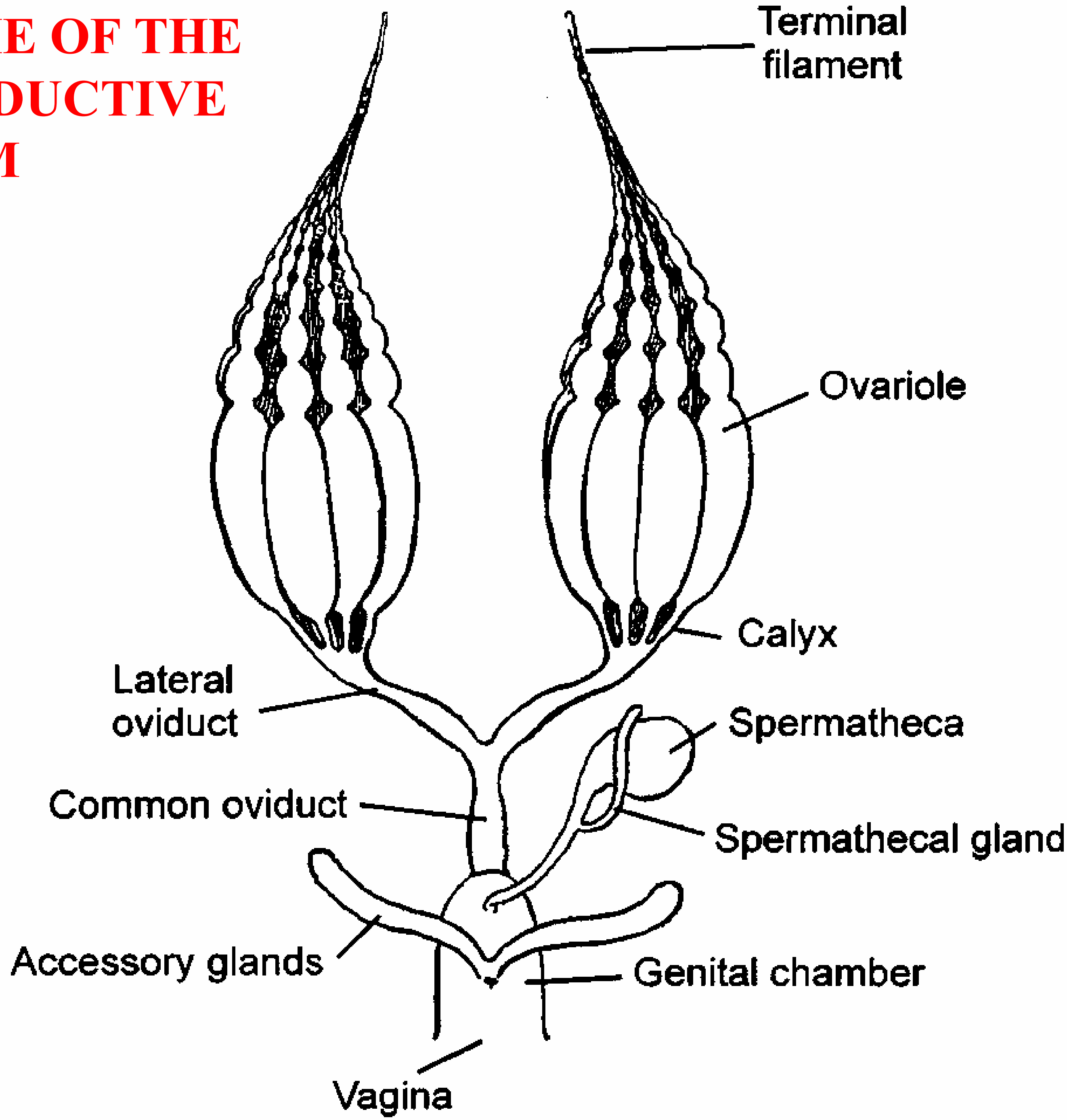
RERODUCTIVE SYSTEM

FUNCTIONS OF THE REPRODUCTIVE SYSTEM

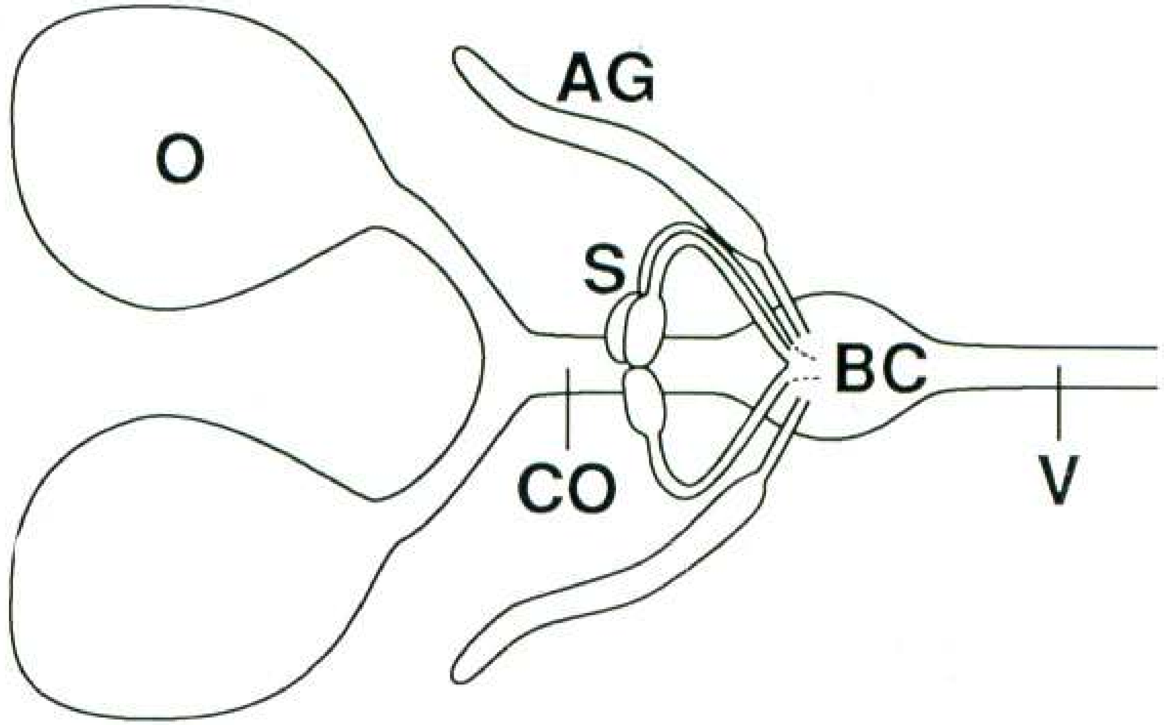
1. Continuation of the species
2. Production of gametes
3. Production of eggs
4. Fertilization of the eggs
5. Laying the eggs
6. Development of the embryo

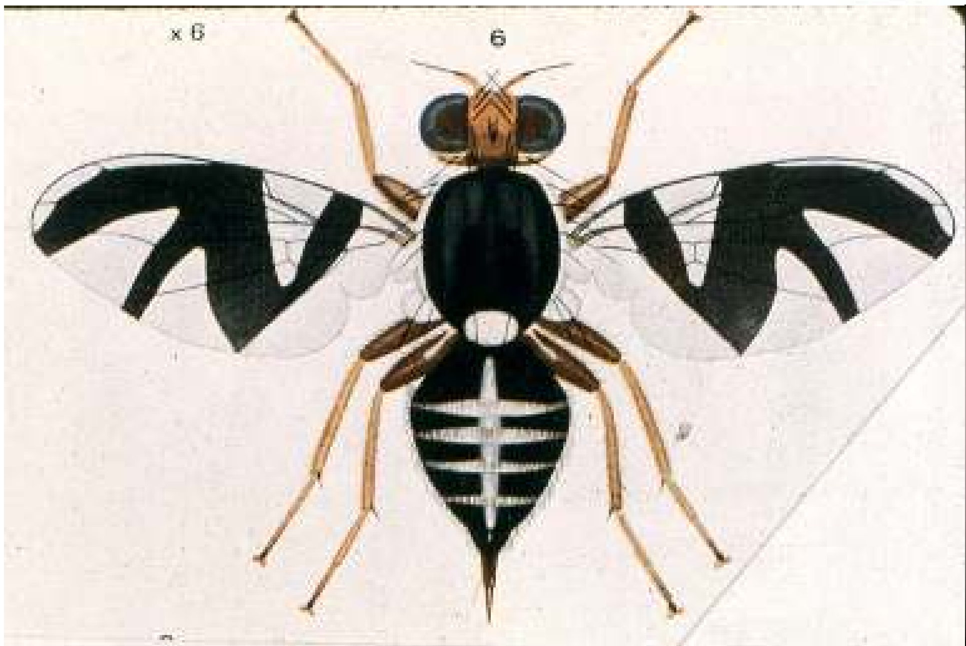
GENERIC SCHEME OF THE FEMALE REPRODUCTIVE SYSTEM

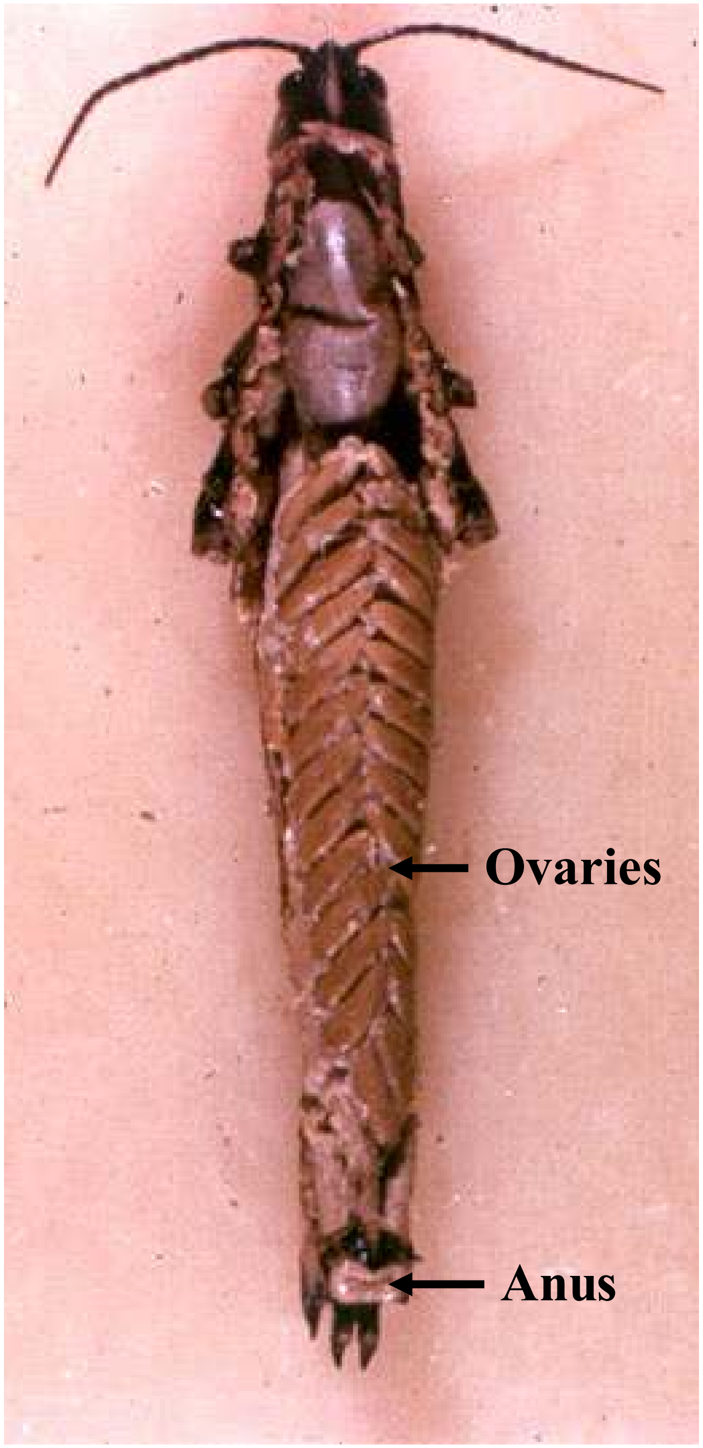


Reproductive system of the female apple maggot, *Rhagoletis pomonella*

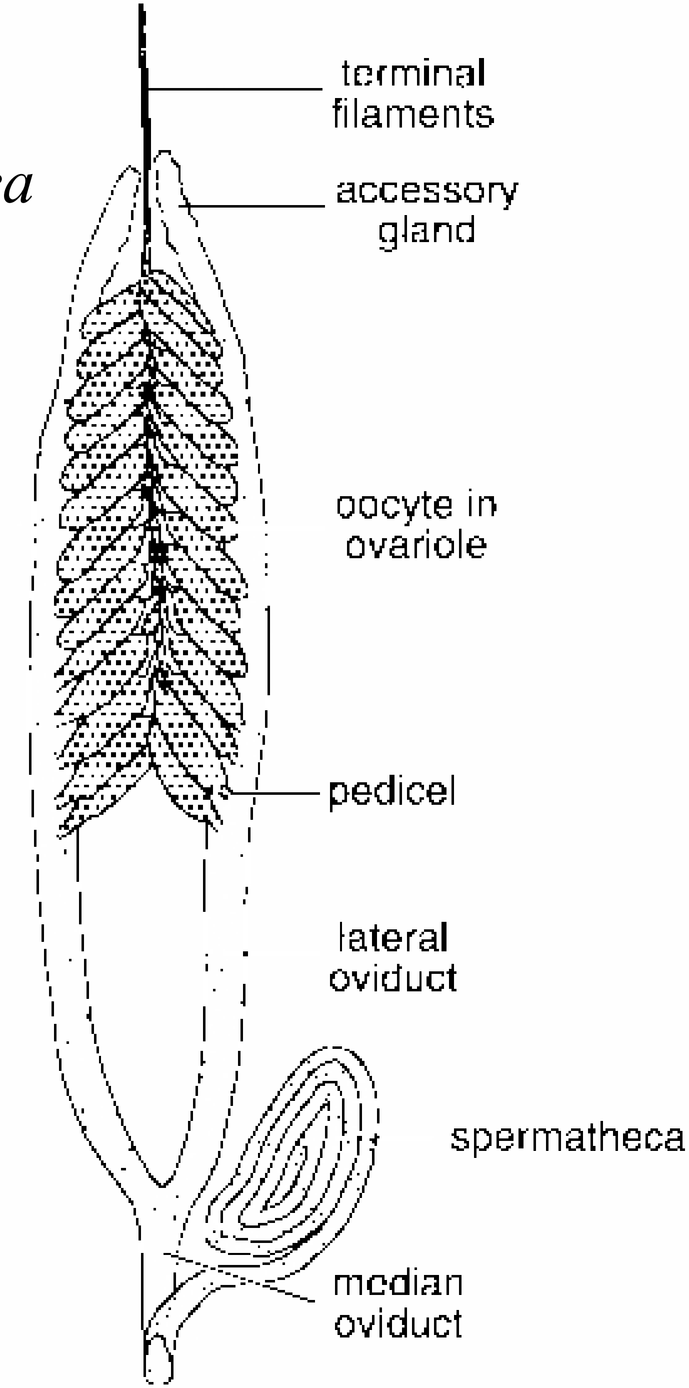
O=OVARY
AG=ACCESSORY GLANDS
S=SPERMATHECAE
CO=COMMON OVIDUCT
BC=BURSA COPULATRIX
V=VAGINA



