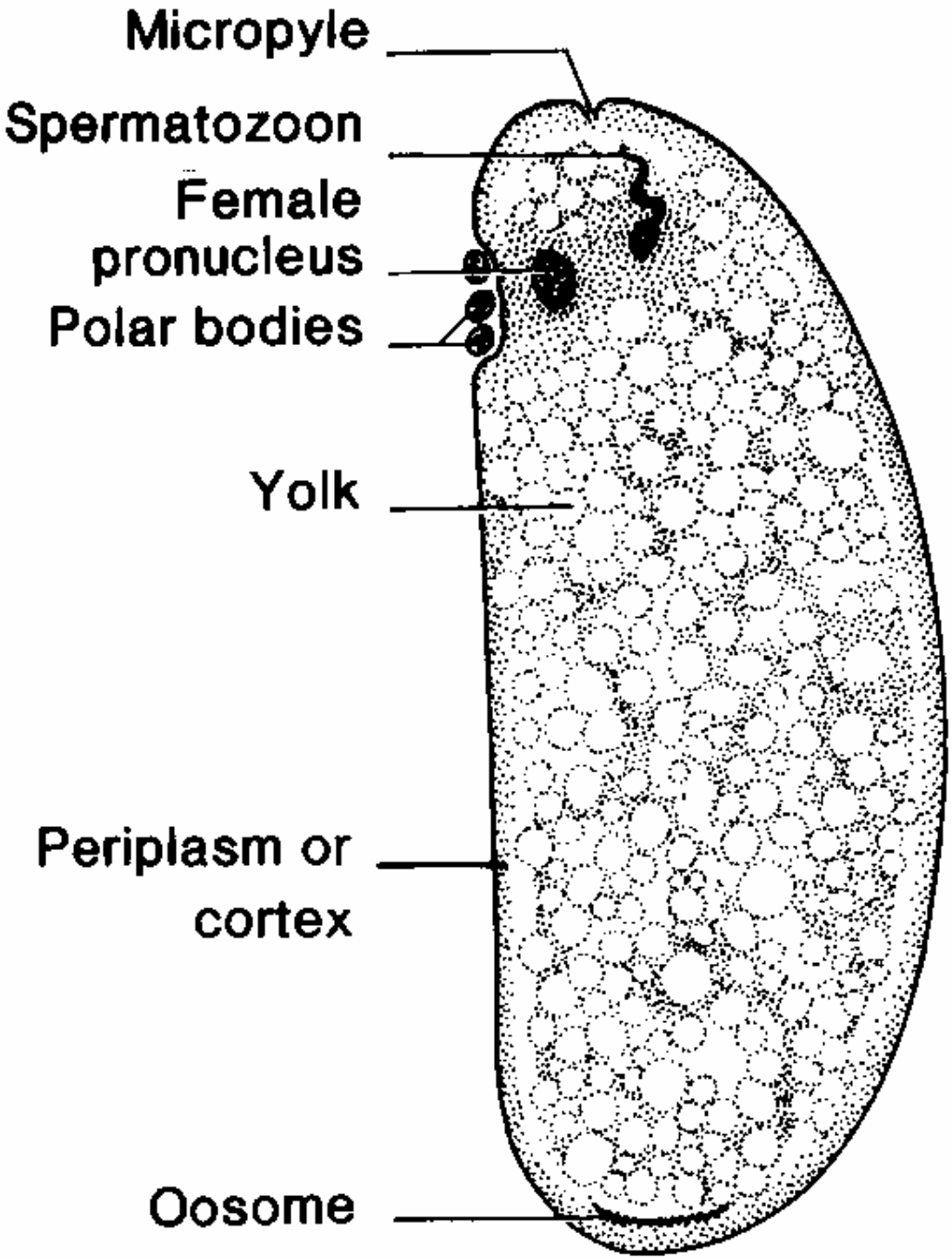






Aristapedia in *Drosophila* due to a mutation in a homeotic gene



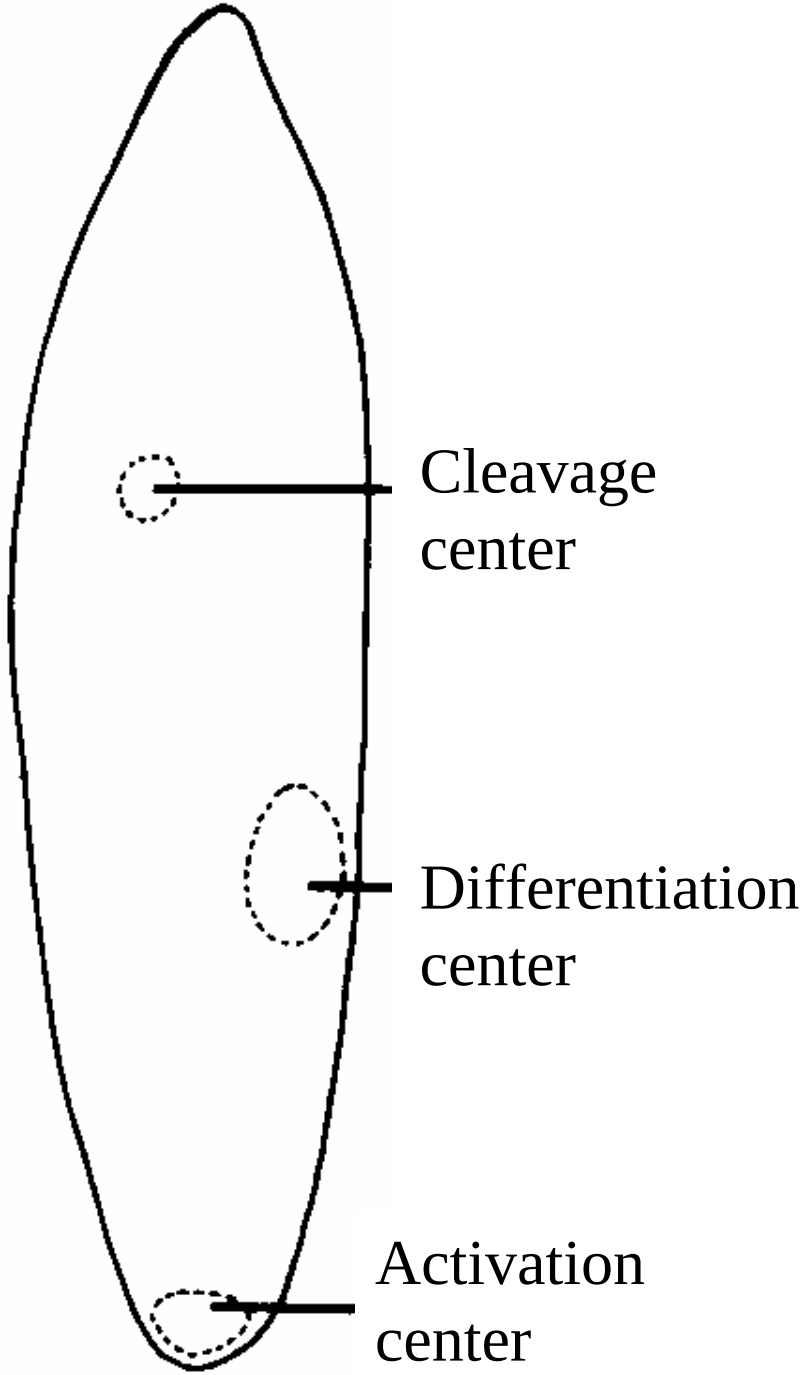


The female nucleus undergoes meiotic divisions forming 4 nuclei, 3 of which migrate to the periplasm area of the anterior and form the polar bodies leaving 1 female pronucleus. This female pronucleus now moves towards the center of the egg and accumulates cytoplasm as it moves to the center. As the sperm nucleus enters it moves to the center to the area called the cleavage center and unites with the female nucleus restoring the 2N number. In parthenogenetic insects one of the polar body nuclei unites with the female pronucleus again restoring the 2N number of chromosomes.

FERTILIZATION

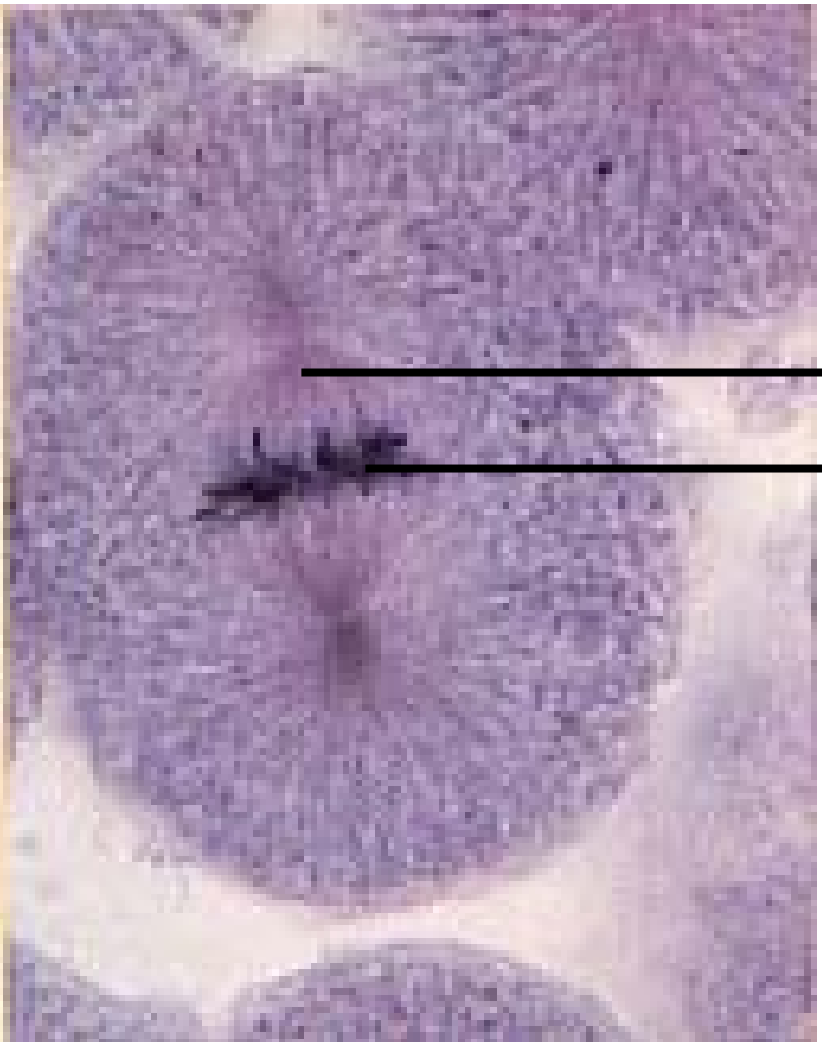
In the cleavage center, the zygote undergoes cleavage or a process of mitosis in which a number of nuclei are formed. These nuclei are called **energids** and are surround by a small amount of yolk. After a series of mitotic divisions, the energids (old literature calls them cleavage nuclei) move to the periplasm and continue to divide there. These continue to initially divide synchronously but soon division stops.

At first, these energids, lacking their own cytoplasm and cell membranes form the **syncytial blastoderm** (see next slide).

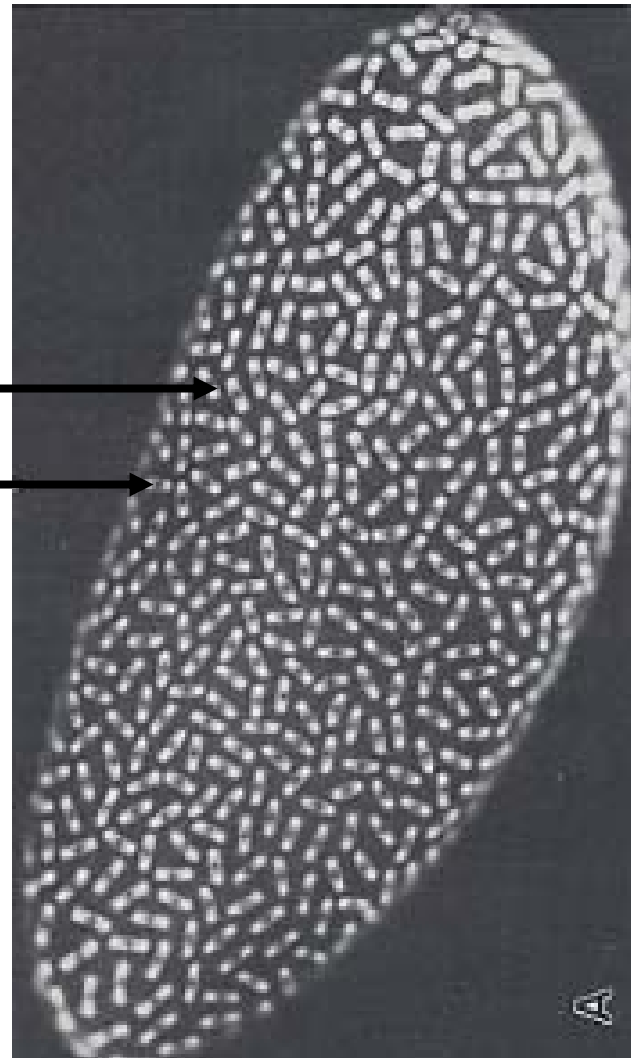


Syncytial blastoderm

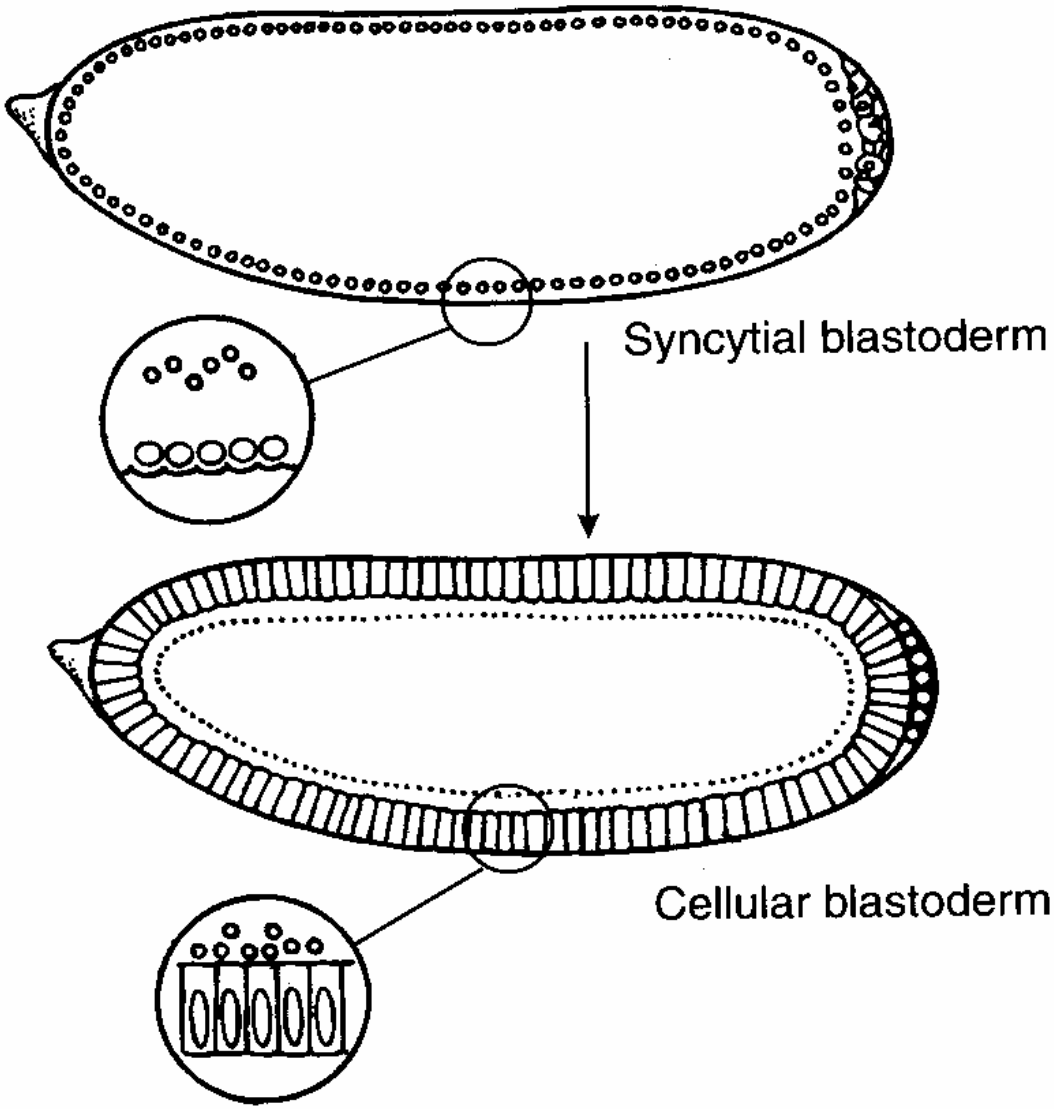
Syncytial blastoderm being formed in *Drosophila* egg (right photo). The photo on the left shows mitosis with the spindle fibers attached to the chromatids. The mitotic patterns shown in the right figure reveal an anti-tubulin staining showing the bipolar mitotic spindles (white) around each anaphase nucleus (black spot).

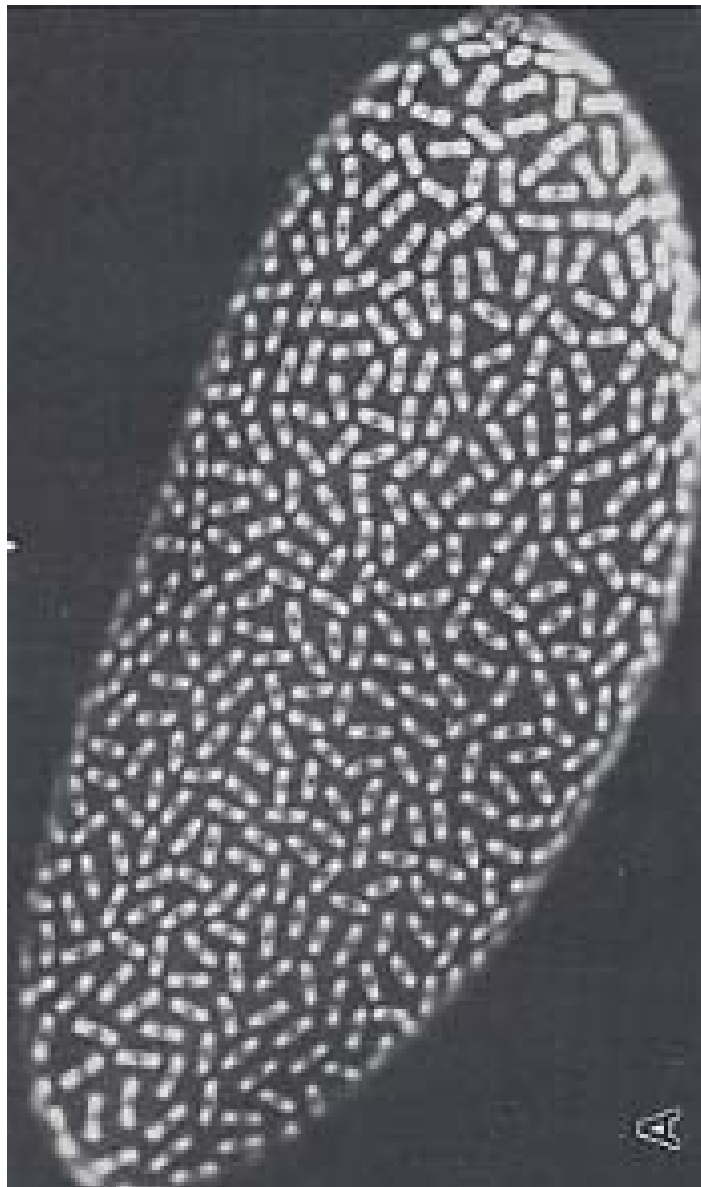


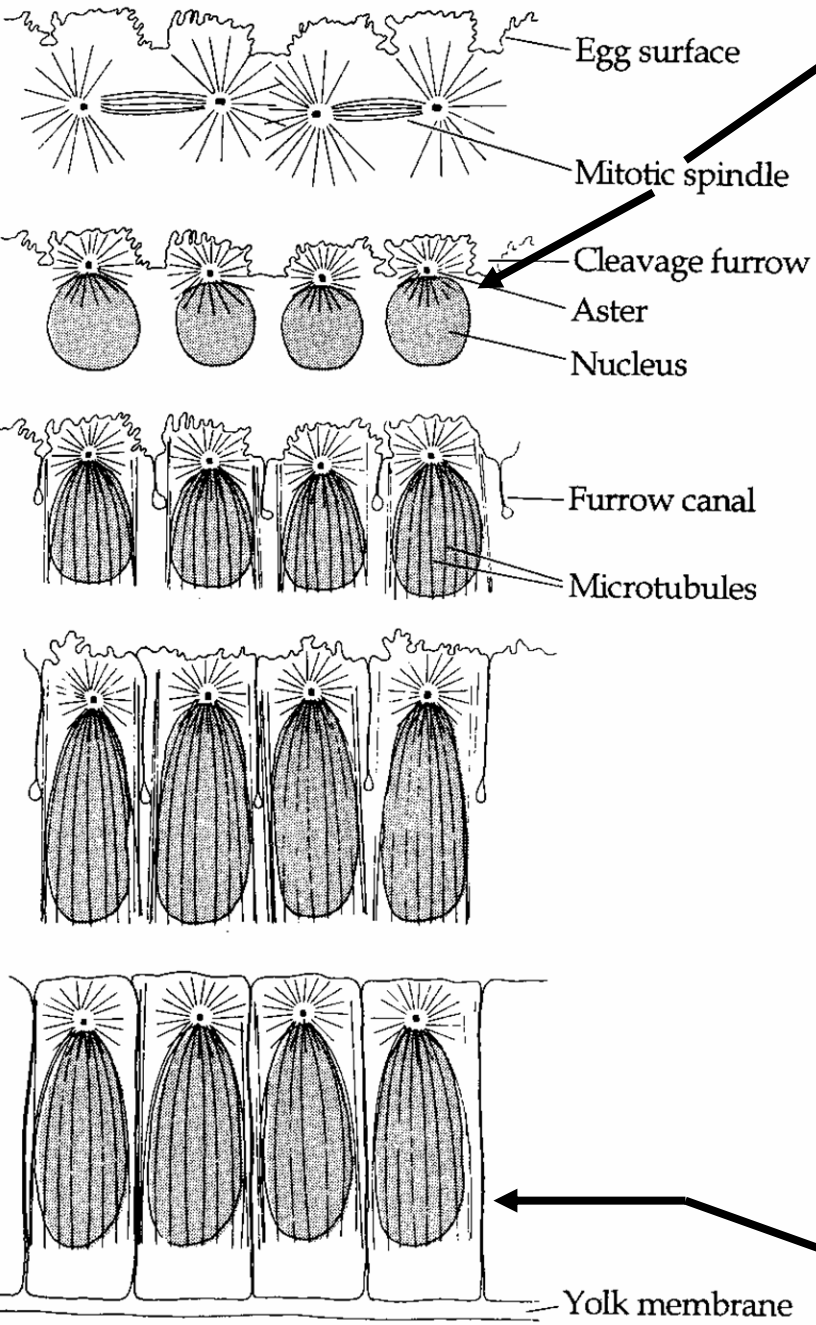
What technique can one use to highlight or show what one sees in the left photo to produce the cells seen in the right photo?



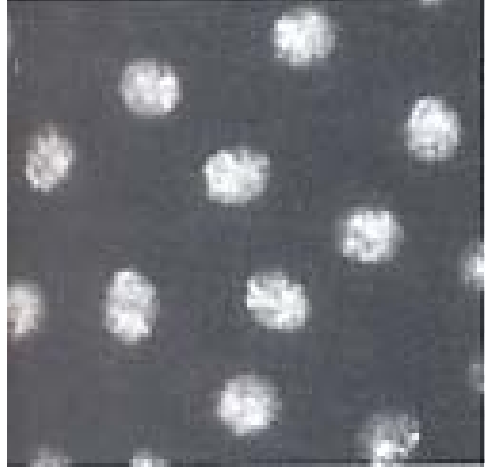
Syncytial blastoderm to the right. These energids lack a cell membrane. They are visualized here using an antibody marker to tubulin. What does syncytial mean?



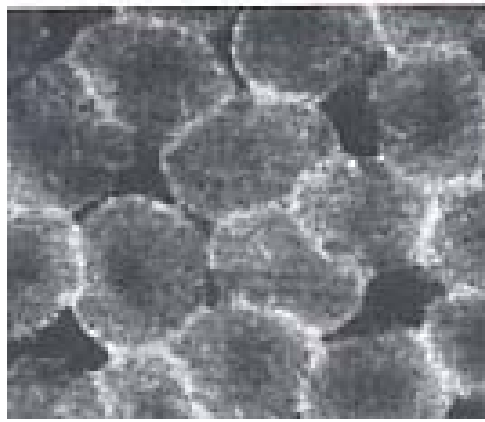




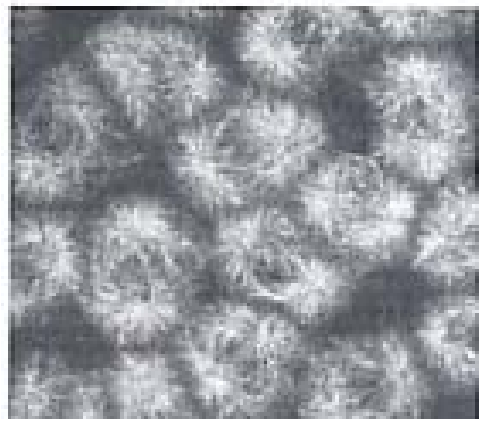
Formation of the **syncytial blastoderm** as seen in top photo where just the energids or nuclei have moved into the periplasm space. Nuclei were stained with a dye specific for DNA.



Microfilaments in middle photo were identified using a fluorescent antibody to actin.



Microtubules were identified in the bottom photo using a fluorescent antibody to tubulin.



These cytoskeletal elements get the nuclei oriented, are part of the scaffolding, and are essential for the formation of the cell membrane around each nucleus and the formation of the **cellular blastoderm**.

BLASTODERM FORMATION

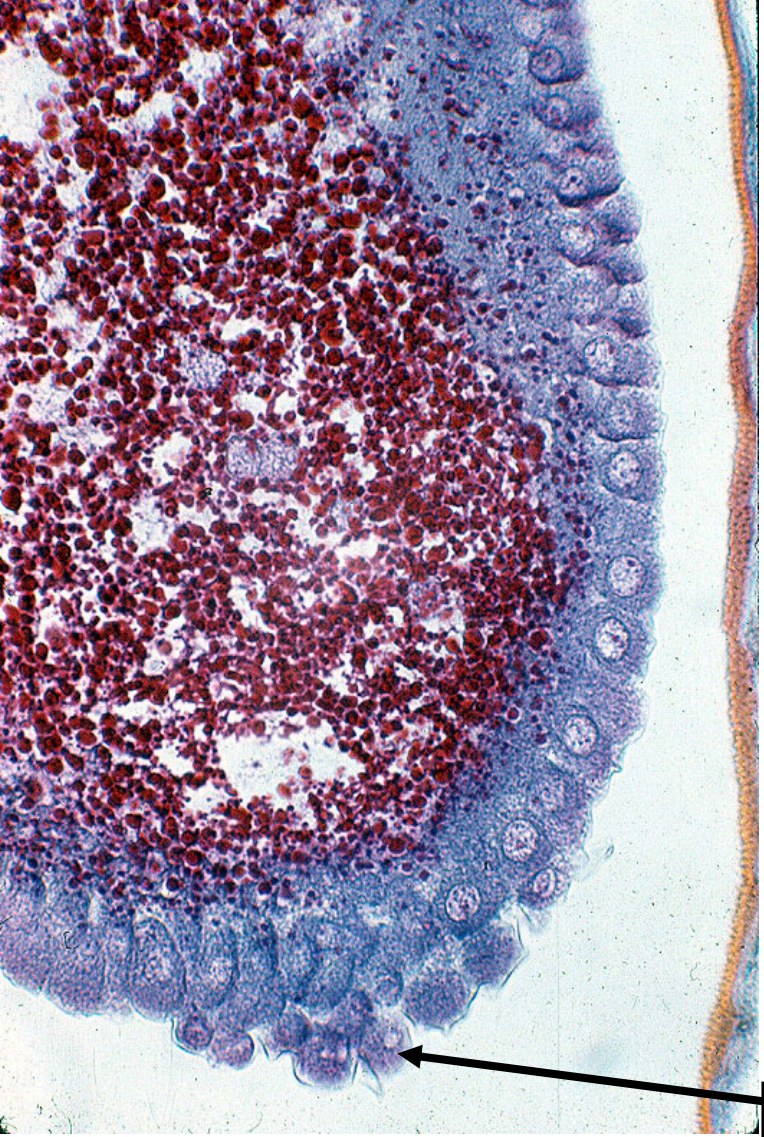
At this stage of development the following has occurred:

1. Syncytial blastoderm develops into cellular blastoderm
2. Vitellophage cells are formed
3. Pole cells are in place

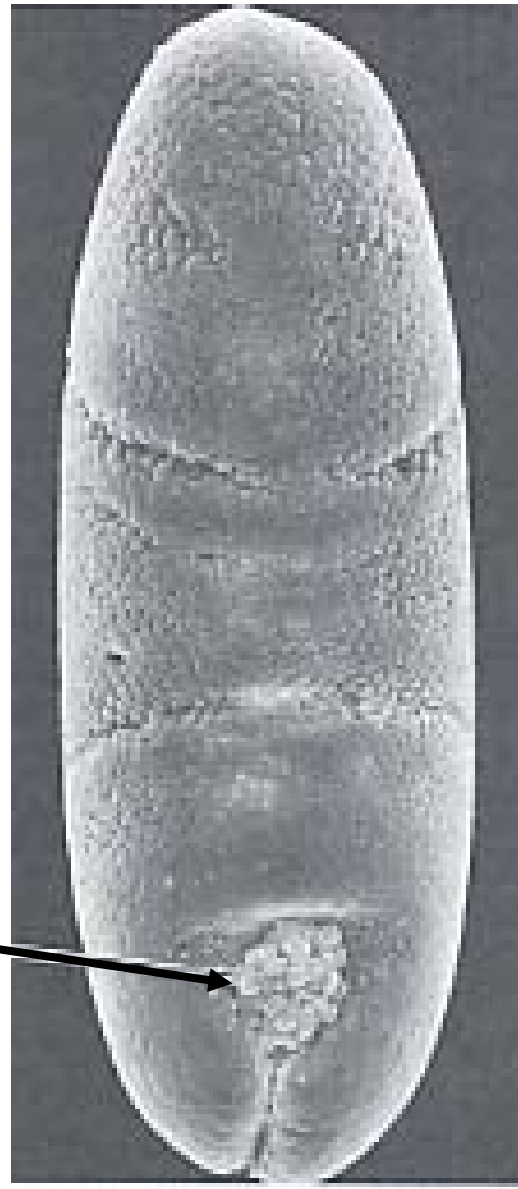
NOW THE FORMATION OF THE GERM BAND FORMS

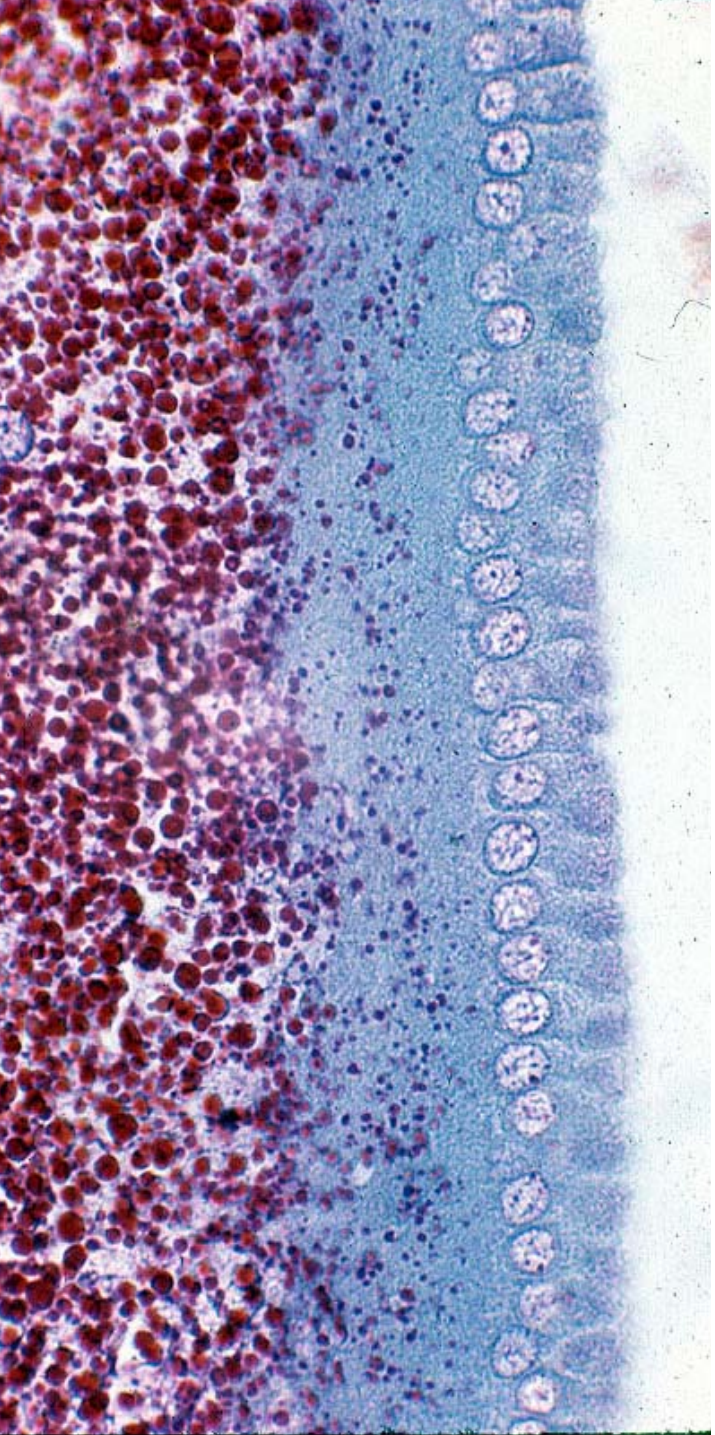
POLE CELLS-

Cells resulting from the migration of some of the energids to the posterior portion of the egg. These cells ultimately develop plasma membranes and remain their during early embryogenesis. During gastrulation and formation of tissue layers, they become internalized and become the germ cells of the adults. With mesodermal tissue they form the reproductive organs of the adult. In the posterior of the egg they are in the microenvironment of the *oskar* gene product that is essential for their development.



Pole cells

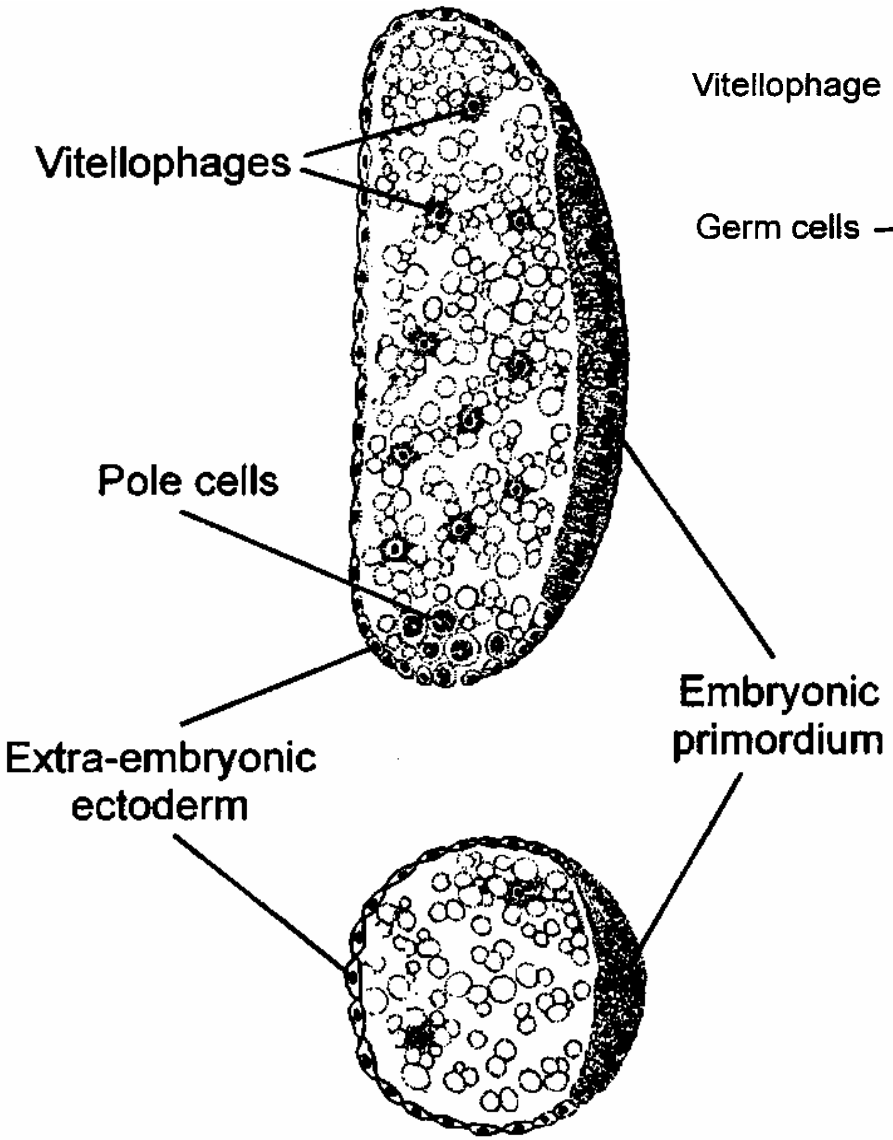


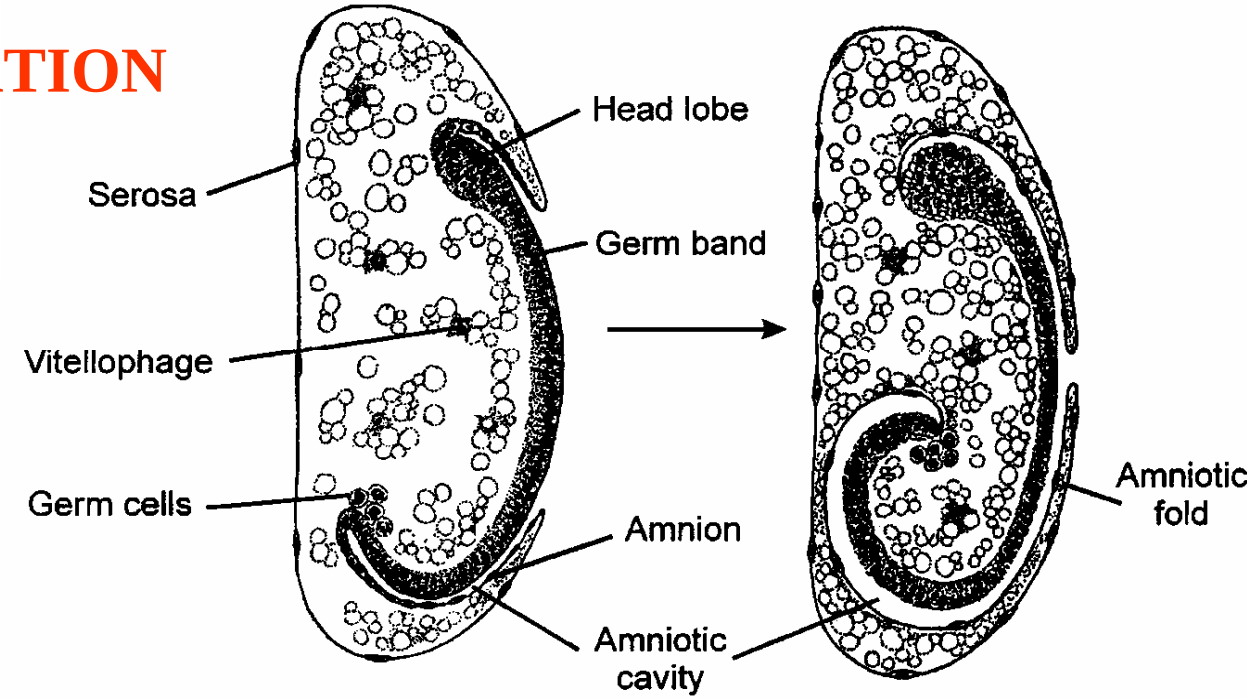


GERM BAND FORMATION

1. **Formation of the germ band**
   - **A. Cells in the ventral part of the egg + due to the morphogens in that area begin to divide and become more columnar in shape.**
   - **B. This thickening forms the embryonic primordium.**
   - **C. The blastoderm cells elsewhere do not become columnar but become the extraembryonic ectoderm.**
   - **D. As the embryonic primordium increases in length it becomes the GERM BAND. This will become the developing embryo.**

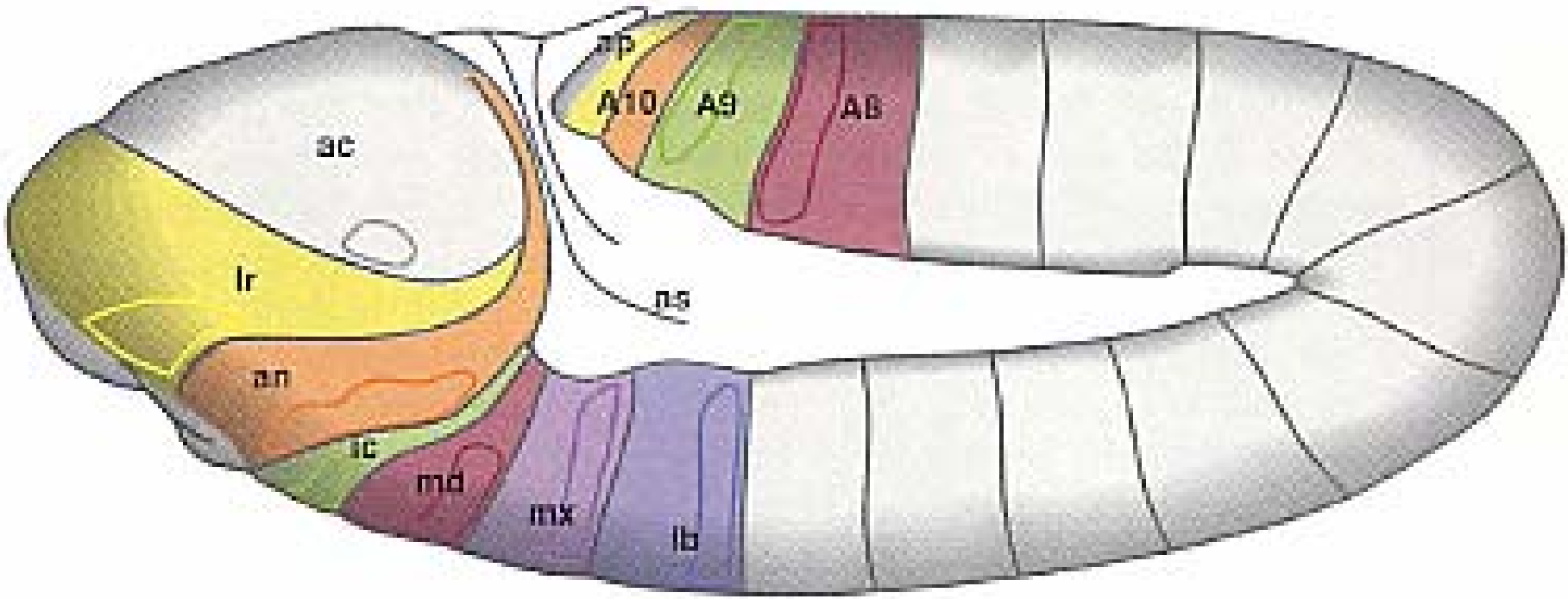
GERM BAND FORMATION



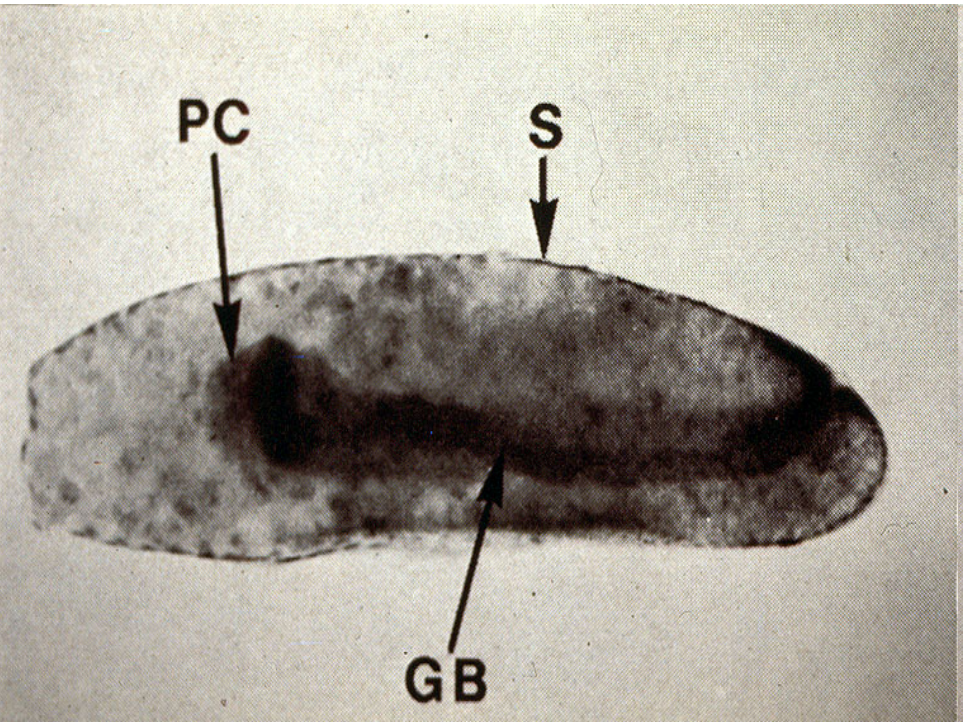


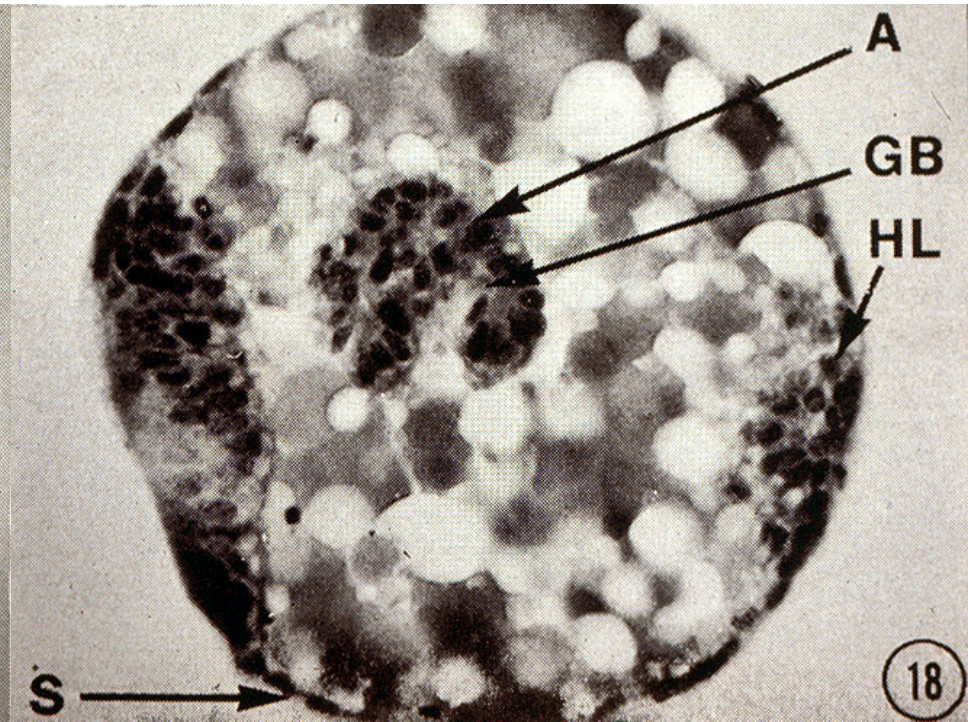
As the embryonic primordium (left) enlongates, it forms the germ band (top photo). As it increases in size it sinks deeper into the yolk mass and continues to develop. IT IS STILL A SINGLE LAYER OF CELLS. Note the rest of the blastoderm cells do not become columnar but become the membranes surrounding the embryo (i.e., serosa and amnion). The amniotic fold eventually closes separating the embryo from the serosa.

Metameric (body segmentation) organization of the *Drosophila* embryo at the late extended germ band stage.



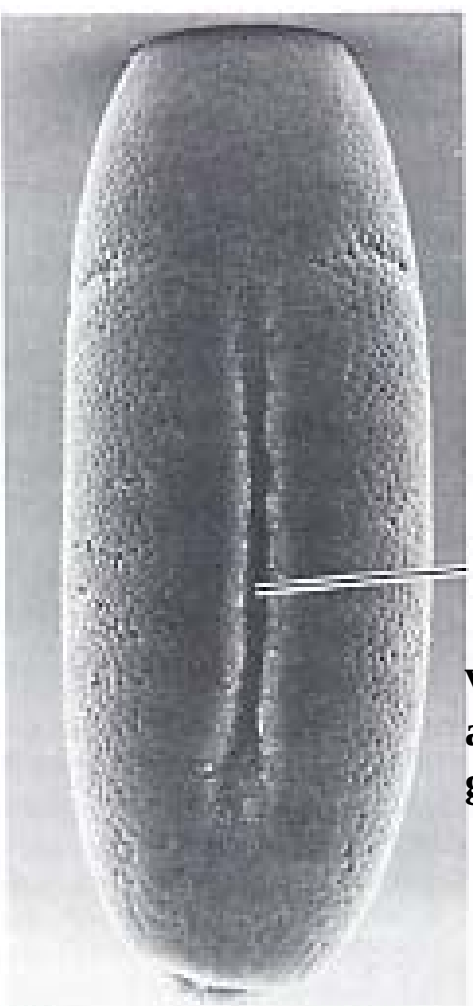
Below are sections of eggs showing on the left the germ band (GB), the serosal (S) membrane. Note the dark spheres in the cross-section of the egg on the right, which are protein yolk while the light colored spheres are lipid.

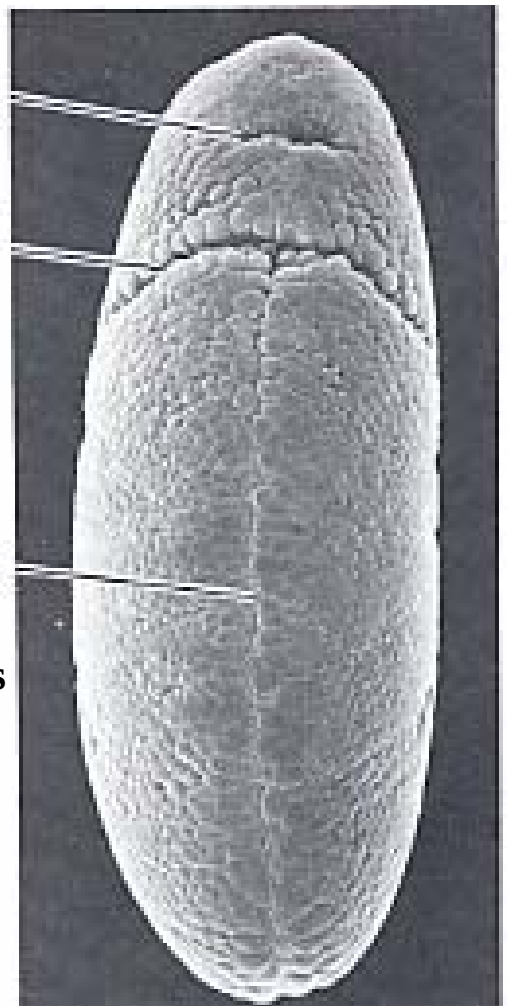




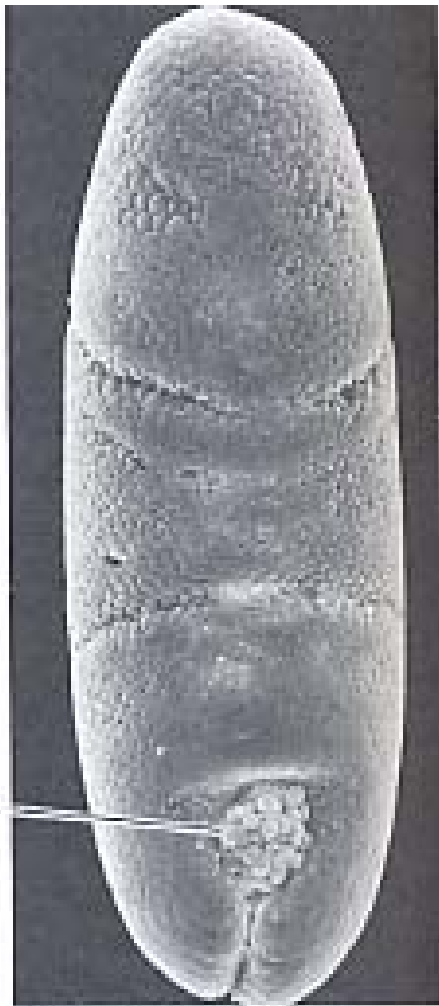
FROM A ONE-CELLED GERM BAND TO TWO LAYERS

1. Gastrulation-the process where the mesoderm and endoderm are invaginated within the ectoderm.

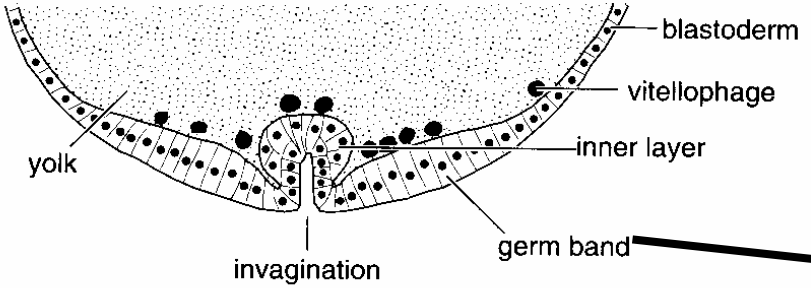




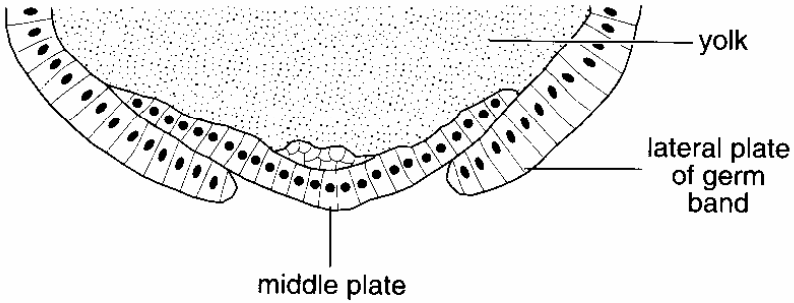
ventral furrow is also called the gastral groove



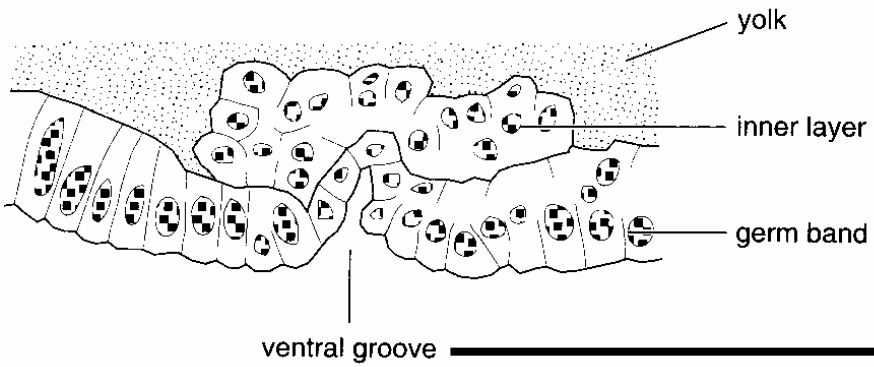
a) *Clytra*

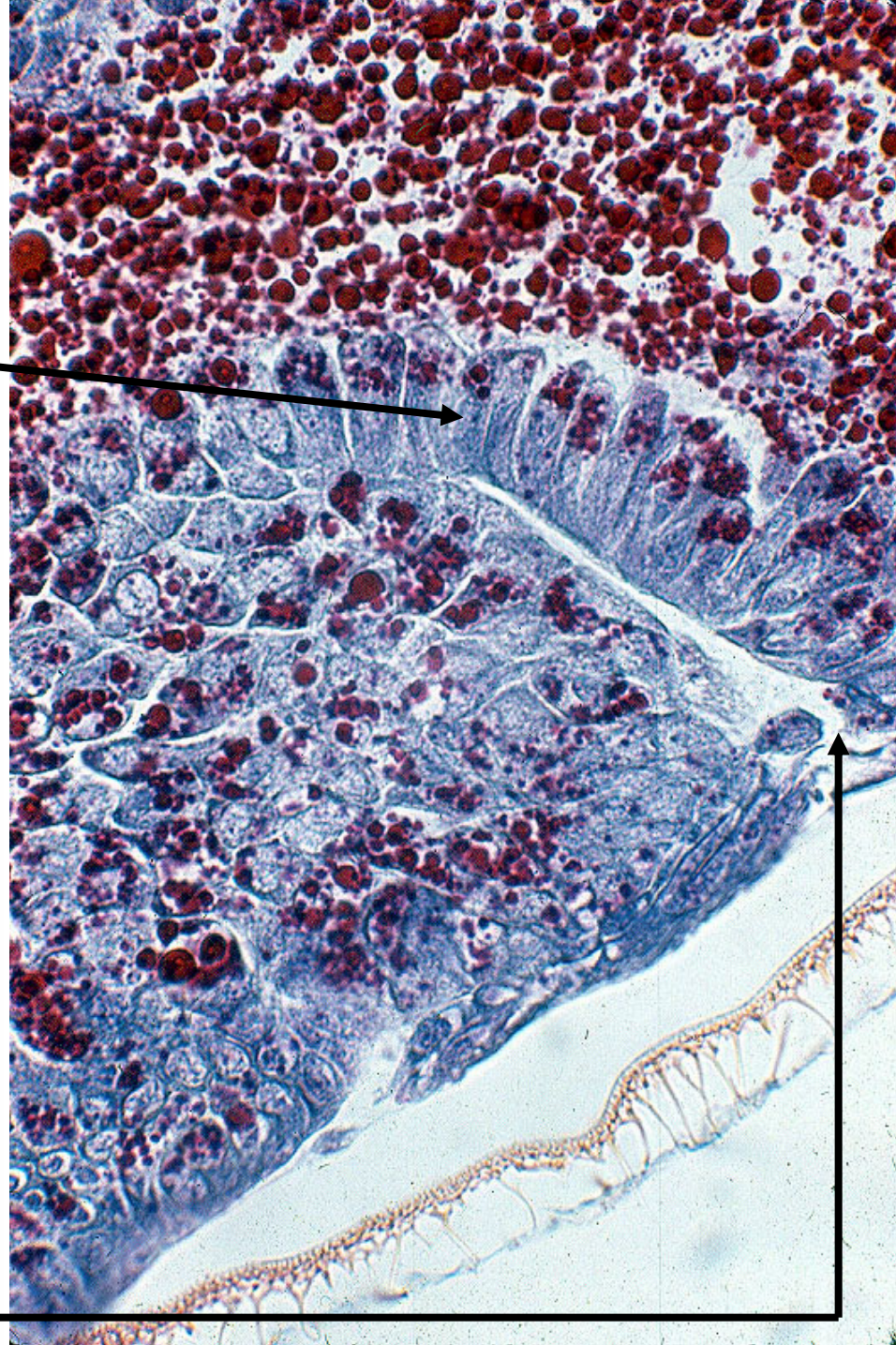


b) *Apis*



c) *Locusta*

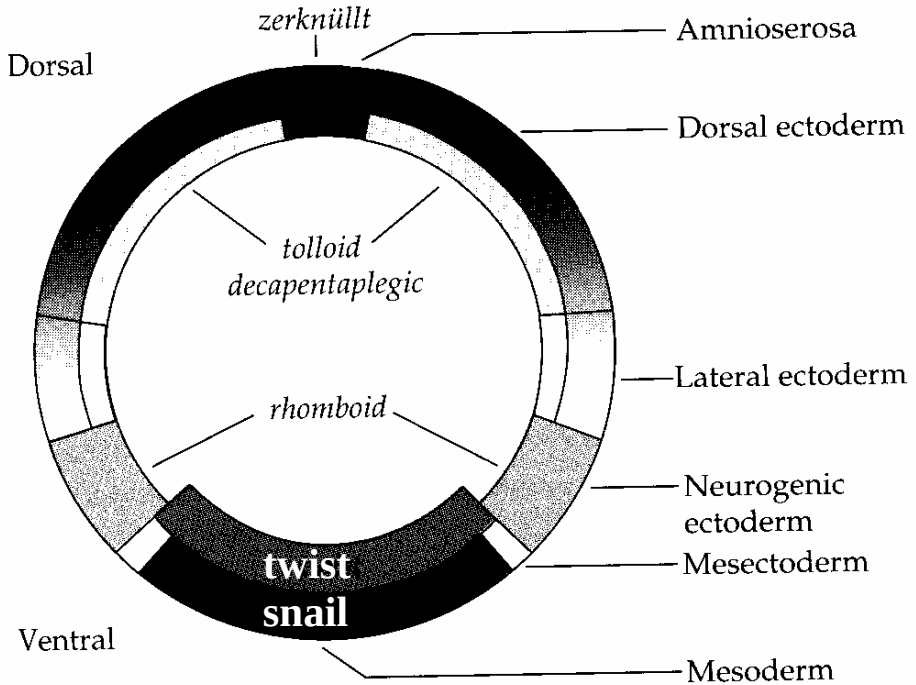


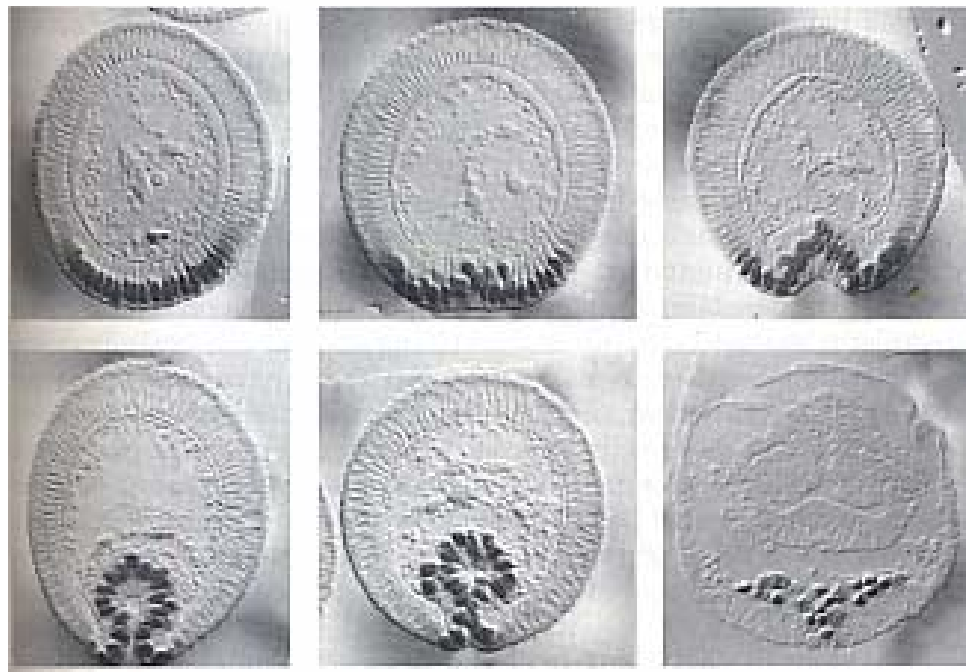


How can we demonstrate what controls gastrulation in *Drosophila* eggs?

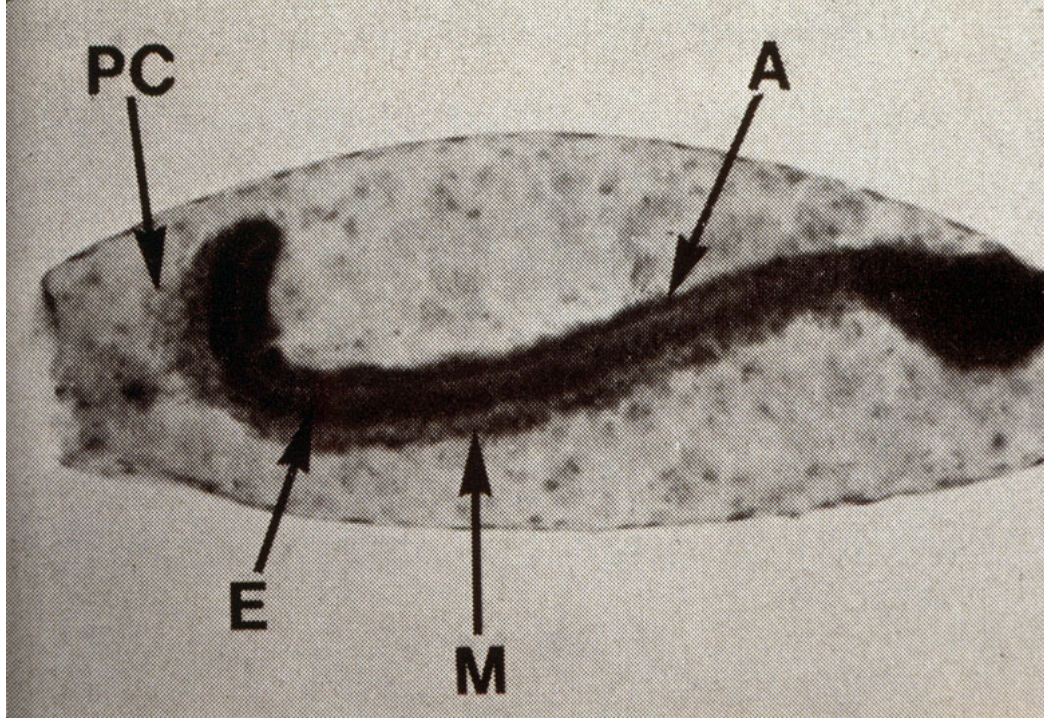
In the photo on the right it was shown that the dorsal protein activates the zygotic genes, one of which is twist (see figure on right).

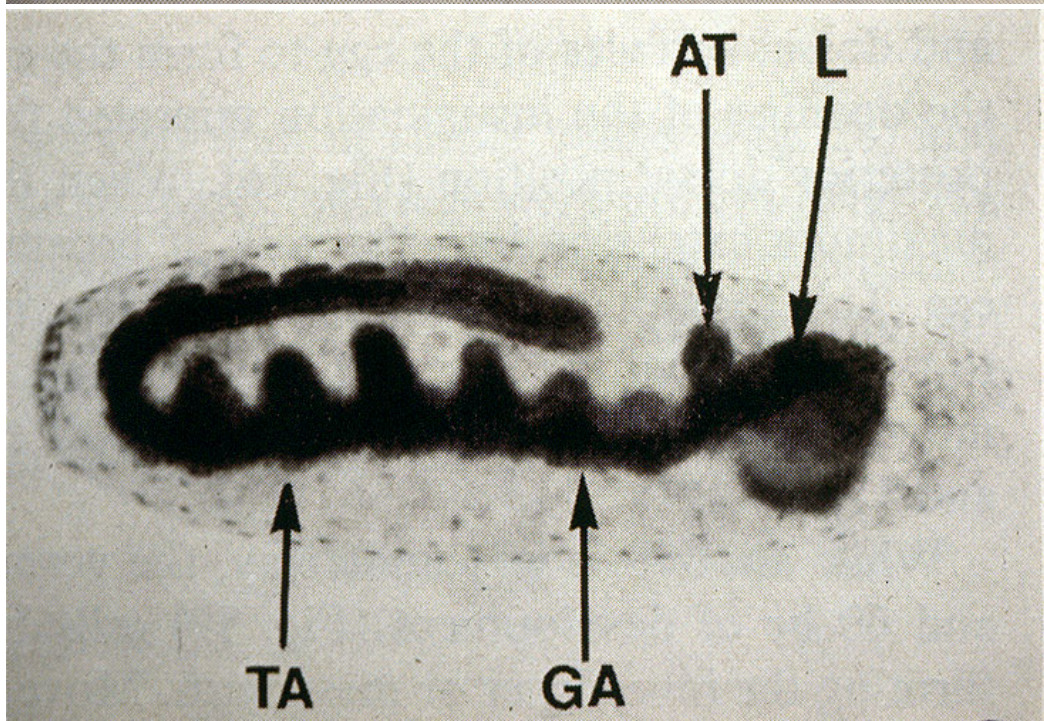
By making an antibody to the twist protein, it was possible to show the involvement of the twist gene and twist protein in gastrulation process (photo on right).



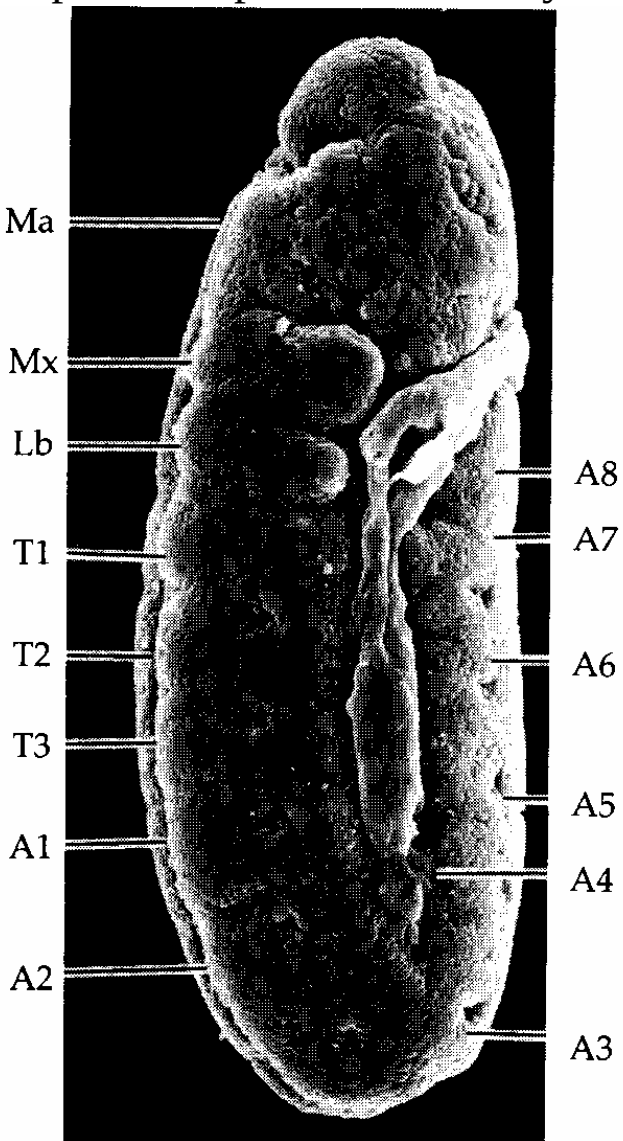


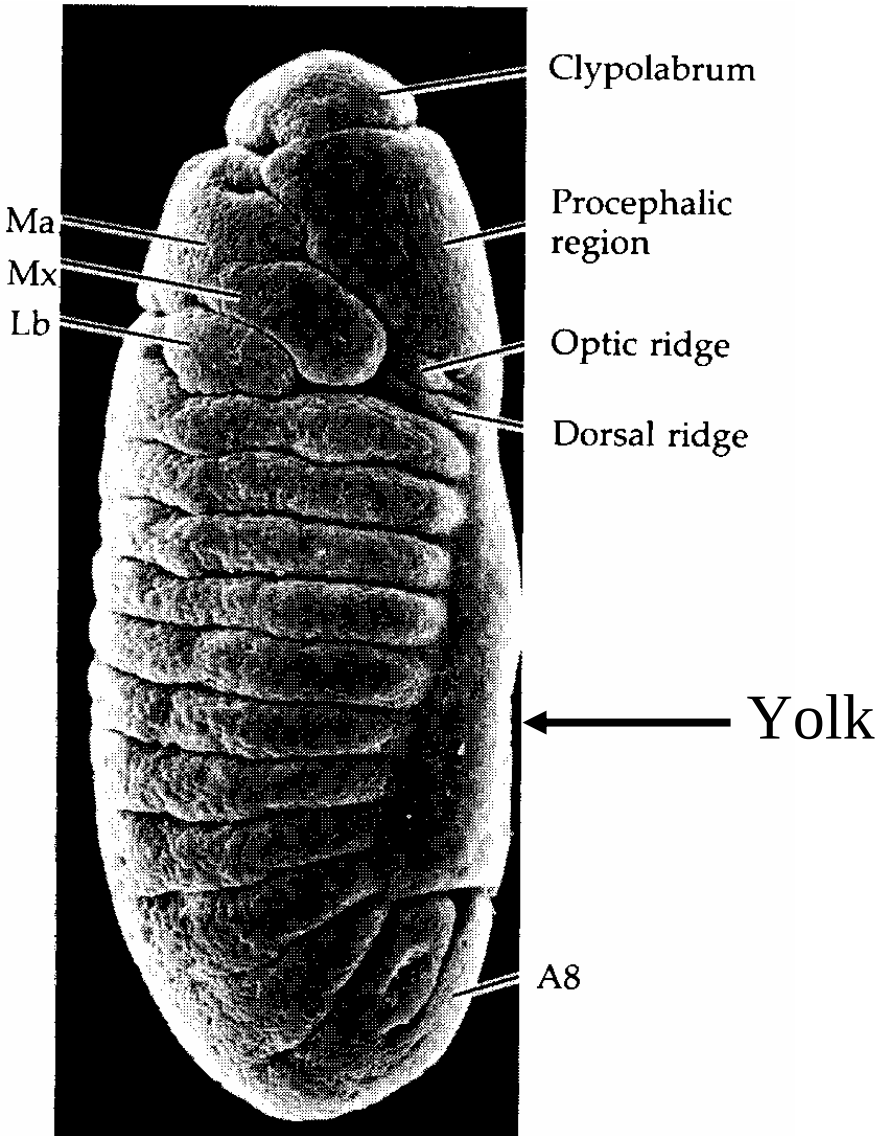
Photos of embryos (E) within the egg. Note in the top photo the presence of the pole cells. In the bottom photo note that segmentation is now a little more evident. Once can see the labrum area (L), antenna (AT), the gnthosegment area (GA) and the thoracic segment area (TA).





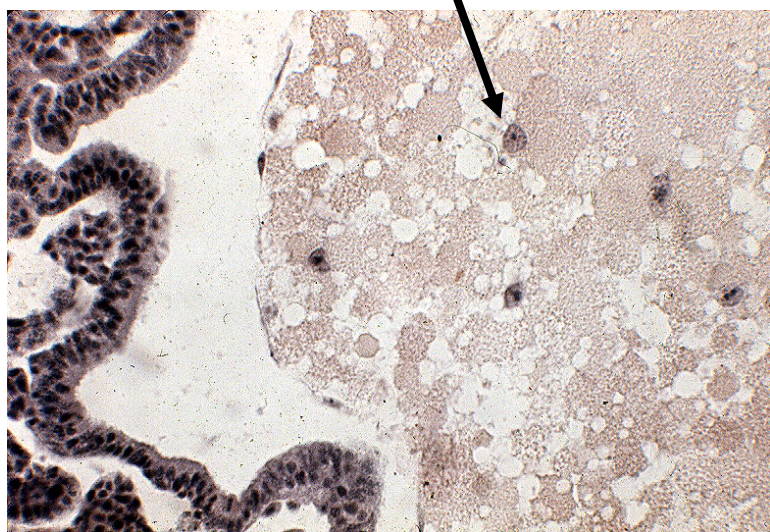
Note in the photo on the left that the terminal segment A8 is just posterior to the head or clypolabrum region. Most embryos within the egg go through a movement known as **blastokinesis** where they reorient themselves such that the yolk is now internalized in the ventral region of the developing embryo. Notice in the photo on the right that now the A8 segment is at the posterior part of the embryo and the yolk area is in its "belly" or ventral abdominal area.