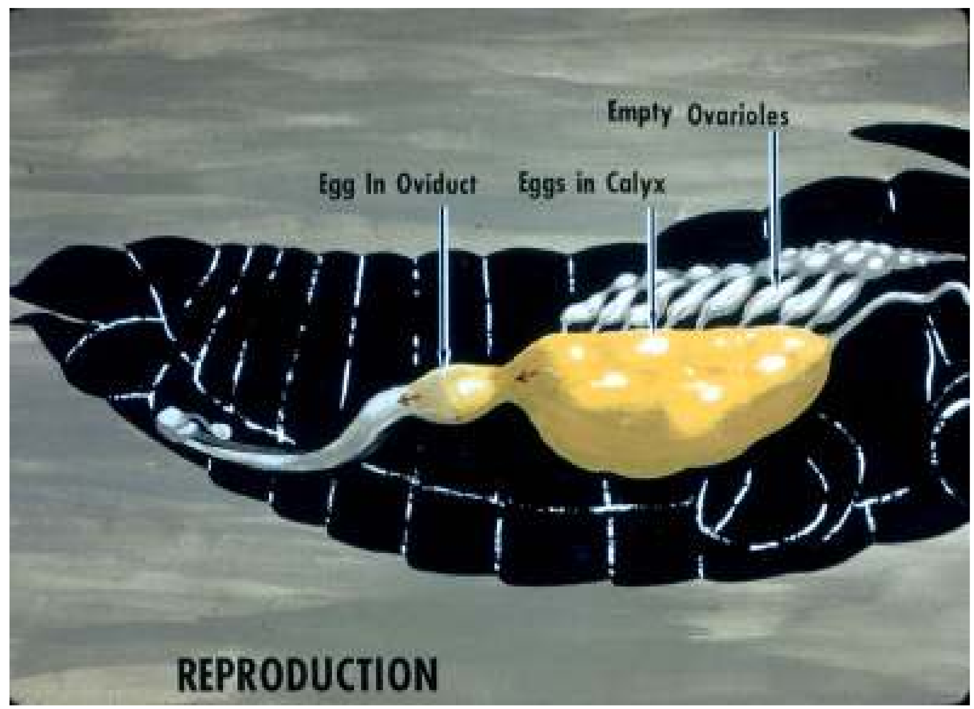




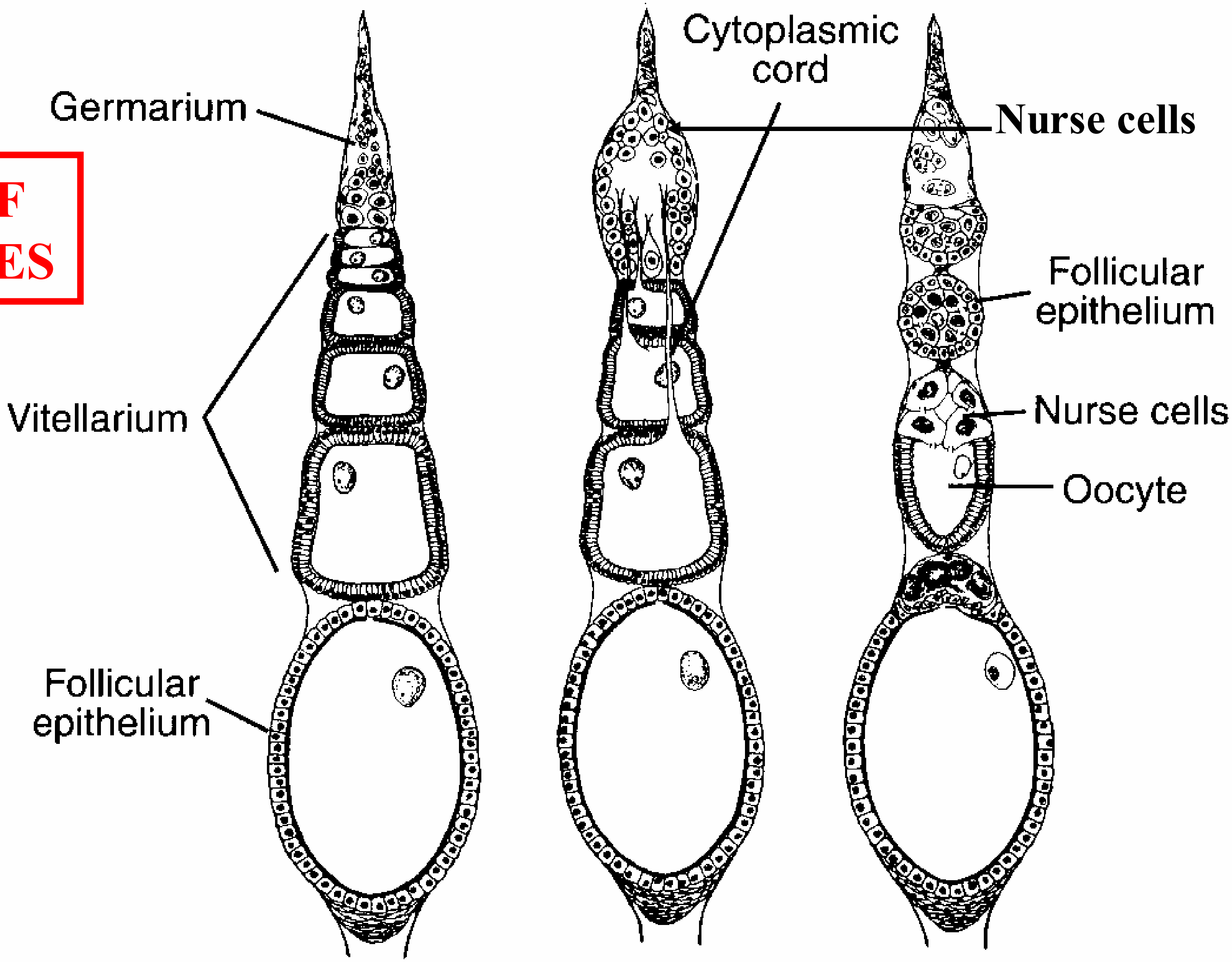
Reproductive tract of *Romalea* on the left and *Schistocerca* on the right. Notice the large volume in the female taken up by the mature eggs.



The drawing on the top right shows a female *Romalea* with eggs developing in each ovariole. In the drawing on the bottom the mature eggs are being laid and are passing down into the lateral oviduct where they will then pass into the common oviduct into a position just below the spermathecae where sperm will be deposited on each egg as it passes through this area.



TYPES OF OVARIOLES



Teleotrophic Polytrophic

Panoistic
(no nurse cells)

Meroistic
(have nurse cells)

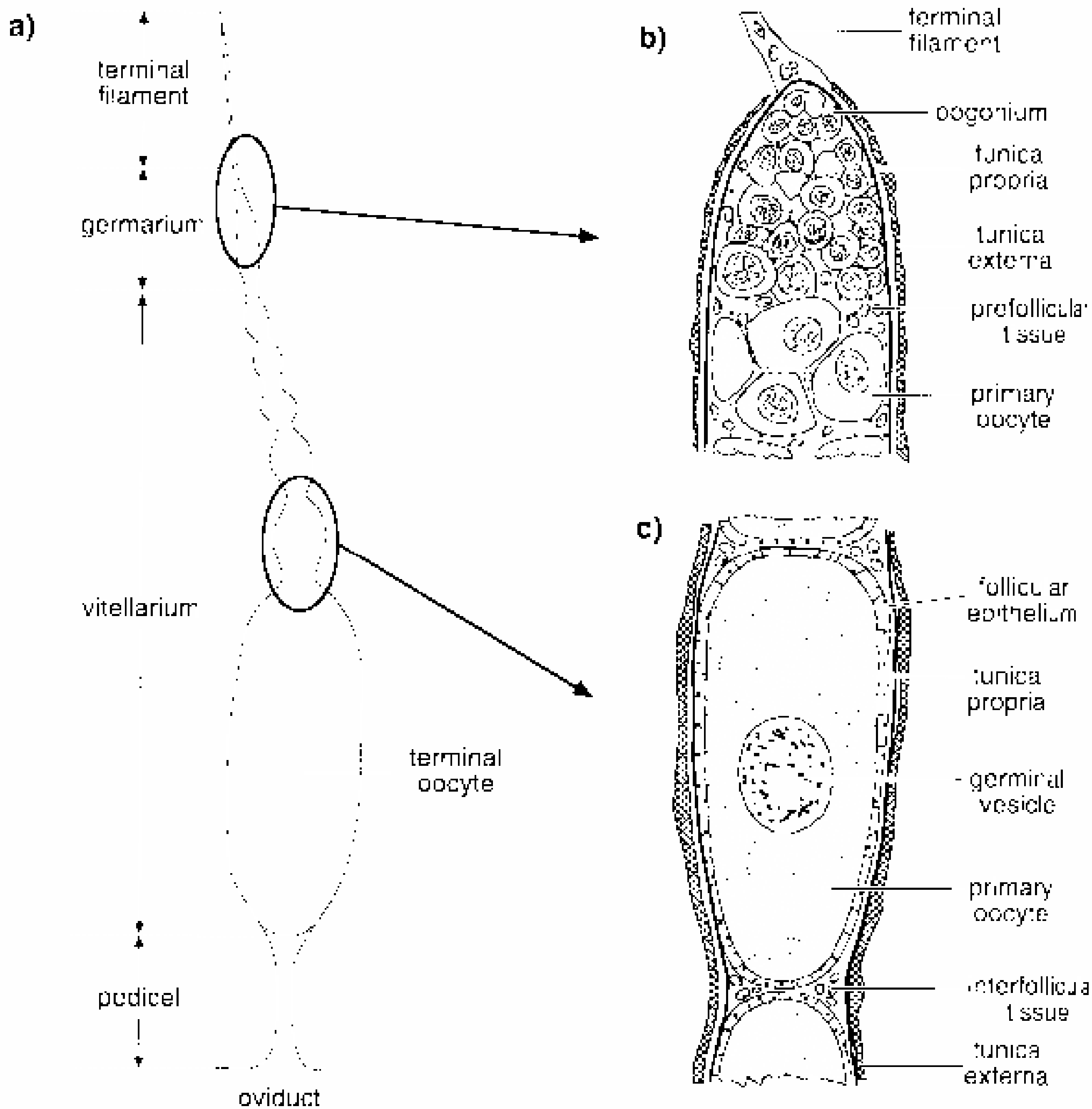
Panoistic ovariole

NO NURSE CELLS OR TROPHOCYTES.

Found in primitive orders:

Protura
Collembolla
Diplura
Thysanura
Odonata
Plectoptera
Orthoptera
Isoptera
Siphonaptera-only holometabolous group to have ovarioles like this

Nearly all of the RNA contained in the mature egg is produced by the oocyte.



MEROISTIC OVARIOLE

Teleotrophic ovariole

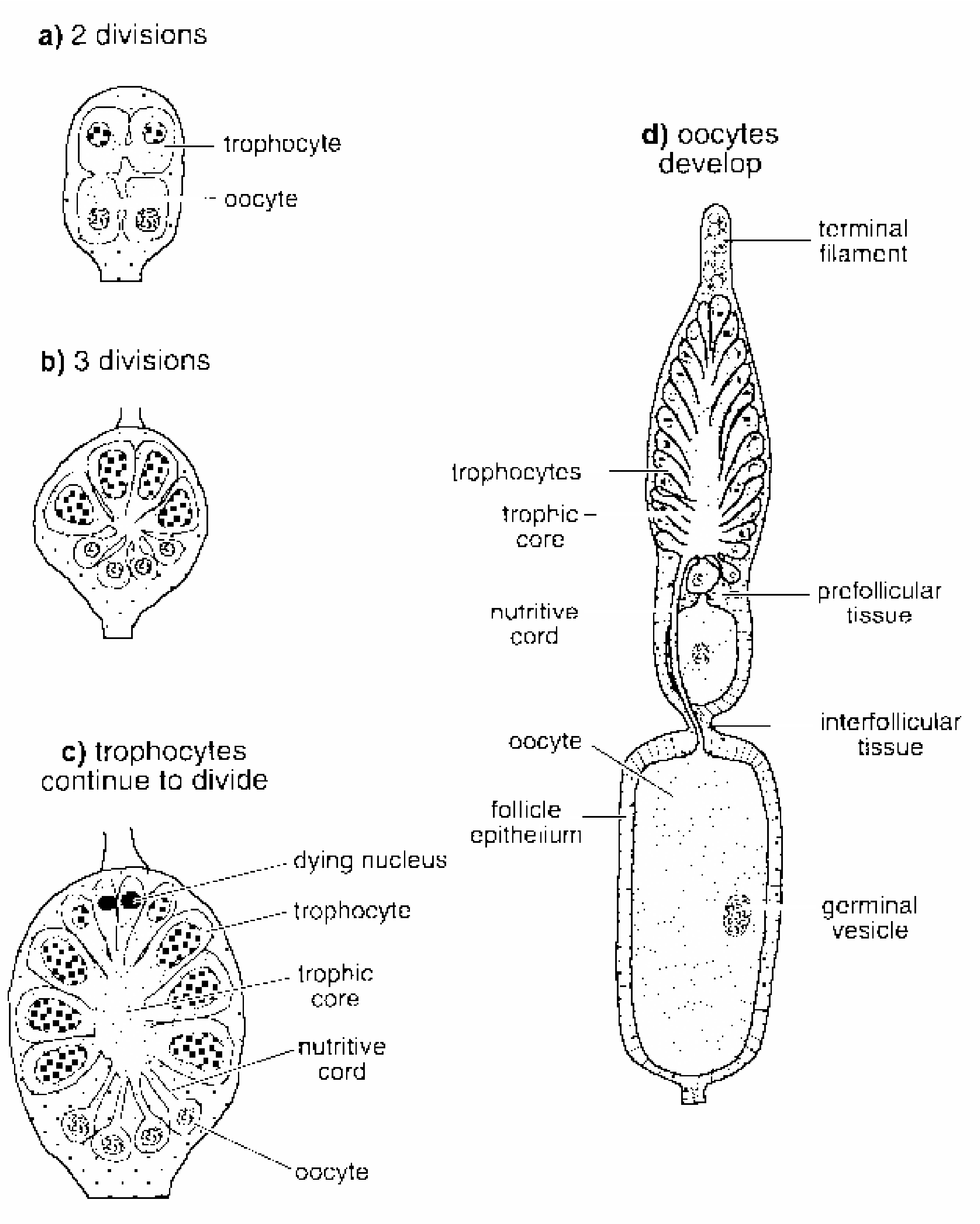
NURSE CELLS/TROPHOCYTES PRESENT BUT HAVE A NUTRITIVE CORD AT A DISTANCE (THUS TELEO) TO PROVIDE NUTRIENTS

Found in:

Hemiptera

Coleoptera

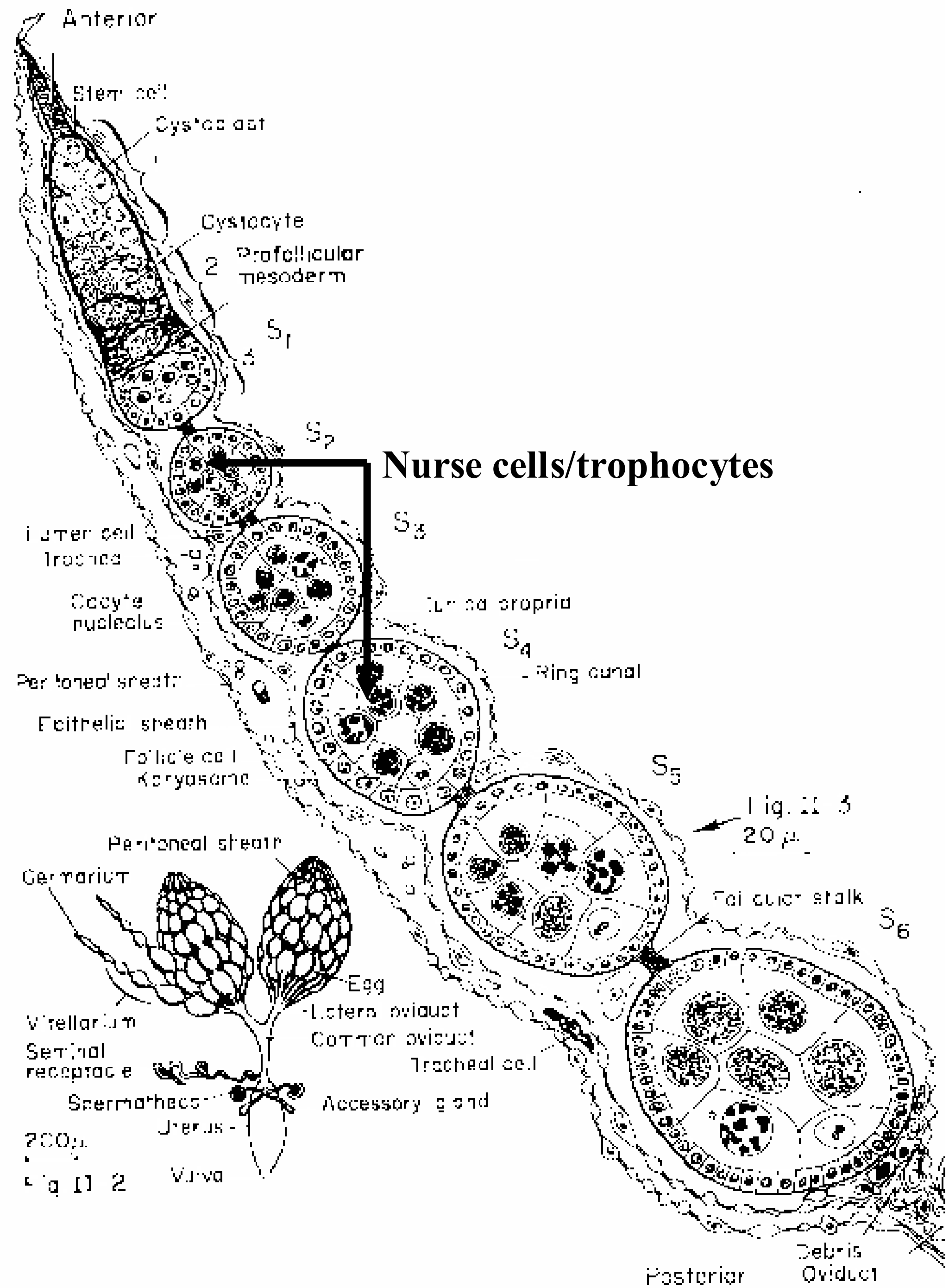
Nearly all of the RNA contained in the mature egg is produced by the trophocytes or nurse cells



MEROISTIC OVARIOLE
Polytrophic ovariole

NURSE CELLS/TROPHOCYTES PRESENT AND FOUND IN EACH DEVELOPING FOLLICLE

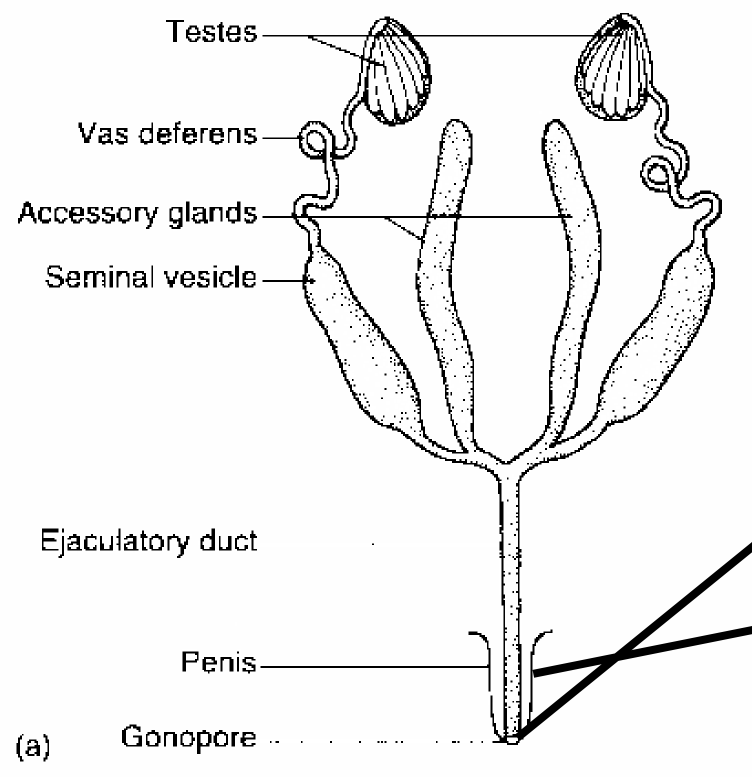
Found in:
Dermaptera
Psocoptera
Phthiraptera
Holometabolous orders except
  Coleoptera and Siphonaptera



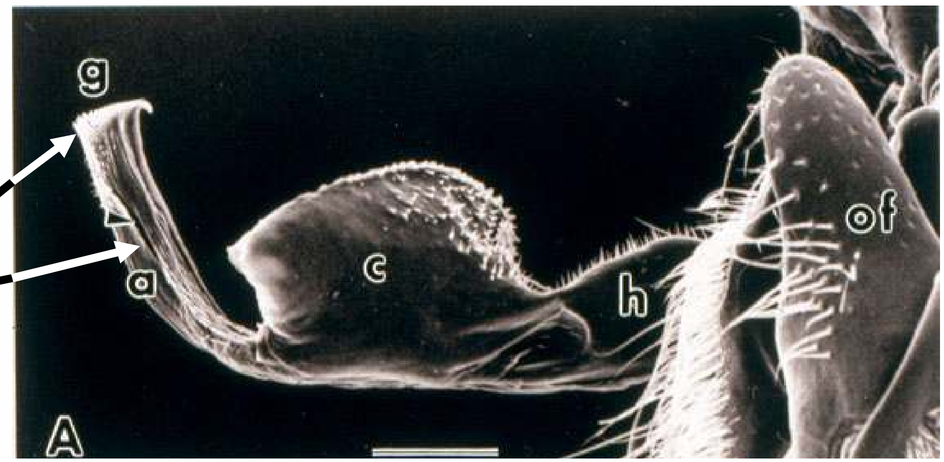
Nearly all of the RNA contained in the mature egg is produced by the trophocytes or nurse cells