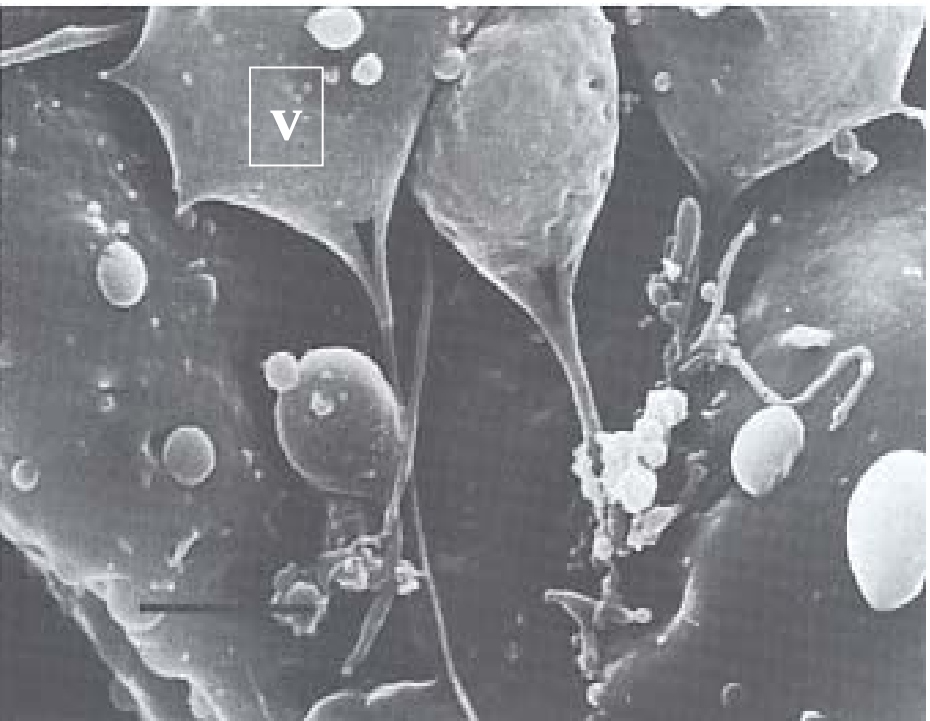
Cellular blastoderm in a *Phormia* egg. Note the nuclei are surrounded by a cell or plasma membrane. Note the red droplets of yolk (vitellin) and the presence of vitellophages.

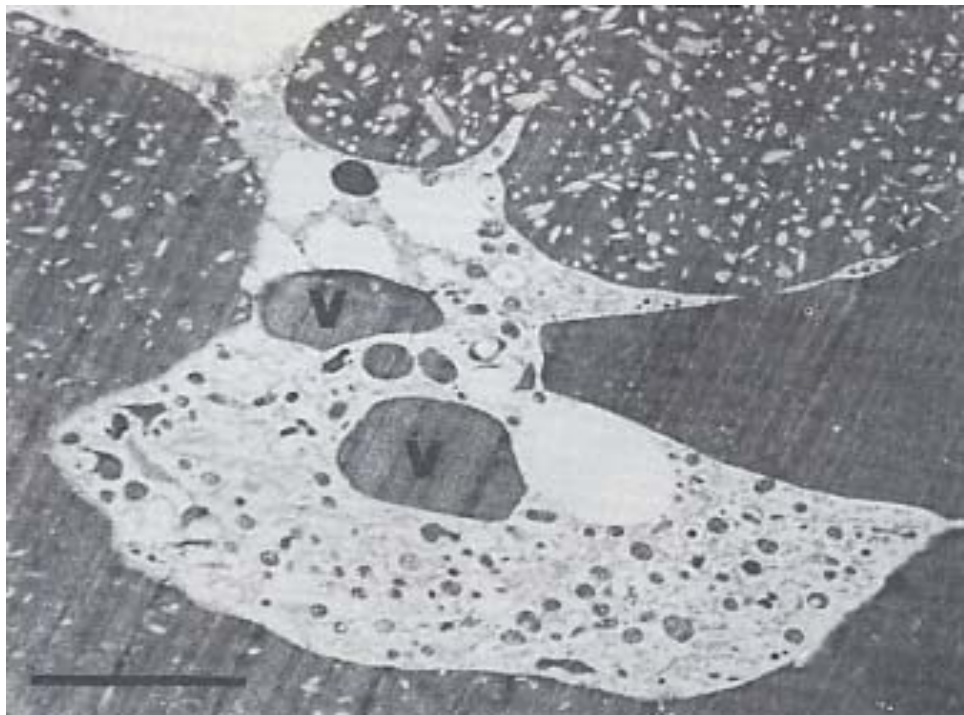
Vitellophage cells



VITELLOPHAGE CELLS-

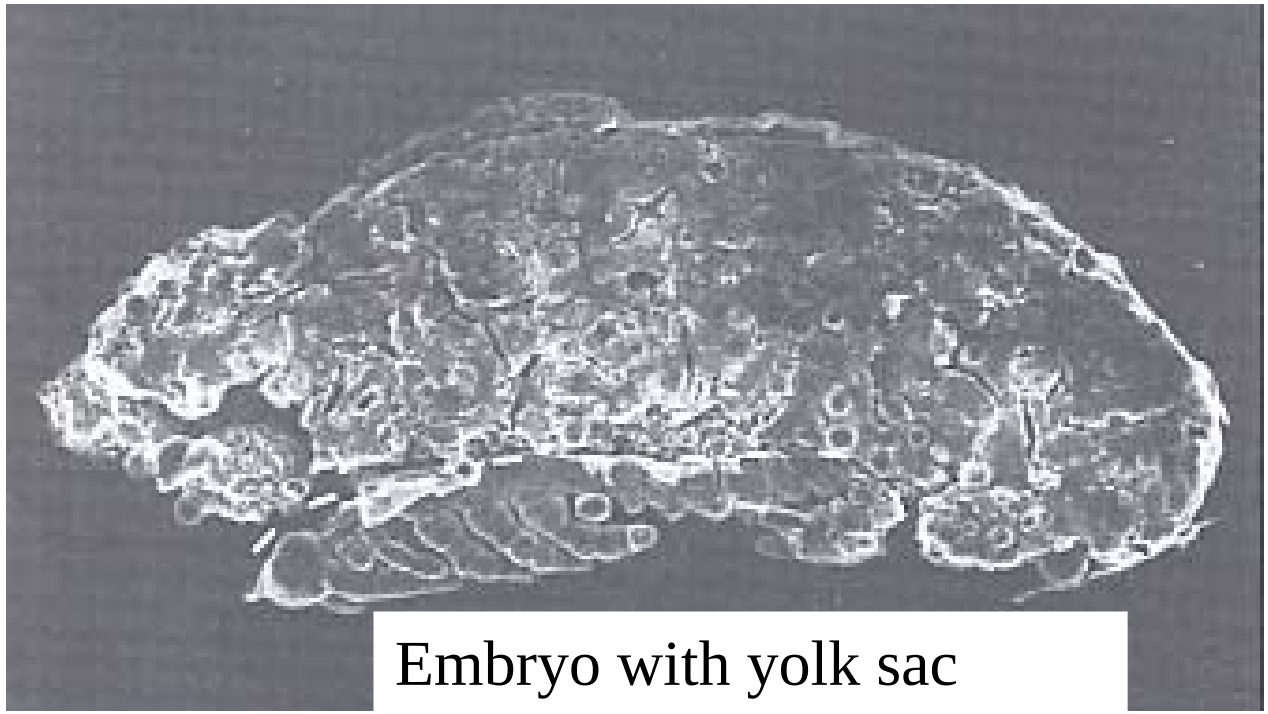
Cells that are formed from energids that remain in the yolk area and/or surround the yolk mass. Their main function appears to be the degradation of yolk spheres, making it useable to the developing embryo. SEM of 6 day old cockroach embryo showing vitellophagic cells lying over a yolk granule with several filopodia extended over the yolk surface. TEM of same embryo showing vitellophage deeply lodged within a yolk granule. The presence of multivesicular bodies suggests digestion of the yolk by the vitellophagic cells (v).



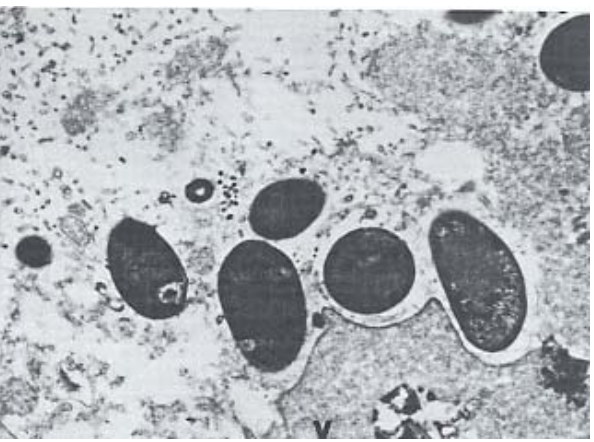


Yolk degradation by vitellophage cells and endosymbiotic bacteria in *Blatella germanica*.

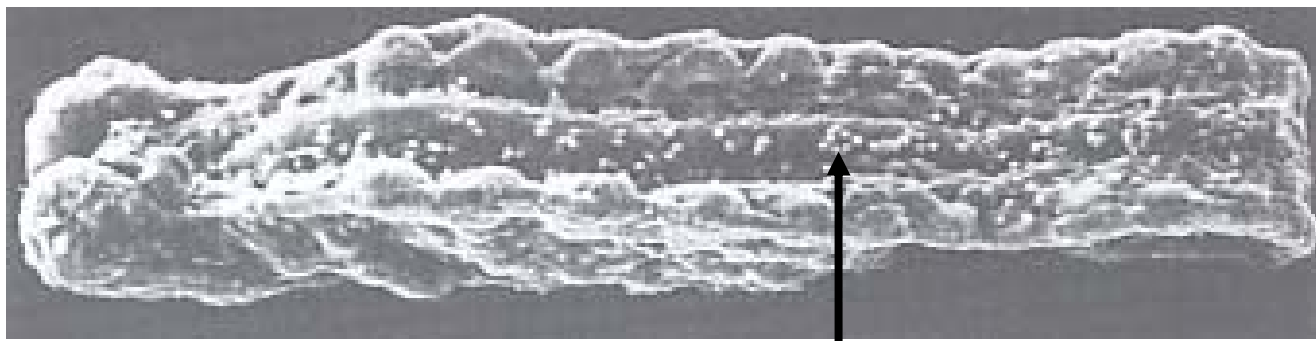
Giorgi and Nordin. 1994. J.Insect Physiol. 40: 1069-1076.



Embryo with yolk sac

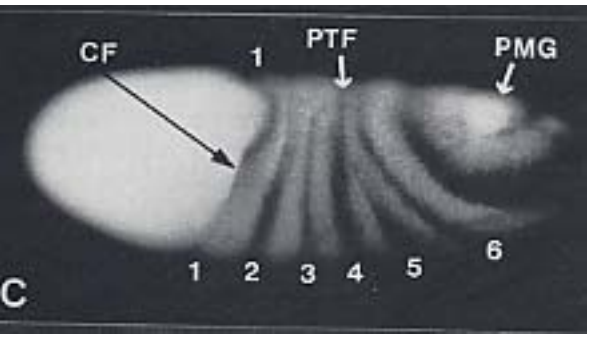


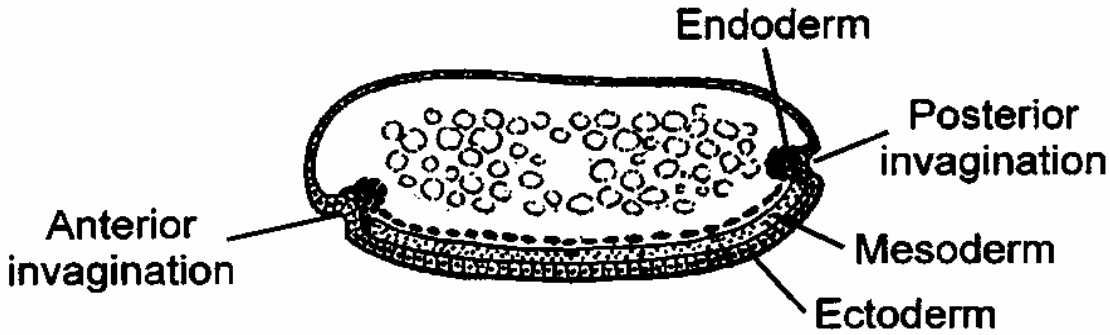
Blattabacterium cuenoti **reside in the ovarian follicles of blattids**



Embryo showing vitellophage cells over the mesodermal layer of the embryo. The vitellophage cells are also believed to be involved in the formation of the midgut epithelium

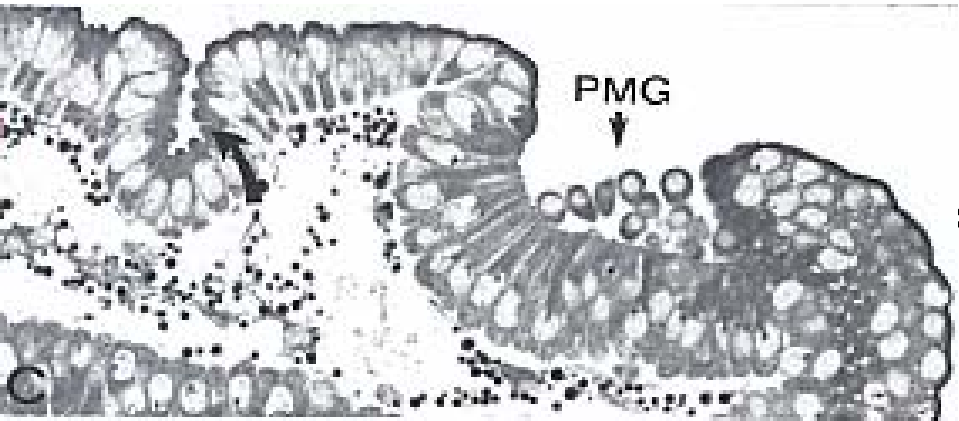
MIDGUT FORMATION IN THE EMBRYO

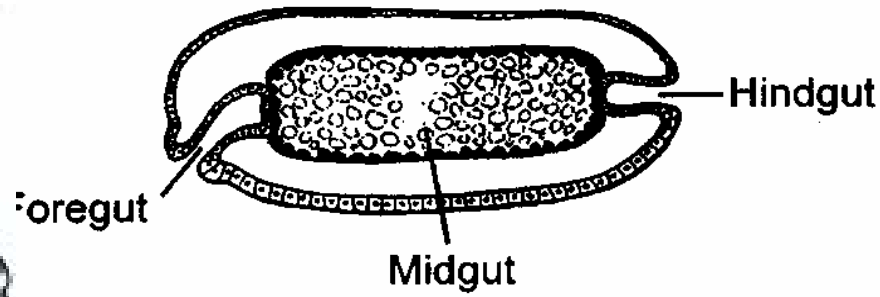


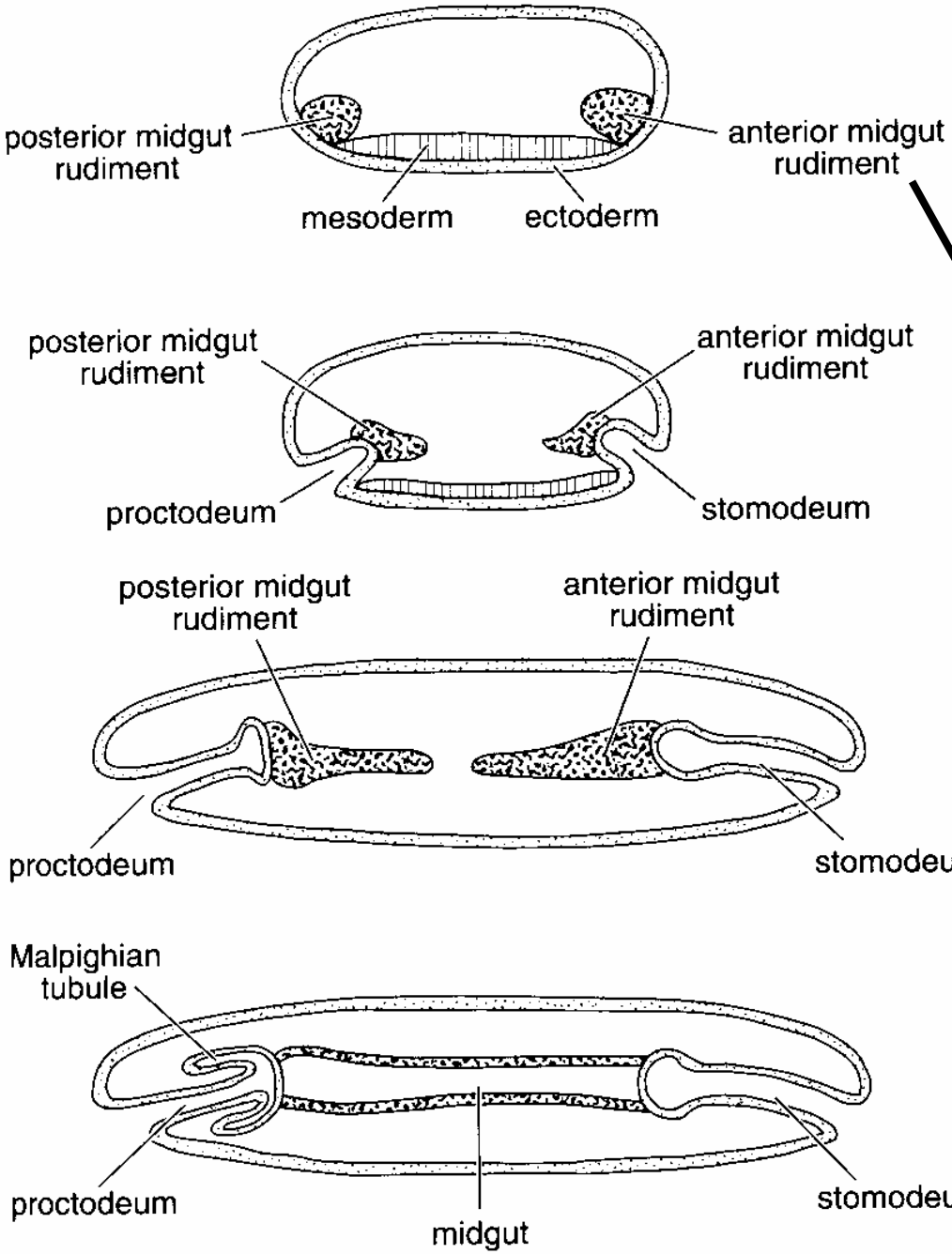


PMG in the above embryo shows the posterior midgut invagination cup where the posterior invagination shown in the drawing on the right will take place. Photo of *Drosophila* embryo. In the slide below of the PMG, note the group of cells within the indented area. **What are these cells and what is their fate?**

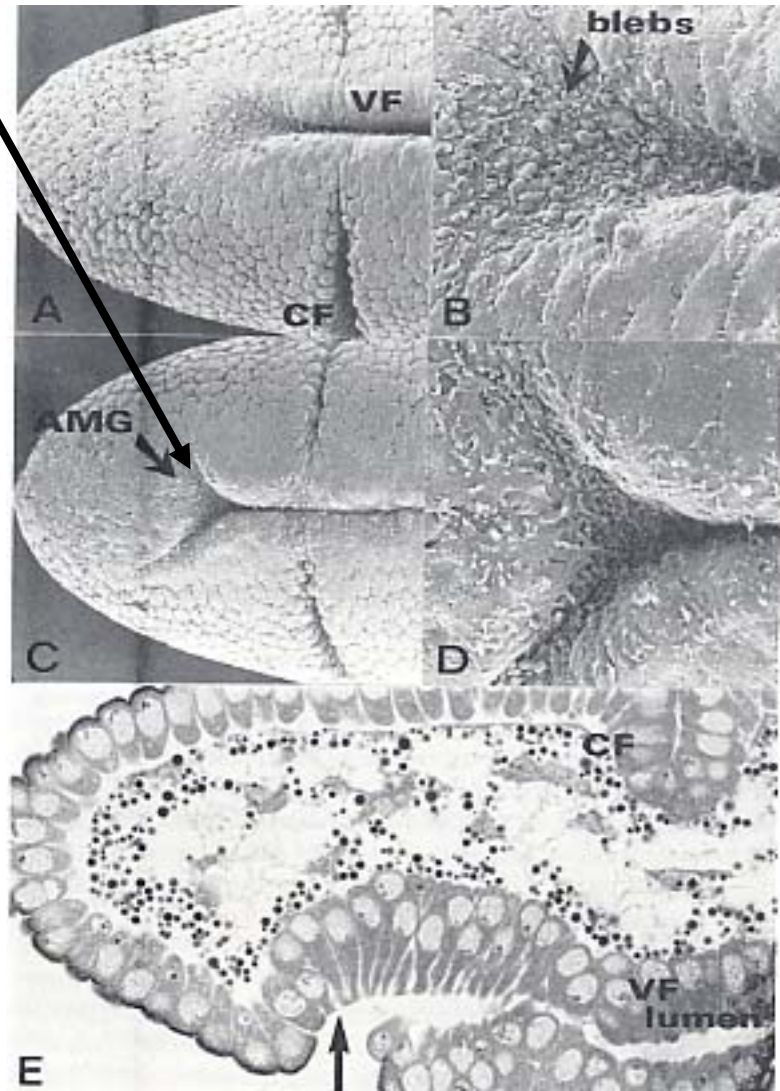
The pole cells-become germ cells

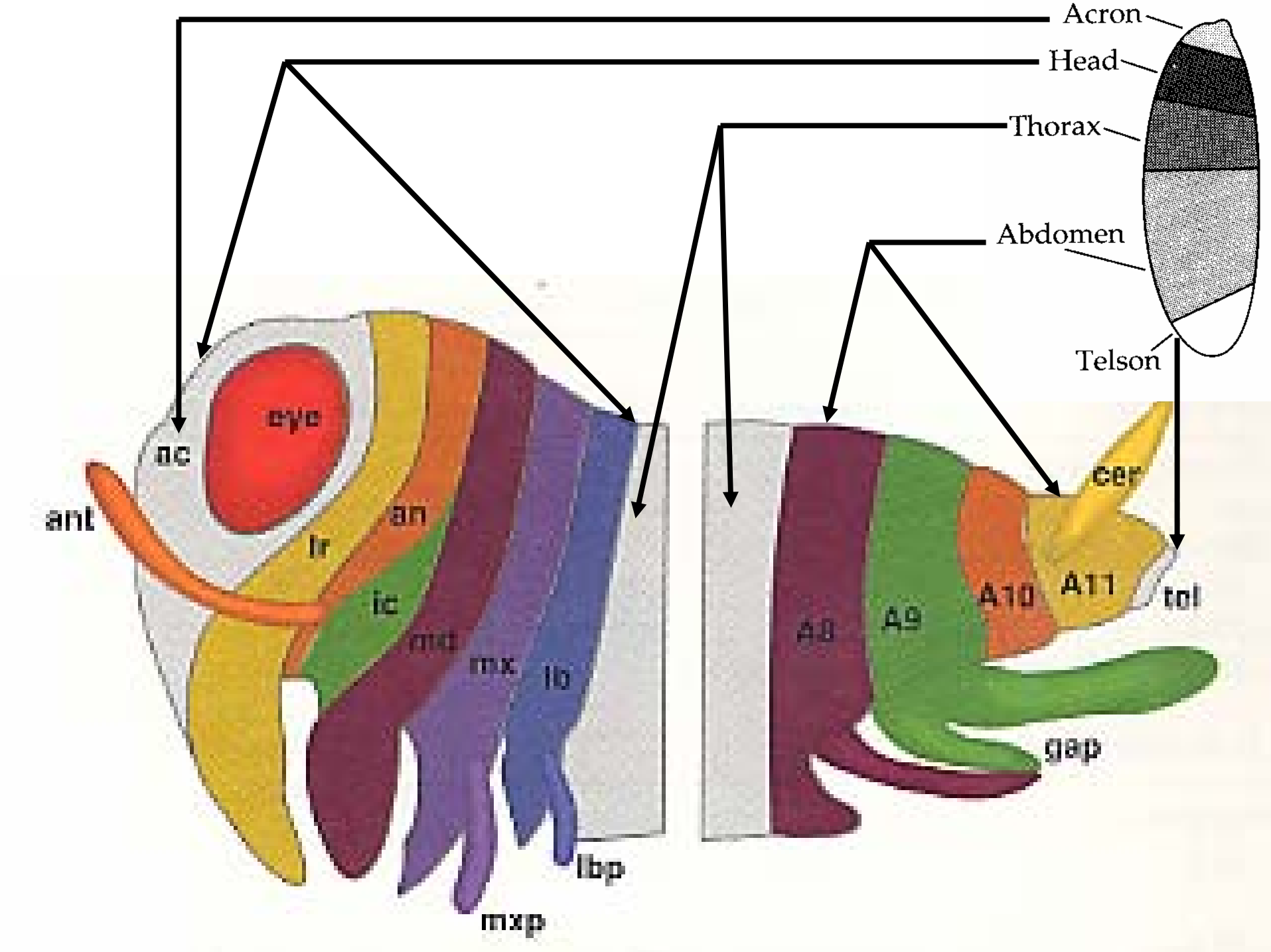




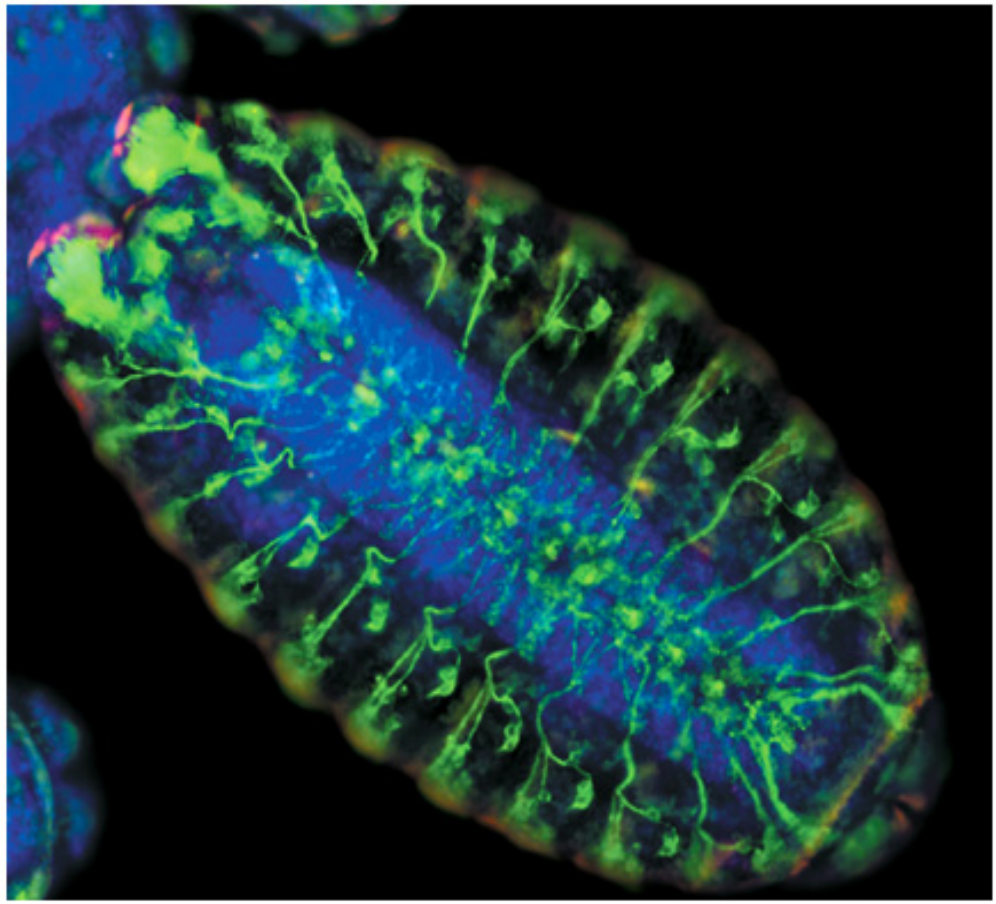


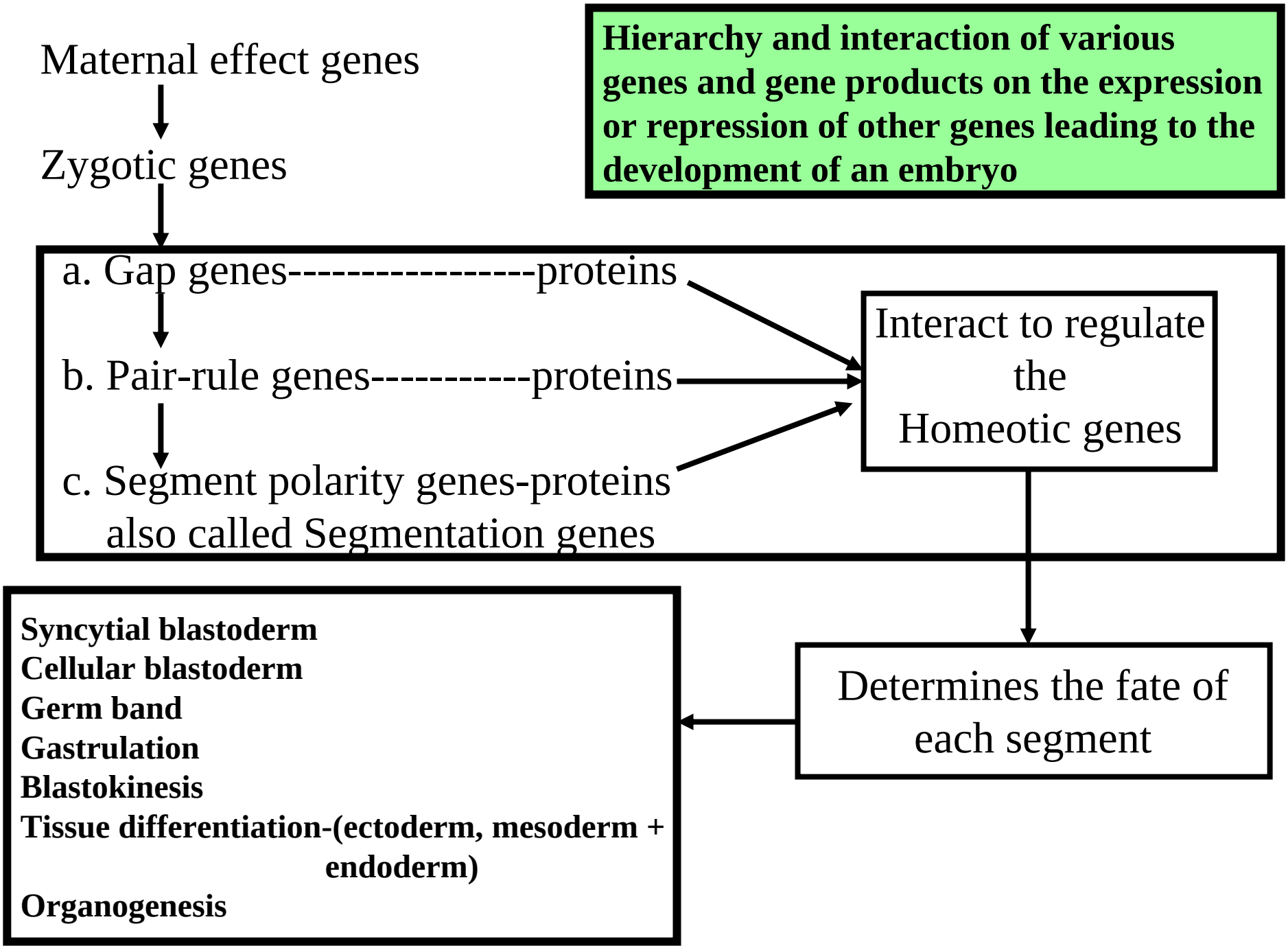
Anterior midgut invagination. In the bottom photo note the invagination starting forming the ventral furrow lumen (VF)



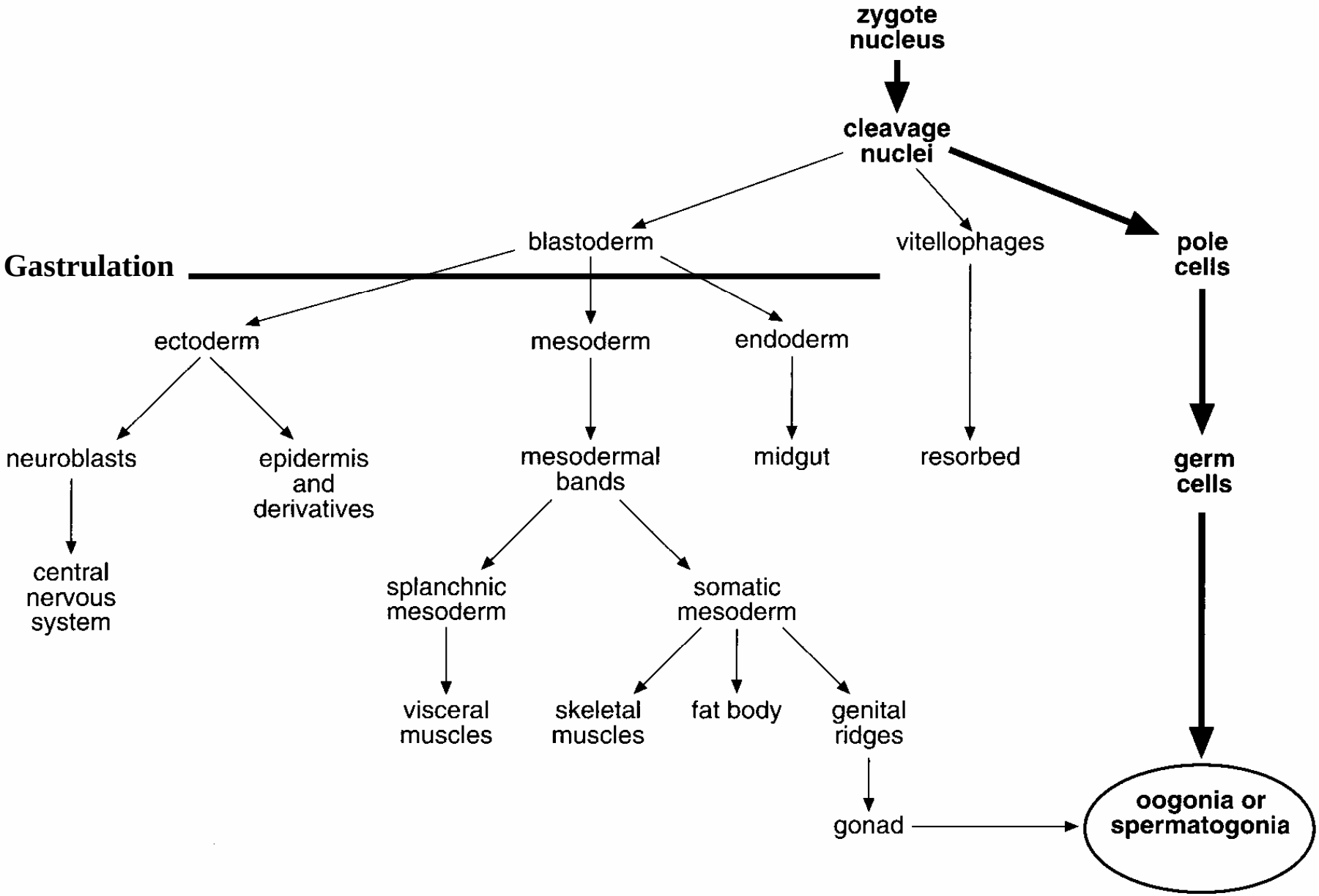

Acron
Head
Thorax
Abdomen
Telson
ac
eye
ant
lr
an
ic
mxp
mx
lb
lbp
mxp
A8
A9
A10
A11
cer
tel
gap

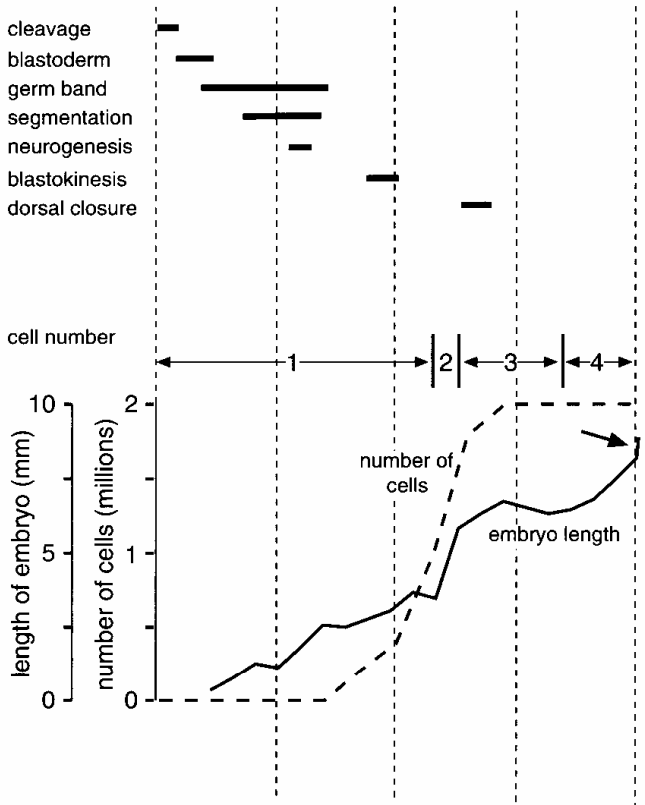
The peripheral nervous system of a wild-type (Canton-S) *Drosophila melanogaster* embryo labeled with the monoclonal 22c10 antibody (which detects a microtubule-associated protein) and subsequently visualized using green-fluorescent Alexa Fluor 488 rabbit anti–mouse IgG antibody (A11059). The actively dividing cells of the developing denticle bands were labeled with a rabbit anti–histone-H3 antibody and visualized using red-fluorescent Alexa Fluor 594 goat anti–rabbit IgG antibody (A11012). Finally, the nuclei, which are concentrated in the central nervous system, were counterstained with blue-fluorescent DAPI (D1306, D3571 D21490). Image contributed by Neville Cobbe, University of Edinburgh.