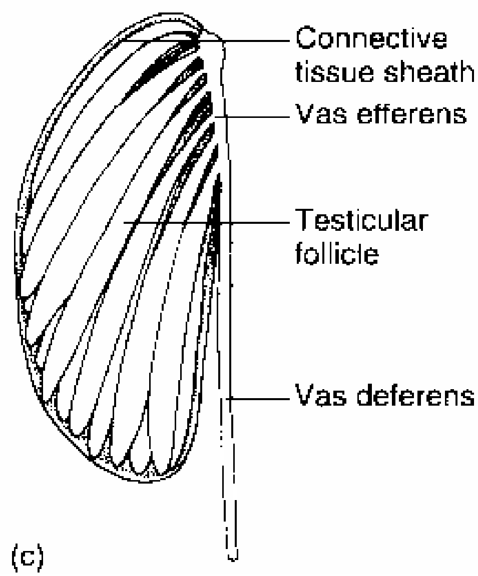
Ovariole system of *Drosophila*

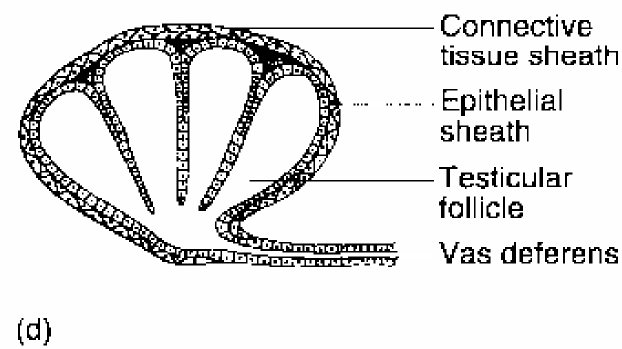
GENERIC SCHEME OF A MALE INSECT



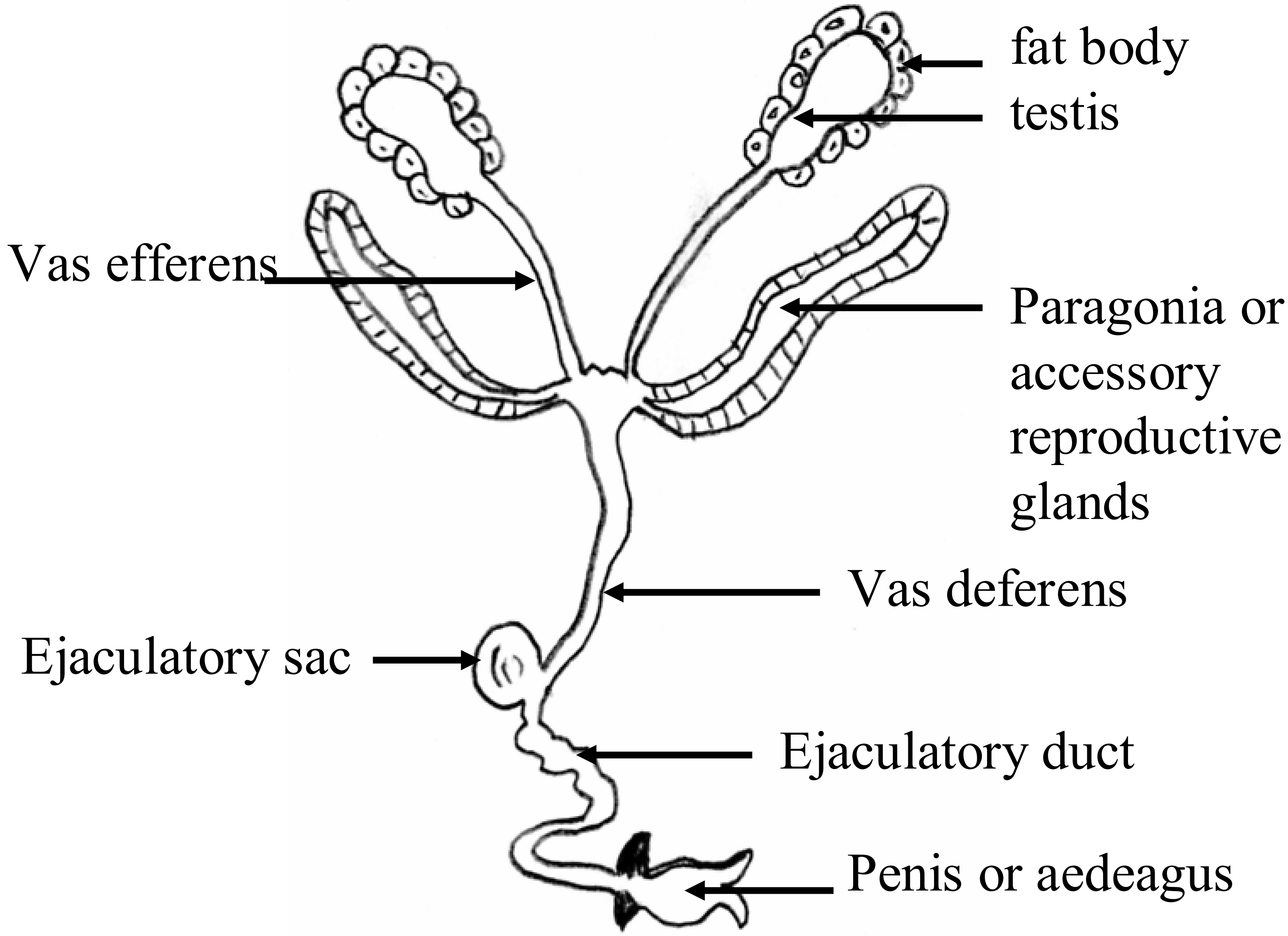
Aedeagus of male *Phormia regina*

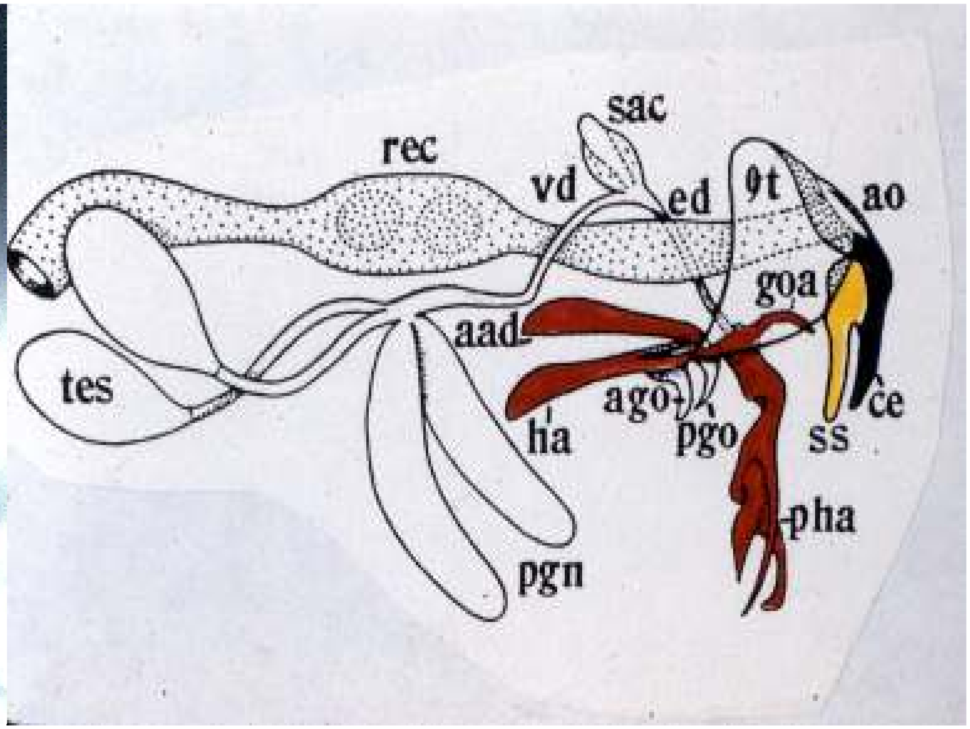




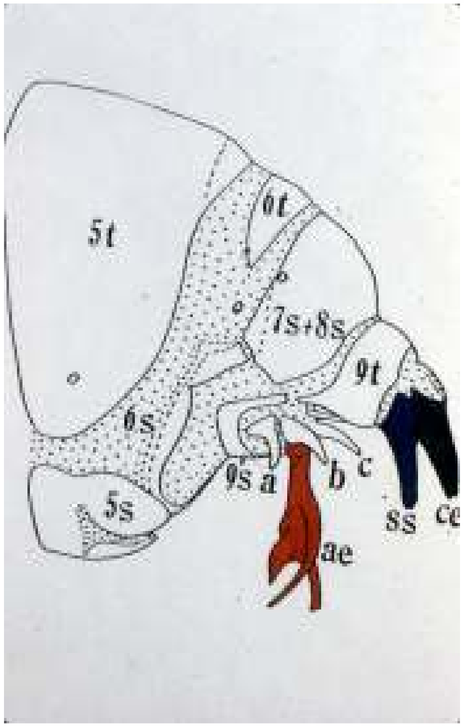


MALE BLOWFLY REPRODUCTIVE TRACT

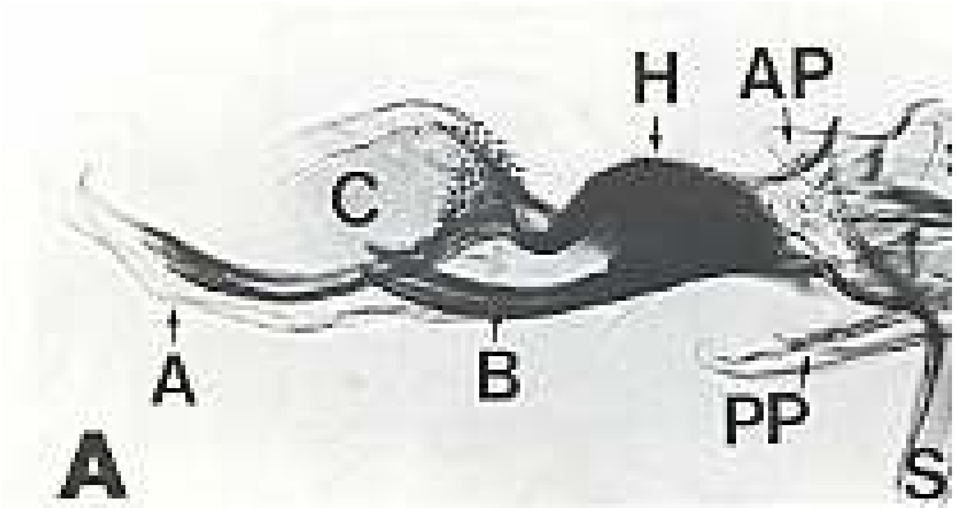


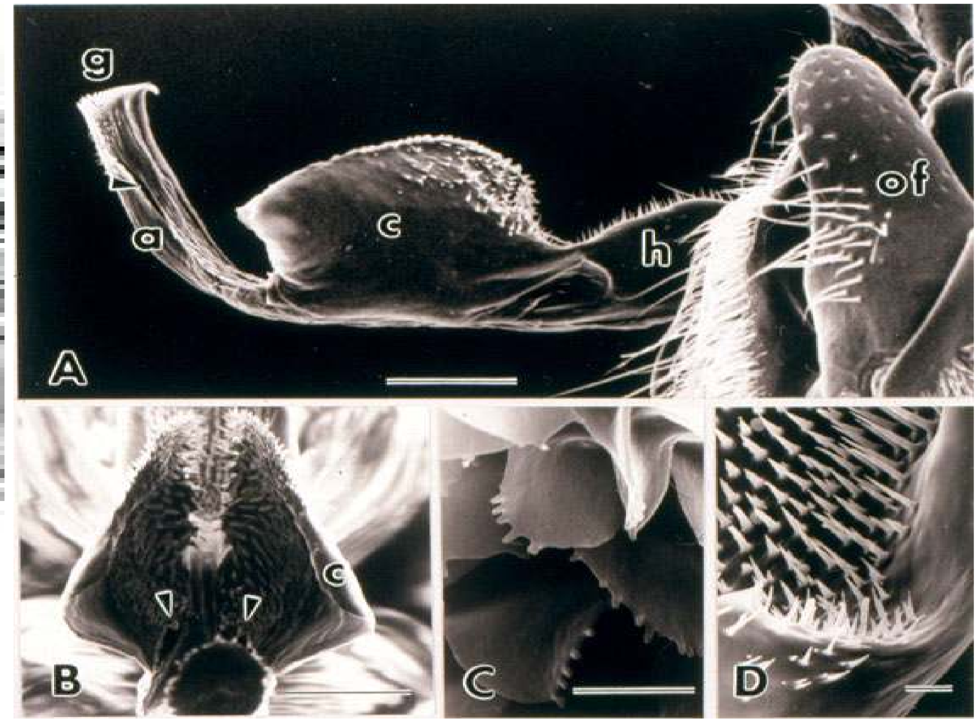


Phormia regina mating

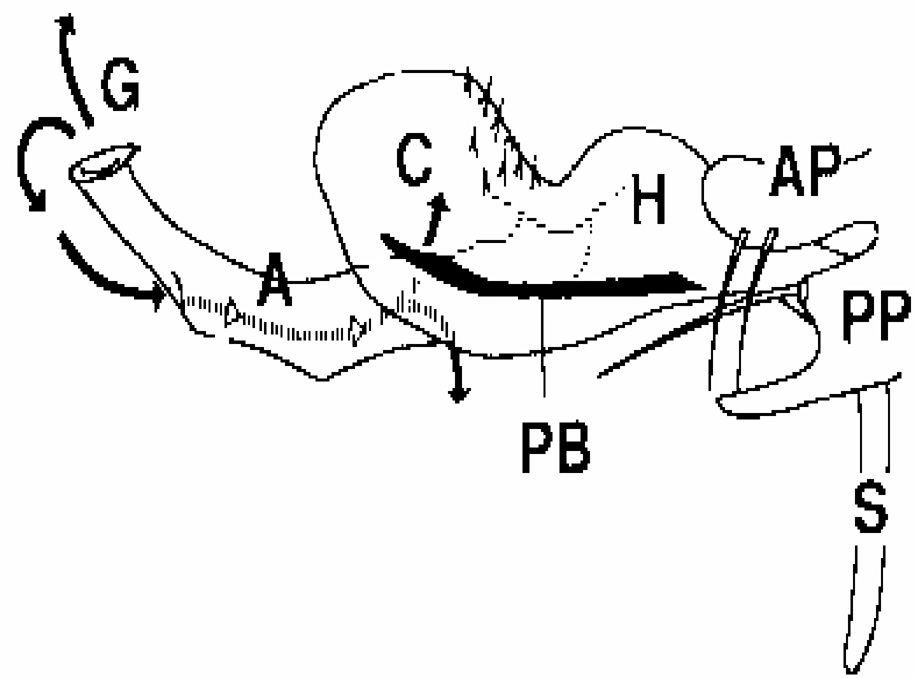


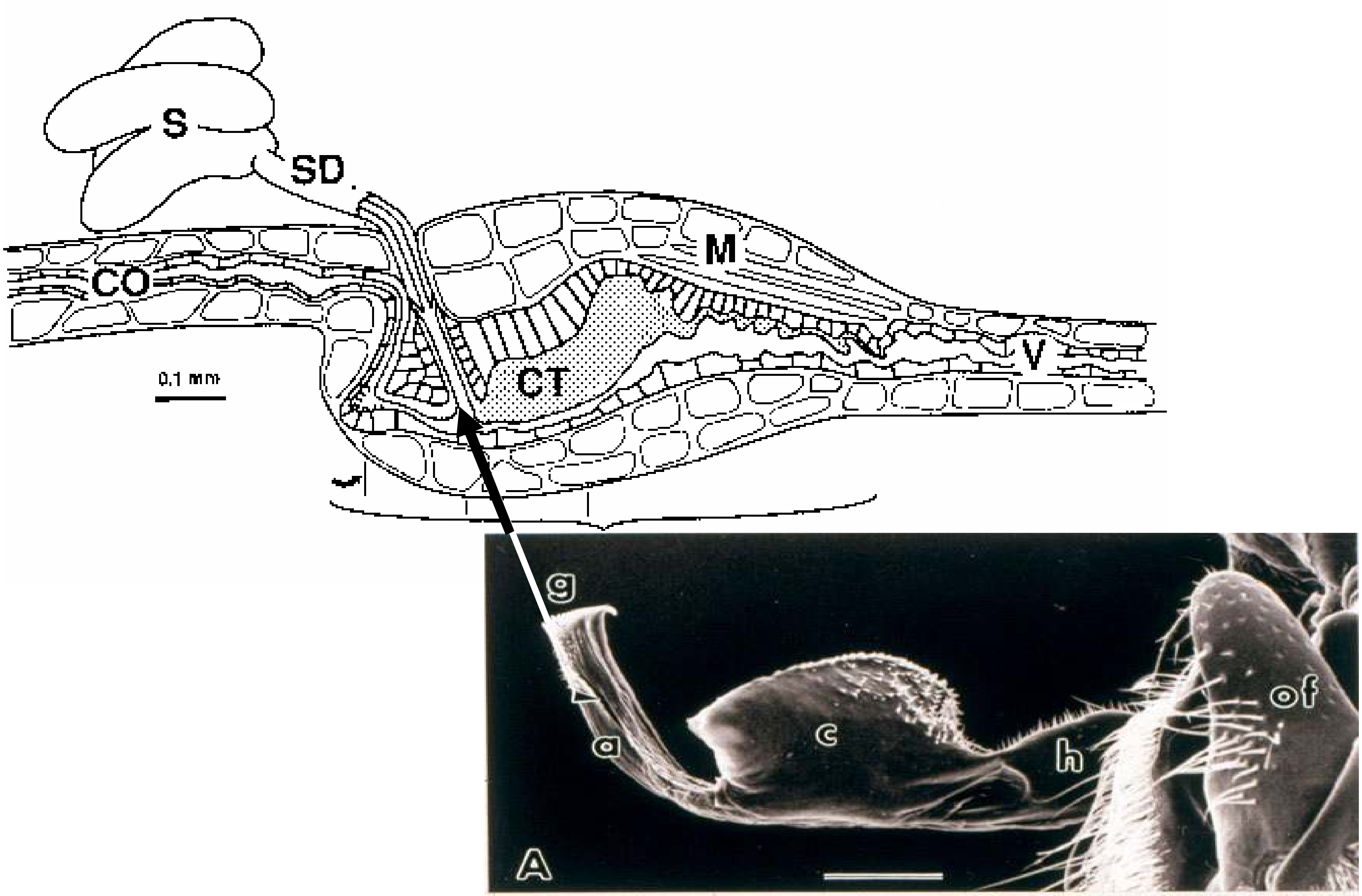
Merritt, D., C.-M. Yin and J.G. Stoffolano, Jr. 1994. Copulatory apparatus and deposition of male accessory secretion in *Phormia regina* (Diptera: Calliphoridae). Ann. Ent. Soc. 87:97-103.



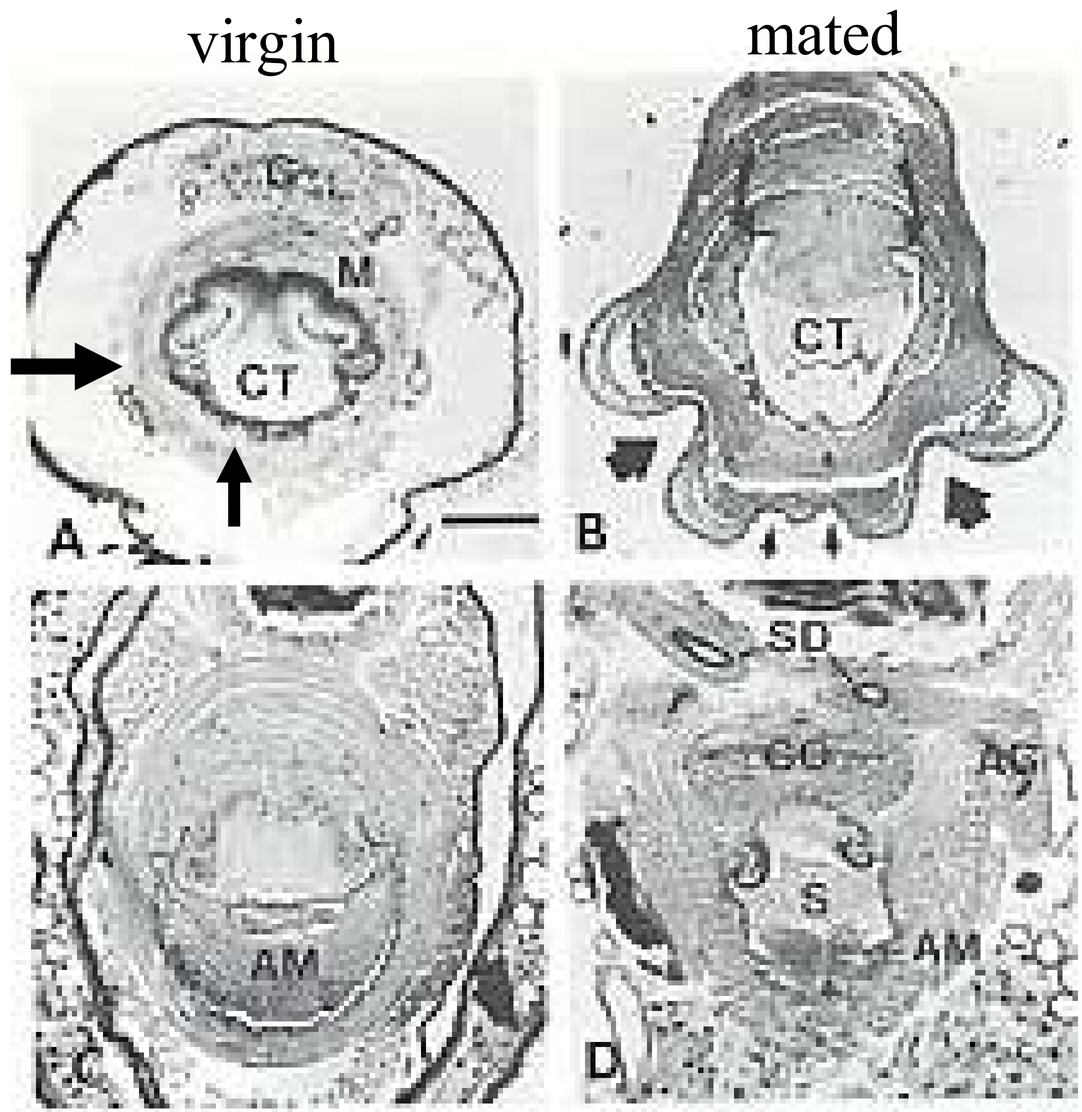


Aedeagus of male, ***Phormia regina***. Note the opening of the gonopore (g) where the sperm and ARG fluid exits from the male. This structure A,C,B,H, called the phallosome, fits into the female's bursa. The ARG fluid then is channeled into the accessory duct inlets (stippled arrows in bottom right). It then goes up into the 'dish-like' cornua that serves as a 'plug' to prevent fluids from escaping.





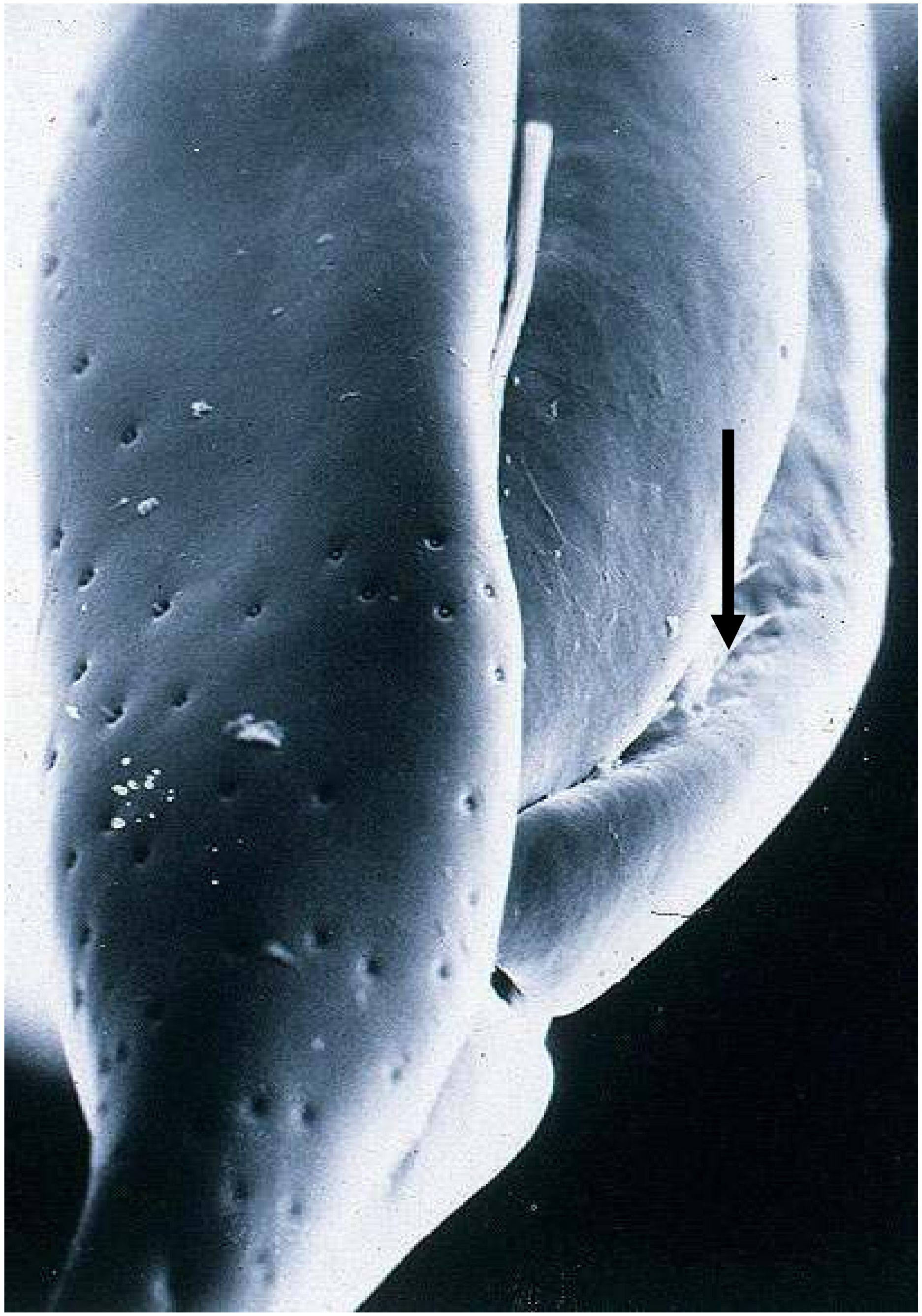