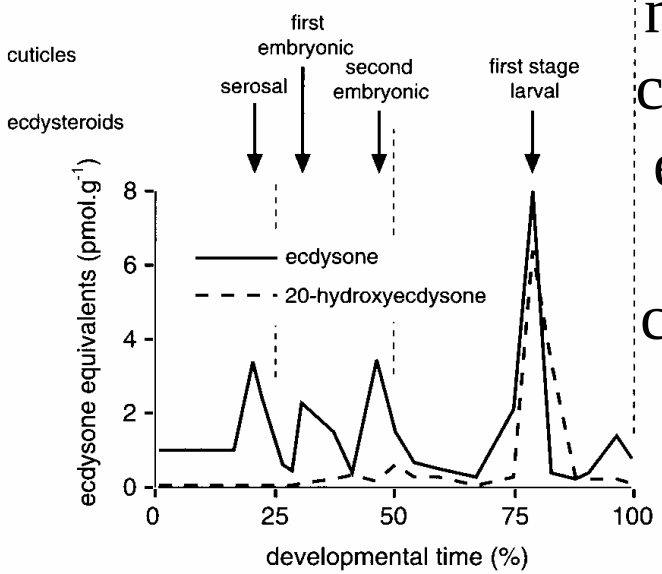



Hierarchy and interaction of various genes and gene products on the expression or repression of other genes leading to the development of an embryo
Maternal effect genes
Zygotic genes
a. Gap genes----------proteins
b. Pair-rule genes----------proteins
c. Segment polarity genes-proteins
also called Segmentation genes
Interact to regulate the Homeotic genes
Determines the fate of each segment
Syncytial blastoderm
Cellular blastoderm
Germ band
Gastrulation
Blastokinesis
Tissue differentiation-(ectoderm, mesoderm + endoderm)
Organogenesis

Flowchart showing embryonic events starting with fertilization to various tissue formation.

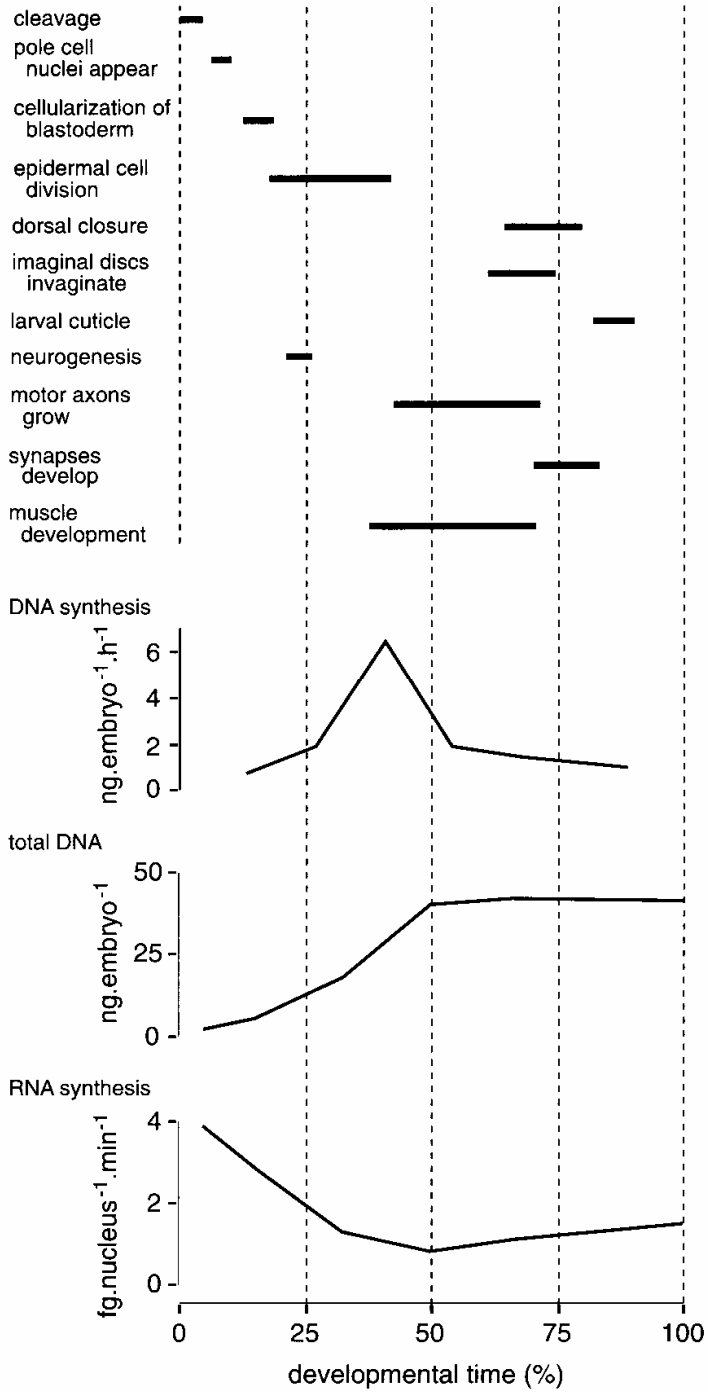






Embryonic scheme for *Locusta* on the left and *Drosophila* on the right

Some insects, such as the locust may produce new cuticles during the embryonic stage. These molts and cuticle production are control by ecdysteroids



HORMONES DURING EMBRYOGENESIS

Our knowledge concerning hormones during embryonic development is limited. Because it is difficult to perform certain gland removals and replacement therapy in embryos, as is done in post-embryonic development, what we know is based mainly on our use of assays for specific hormones.

Both ecdysteroids and JH are present in the embryo and are contributions from the female.

Where do embryonic hormones come from since the embryonic endocine glands don't start to form until after blastokinesis?

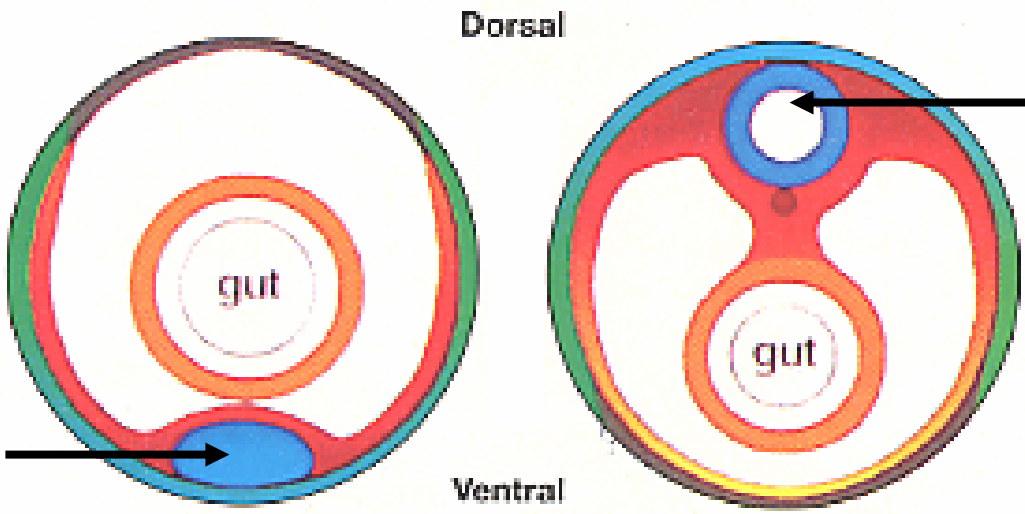
a. Packaged into the embryo by female as inactive hormone conjugates and released from the conjugates as active hormones when required.

Embryonic diapause-Due to lack of ecdysteroids

The Ghost of Geoffroy Saint-Hilaire

Frog and fly genes revive the ridiculed idea that vertebrates resemble upside-down insects

By JOHN TRAVIS



Central nervous system

Central nervous system

Arthropood **Vertebrate**

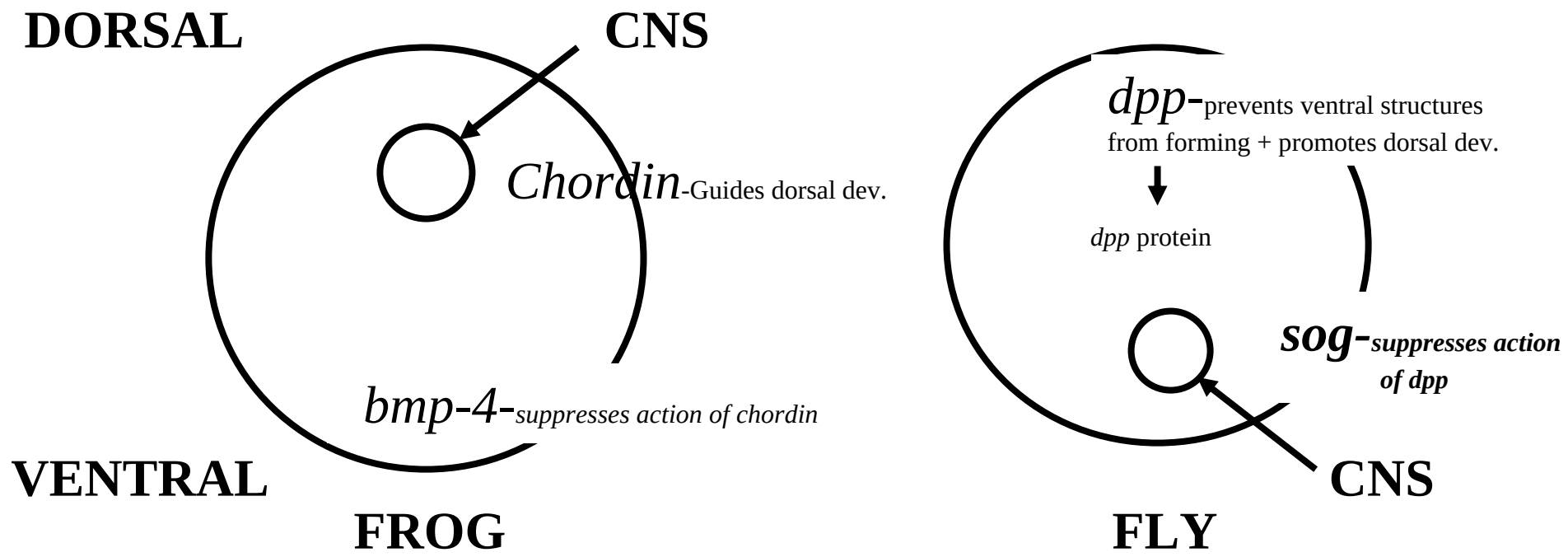
Science News, vol. 148; Sept. 30, 1995

Saint-Hilaire in 1822, decades before Darwin proposed his theory theorized that all animals share a fundamental body plan upon which nature has imposed dramatic variations. Think about the **homeobox!**

Even more exciting than demonstrating the presence of homeotic genes is the evidence that most homeotic genes studies to date-whether they are from earthworms, centipedes, insects, frogs, or humans-have a highly conserved (retained through evolution) region of the DNA that encodes a sequence of 180 nucleotide pairs. This is known as the **homeobox** and represents a highly conserved and uniform sequence of DNA. The reason this regions has been so resistant to evolutionary change remains speculative.

1. a. Fly *sog* gene directs ventral development
   b. Frog *chordin* gene directs dorsal development

2. Fly *sog* and frog *chordin* are homologous genes
3. The two genes can be substituted for one another
   a. Injecting sog into a frog it promoted ventral development
   b. Injecting chordin into fly it promoted dorsal development



POST-EMBRYONIC DEVELOPMENT

a. Growth

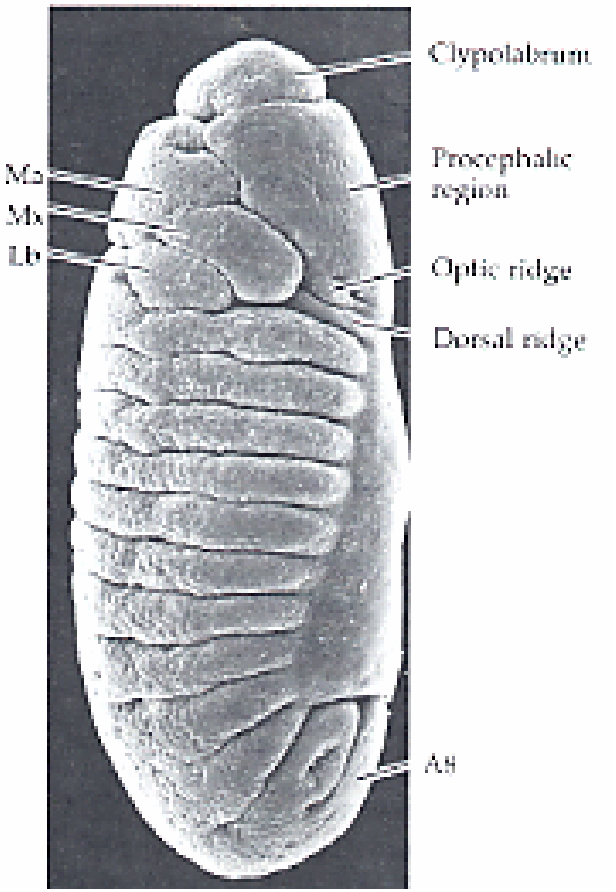
b. Molting

c. Metamorphosis

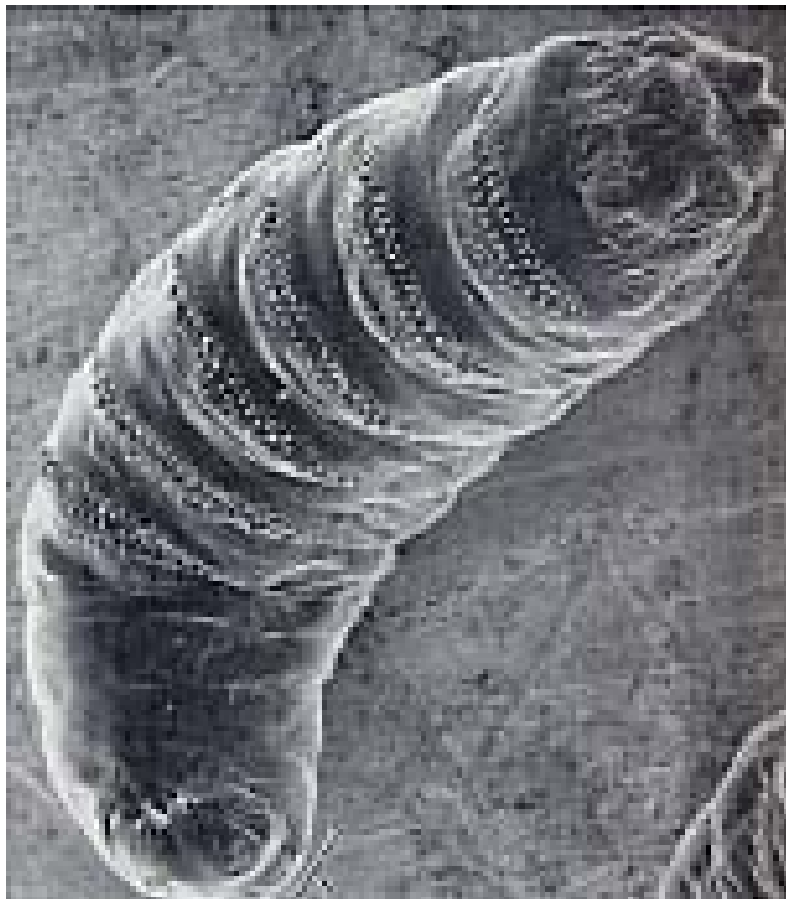
d. Aging

a. Growth-an increase in either cell size and/or cell number

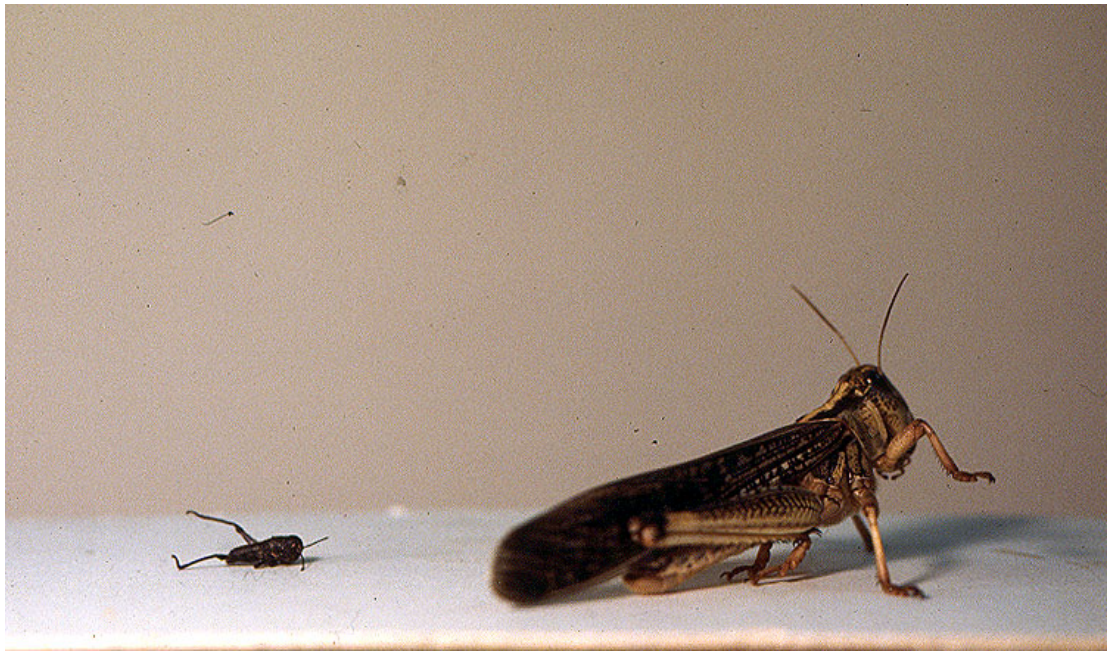
Embryo

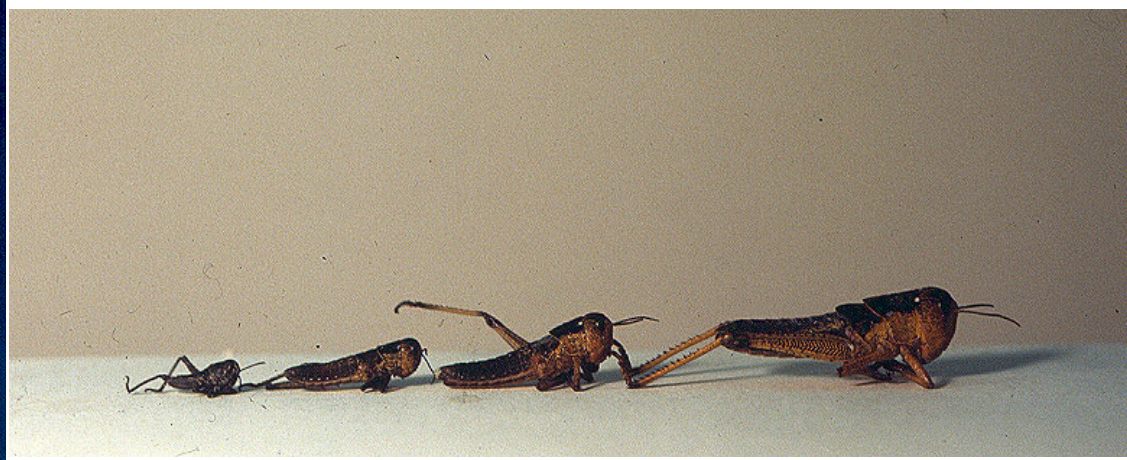


1st instar larva



Growth-an increase in either cell size and/or cell number





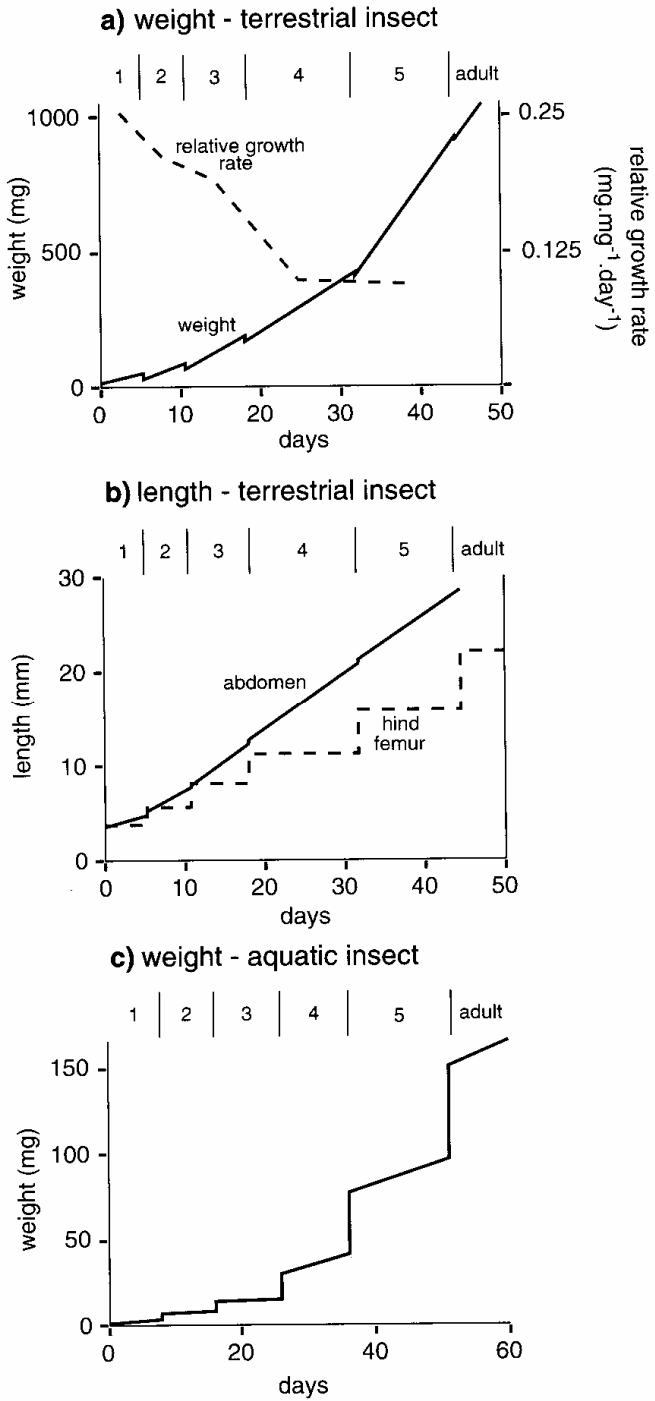
GROWTH PATTERNS

Larval growth patterns in hemimetabolous insects. Numbers at the top indicate the number of successive larval stages and vertical lines represent the following:

TOP-change in weight and relative growth rate of *Locusta*.

MIDDLE-Changes in linear dimensions of a terrestrial insect. Note difference in changes in abdomen having arthrodial membranes and the hind femur that is rigid.

BOTTOM-Change in weight of an aquatic insect.



Growth occurs mainly when the insect has exited its old exoskeleton and the next apolysis.

The number of instars a group has varies from many in primitive groups to very few in more advanced groups.

In some groups, but not in the Diptera, the number of instars is fixed and cannot be changed by diet, etc.

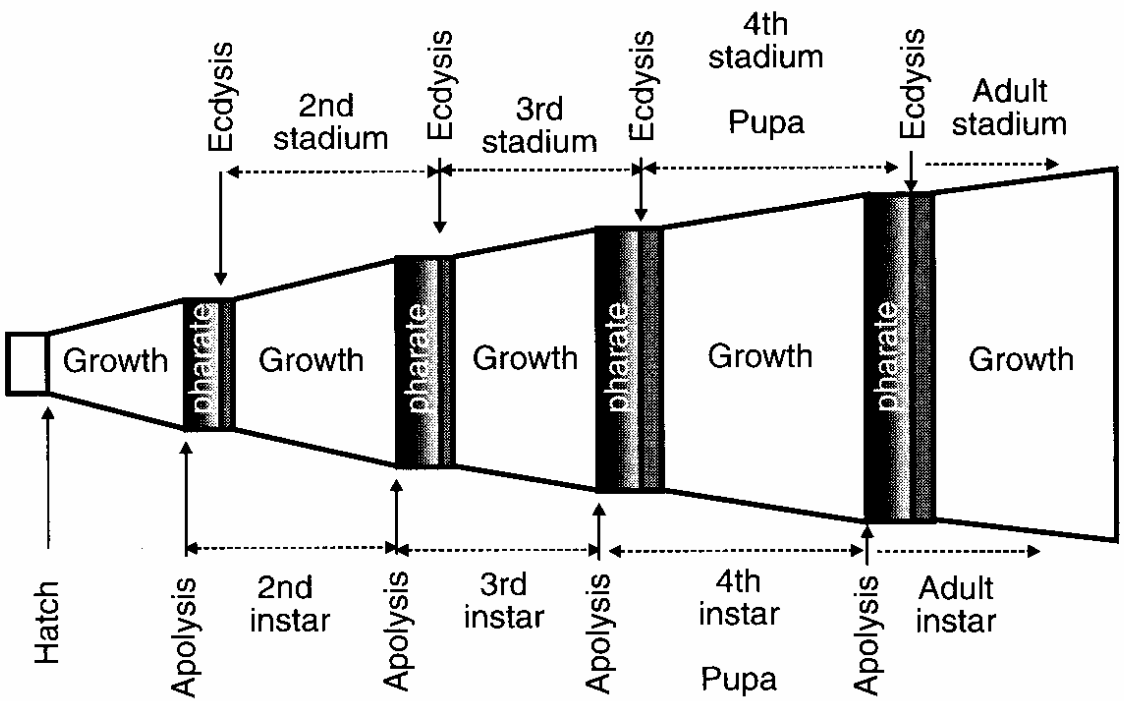


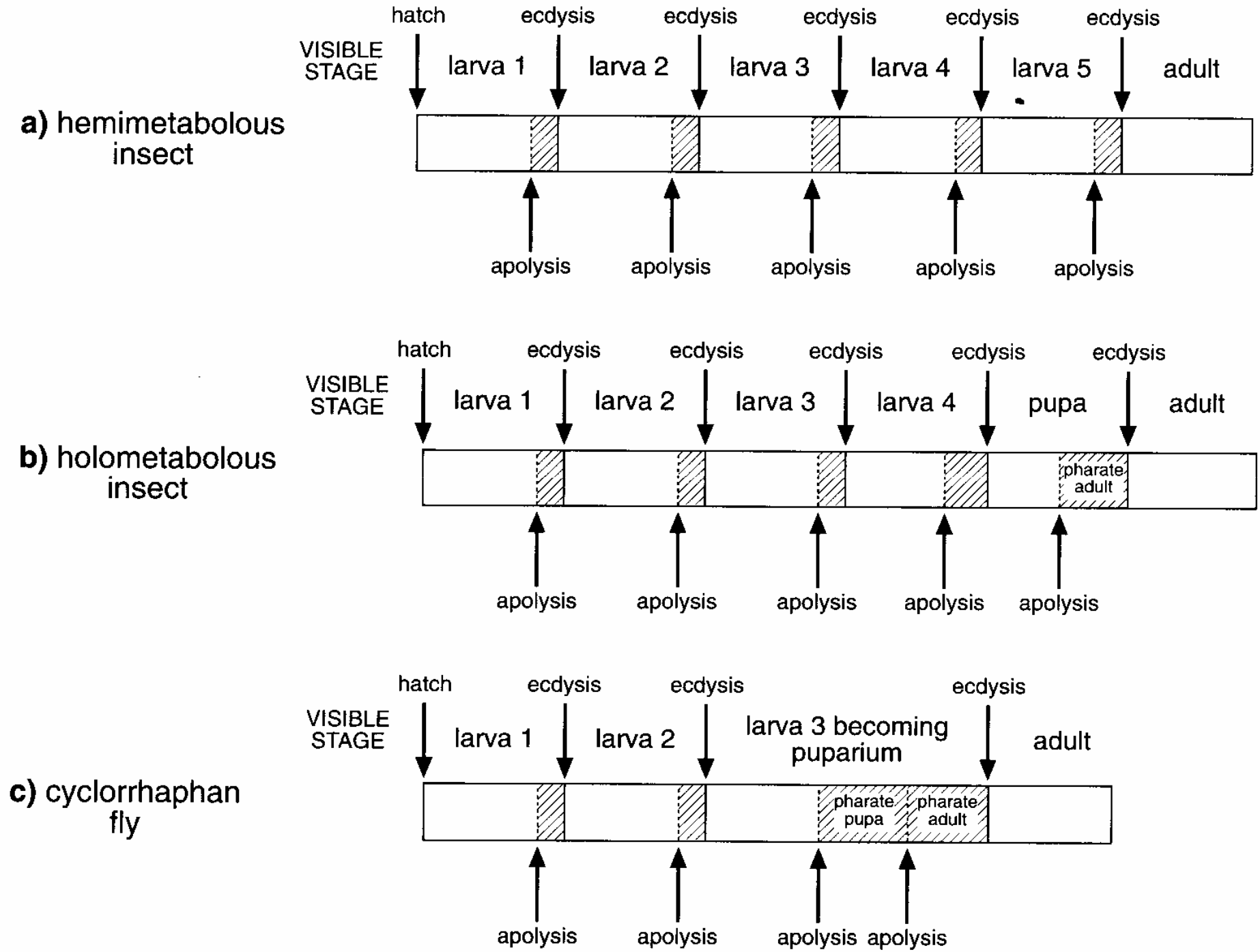
Table 15.1. *Numbers of larval stages in different orders of insects. The commonly occurring numbers are given. Numbers outside this range may occur under some conditions*

Order	Common name	Number of larval stages
Archaeognatha		10–14
Thysanura	Bristletails	9–14
Ephemeroptera	Mayflies	20–40
Blattodea	Cockroaches	6–10
Mantodea	Mantids	5–9
Grylloblattodea	Rock crawlers	8
Orthoptera	Crickets	5–11
Phasmida	Stick insects	8–12
Isoptera	Termites	5–11
Dermaptera	Earwigs	4–6
Embioptera	Webspinners	4–7
Plecoptera	Stoneflies	22–23
Hemiptera	Bugs	3–5
Thysanoptera	Thrips	5–6
Psocoptera	Book lice	6
Phthiraptera	Lice	3–4
Neuroptera	Lacewings	3–5
Mecoptera	Scorpion flies	4
Siphonaptera	Fleas	3
Diptera	Flies	3–6
Trichoptera	Caddis flies	5–7
Lepidoptera	Butterflies	5–6
Coleoptera	Beetles	3–5
Hymenoptera	Bees	3–6

Do the insects with the largest number of molts have the largest adults; and, if so why do they? If not, why to the others have the largest adults?

The largest insects are those that have the least number of molts? How can this happen?

Those insects, such as the holometabolous insects, have larva with cuticles that can expand, thus permitting growth in an expandable cuticle while the hemimetabolous insects are in a more rigid exoskeleton.


a) hemimetabolous insect
hatch
ecdysis
ecdysis
ecdysis
ecdysis
ecdysis
VISIBLE STAGE
larva 1
larva 2
larva 3
larva 4
larva 5
adult
apolysis
apolysis
apolysis
apolysis
apolysis
b) holometabolous insect
hatch
ecdysis
ecdysis
ecdysis
ecdysis
ecdysis
VISIBLE STAGE
larva 1
larva 2
larva 3
larva 4
pupa
adult
pharate adult
apolysis
apolysis
apolysis
apolysis
apolysis
c) cyclorrhaphan fly
hatch
ecdysis
ecdysis
ecdysis
VISIBLE STAGE
larva 1
larva 2
larva 3 becoming puparium
adult
pharate pupa
pharate adult
apolysis
apolysis
apolysis
apolysis

Suggestions for making measurements about insects for experimental purposes whether insects are from the field or laboratory

1. Weight can be a misleading measurement, especially in an adult insect

2. If one wants to get differences in sizes between adults it is best to measure a structure such as the wing length or head width

b. Molting-the process of shedding or getting rid of the old cuticle or exoskeleton.

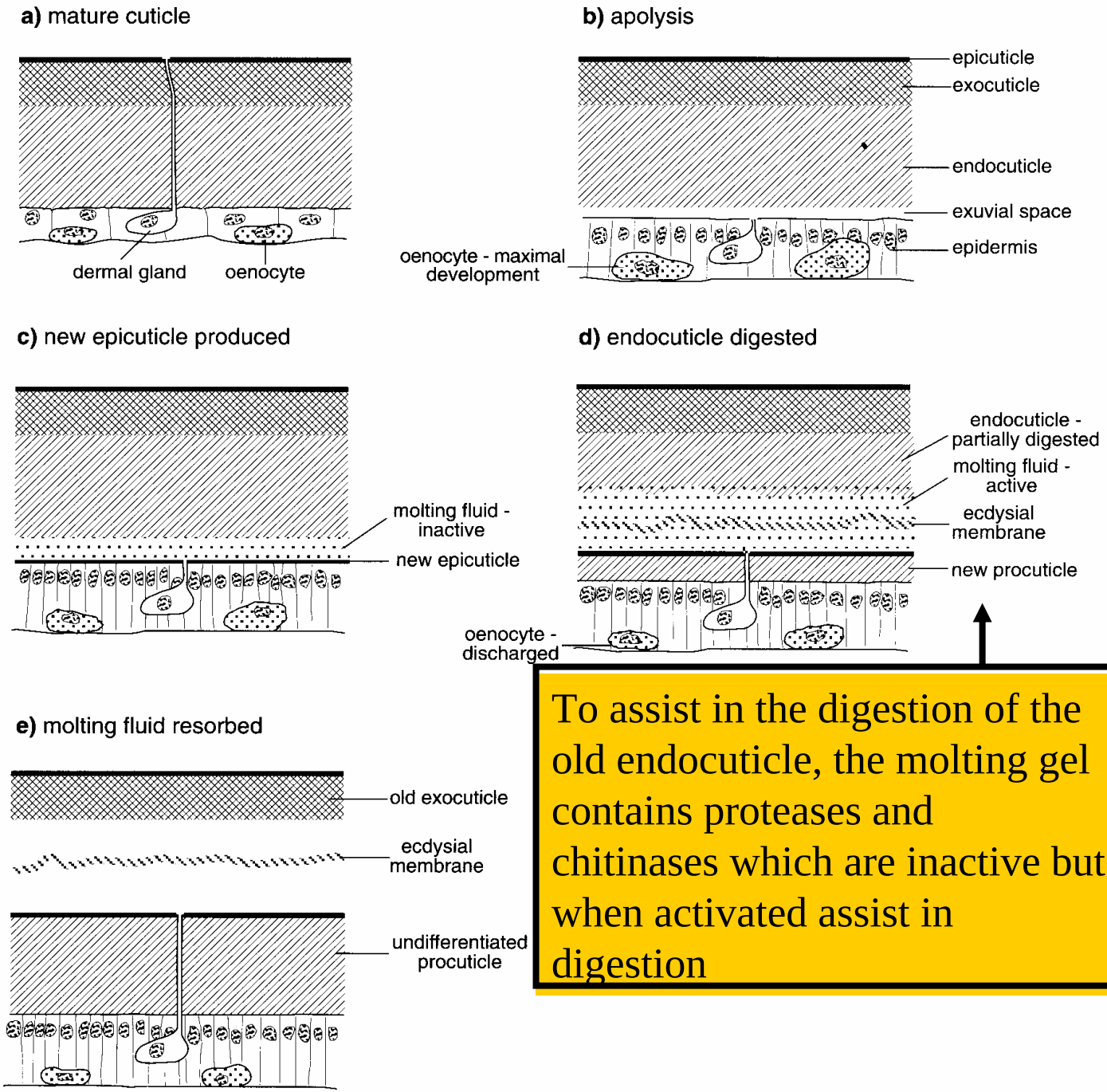
b

1. All arthropods have exoskeletons made of chitin.
2. In order to grow, arthropods must shed or get rid of their old exoskeleton
3. This process is called molting and is composed of 3 main events
   a. **Apolysis**-separation of epidermis from cuticle
   b. **Digestion + resorption** of old endocuticle
   c. **Ecdysis**-Shedding of old cuticle

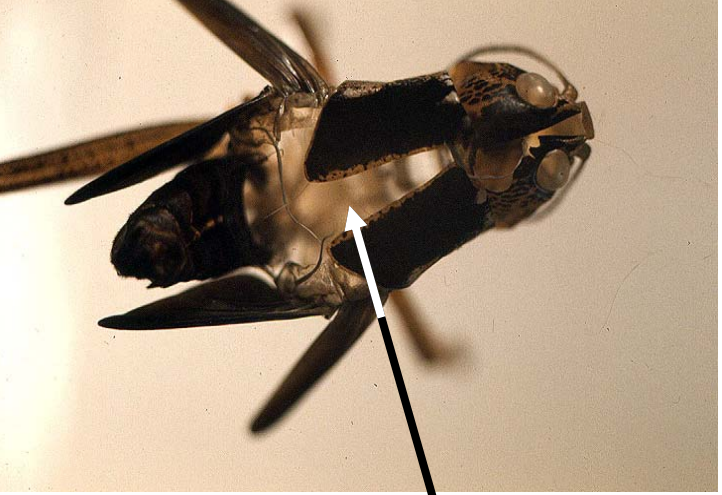
ECDYSONE

THE MOLTING HORMONE INITIATES THE MOLT

1. Apolysis
2. Digestion + Resorption
3. Ecdysis

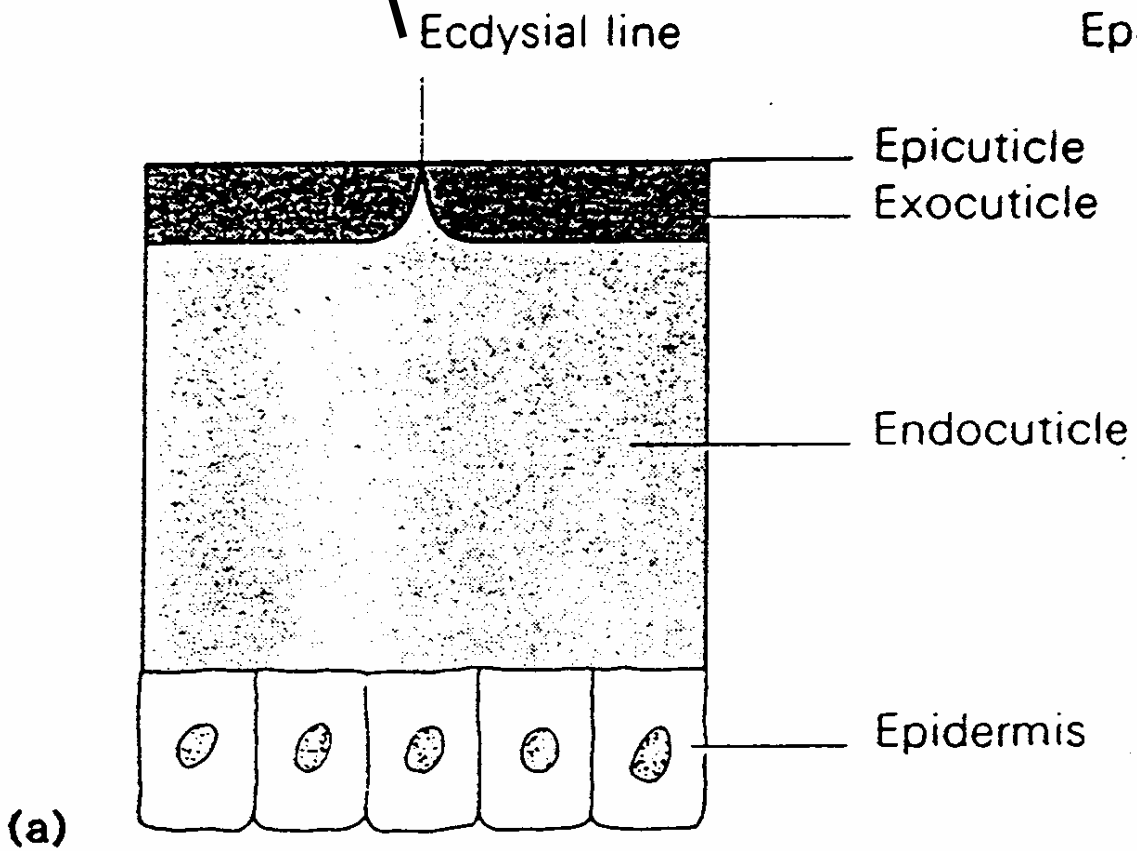


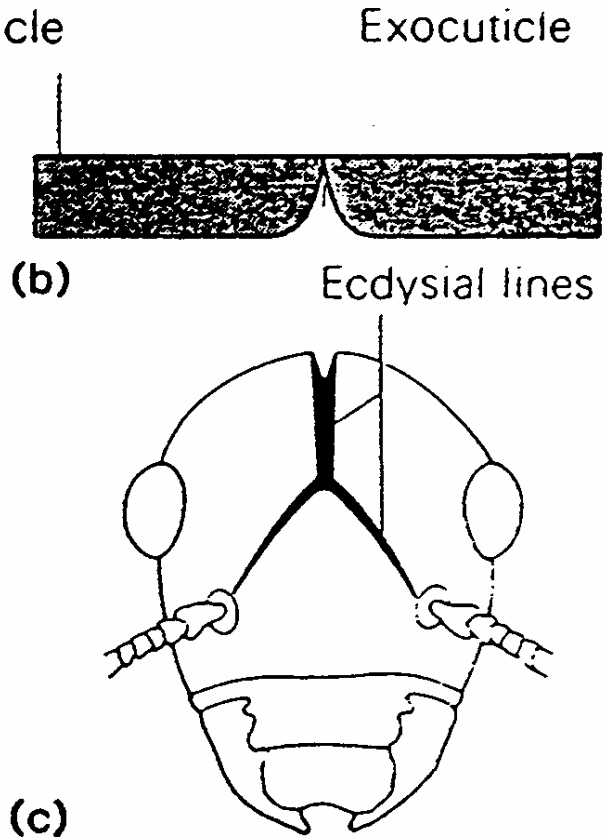
To assist in the digestion of the old endocuticle, the molting gel contains proteases and chitinases which are inactive but when activated assist in digestion



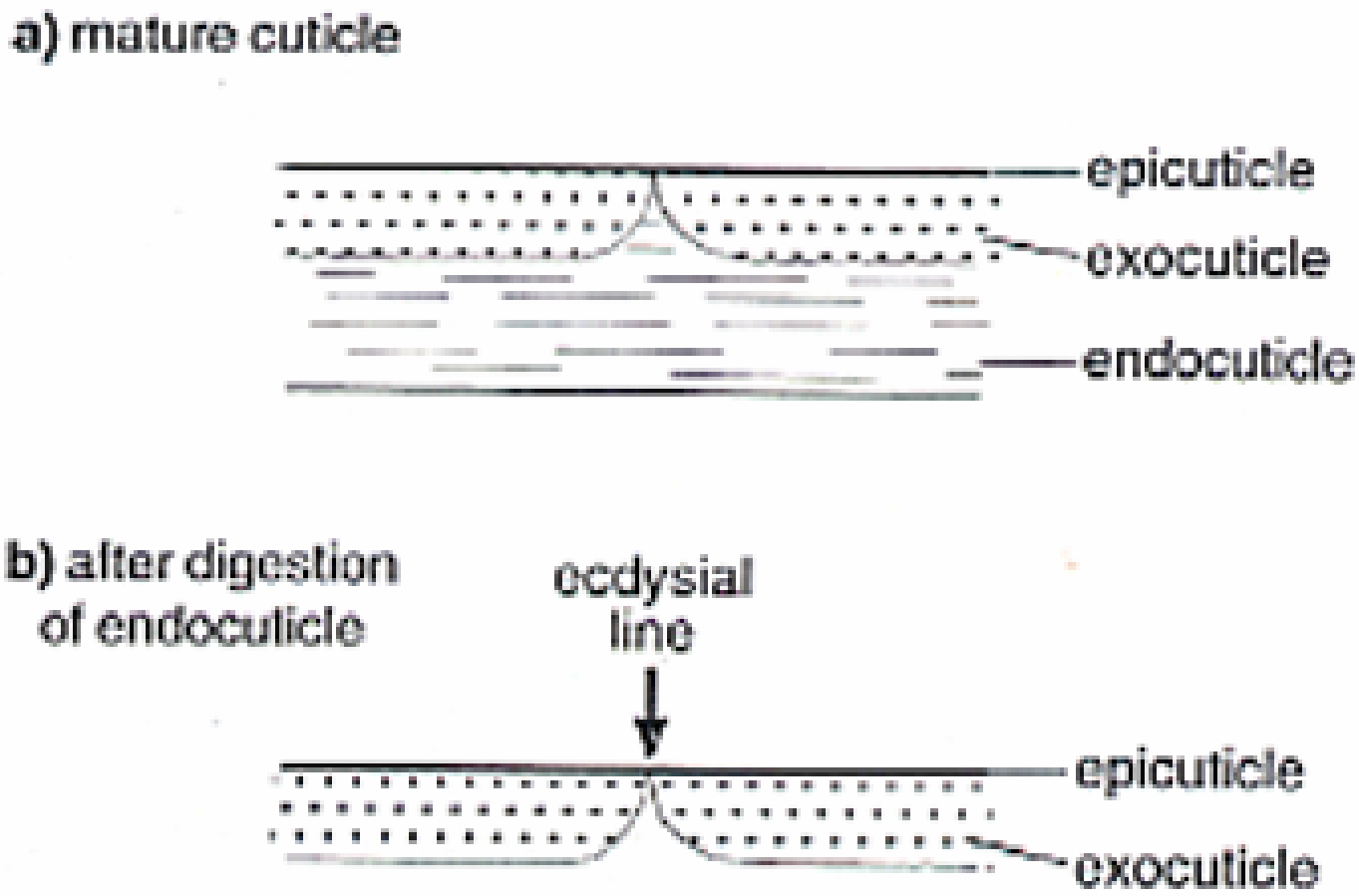
Eclosion=emergence of immature from the egg

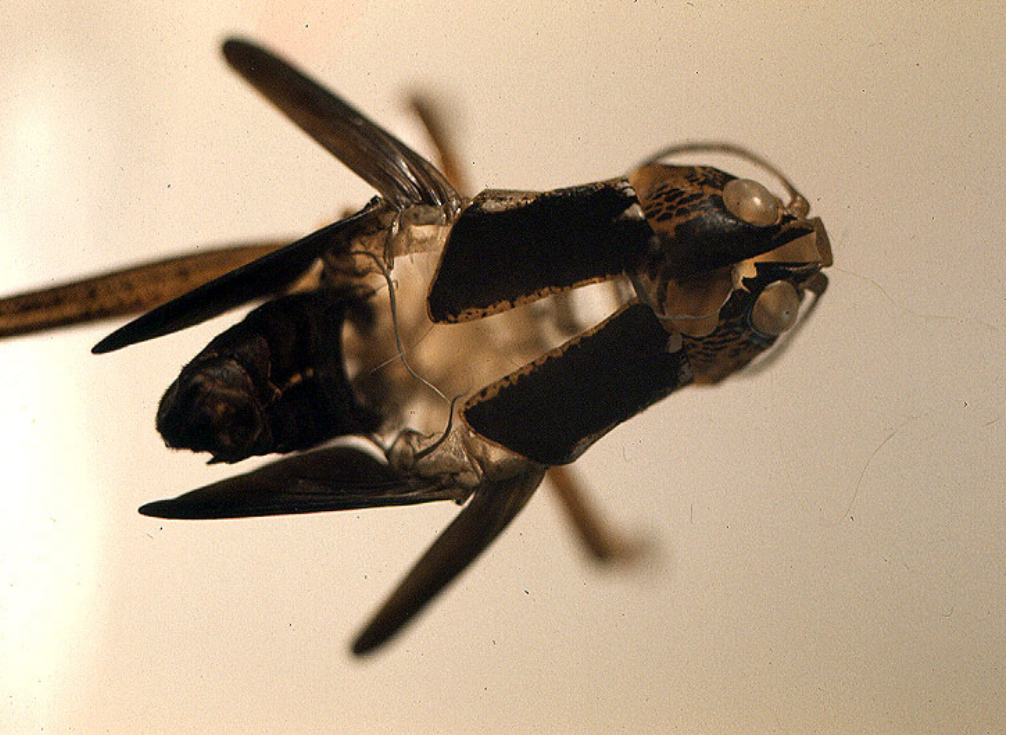
Eclosion is now used to mean the pupal-adult ecydisis





Molting and ecdysial suture





Note in the photo above that the **ecdysial suture** goes from the head and down the thorax in most insects. It is usually a gravity escape from the old cuticle.

What cuticular layer is absent at the ecydsial suture?

Pore canals

Old endocuticle

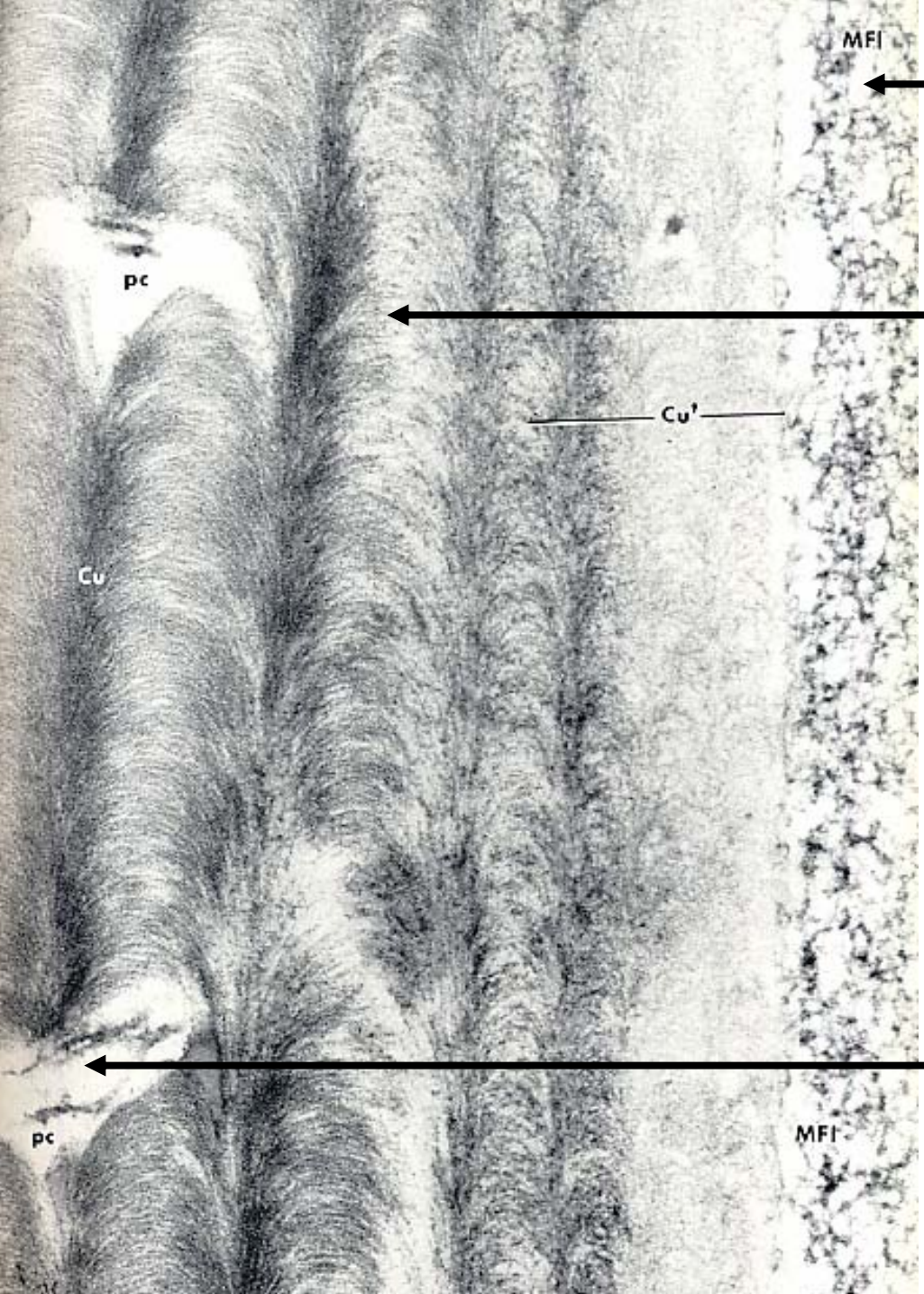
Exuvial space formed at apolysis

New cuticle being formed by the epidermal cells (Cu_2)

Epidermal cell

TEM through integument of a larva or nymph of milkweed bug shortly before the molt to the adult.

Molting fluid



Molting fluid produced by the epidermal cells, which contains chitinase and proteases

Note parabolic shape and pattern of the chitin-protein microfibrils. Between the lines on both sides of the Cu' you will see that this shape and pattern has been altered due to the digestive action of both enzymes.

Pore canal

Enlargement of previous slide

What triggers molting?
Growth and nutrition are important

1. The kissing bug, *Rhodnius prolixus* and Wigglesworth
   a. *Rhodnius* initiates apolysis 2-3 days following blood meal
   b. Beckel and Friend (1964) fed *Rhodnius* saline with ATP that causes a similar stretch and got the apolysis cue.
   c. Is it due to stretch receptors in the abdomen responding to the large bloodmeal?
   d. Wigglesworth cut the VNC after a blood meal and no molt. He concluded it was due to stretch receptors.
   e. Nijhout (1984) and Chiang and Davey (1988) showed it was not due to the stretching of the stretch receptors but due to pressure from the bloodmeal applying pressure on these receptors and not stretch.
   f. A similar situation occurs in *Dipetalogaster*, another reduviid. Here it is the stretching of the main trunk of the abdominal nerves that produces a continued and persistent train of action potentials.

What triggers molting?

Growth and nutrition are important

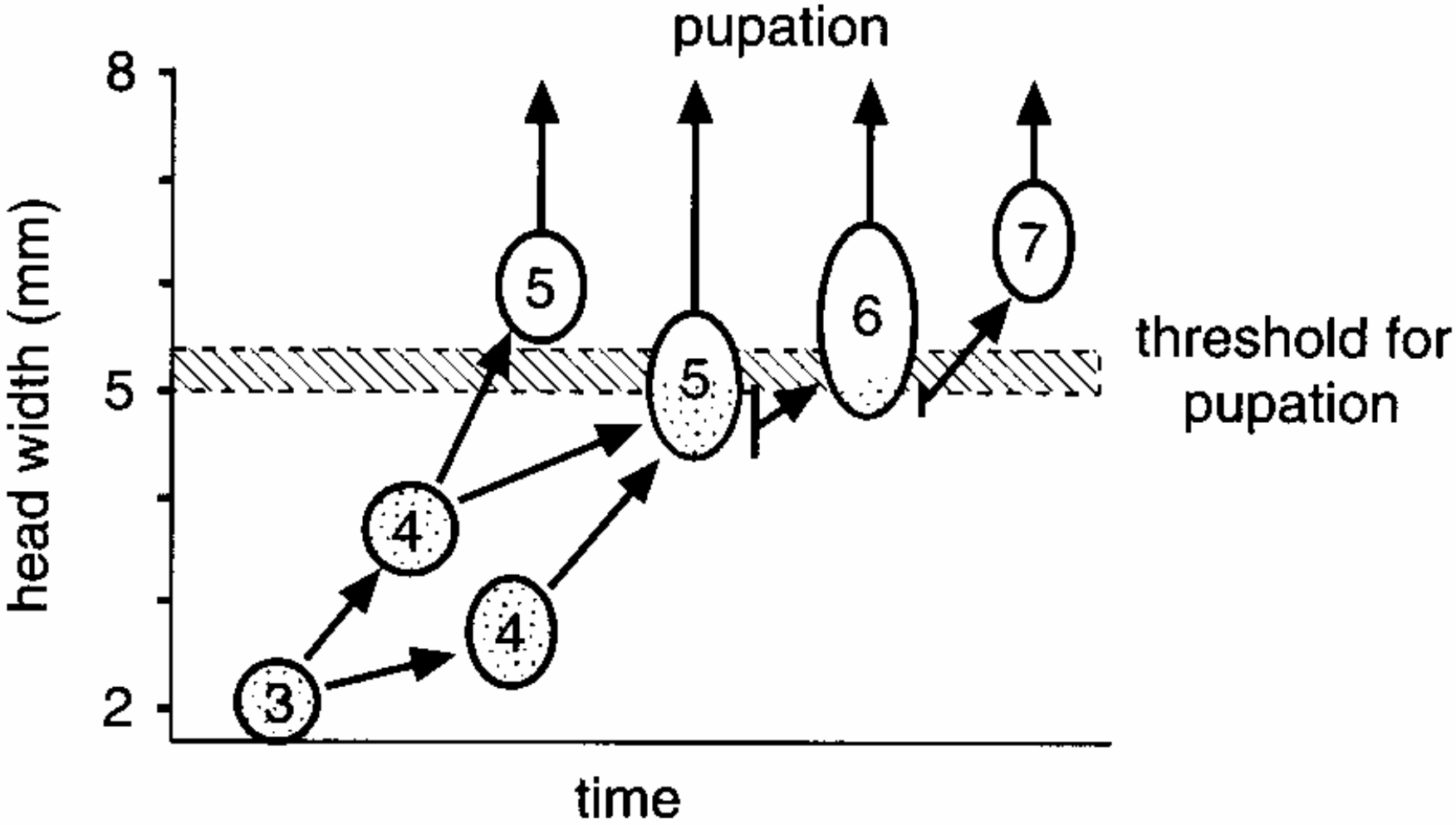
2. *Oncopeltus fasciatus* initiates a molt only when they have reached a critical weight in each instar (Nijhout, 1979, 1981).

a. Nijhout was able to get subcritical weight bugs to molt by injecting them with saline. This increased their weight and presumably the stretch of the abdomen. The stretch receptors in this insect have not yet been identified.

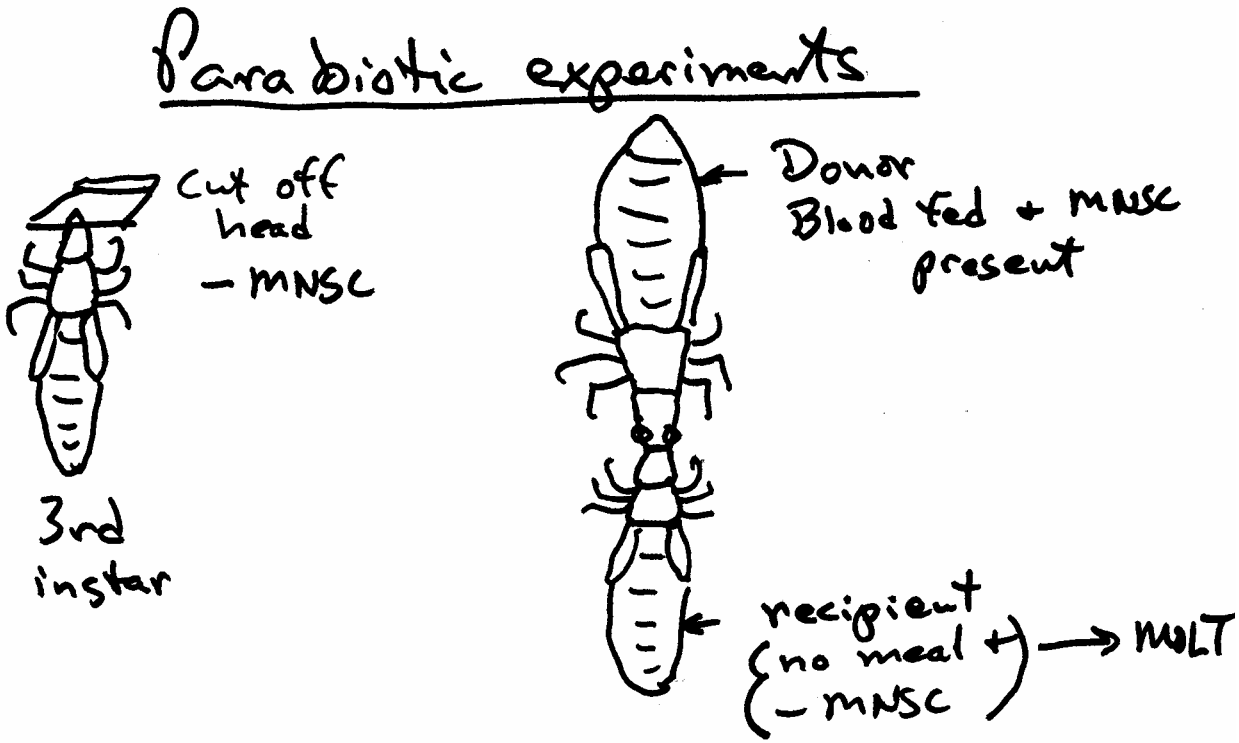
3. In *Manduca* the caterpillar somehow is able to monitor a critical weight that inititates the molt.

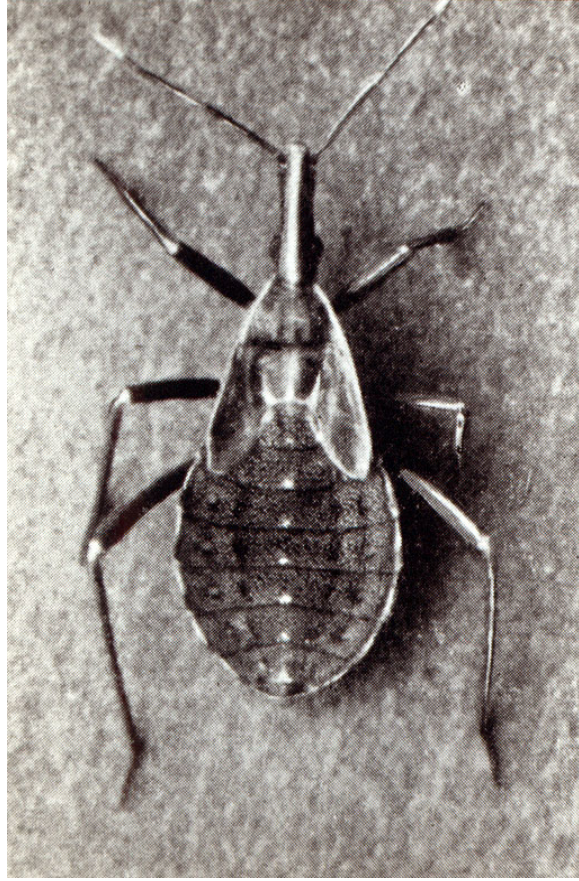
These photos are from yesterday's lab in which the ventral nerve cord was cut in *Phormia regina* and the fly was then fed on a sucrose soln. Note that the fly has become extremely hyperphagic because it has lost the negative input or feedback from the stretch receptors in the nerve net that overlays the crop. Photos and dissection by Roger.

Importance of critical head width or size for pupation in *Manduca sexta*. If the headwidth is less than the threshold immediately following the molt (shaded areas within the elipses) the caterpillar will molt into another larval stage.



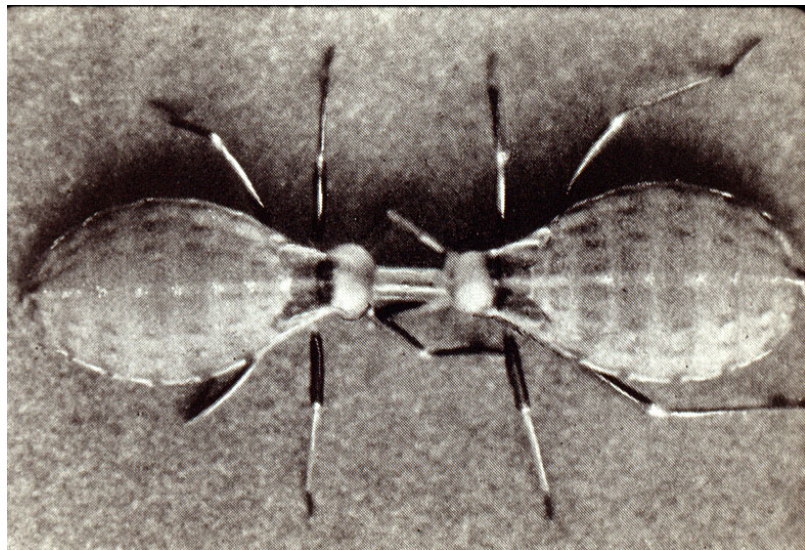
Control of molting-Neural or hormonal?





ROLE OF NEUROSECRETION IN THE BRAIN

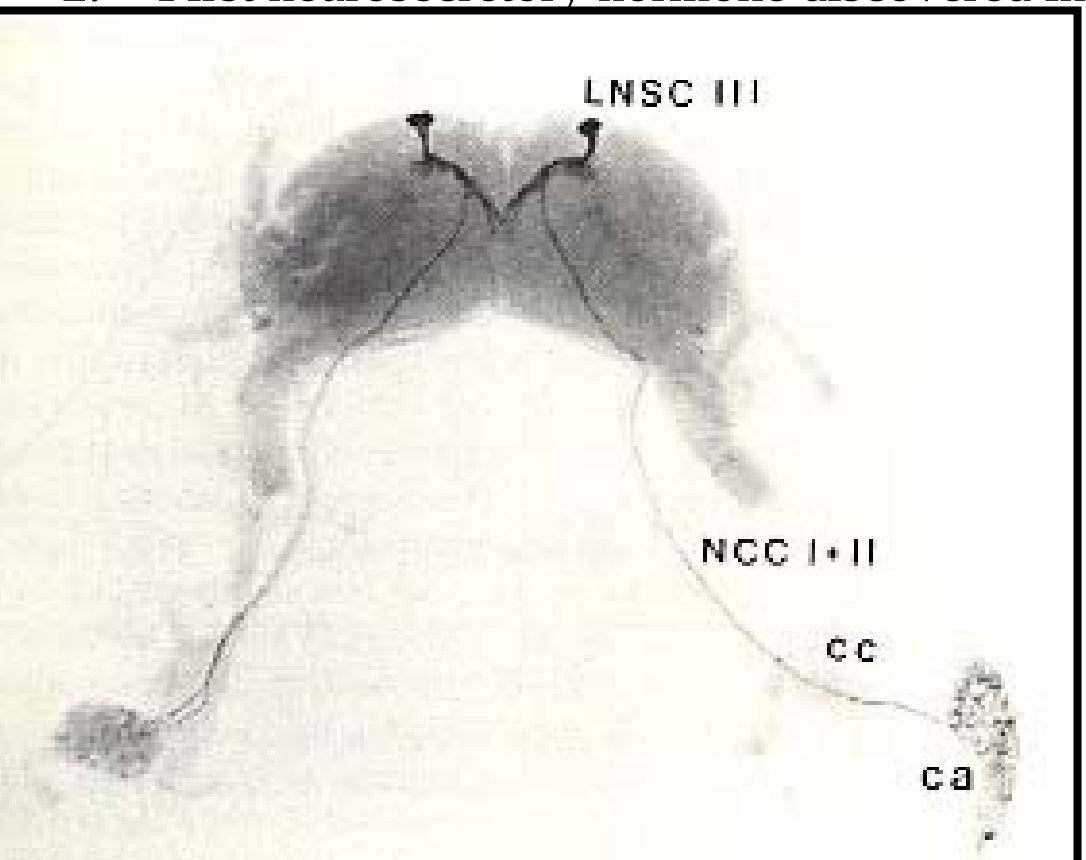
Wigglesworth (1934) demonstrated using parabiosis experiments with *Rhodnius* that a brain factor was involved in initiating the molt.



HORMONES INVOLVED IN MOLTING

Prothoracicotropic hormone (a neuropeptide)

1. First hormone discovered in insects by Kopec (1917, 1922) doing ligation exps. on gypsy moth. Ligation in middle of caterpillar resulted in anterior half pupating but not the posterior half. He then removed the brain that in turn inhibited pupation. He said it was something in the brain and for a long time hormones from this area were just called ‘brain hormones.’
2. First neurosecretory hormone discovered in any animal



Using an antibody to PTTH it was demonstrated that the LNSC of group III are the primary source of PTTH. When released, it travels down the NCC I+II through the CC and into the CA and released. Thus, the CA for *Manduca sexta* is the neurohemal organ.

HORMONES INVOLVED IN MOLTING

Ecdysteroids

1. Shown by Carroll Willaims (1947). Using ligation and implantation experiments with *Hyalophora cecropia* he showed that when active brains were implanted in bisected pupae the anterior half molted into a normal adult but the posterior did not. He then found that the posterior half could molt normally and become an adult if it received both active brains and the prothoracic glands. Thus, he concluded that the brain hormone stimulated the prothoracic glands to secrete a hormone that induced the molt.

MOLTING

PTTH

↓

ECDYSIAL GLAND

↓

ECDYSTEROIDS

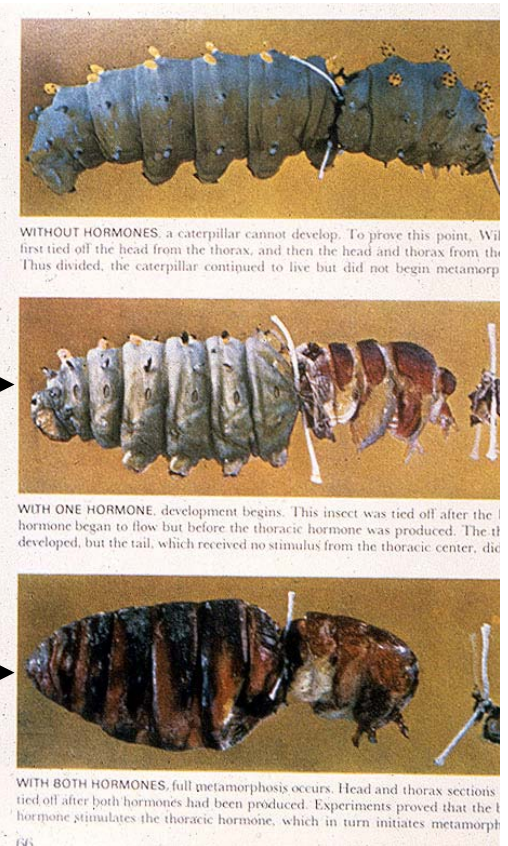
↓

EPIDERMAL CELLS

With no hormone →

With brain hormone into the thoracic area →

With brain hormone and prothoracic gland implants into the abdominal section →



WITHOUT HORMONES, a caterpillar cannot develop. To prove this point, Wi
first tied off the head from the thorax, and then the head and thorax from th
Thus divided, the caterpillar continued to live but did not begin metamorp

WITH ONE HORMONE, development begins. This insect was tied off after the
hormone began to flow but before the thoracic hormone was produced. The th
developed, but the tail, which received no stimulus from the thoracic center, did

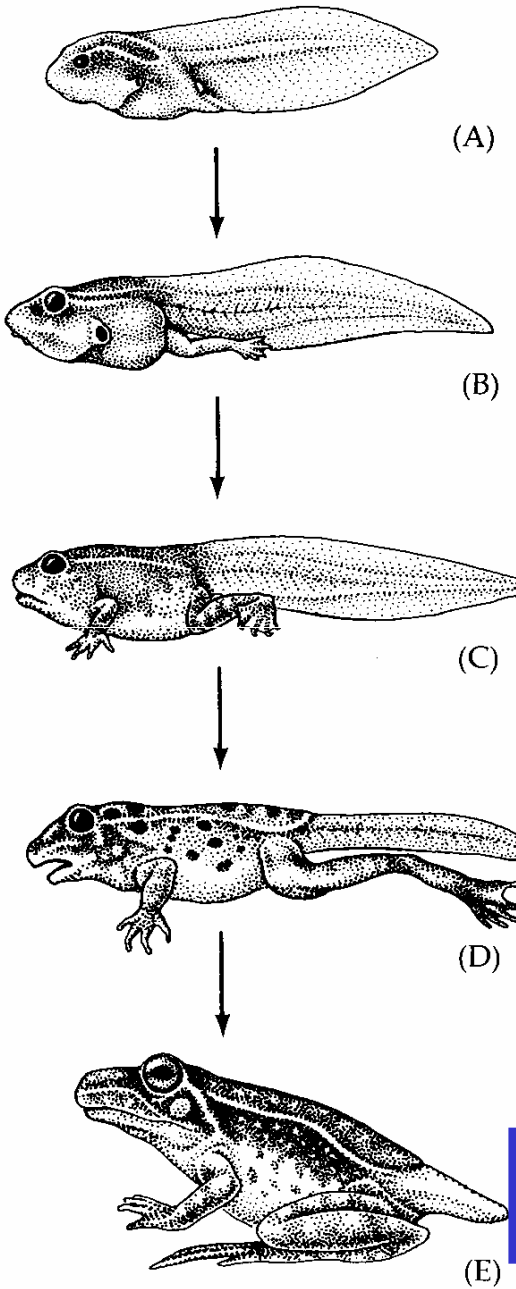
WITH BOTH HORMONES, full metamorphosis occurs. Head and thorax sections
tied off after both hormones had been produced. Experiments proved that the
hormone stimulates the thoracic hormone, which in turn initiates metamorph

METAMORPHOSIS-Change in shape and/or form.