During copulation the male inserts his phallosome into the female's bursa. Note that the gonopore (g) lines up with the opening of the spermathecal ducts. Also note that during egg laying, the egg passed out of the common oviduct (CO) and as it passes down is in the correct position just under the spermathecal duct for sperm to be deposited.

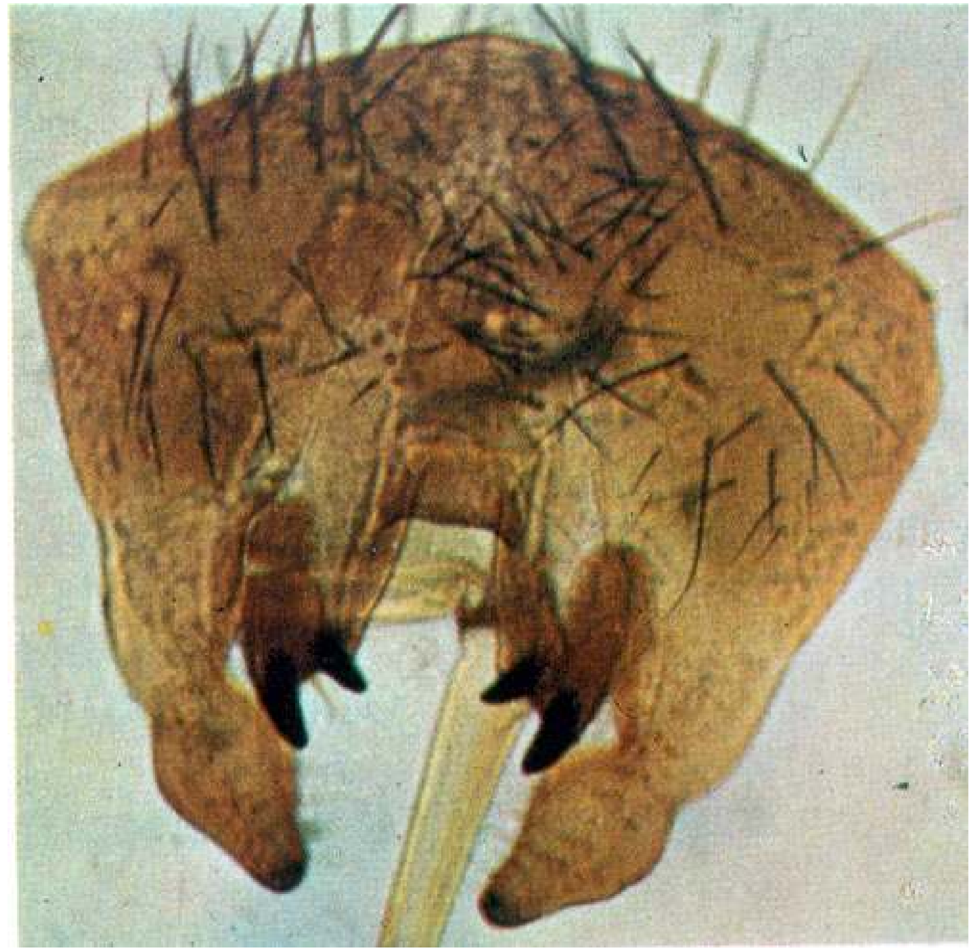


Photos to the right are transverse sections through the bursa of a female ***Phormia regina*** that were fixed 1 min after copulation and the male was removed. In A, note that in this virgin female the walls of the bursa are folded (see arrow) + the cuticular tonsil (CT) fills the lumen of the bursa, thus no lumen is showing (smaller arrow) but, in B one can see the lumen (L) of the mated female. The male's phallosome was removed before this female was sectioned. The remnants of the male's cornua are seen in B (curved arrows). Also note the shorter arrows, especially the thicker ones showing where the male's claspers were holding onto the female [compare fig. A (virgin) with B (mated)]. In fig. C. the lumen is now filled with accessory reproductive material (AM) while in D, one can see the ARG material and sperm (S) in the lumen.