Greek, meta=change in; morphe=shape

Metamorphic rock

METAMORPHOSIS



Comparison between the frog larva and adult

System	Larva	Adult
Locomotory	Aquatic; tail fins	Terrestrial; tailless tetrapod
Respiratory	Gills, skin, lungs; larval hemoglobins	Skin, lungs; adult hemoglobins
Circulatory	Aortic arches; aorta; anterior, posterior, and common cardinal veins	Carotid arch; systemic arch; jugular veins
Nutritional	*Herbivorous*: Long spiral gut—intestinal symbionts; small mouth—horny jaws, labial teeth	*Carnivorous*: Short gut—proteases; large mouth—long tongue
Nervous	Lack of nictitating membrane, porphyropsin, lateral line system—Mauthner's neurons	Development of ocular muscles, nictitating membrane, rhodopsin, loss of lateral line system—degeneration of Mauthner's neurons; tympanic membrane
Excretory	Largely ammonia, some urea (ammonotelic)	Largely urea, high activity of enzymes of ornithine–urea cycle (ureotelic)
Integumental	Thin, bilayered epidermis with thin dermis; no mucus glands or granular glands	Stratified squamous epidermis with adult keratins; well-developed dermis contains mucus glands and granular glands secreting antimicrobial peptides

Thyroxine is the control hormone

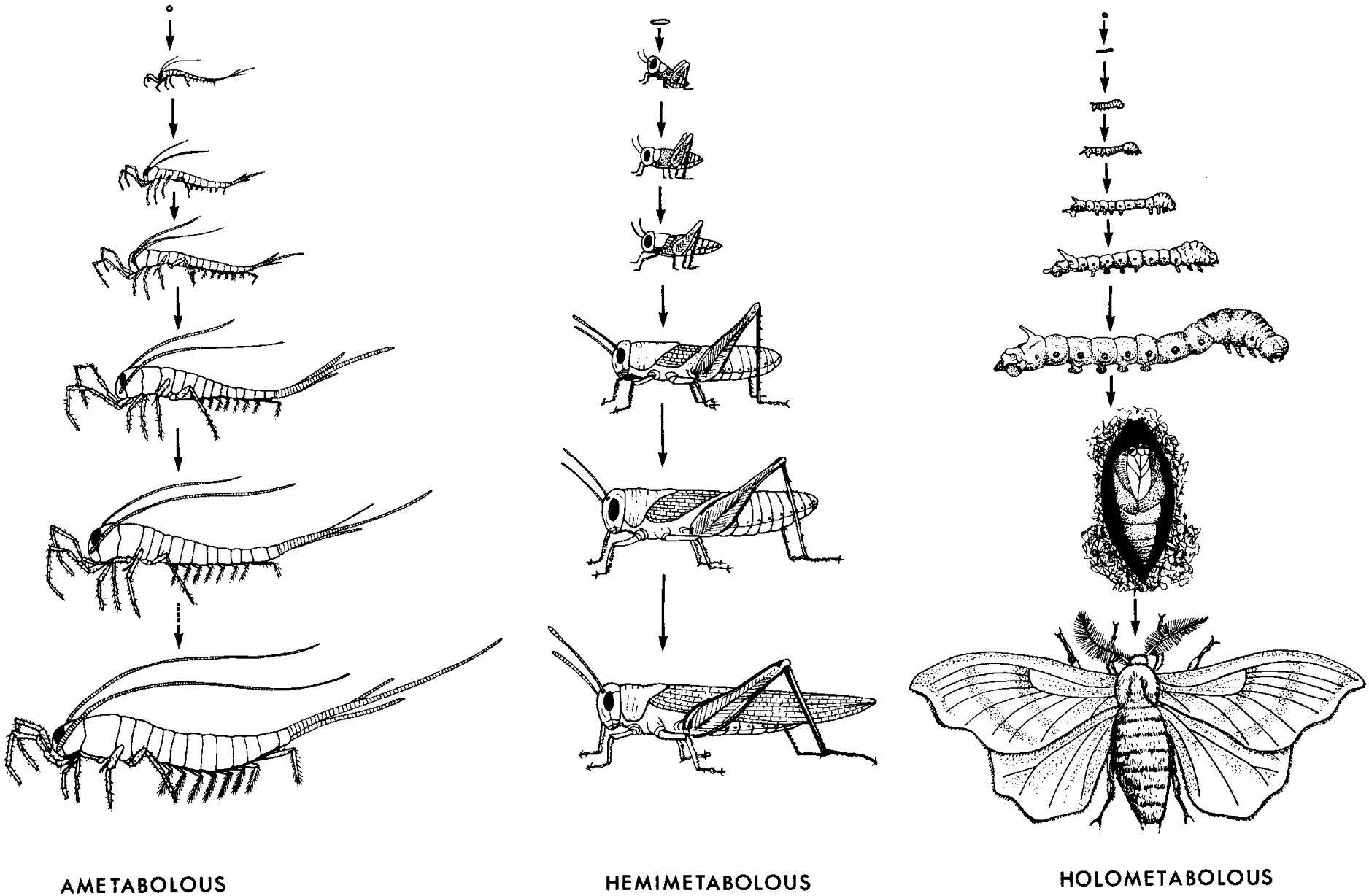
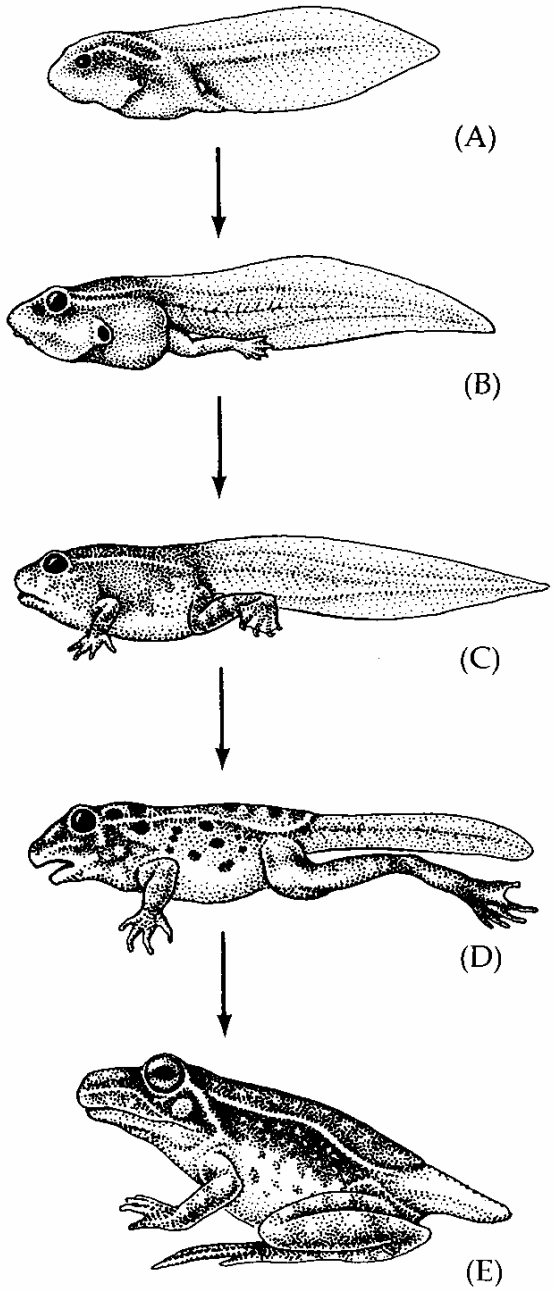


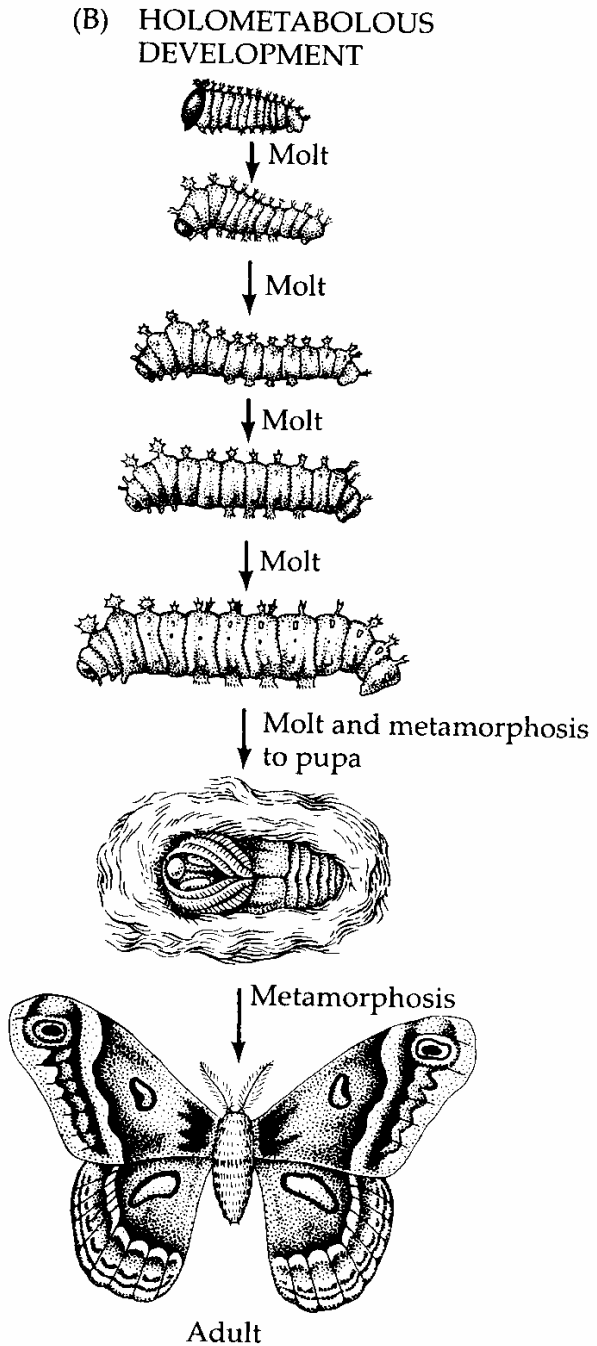
FIGURE 21.4. Basic types of development in insects. Broken arrow indicates several molts.

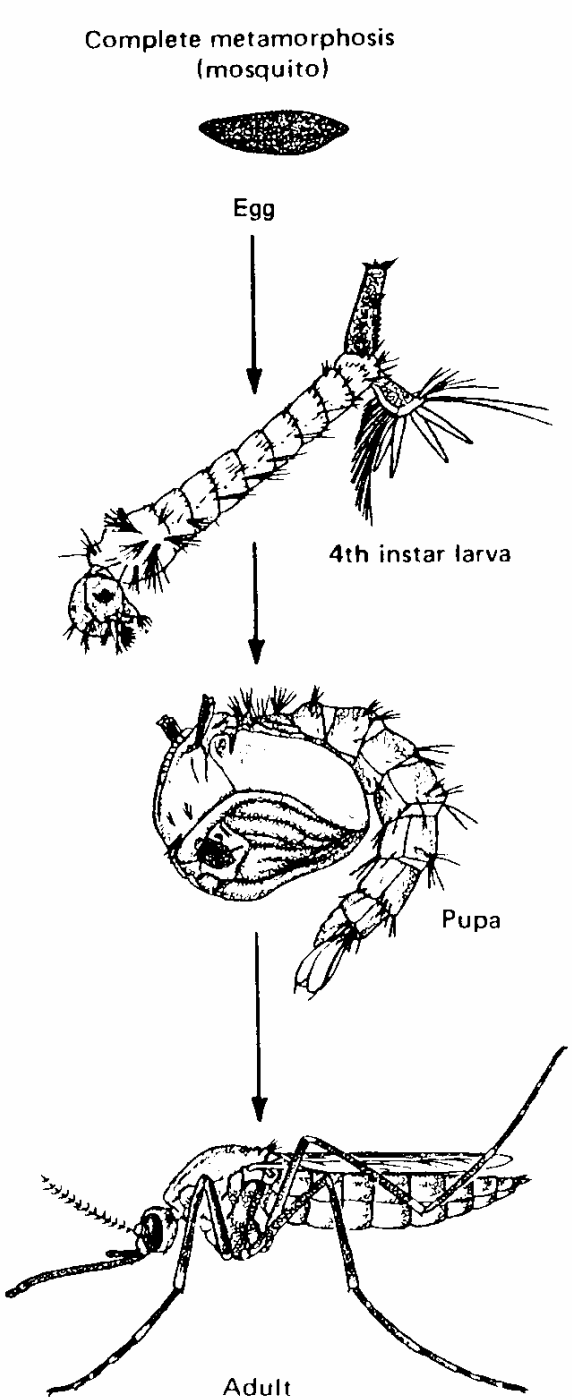
c. Metamorphosis

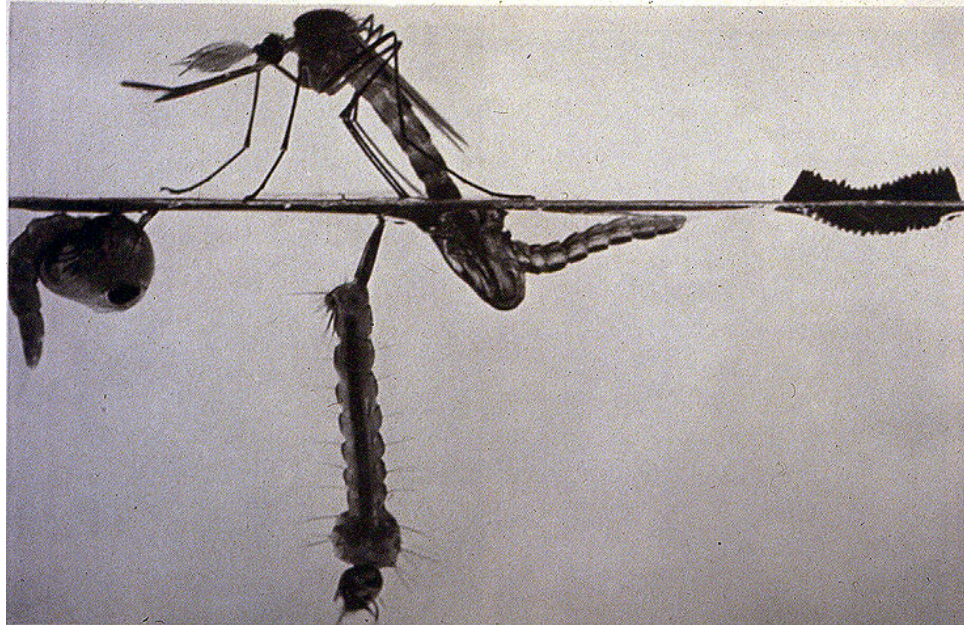
FOOD DIFFERENCES

In the case of the frog and for complete insect metamorphosis, there is an important advantage in that the food of the immature and the adult are very different. Thus, they do not compete for the same food.





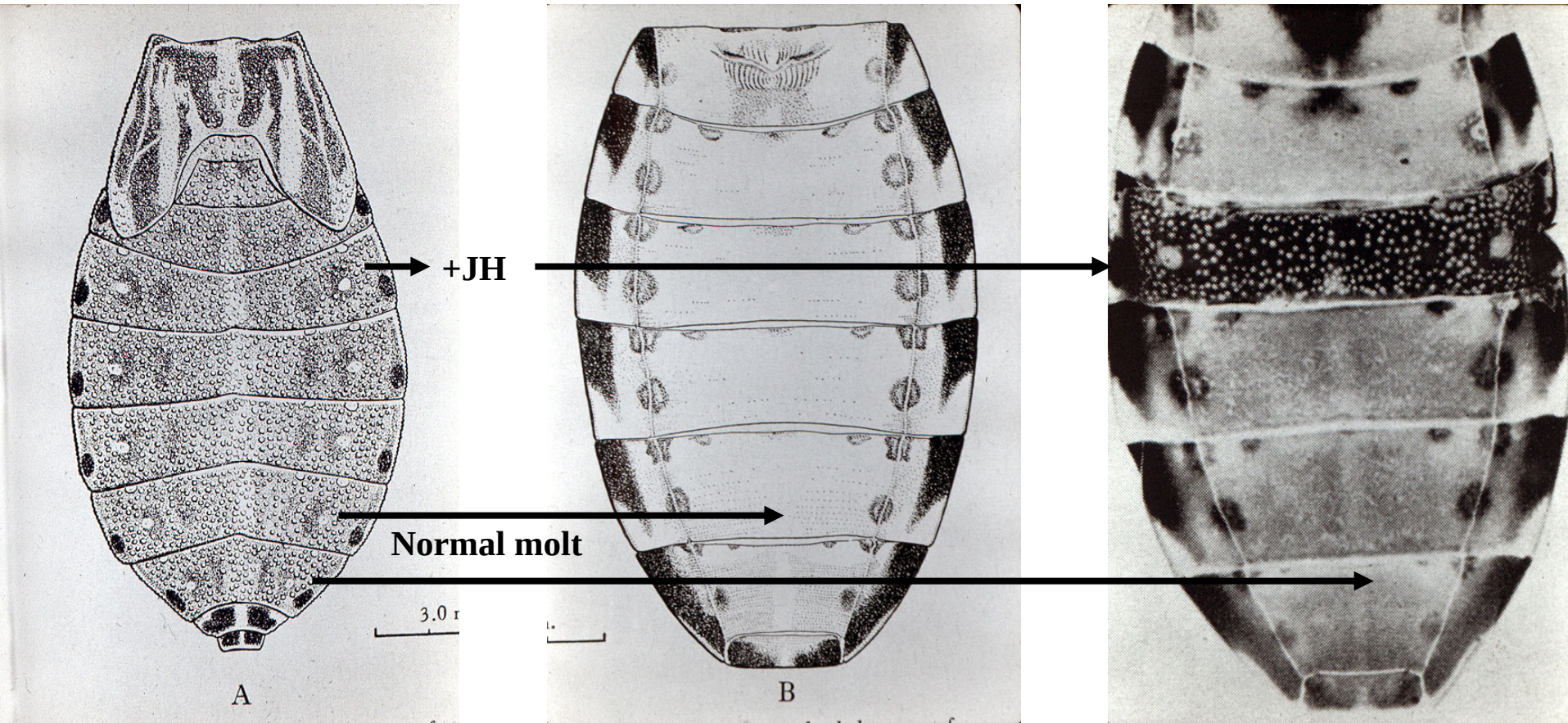




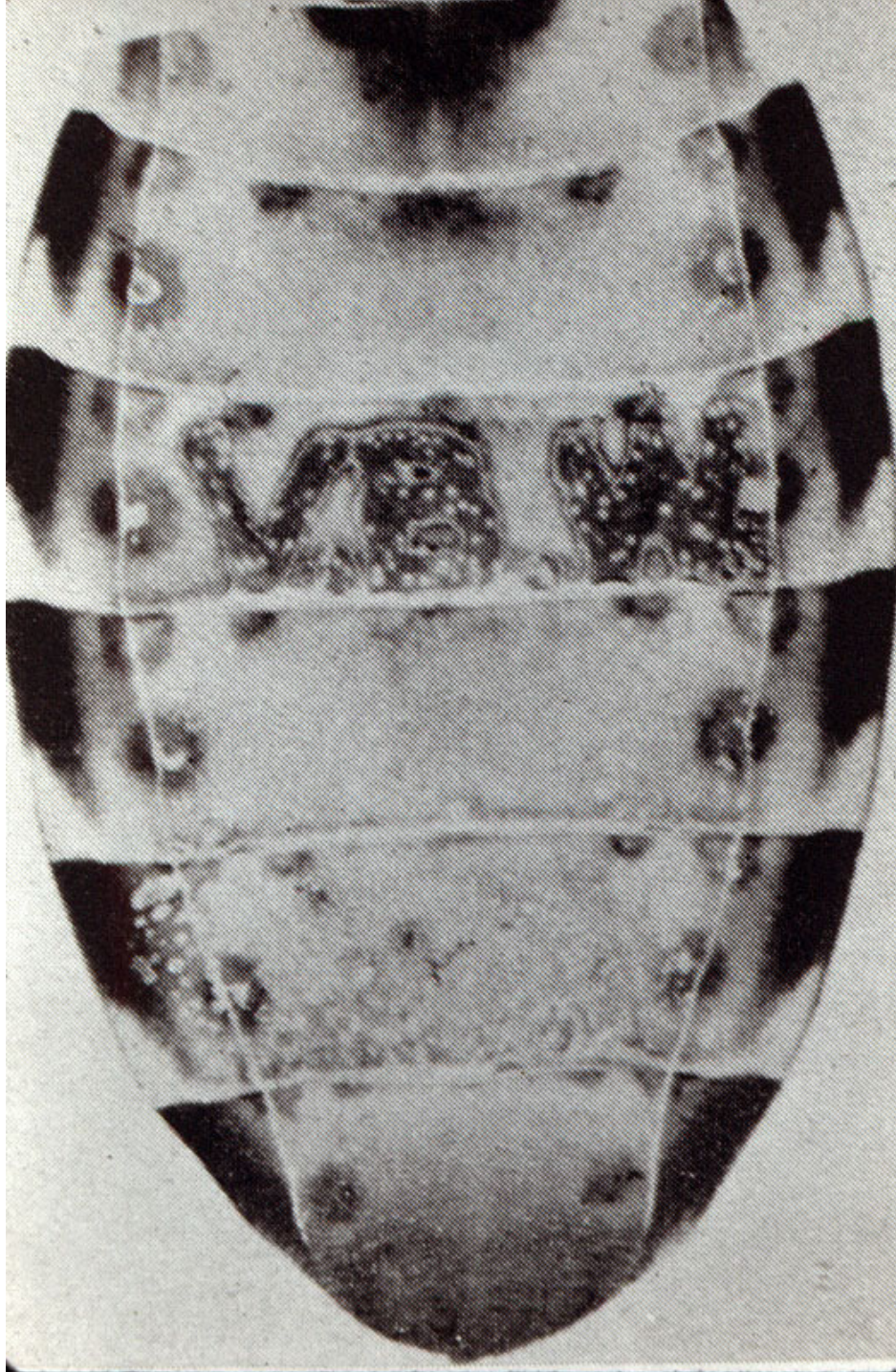
HABITAT DIFFERENCES

Another advantage of complete metamorphosis is that the adults and the immatures may live in completely different habitats, thus avoiding competition for habitats not just food.

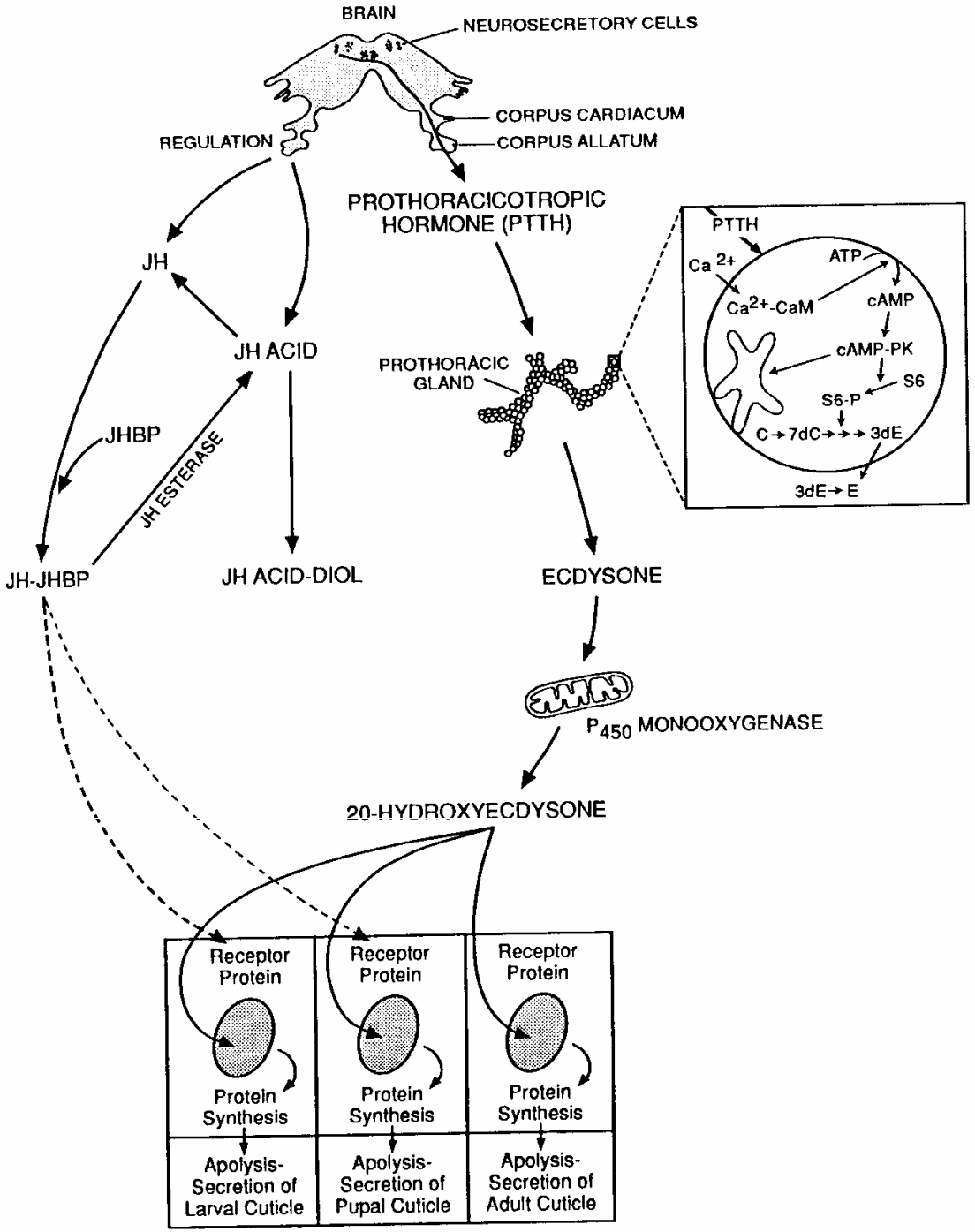
Wigglesworth showed the importance of JH at the molt from the last larval instar to the adult by topically adding JH to one of the abdominal sclerites of the larval instar that was ready to molt. Normally the cuticle produced is that of the adult (middle photo) but when JH was added, the segment it was added retained the larval cuticle and an adult cuticle was not produced (photo on right).



When the last larval instar was getting ready to molt into the adult, Wigglesworth painted JH onto the 3rd abdominal ventral segment. Rather than molting and producing an adult cuticle, the area painted with JH produced another larval cuticle and spelled out his name.

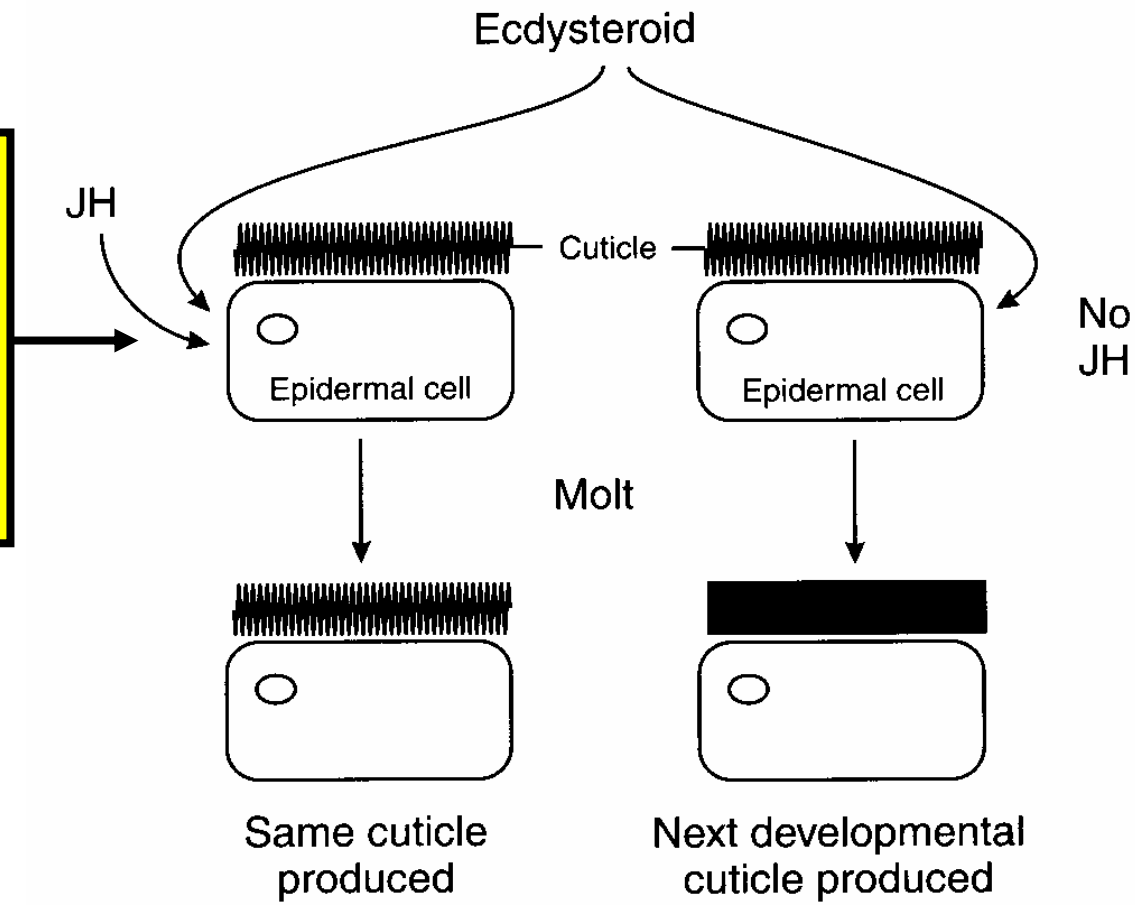


The scheme at the right represents the old scheme based on gland removal and gland implants. Thus, the idea that the pupal to adult molt was in complete absence of JH was both incomplete and incorrect. It was not until physiologists could determine the titers of both ecdysteroids and juvenile hormones did one get a more accurate picture of hormonal involvement in metamorphosis.

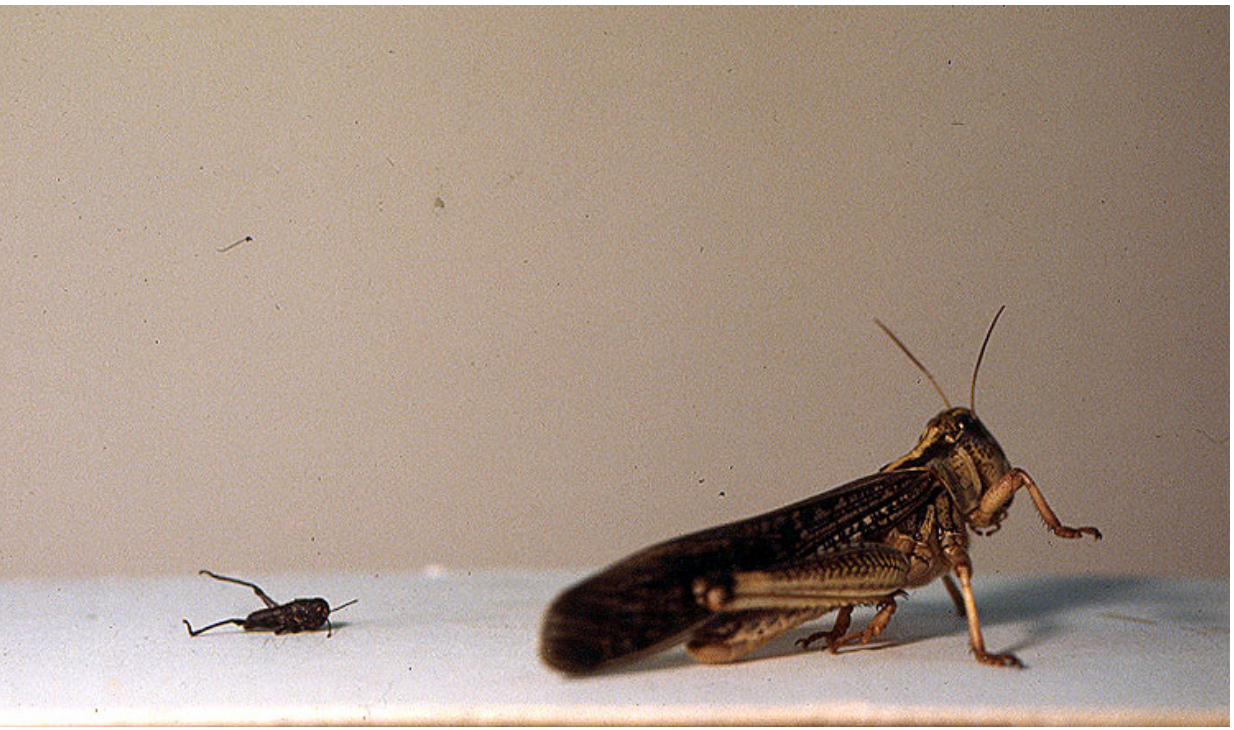


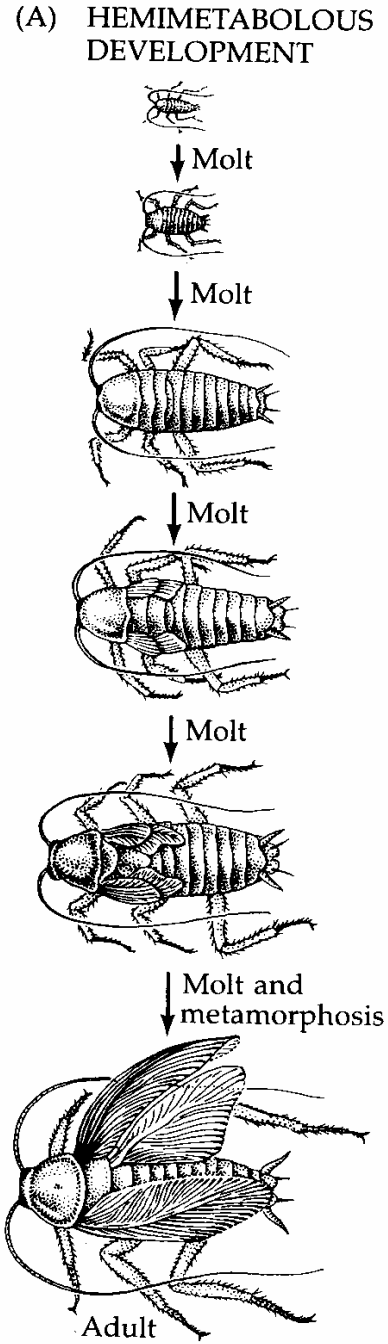
JH must be completely absent during the JH-sensitive periods or the JH-critical period of the last larval instar for hemimetabolous insects or during the JH-sensitive period of the pupal to adult molt for holometabolous insects in order for the normal developmental switches associated with metamorphosis to occur.

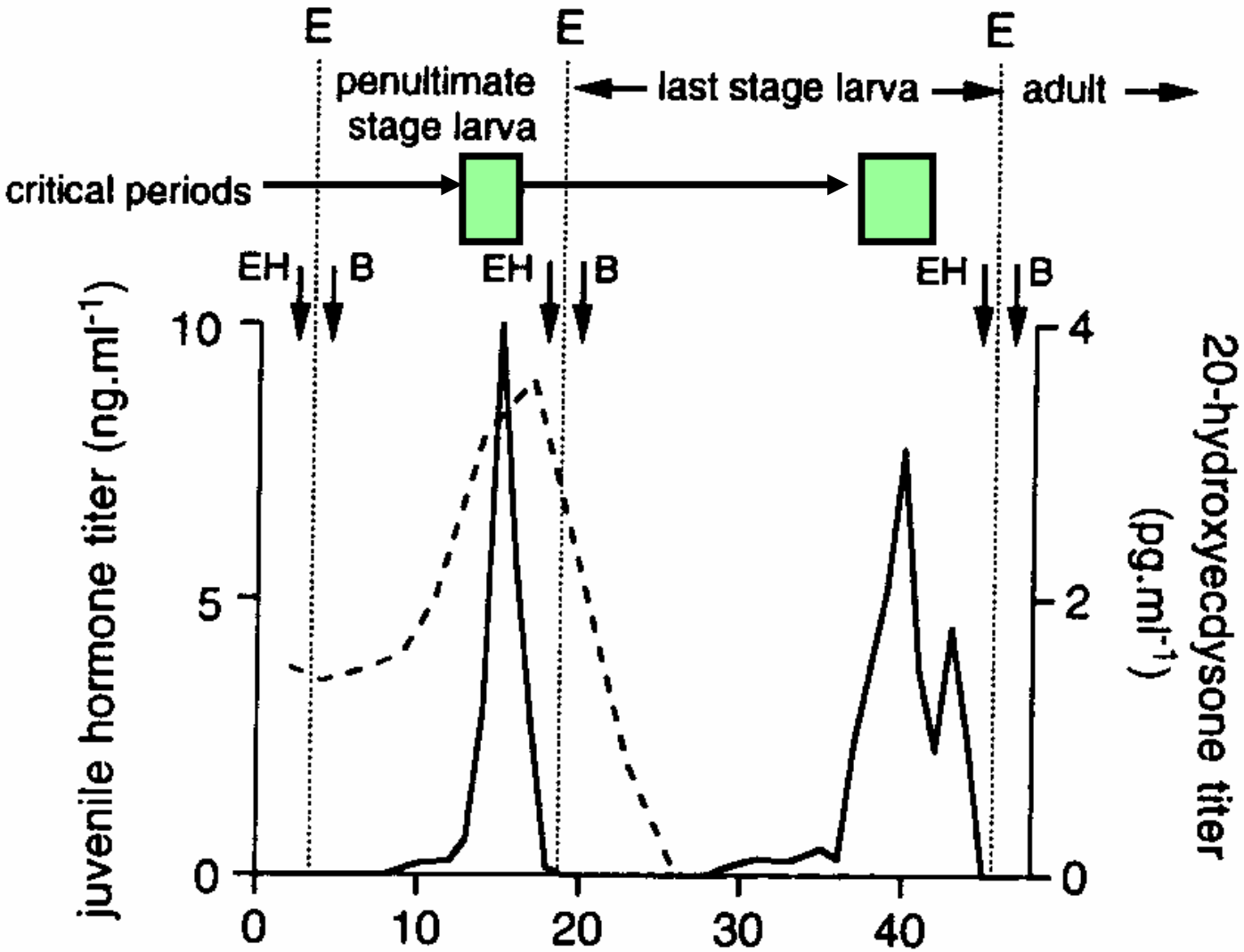
Epidermal cells experiencing JH or lack of JH during the JH-sensitive period.



CONTROL OVER METAMORPHOSIS IN HEMIMETABOLOUS INSECTS







The early idea that JH was completely absent during the last immature molt before becoming an adult was not true. It wasn't until one could determine JH titers that it was shown that JH was present during that molt but NOT during the critical JH period.

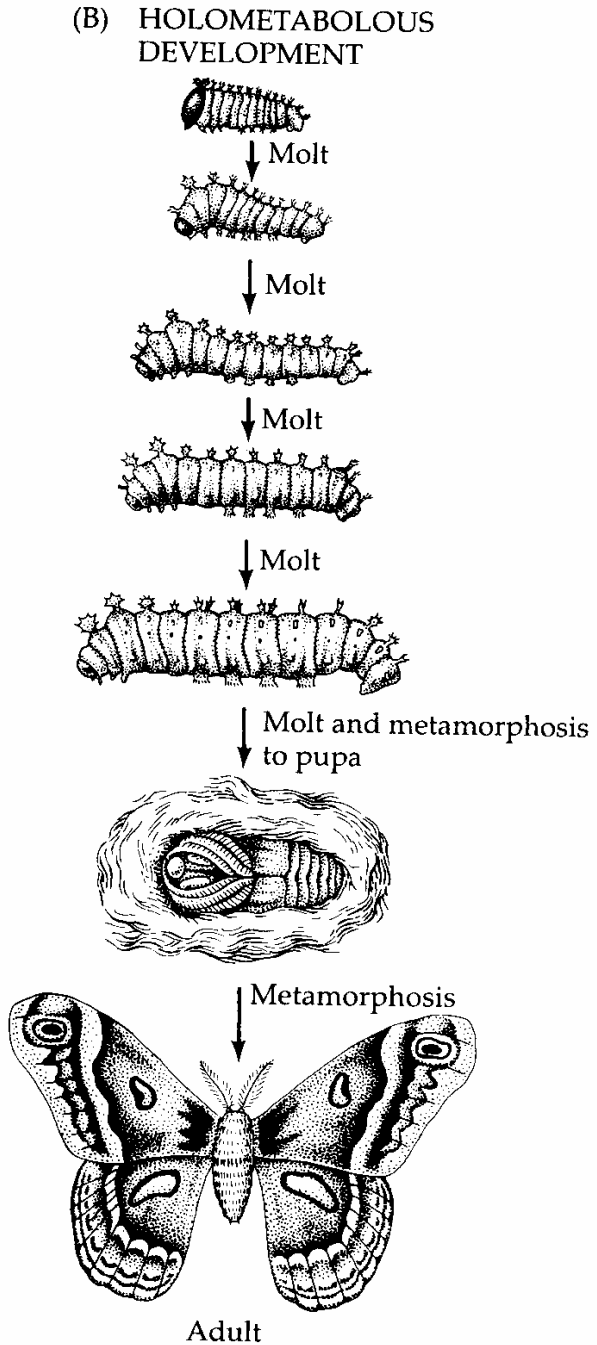
E=ecdysis

EH=eclosion hormone

B=Bursicon

Generalized scheme for changes in hormone titers regulating molting and metamorphosis in **hemimetabolous** insects

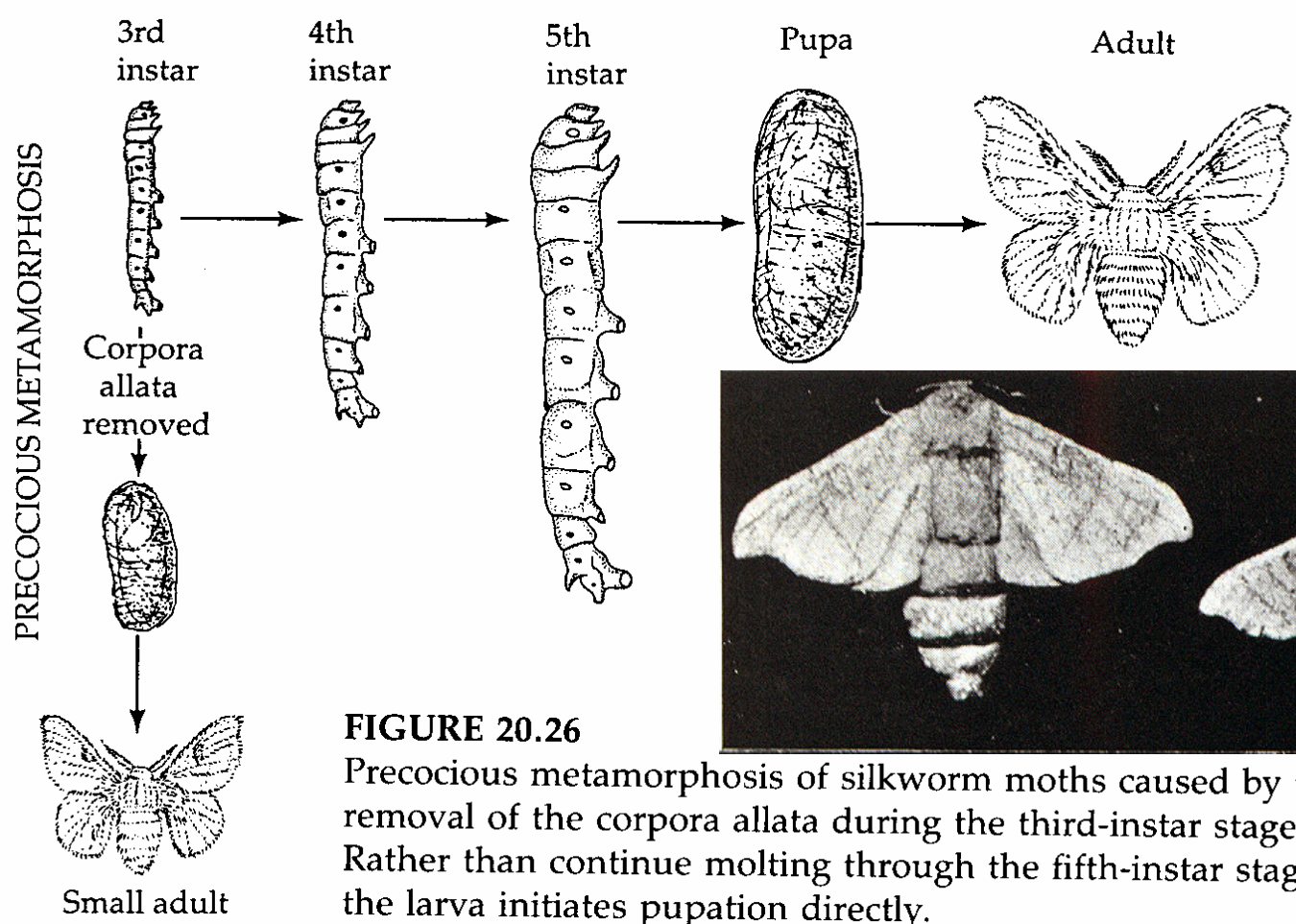
CONTROL OVER METAMORPHOSIS IN HOLOMETABOLOUS INSECTS



Metamorphosis in holometabolous insects involves some major differences when compared to hemimetabolous insects

What are some of these differences?

1. Pupal stage in holometabolous insects
2. Presence of imaginal discs



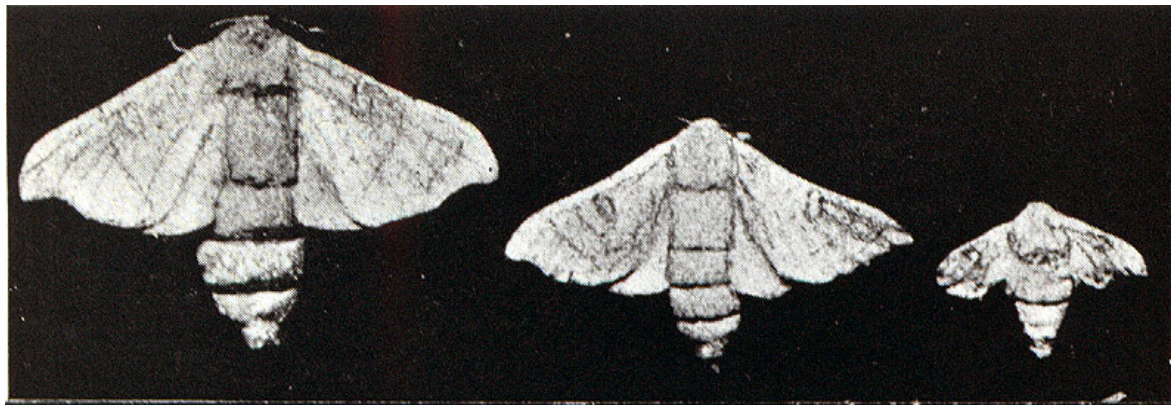
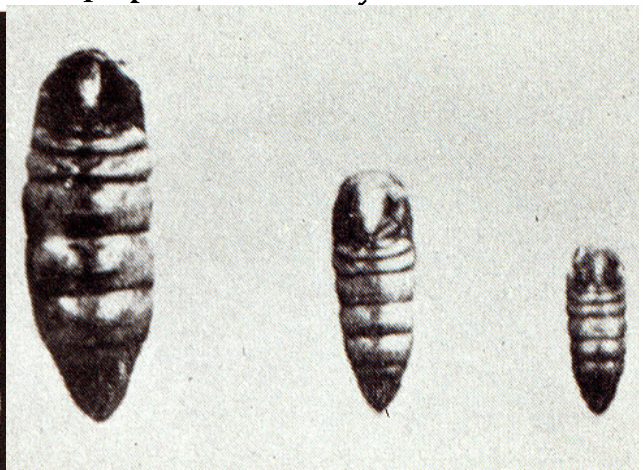


FIGURE 20.26
Precocious metamorphosis of silkworm moths caused by the removal of the corpora allata during the third-instar stage. Rather than continue molting through the fifth-instar stage, the larva initiates pupation directly.



WHAT DOES THIS TELL YOU ABOUT THE FATE OF THE IMAGINAL DISCS?

IMAGINAL DISCS

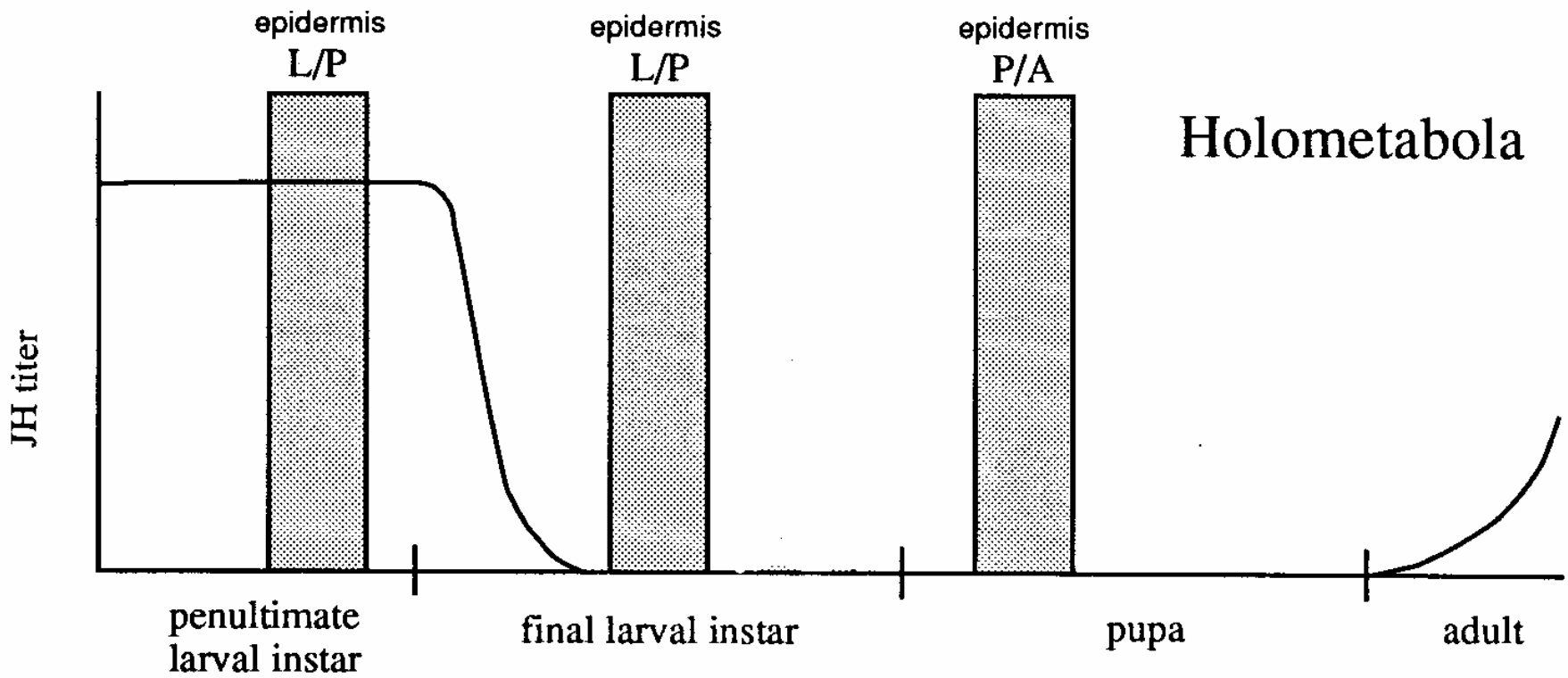
1. The previous slide demonstrated that the imaginal discs and the insect in general is programmed to proceed to adulthood without any problems if JH is removed.

2. In holometabolous insects, however, the situtation is different. Now the insect must go through 2 extra molts not seen in the hemimetabolous insects.
   a. Larva to pupa
   b. Pupa to adult

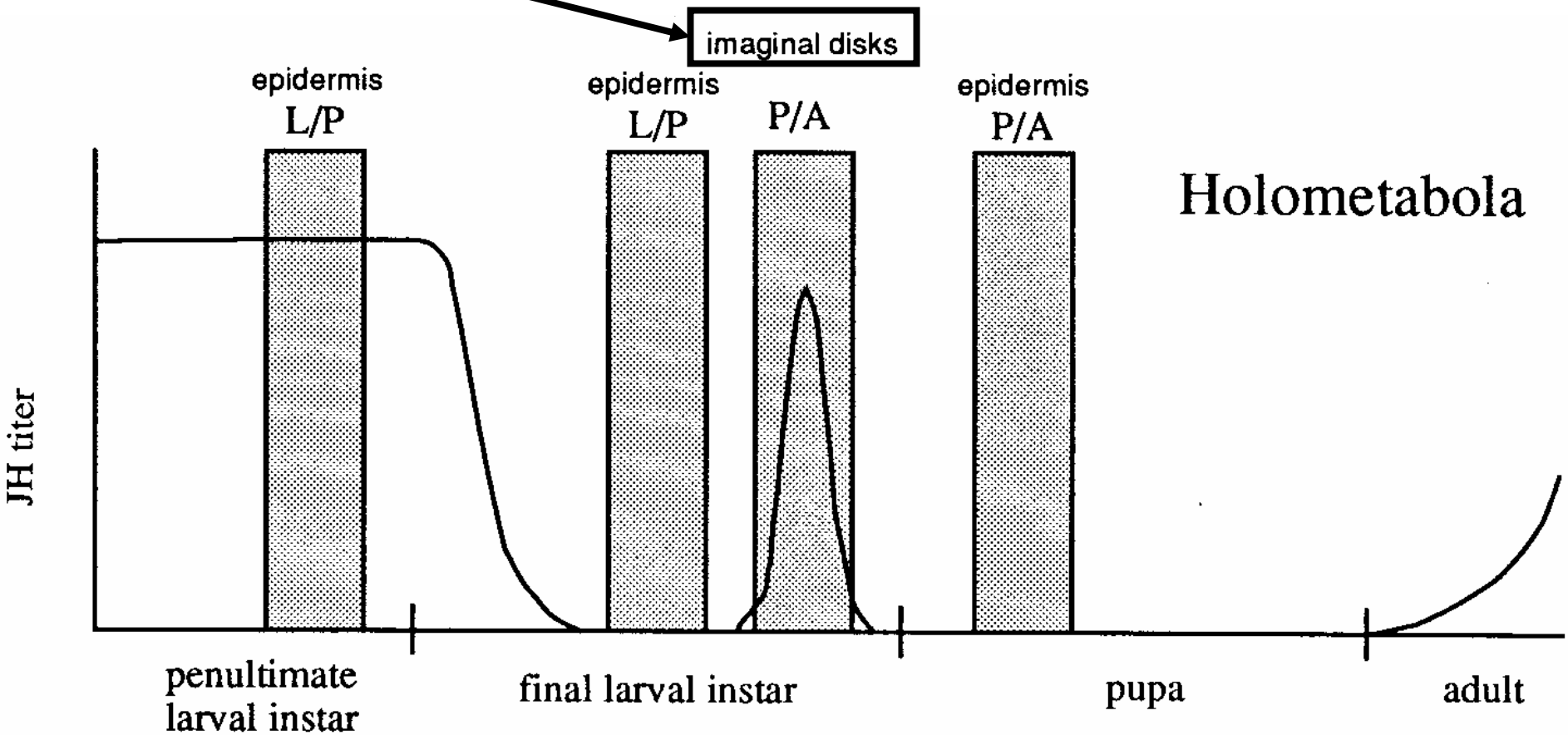
Below is a scheme for homometabolous insect development based on hormone titer for JH. The grey bars are the JH-sensitive or critical periods. If JH is seen during that period the insect at the molt remains in that stage. Thus, in the first penultimate larval instar JH is 'seen' during the JH-sensitive period thus the epidermal cells produce the final larval instar. Since JH is not see during that instar a larval/pupal molt occurs. Finally, the lack of JH during the pupal stage results in the pupal/adult molt.

Besides the epidermal tissue, what other important tissue is not presented in this scheme?

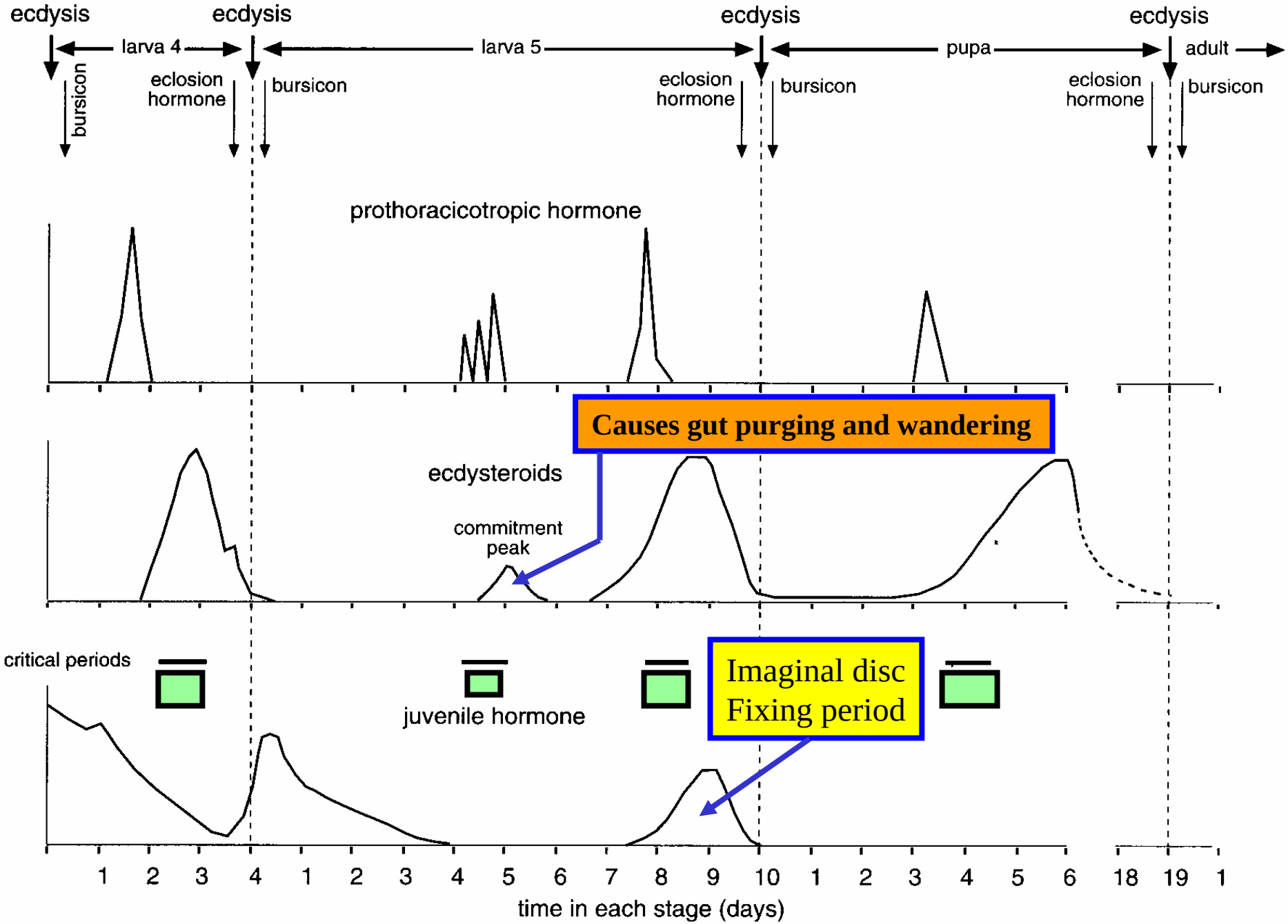
Why doesn't this tissue become adult in the L/P molt since in the absence of JH it should become adult in the L/P molt in the absence of JH?



It is important for the imaginal discs not to undergo premature development during the L/P molt. The JH peak at this time fixes the imaginal discs so that they do not begin development until the P/A molt.

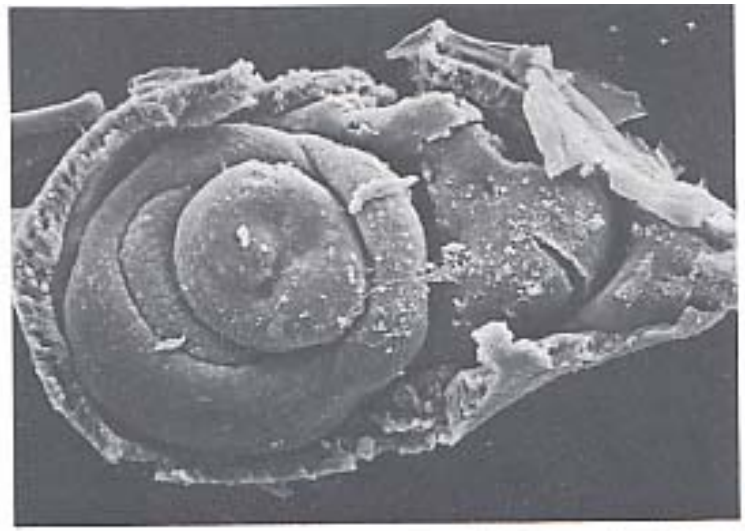


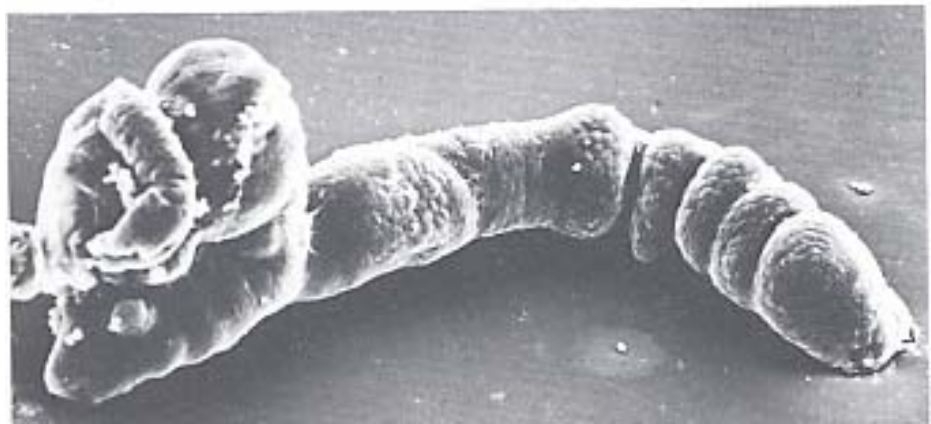
Hormone titers controlling molting and metamorphosis in *Manduca sexta*



LEG IMAGINAL DISC

SEM of unevaginated leg disc (top) with the peripodial membrane removed to show the disc itself and fully evaginated leg disc below





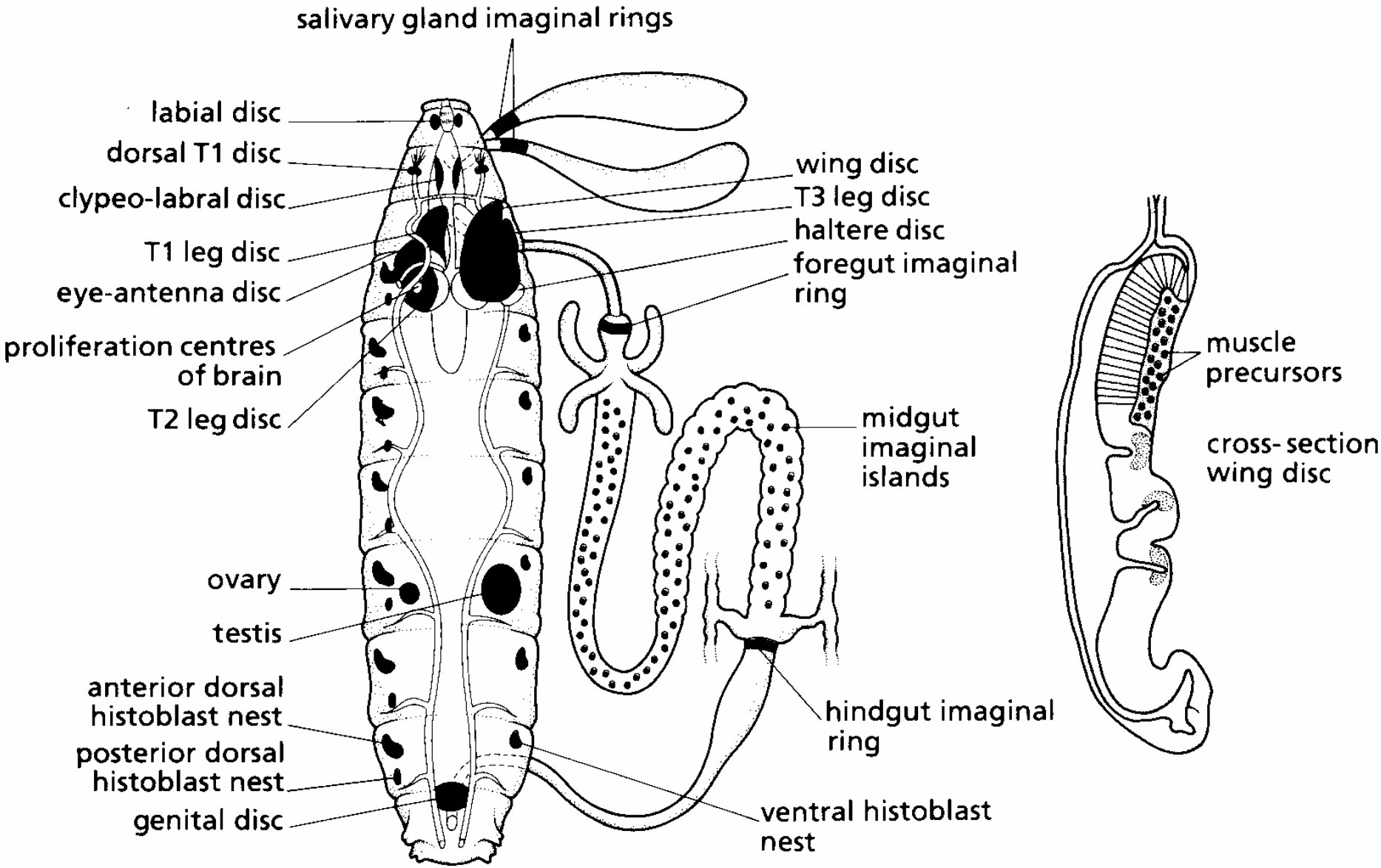
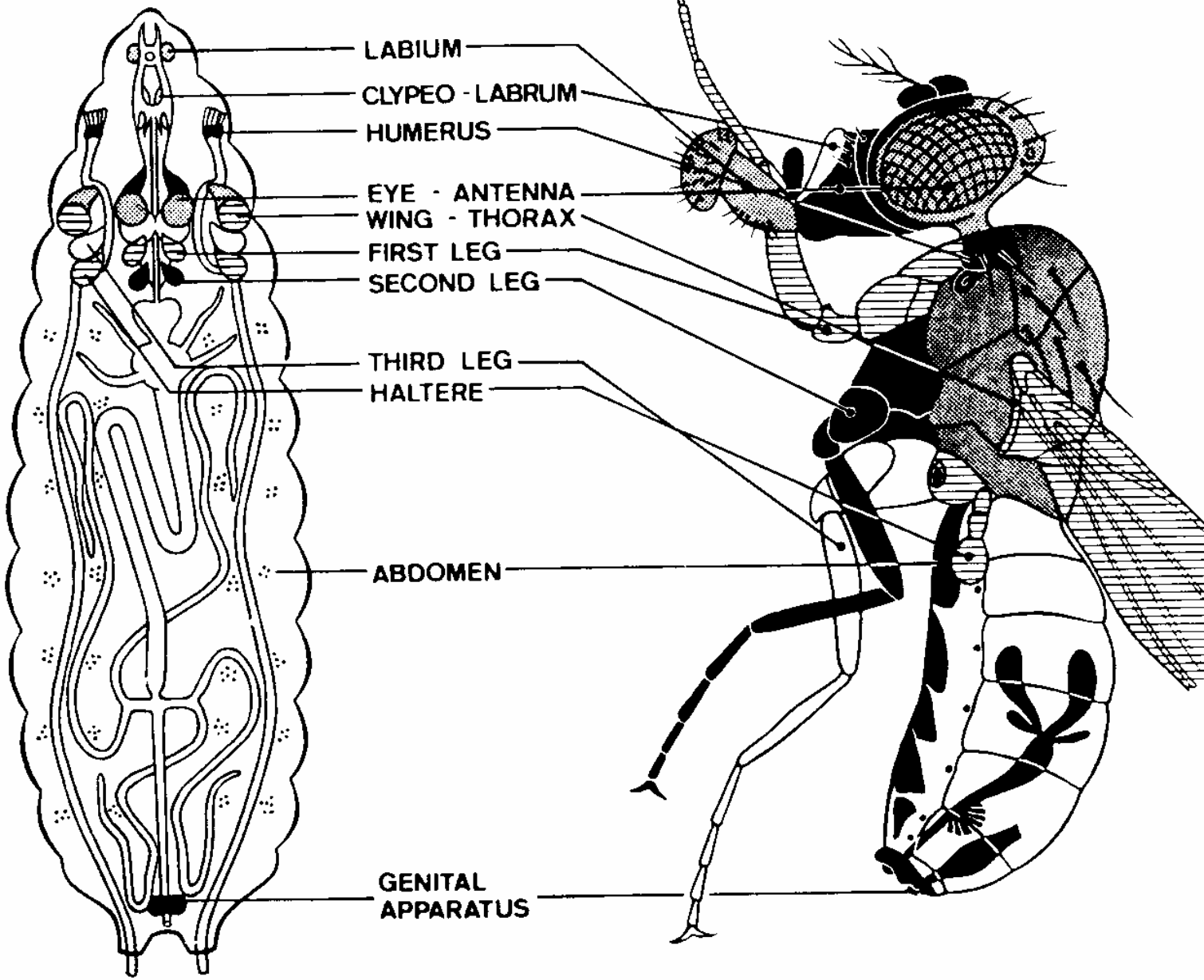
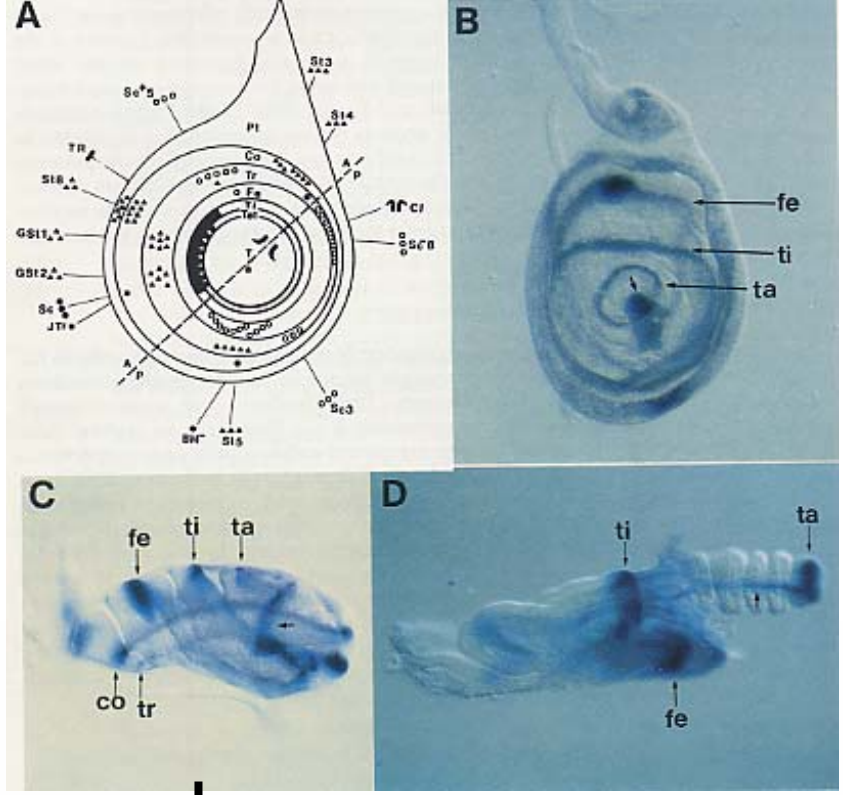


Figure 1.7 The origin of the adult cells **(black)**

In the Cyclorrhaphous Diptera the imaginal
Discs are determine very early in development



1. If you take a leg imaginal disc out of a larva and put it into the abdomen of another larva it will become a leg (as seen below in this fly)


A
B
fe
ti
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C
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co
tr
D
ti
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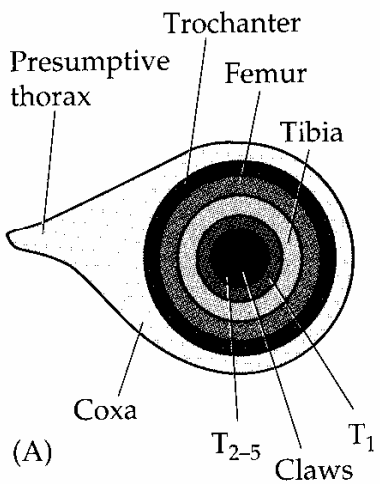
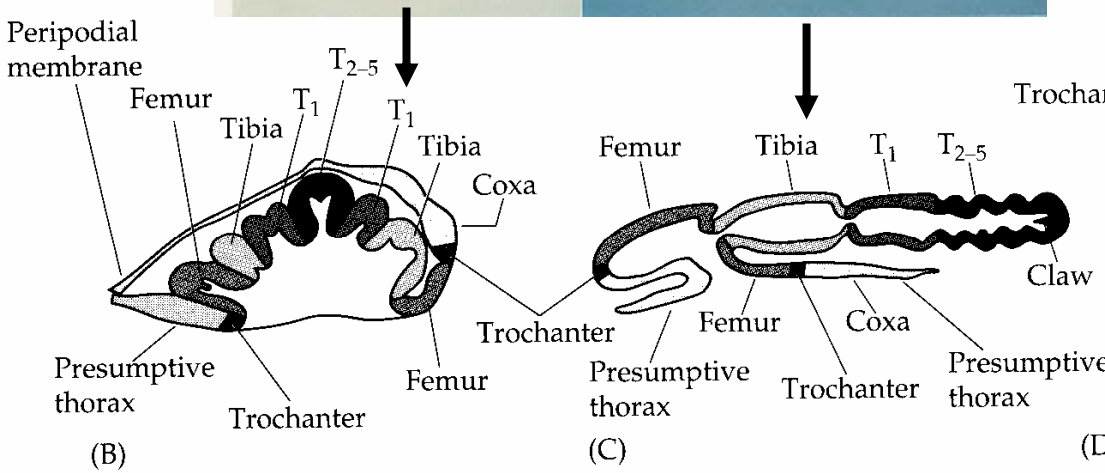
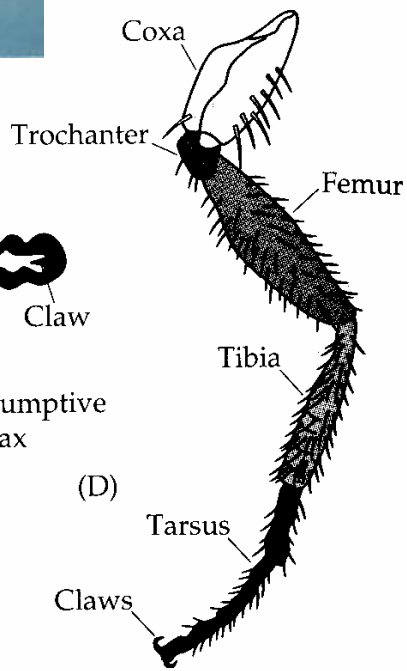
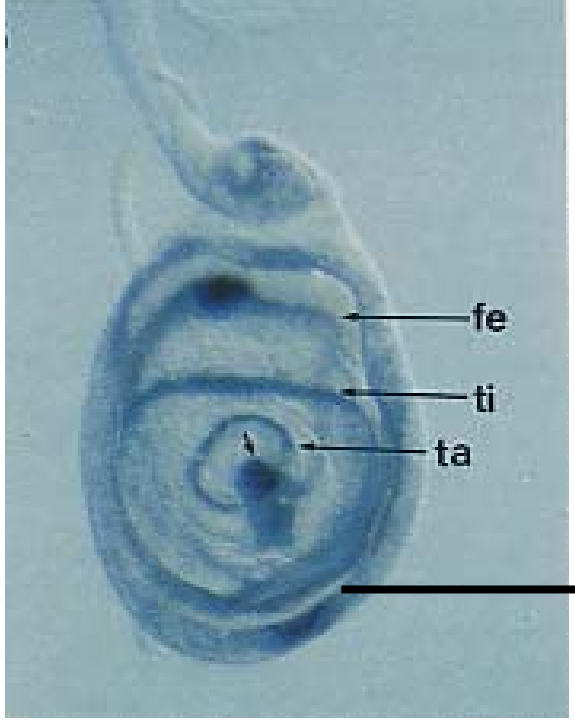
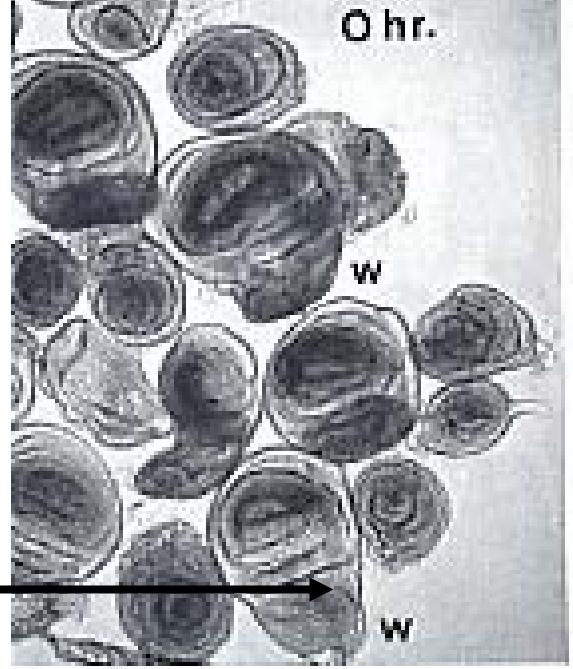
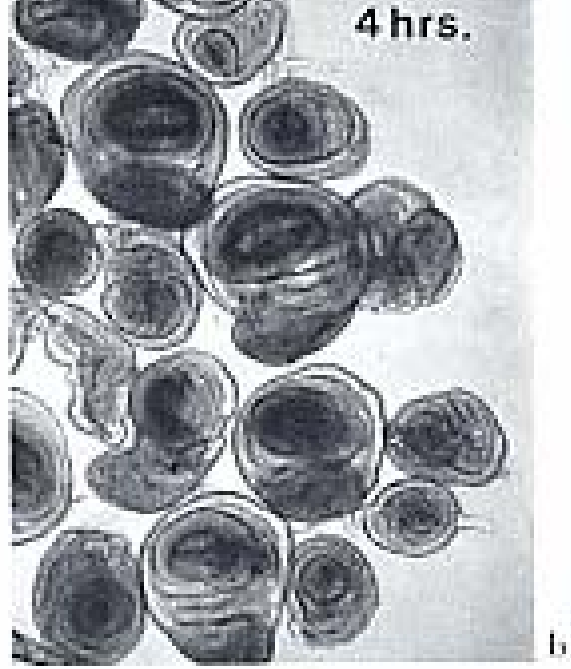

Trochanter
Presumptive thorax
Femur
Tibia
Coxa
T_{2-5}
T_1
Claws
(A)


Peripodial membrane
Femur
Tibia
T_1
T_{2-5}
T_1
Tibia
Coxa
Presumptive thorax
Trochanter
Femur
(B)
Trochanter
Femur
Tibia
T_1
T_{2-5}
Claw
Presumptive thorax
Femur
Coxa
Presumptive thorax
Trochanter
(C)

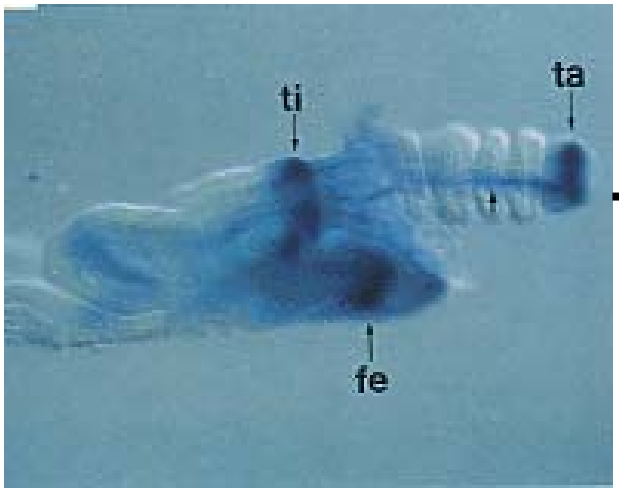

Coxa
Trochanter
Femur
Tibia
Tarsus
Claws
(D)