As you could in D of the previous slide, the male cornua with the spines grabs onto the female's cuticular tonsil and between the two form a plug-like structure that prevents fluids from escaping while the males pump is working. The sperm comes out first and is deposited in the region of the spermathecal duct opening. Following and after most sperm fill the spermatheca, the ARG fluid is released. It takes a different path than the sperm and moves back into the coruna because of special cuticular pathways. Thus, the sperm is anterior while the ARG is found in the posterior. Two days following mating, the sperm are all in the spermatheca and the ARG fluid is absent. It is believed that this passes through the bursa into the female's hemolymph where it acts to inhibit further matings, tells her to terminate the copulation and also that she is mated and can begin laying eggs. The ARG's fluid contains a refractory inhibiting substance (RIS) that renders the female unreceptive to other male mating attempts. More will be said about this later.

MALE CLASPERS



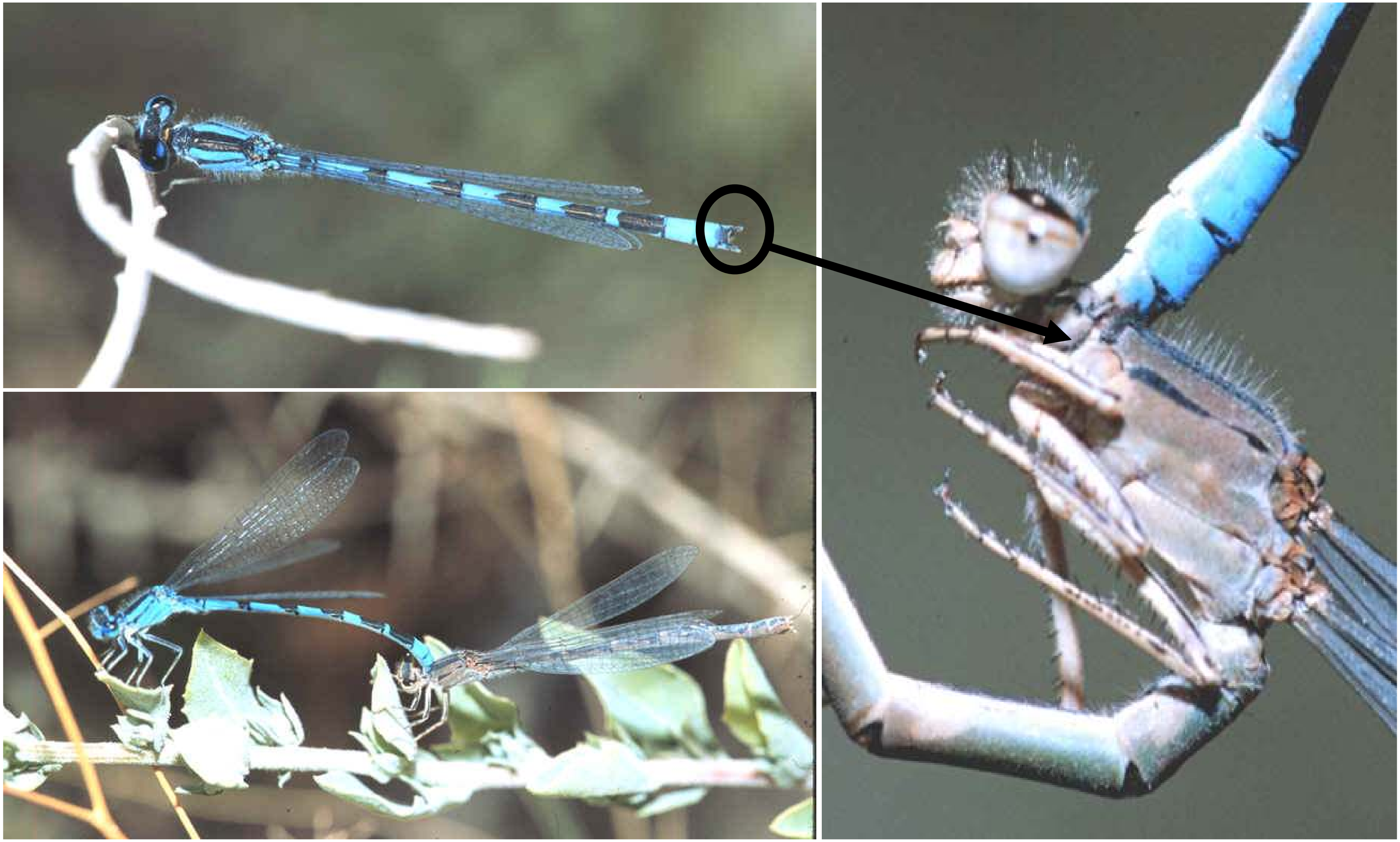


Right SEM shows ovipositor of the female apple maggot. Note larger peg campaniform sensilla that monitor oviposition position within the fruit. Towards the tip they are classical cap campaniform sensilla. Right photo shows the male's claspers that fit around and hold onto the female while he inserts the aedeagus into her vagina (see arrow in photo on the left).

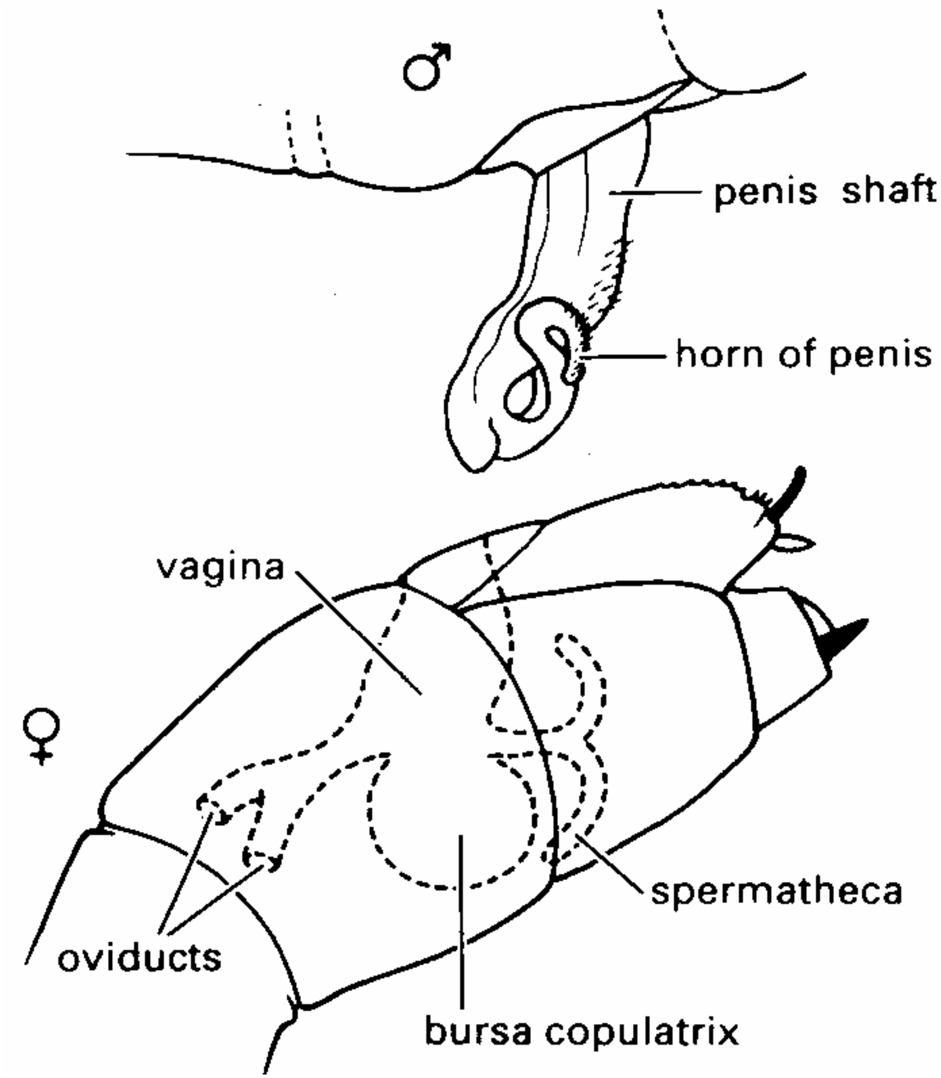
Mate guarding-Several insect species use this form of behavior to ensure that it is their sperm that is used to fertilize the eggs of the female. To accomplish this, the male clasps the female around the neck using claspers. Following mating he even remains with the female while she is laying eggs (see photo to the bottom right).

Go to the website below to see videos of mating
http://www.windsofkansas.com/dsa20032.html

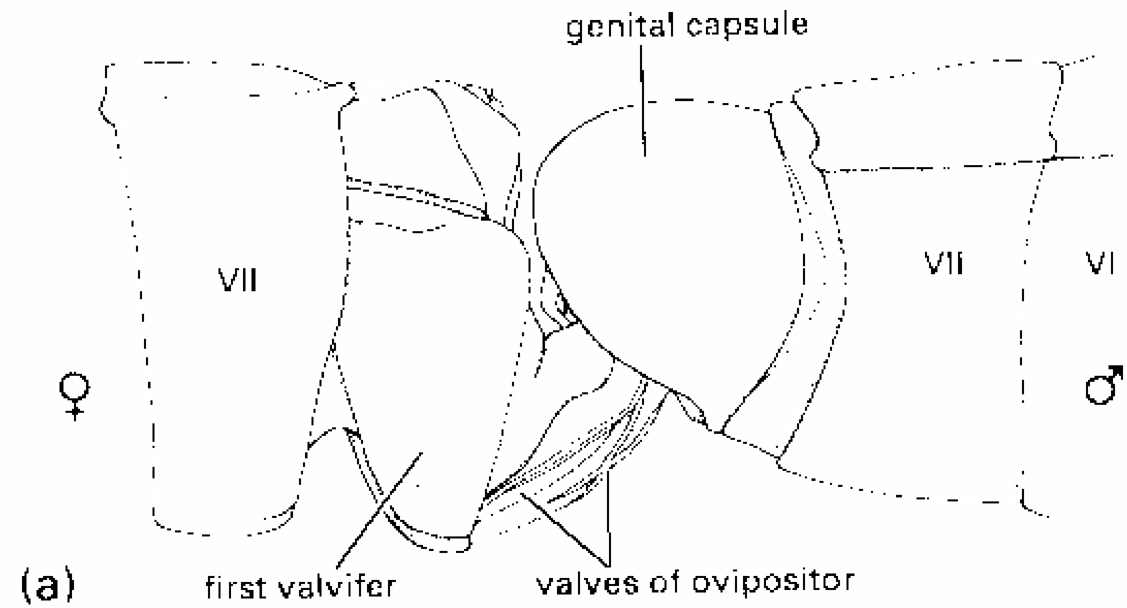
Note claspers of the male dragonfly holding onto the female in the neck region. He will hold onto her until she has finished laying eggs, thus assuring his sperm transfer

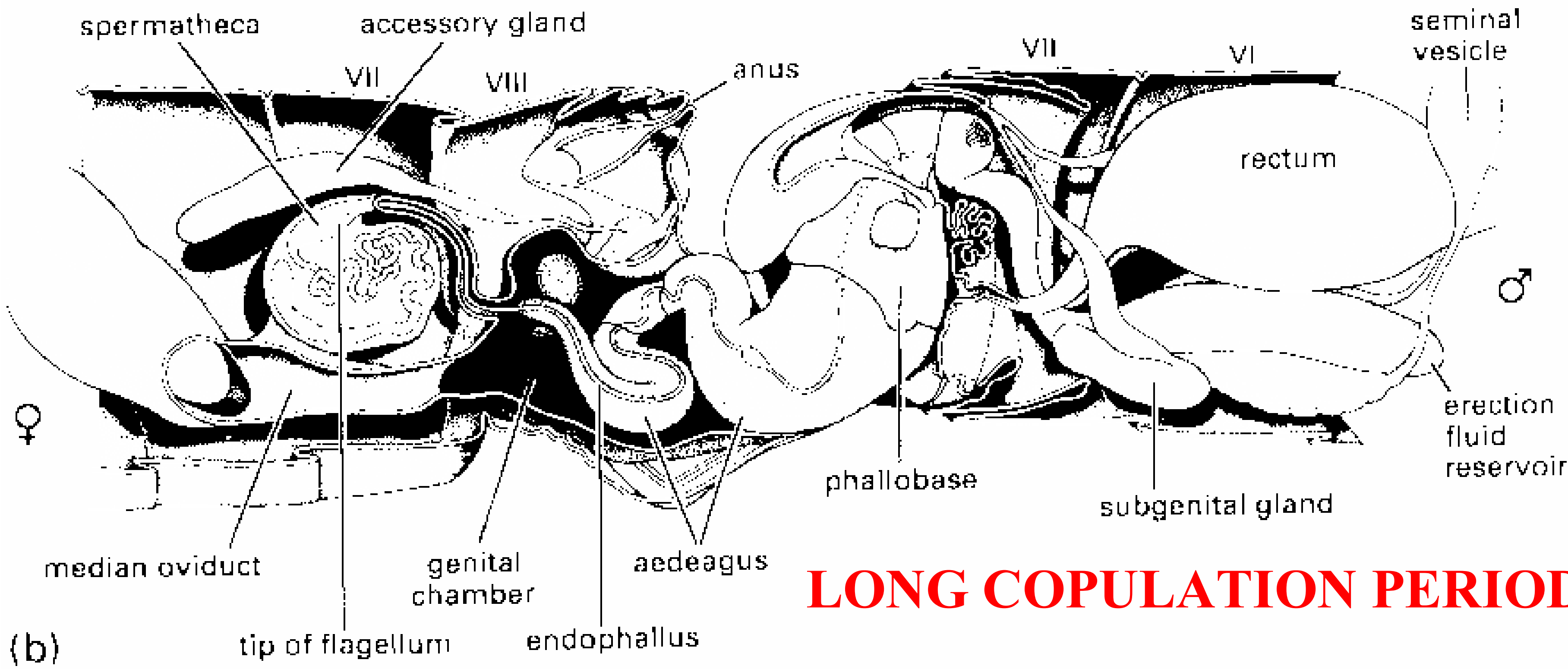


Because the male is mate guarding the female by using the tip of his abdomen and his claspers, he is unable to transfer sperm the normal way. Instead, Odonata have evolved a novel way for sperm transfer. The male transfers sperm to a special pouch located on the 2nd ventral abdominal segment. While holding onto the female, the female bends her abdomen forward and makes contact with this pouch. Sperm transfer takes place here and in this position. Male dragonflies have also evolved a special aedeagus that aids in either pushing his sperm to the front of a previous males or actually scooping the previous male's sperm out.