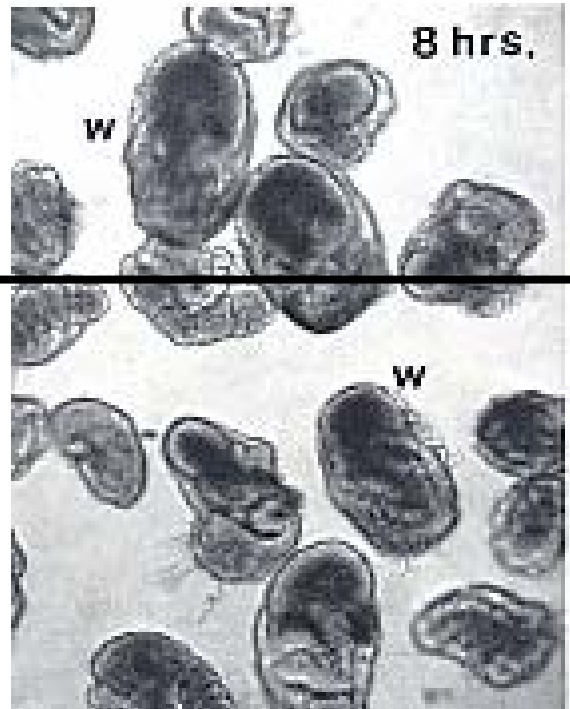


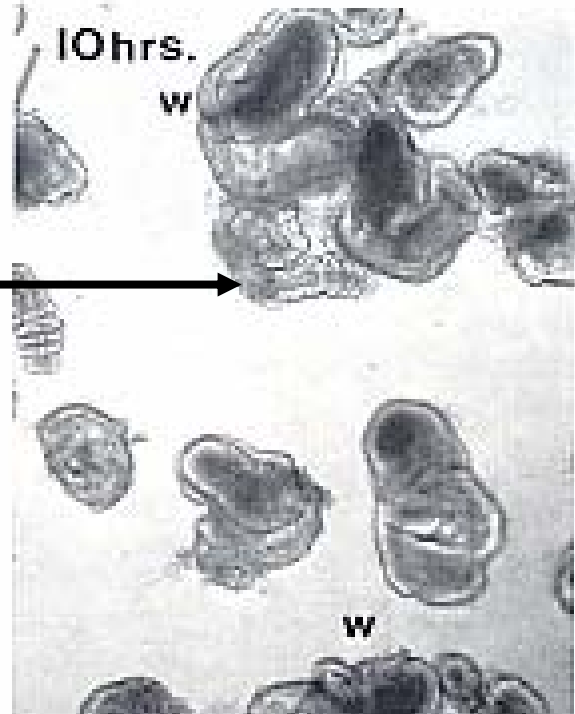




Leg imaginal discs cultured

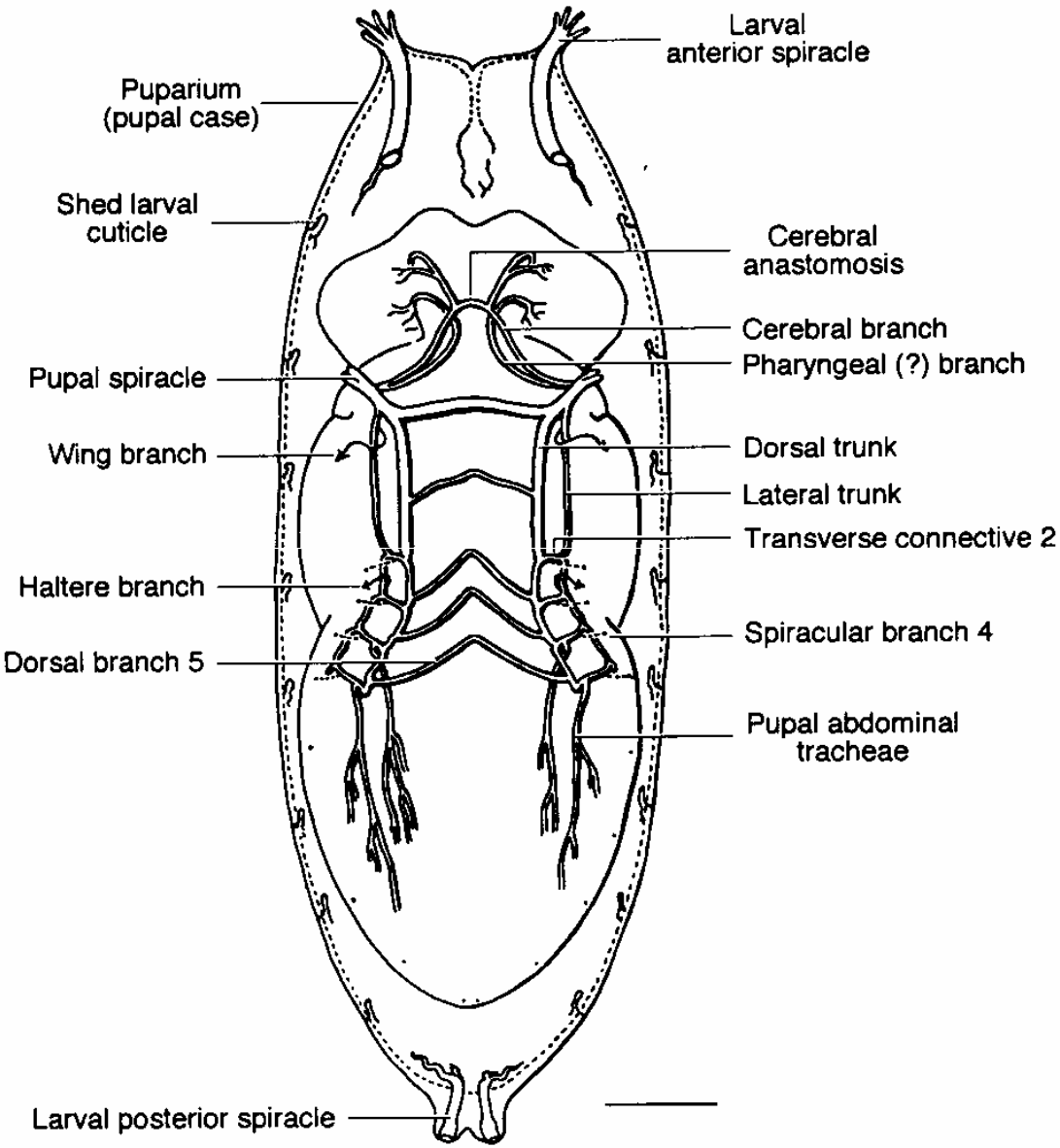






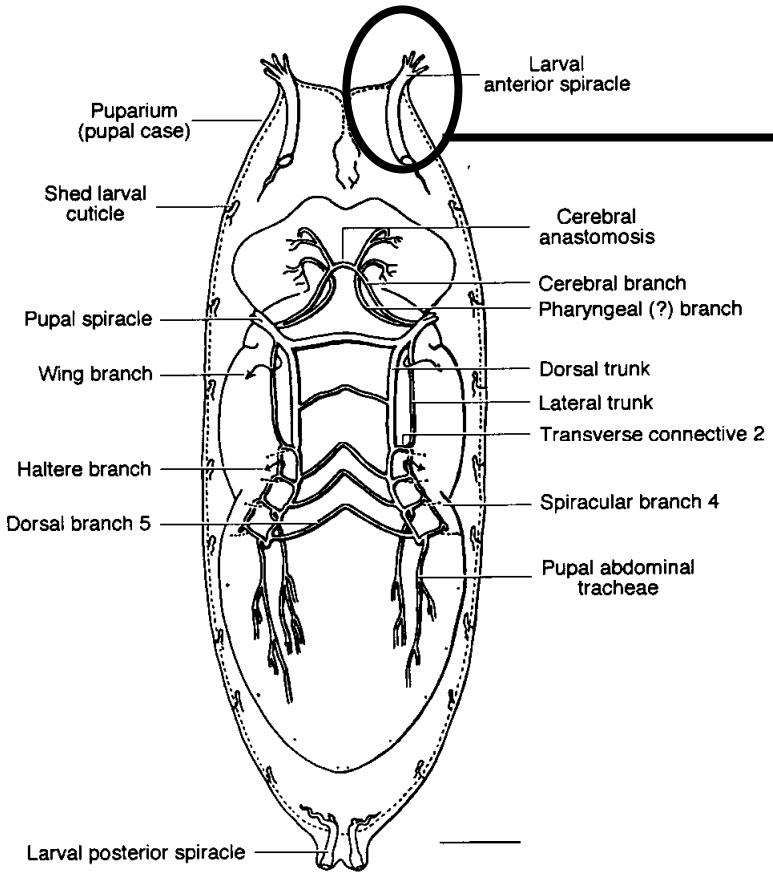
Ecdysteroids added to the culture media causes eversion

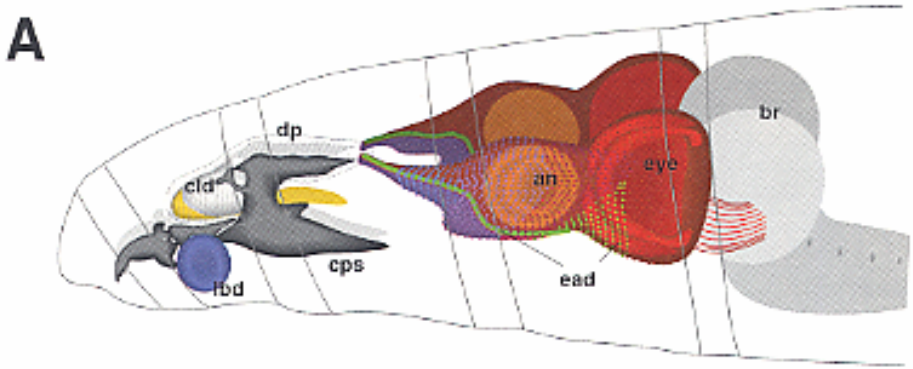
RESTRUCTURING DURING COMPLETE METAMORPHOSIS

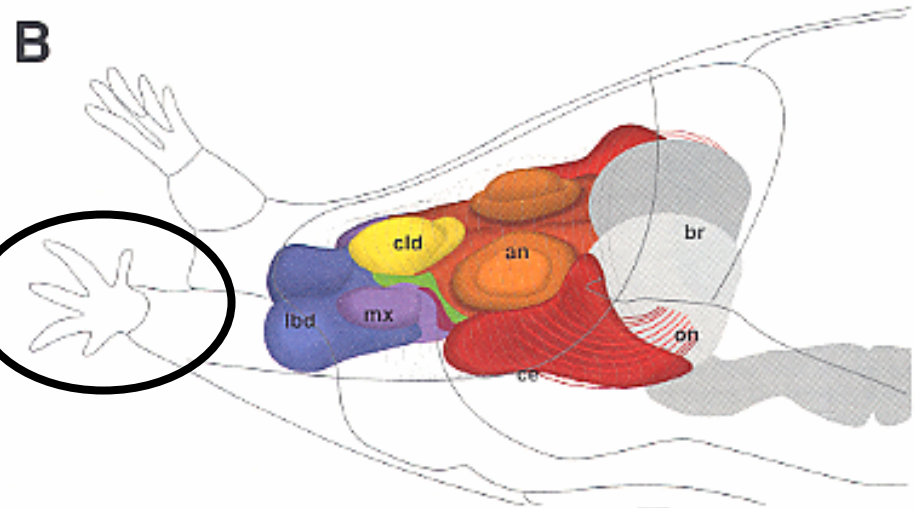


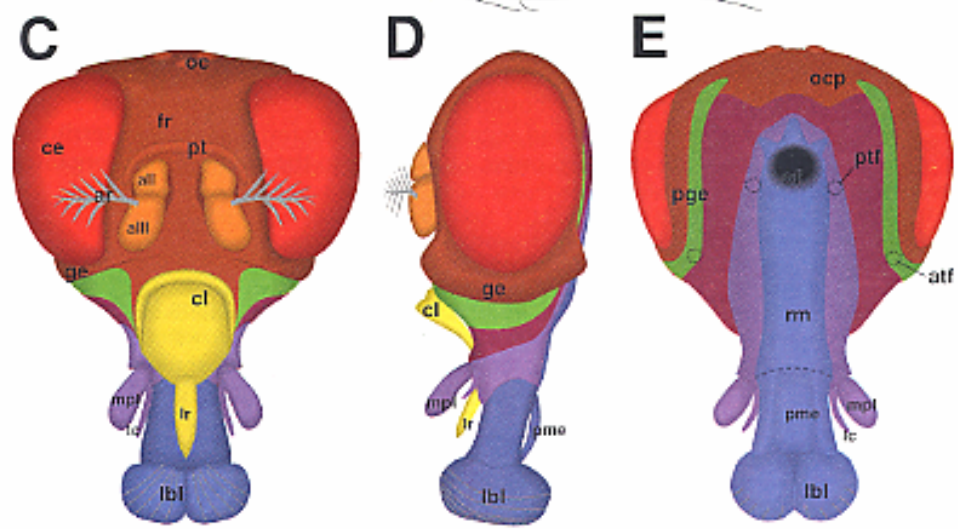
Note in this drawing that in A or the larva there are anterior spiracles. In B we see a late pre-pupa, about 10 hrs after pupation started. Note the extension forward of the anterior spiracles.

Below you can see how these larval structures in B remain with the last larva cuticle, which in the Diptera becomes the pupal case.

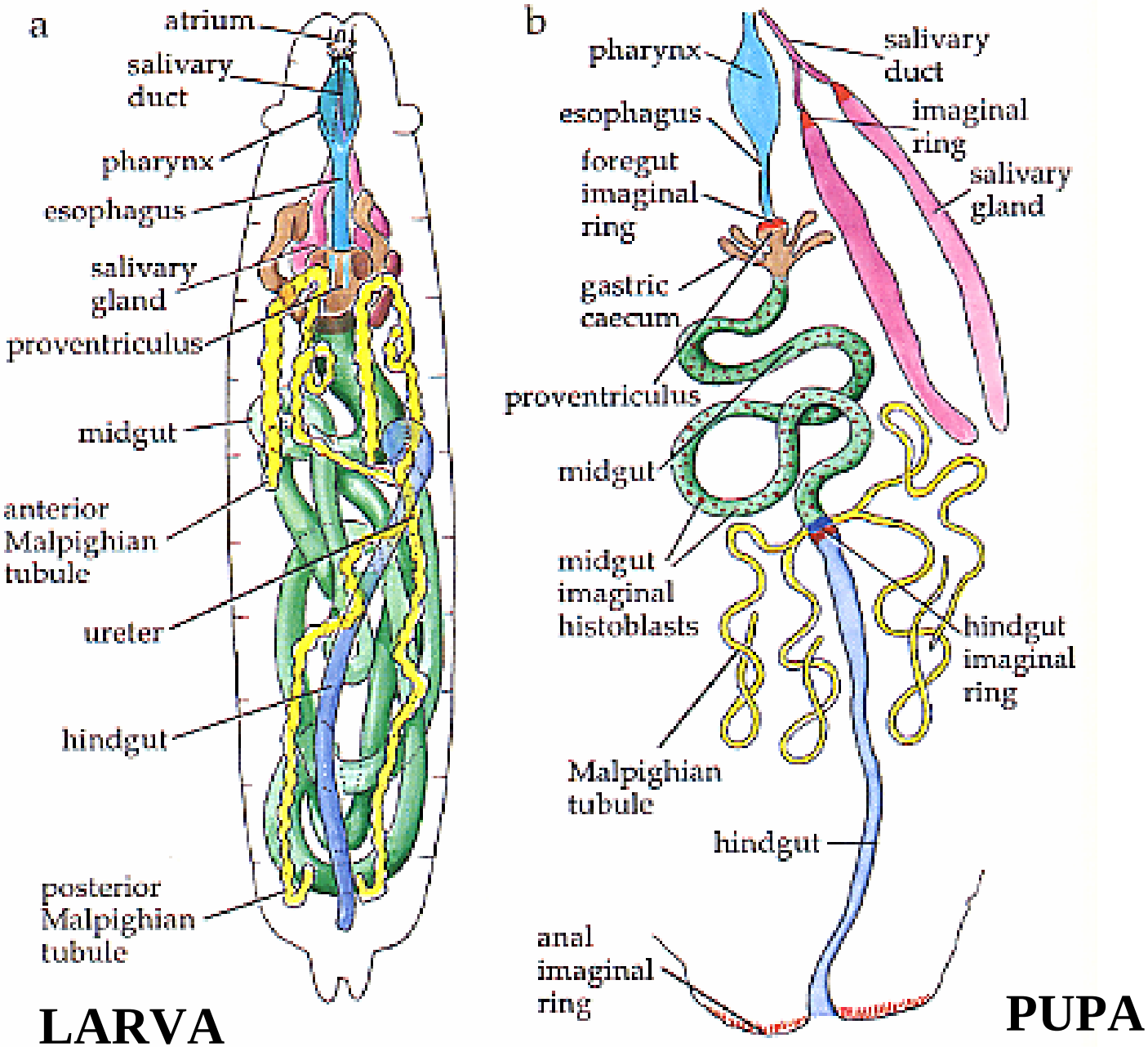




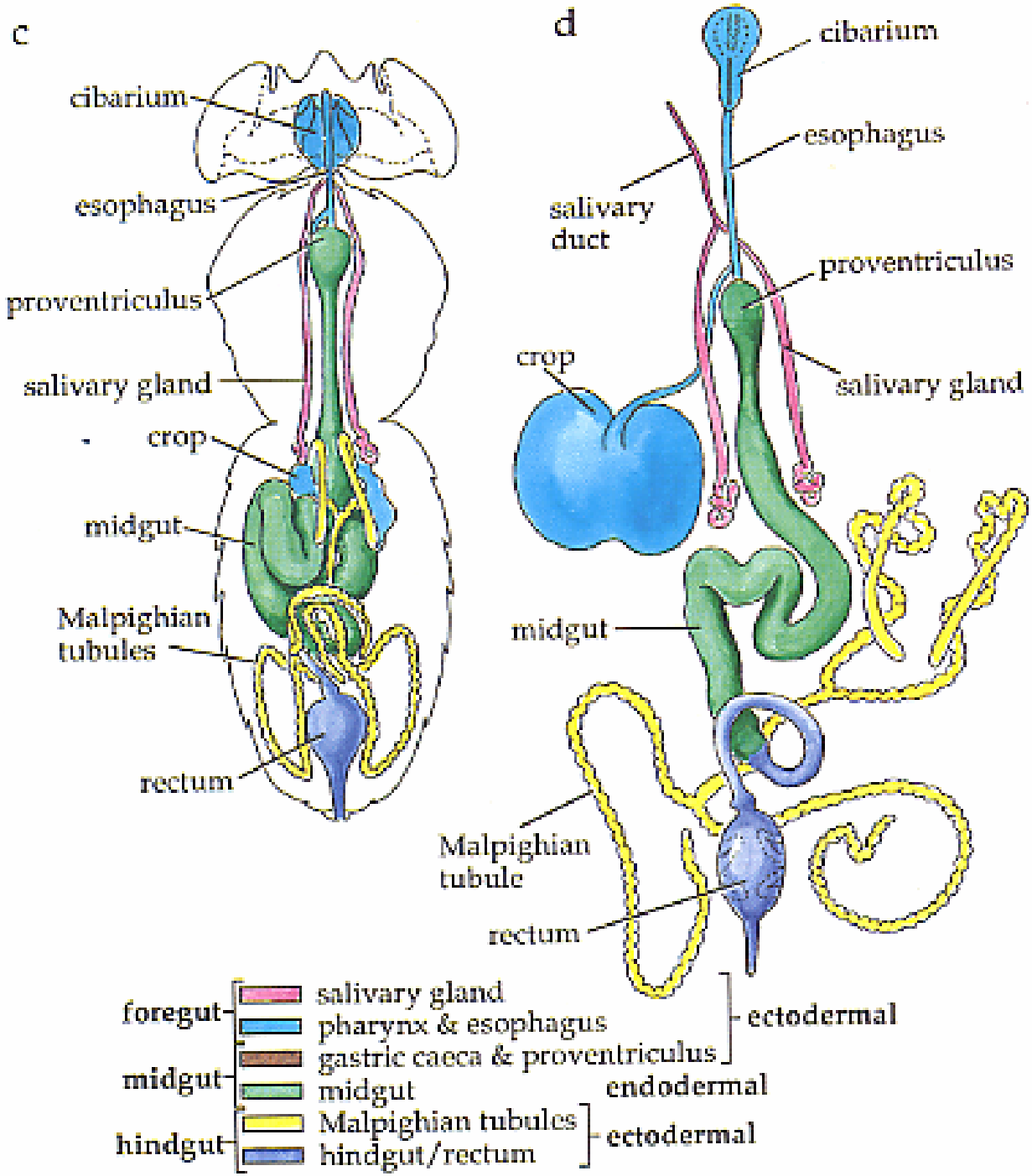


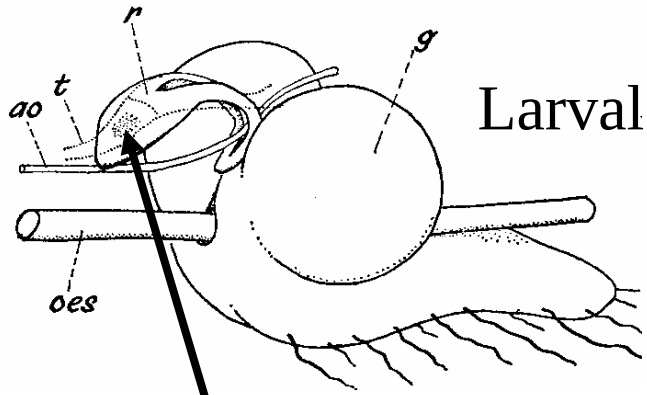


Restructuring of the digestive tract of *Drosophila* and other Diptera



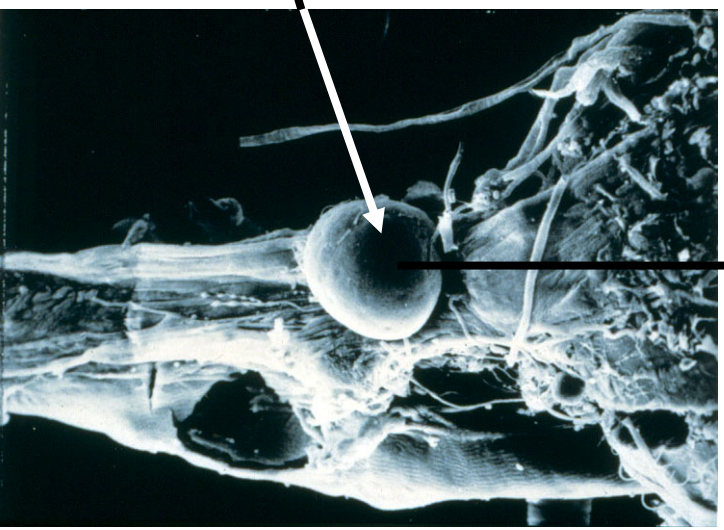
ADULT

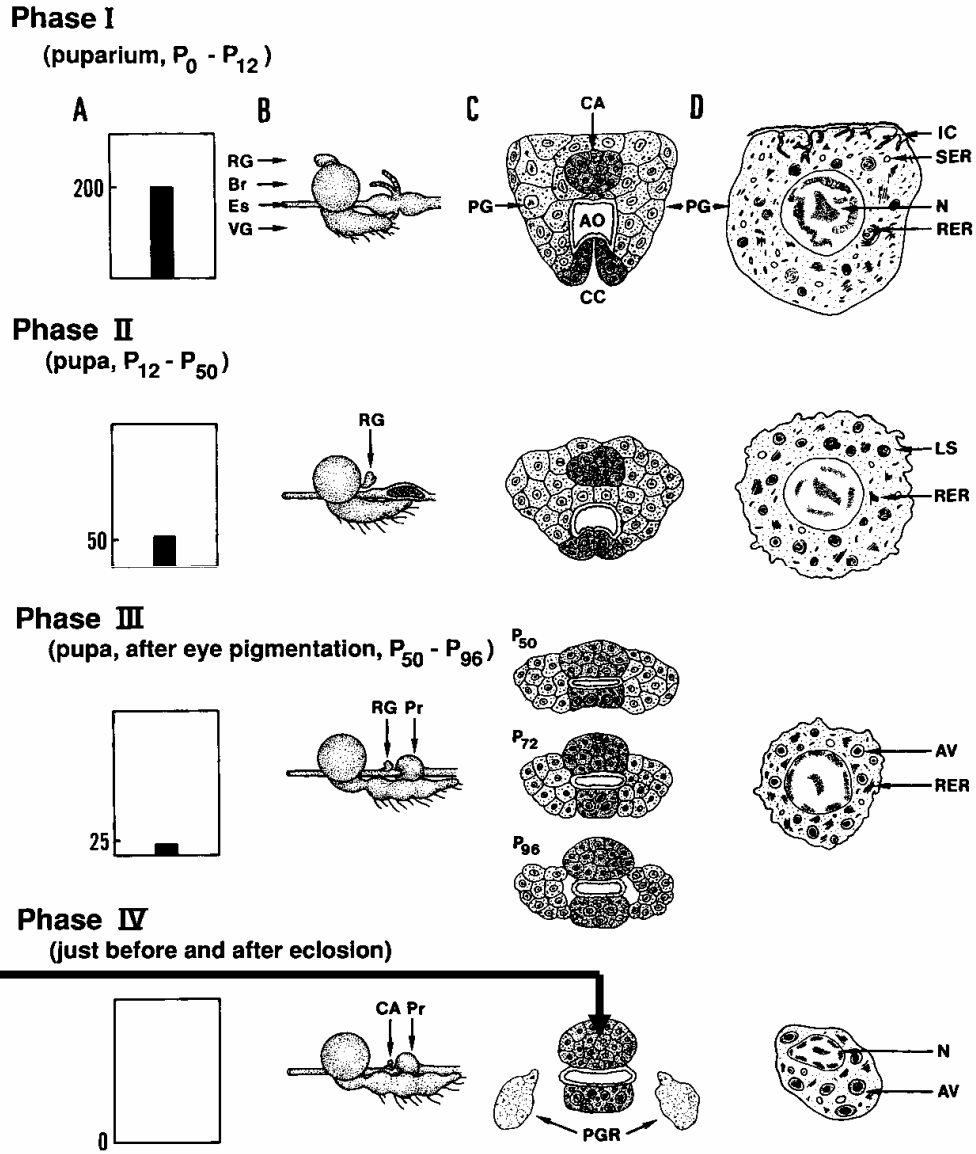

c
cibarium
esophagus
proventriculus
salivary gland
crop
midgut
Malpighian tubules
rectum
d
cibarium
esophagus
salivary duct
proventriculus
crop
salivary gland
midgut
Malpighian tubule
rectum
foregut
midgut
hindgut
salivary gland
pharynx & esophagus
gastric caeca & proventriculus
midgut
Malpighian tubules
hindgut/rectum
ectodermal
endodermal
ectodermal



Larval ring gland

Adult Corpus allatum





Restructuring of the ring gland from larva to adult in the Diptera

THE ADULT INSECT

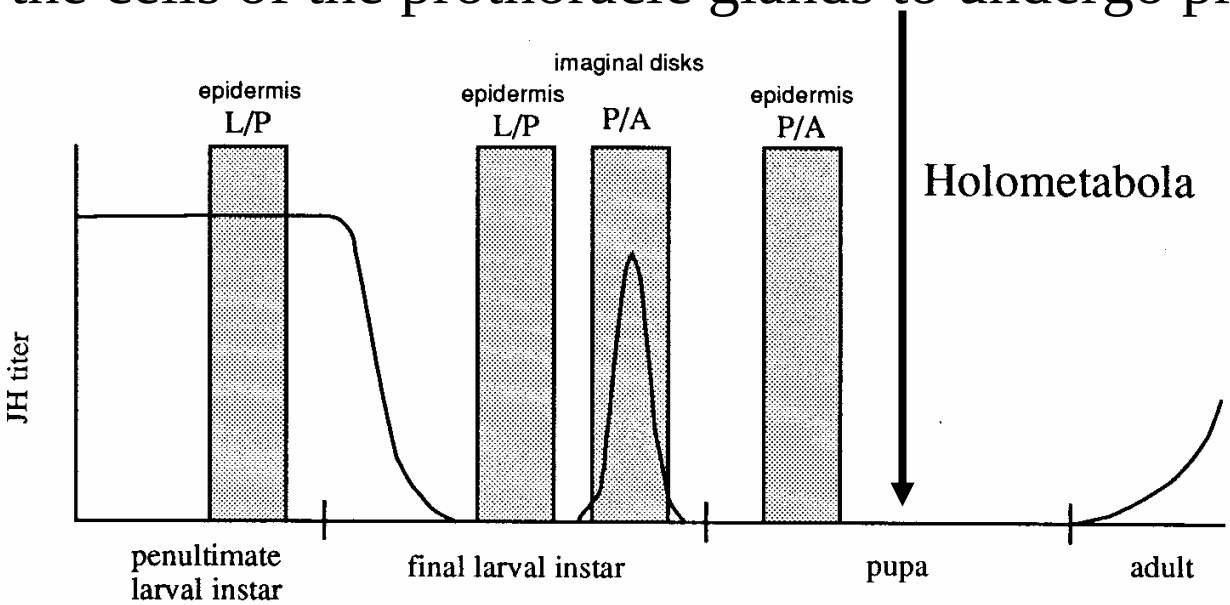
WHY DON'T ADULT INSECTS CONTINUE TO MOLT?

The prothoracic or ecdysial glands degenerate during the

1. 1st few days of adult development in hemimetabolous insects
2. Pupal stage in holometabolous insects

Also, the epidermal cells probably lack the ecdysteroid receptors

It is believed that the lack of JH during the pupal period is the signal to the cells of the prothoracic glands to undergo preprogrammed cell death.



ECDYSTEROIDS IN ADULT INSECTS

Fallon et al. (1974) and Hagedorn et al. (1975) demonstrated that in adult mosquitoes ecdysteroids are present in high concentration even without the prothoracic glands present.

Later it was shown that the ovaries were the source of the ecdysone in adult females and the testes in adult males.

d. Aging-the degeneration of specific cell types, glands, tissues, muscles, etc. and/or their histoloysis due to programmed cell death or **apoptosis**

Theories on aging:

1. **Genetic basis-Maximum life span set for each species**
2. **Nutrition and exercise**
3. **Teleomere wearing**
4. **Wear and tear-effects of alcohol and smoking**
5. **Hormonal decline-diabetes in humans, menopause in woman and thyroid gland**

Stoffolano, J.G., Jr. 1976. Insects as model systems for aging studies. In: Experimental aging researach (ed. by Elias, M. et. al.): Progress in biology. EAR, Inc., Bar Harbor, Maine.

Raff, M. C. 1994. Cell death genes: *Drosophila* enters the field. Science 264: 668-669.

Steroid regulated programmed cell death during *Drosophila* metamorphosis

Changan Jiang*, Eric H. Baehrecke*,† and Carl S. Thummel‡

Development 124, 4673-4683 (1997)

During insect metamorphosis, pulses of the steroid hormone 20-hydroxyecdysone (ecdysone) direct the destruction of obsolete larval tissues and their replacement by tissues and structures that form the adult fly. We show here that larval midgut and salivary gland histolysis are stage-specific steroid-triggered programmed cell death responses. Dying larval midgut and salivary gland cell nuclei become permeable to the vital dye acridine orange and their DNA undergoes fragmentation, indicative of apoptosis. Furthermore, the histolysis of these tissues can be inhibited by ectopic expression of the baculovirus anti-apoptotic protein p35, implicating a role for caspases in the death response. Coordinate stage-specific induction of the *Drosophila* death genes *reaper* (*rpr*) and *head involution*

The Endocrine Regulation of Aging by Insulin-like Signals

Marc Tatar,[1] Andrzej Bartke,[2] Adam Antebi[3]

Reduced signaling of insulin-like peptides increases the life-span of nematodes, flies, and rodents. In the nematode and the fly, secondary hormones downstream of insulin-like signaling appear to regulate aging. In mammals, the order in which the hormones act is unresolved because insulin, insulin-like growth factor–1, growth hormone, and thyroid hormones are interdependent. In all species examined to date, endocrine manipulations can slow aging without concurrent costs in reproduction, but with inevitable increases in stress resistance. Despite the similarities among mammals and invertebrates in insulin-like peptides and their signal cascade, more research is needed to determine whether these signals control aging in the same way in all the species by the same mechanism.

Experimental Gerontology 39 (2004) 1745–1750

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The neuroendocrine regulation of Drosophila aging☆

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Aging Cell (2002) 1, pp158–160

SHORT TAKE

Impaired ovarian ecdysone synthesis of *Drosophila melanogaster* insulin receptor mutants

Meng-Ping Tu,[1,2] Chih-Ming Yin[2] and Marc Tatar[1]

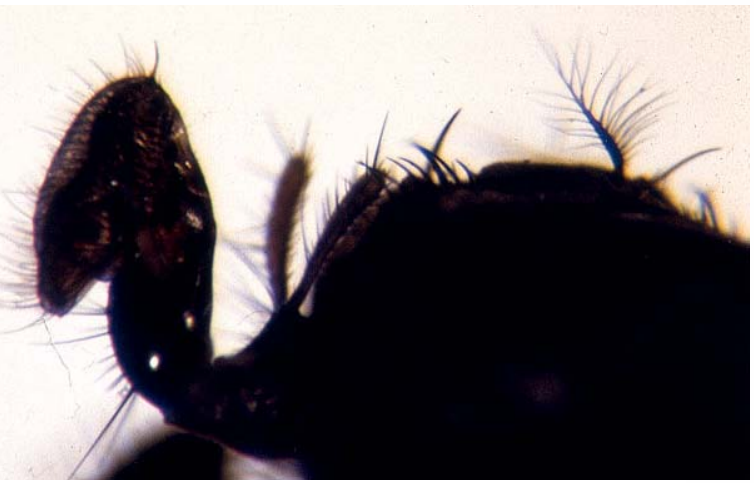
[1]Brown University, Providence, Rhode Island, USA

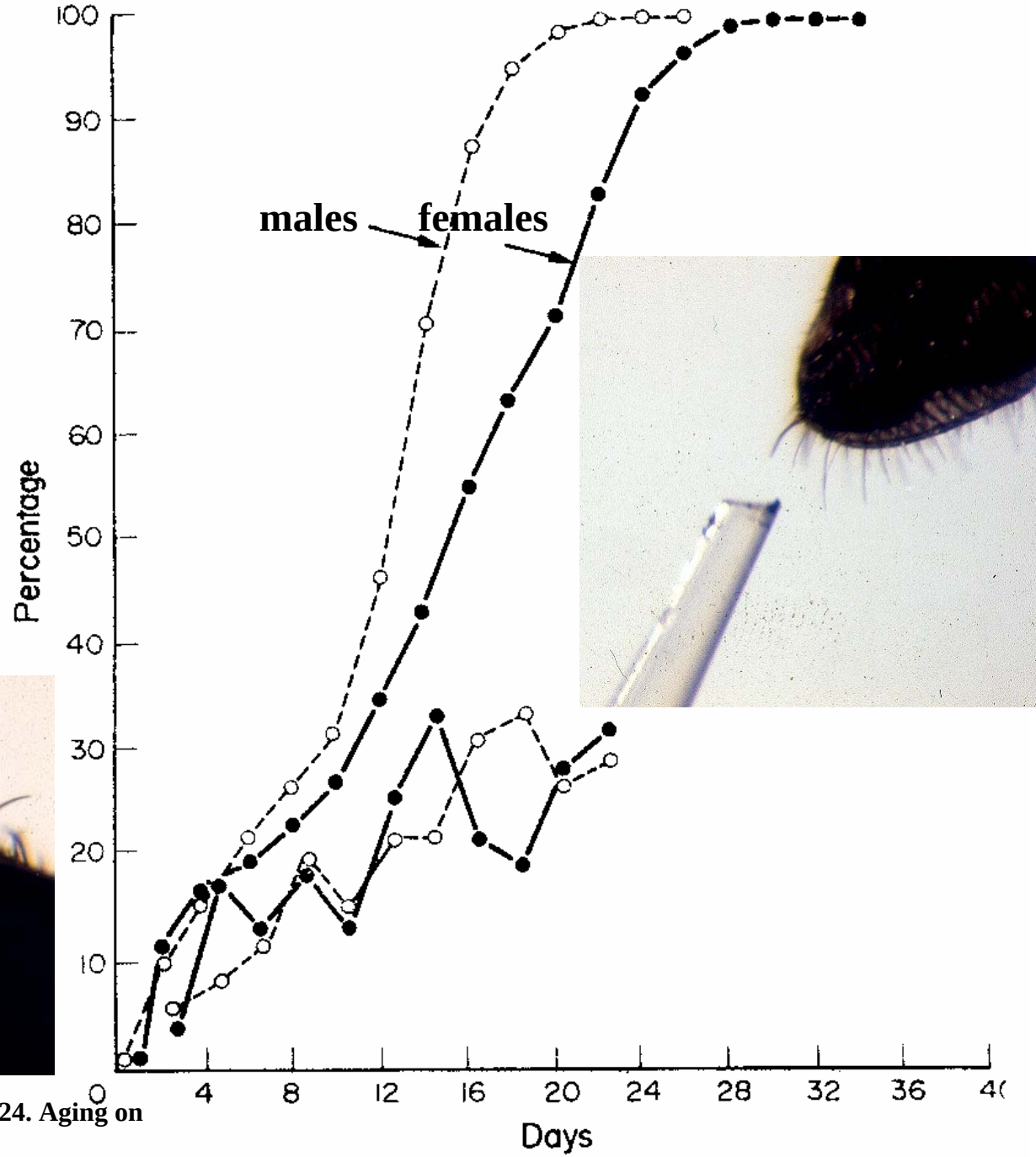
[2]Department of Entomology, University of Massachusetts, Amherst, Massachusetts, USA

Deficient juvenile hormone synthesis is thought to extend adult longevity in insects, including *Drosophila melanogaster*, the Monarch butterfly and several species of Mediterranean grasshopper (Pener, 1972; Herman & Tatar, 2001; Tatar & Yin, 2001).

Although the potential impact of ecdysone upon aging has yet to be reported, from basic studies of insulin function we predict that ovaries of long-lived *D. melanogaster* mutant for *InR* will produce little ecdysone. In *D. melanogaster*, insulin

Arrows point to the mortality curves for both sexes of *Phormia regina*. Lower lines indicate the change in the % of labellar salt chemoreceptor sensilla that remained operative. Notice that it is about 35% degeneration in old flies and in humans it is about 40%.





Stoffolano et al. 1978. Exp. Gerontology 13: 115-124. Aging on peripheral taste receptors in blowflies.

POST-EMBRYONIC DEVELOPMENT

a. Growth
b. Molting
c. Metamorphosis
d. Aging

BASIC & APPLIED RESEARCH ON DEVELOPMENT

BASIC AND MODEL SYSTEM:

1. Role of hormones on cell differentiation and determination
2. Interaction of hormones on cell fate
3. Effects of gene hierarchy and control of cell and tissue fate

APPLIED OR I.P.M. SPIN-OFFS

1. Use of hormone analogues and/or mimics, such as methoprene