The male aedeagus in the Odonata is designed to either push its own sperm to the front of any previously deposted sperm or to actually scoop it out and remove the previous males sperm. The design with the unusual horn helps remove the sperm of a previous male.





LONG COPULATION PERIODS

NUPTUAL GIFTS

Several insect species offer their mate a nuptual gift that often contains nutrient supplements essential for egg production. In other cases, the male may just use this 'gift' as a way to appease the female while he engages in copulation. To the right is a male grasshopper that is feeding and has his pronotal shield raised. This exposes special paranotal glands that contain a secretion that the female will feed on while he inserts his aedeagus and copulates with her.

FACTORS AFFECTING EGG PRODUCTION AND FECUNDITY

1. **Nutritional requirements**
   a. Food quality
   b. Food quantity
   c. Autogeny vs. anautogeny
   d. Mechanisms involved

2. **Mating stimuli**

3. **Environmental factors**
   a. Temperature
   b. Humidity
   c. Photoperiodic influences
   d. Group interactions
   e. Availability of hosts
   f. Interaction of different factors

NUTRITION AND EGG DEVELOPMENT

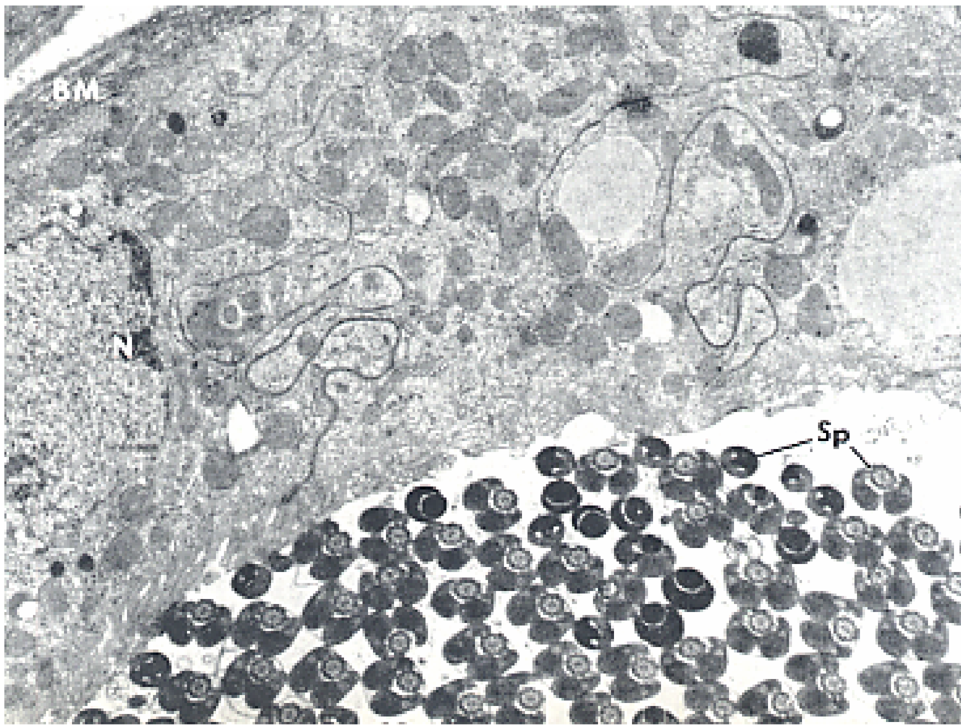
It is well known that insects need protein in their diet to develop eggs. They get this in different ways.

1. Carry over from immature stages
2. Nuptual gifts-some contain essential nutrients for egg development
3. Eating the males
4. Acquiring the protein as adults

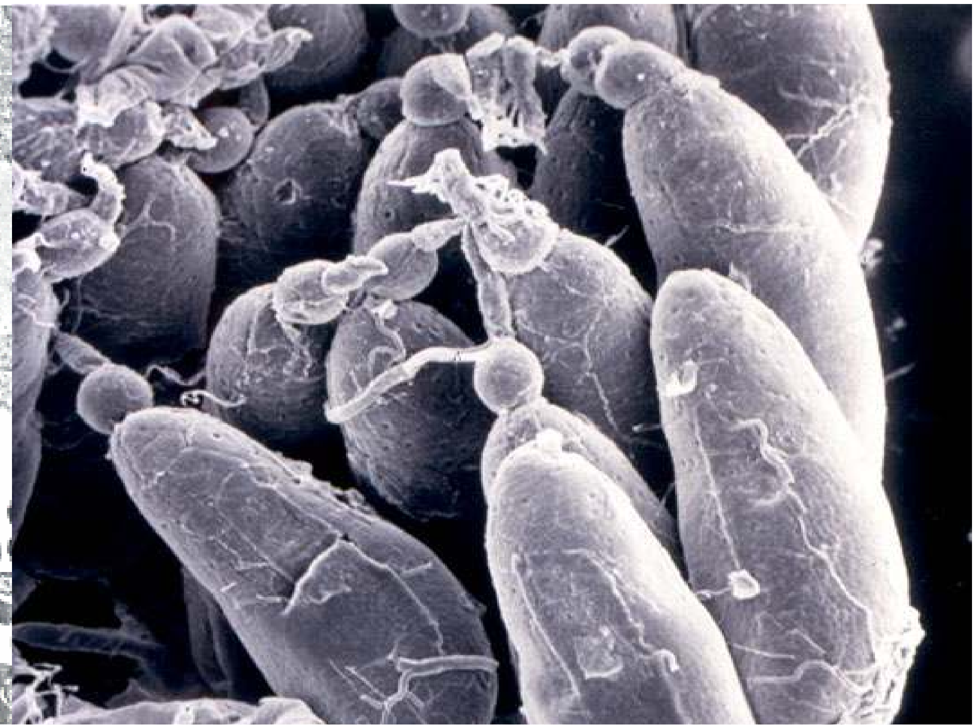
EGG DEVELOPMENT OR OOGENESIS

1. **Gametogenesis (germarium)**
2. Movement of follicle down the ovariole
3. Function of the nurse cells or trophocytes
4. Function of the follicle cells
5. **Vitellogenesis**
   a. Control of yolk production
   b. Yolk uptake (the process)
6. Chorion or egg shell formation

Sperm in vas deferens of milkweed bug



Ovarioles with yolk in *Phormia regina*



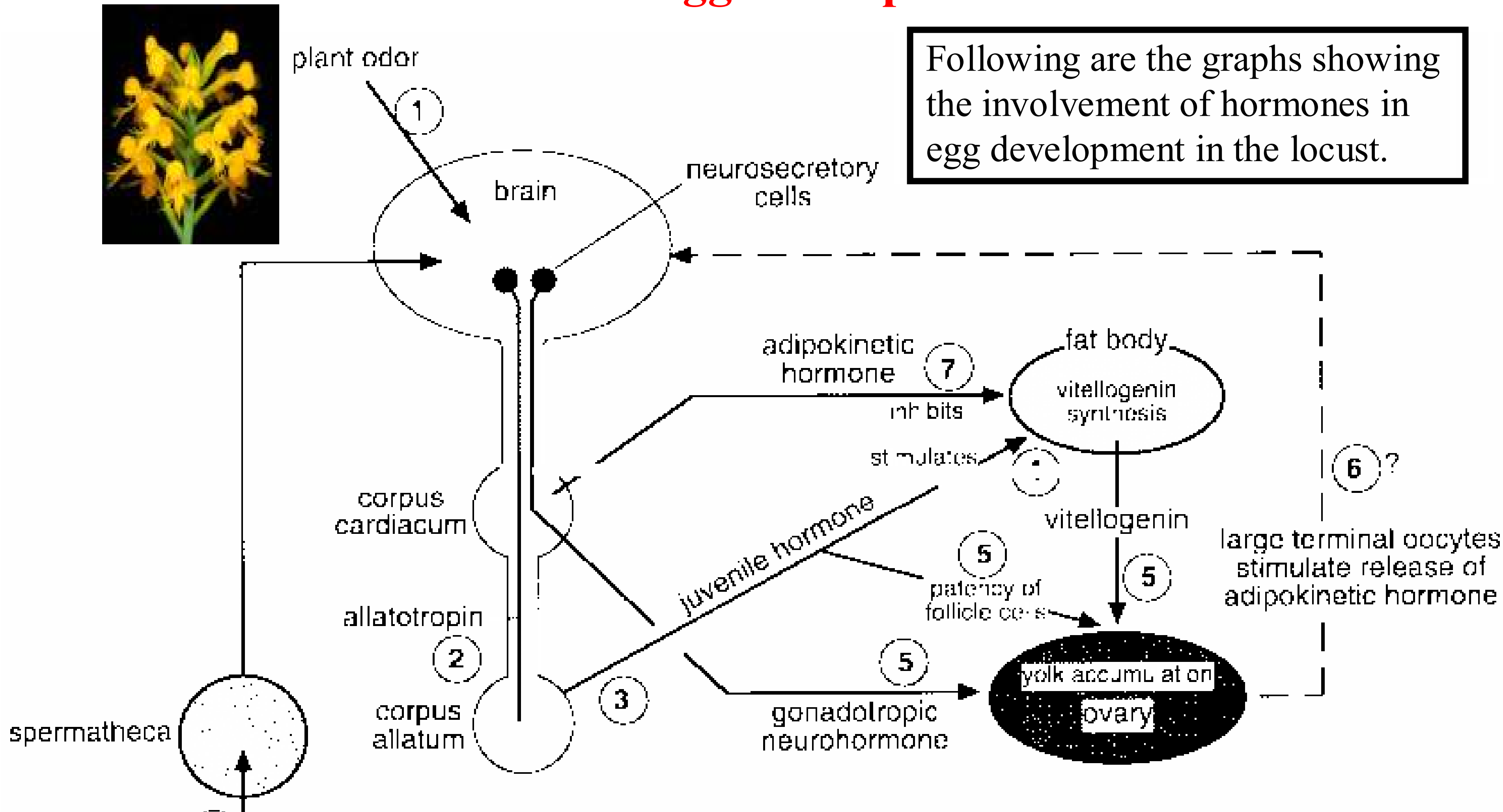
Control of egg development in insects

Control of vitellogenin synthesis-in most insects this is initiated by JH acting directly on the fat body because this is where vitellogenin (Vg) is synthesized from raw materials from the female's hemolymph. Vg is often called the female specific blood protein because on gels it shows up as a special band that is absent in males.

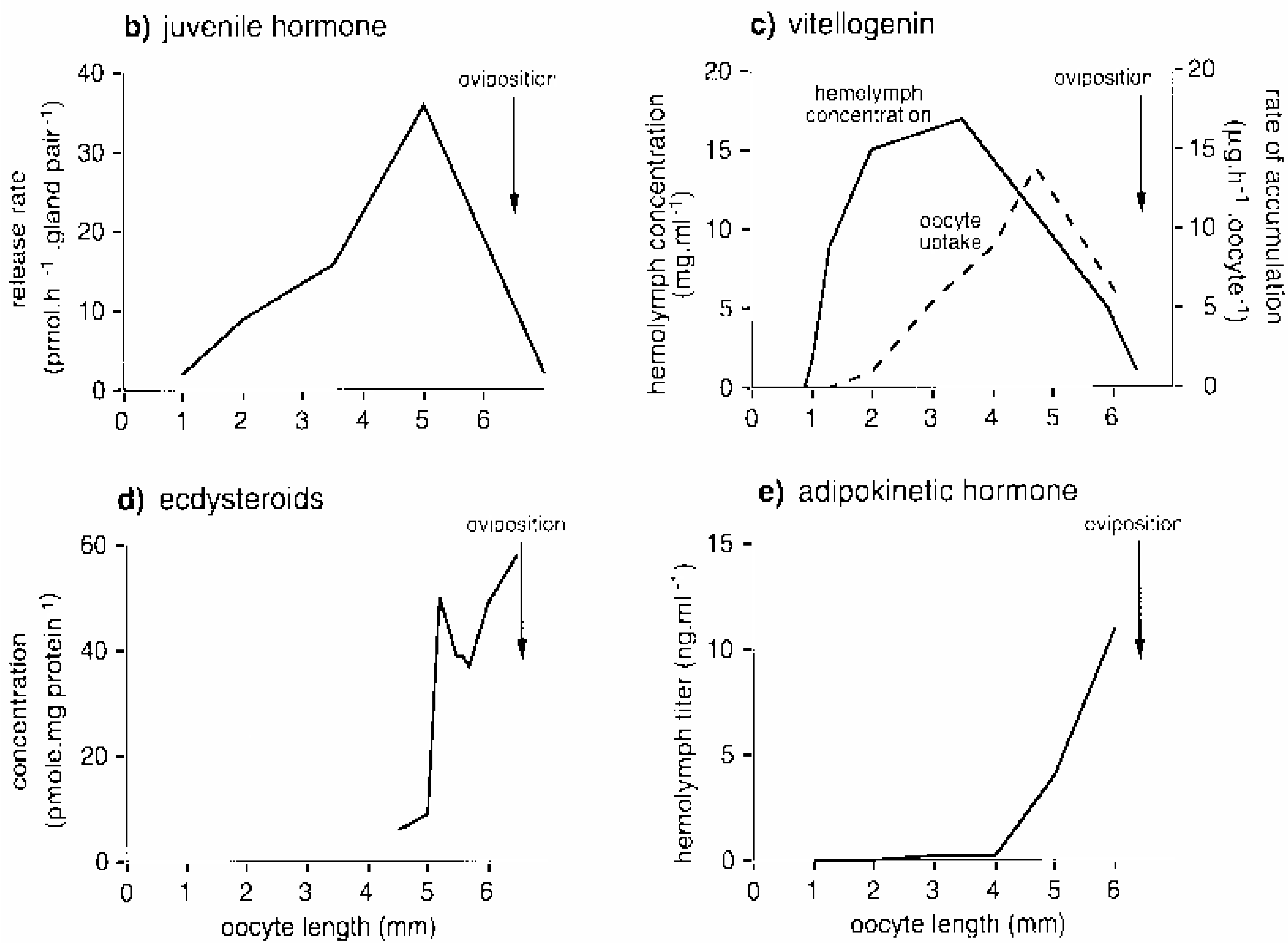
I. Signals from the brain to
   a. Release JH from the CA
      1. Mating and food odors cause this in the locusts- act on neurosecretory cells in the brain
      2. Blood-feeding (stretch + nutrition) in mosquitoes + *Rhodnius*
      3. High-protein diet in *Phormia regina*

Overall scheme for control of egg development in the Locust

Following are the graphs showing the involvement of hormones in egg development in the locust.



Fertility-enhancing substance is from the male's ARG fluid



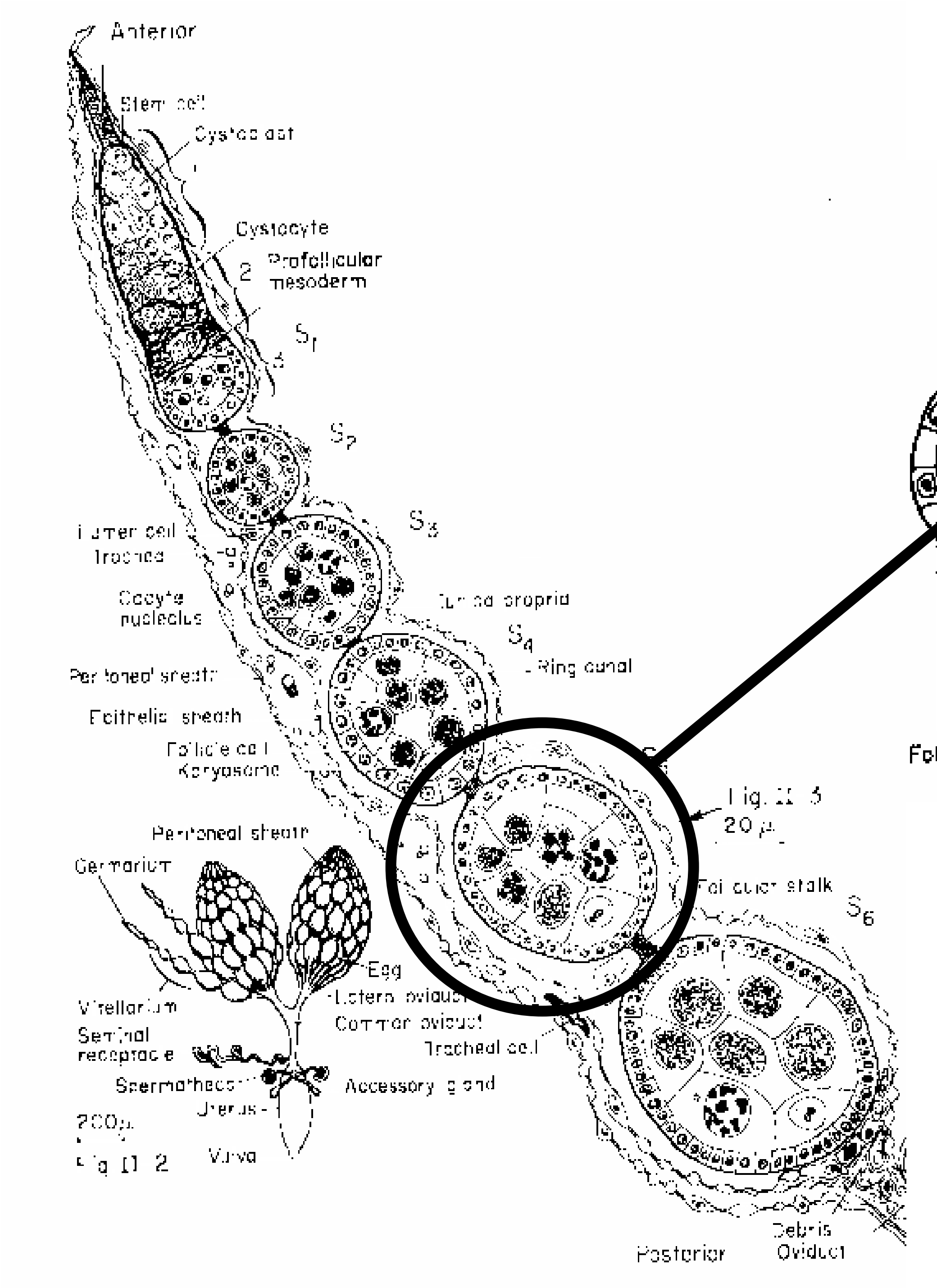
b) juvenile hormone
release rate (pmol.h -1 .gland pair -1)
0
10
20
30
40
oviposition
0
1
2
3
4
5
6
c) vitellogenin
hemolymph concentration (mg.ml-1)
rate of accumulation (µg.h-1 .oocyte-1)
hemolymph concentration
oocyte uptake
oviposition
0
5
10
15
20
0
1
2
3
4
5
6
d) ecdysteroids
concentration (pmole.mg protein 1)
0
20
40
60
oviposition
0
1
2
3
4
5
6
oocyte length (mm)
e) adipokinetic hormone
hemolymph titer (ng.ml-1)
0
5
10
15
oviposition
0
1
2
3
4
5
6
oocyte length (mm)

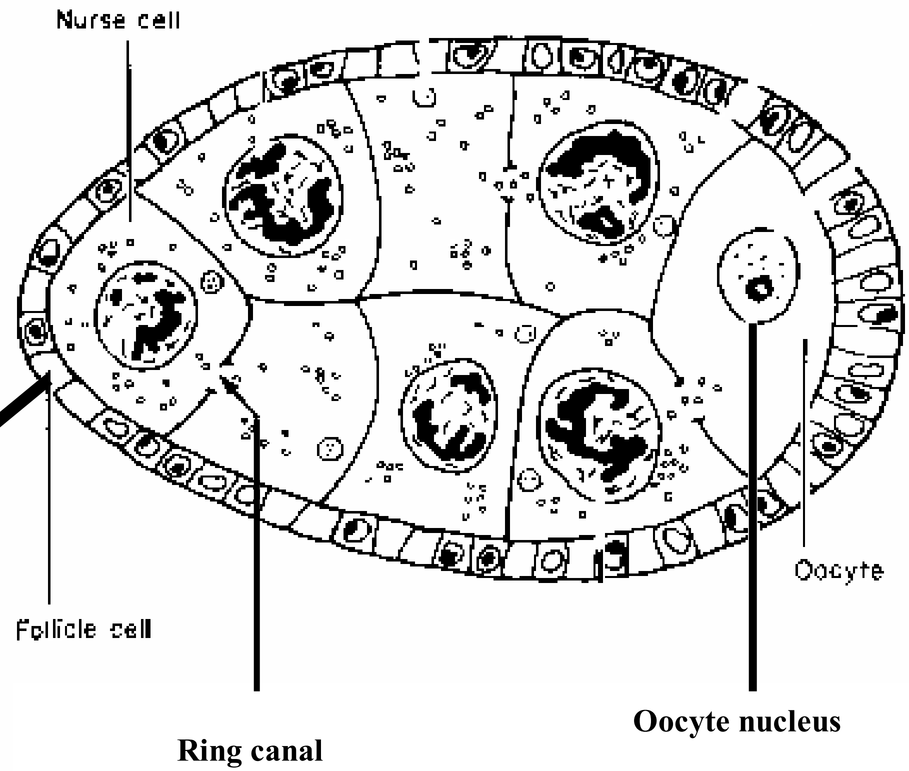
DIETARY INDUCED HORMONAL CONTROL OF TERMINAL OOCYTE DEVELOPMENT IN *PHORMIA REGINA*

CONTROL OF OOGENESIS

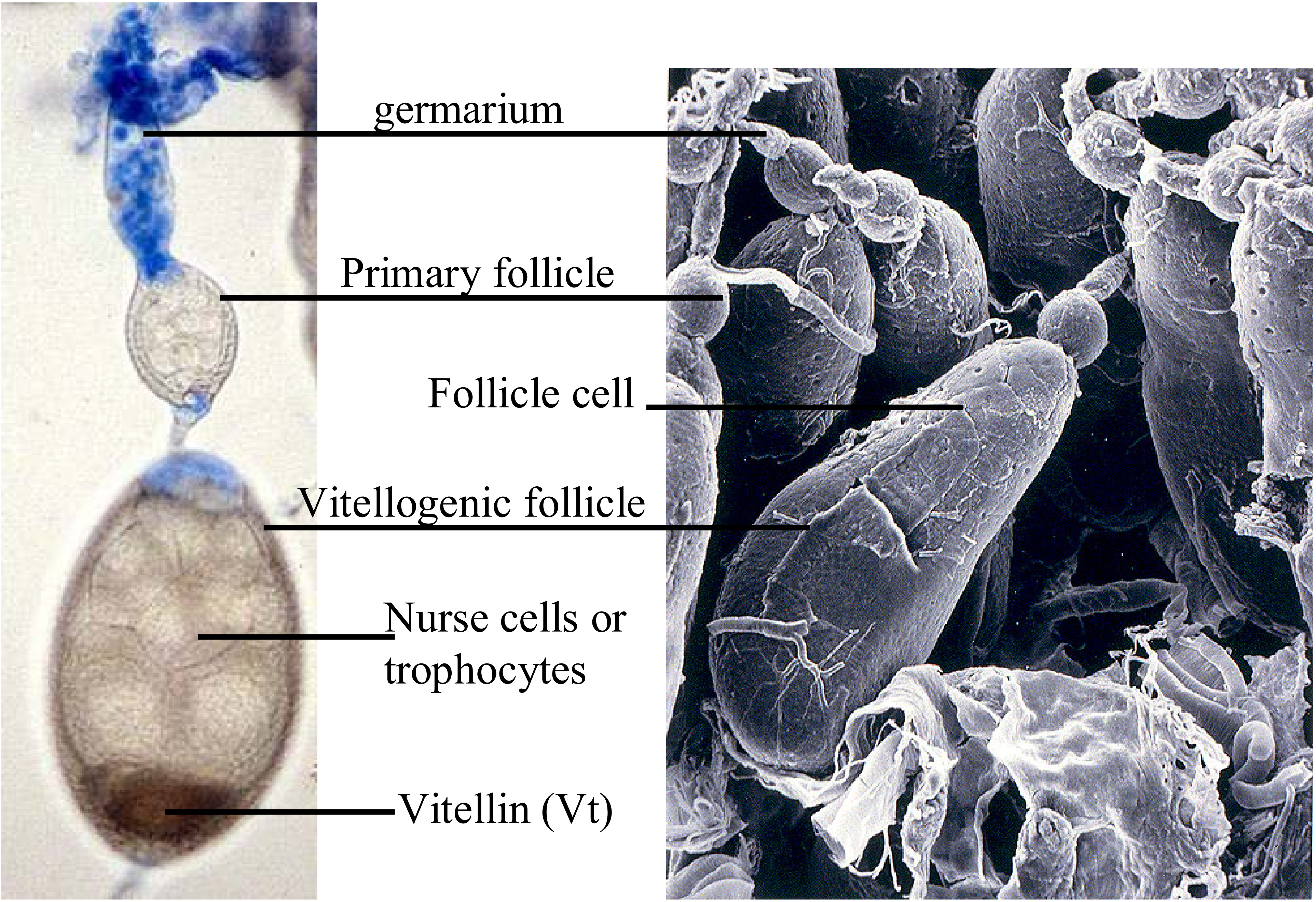
A. Follicle development
B. Nutrient control
C. Hormonal control
D. How is nutrient content of the diet translated into hormonal messengers that control oogenesis?

A. FOLLICLE DEVELOPMENT





A. FOLLICLE DEVELOPMENT in *Phormia regina*



Developing vitellogenic follicles of *P. regina*

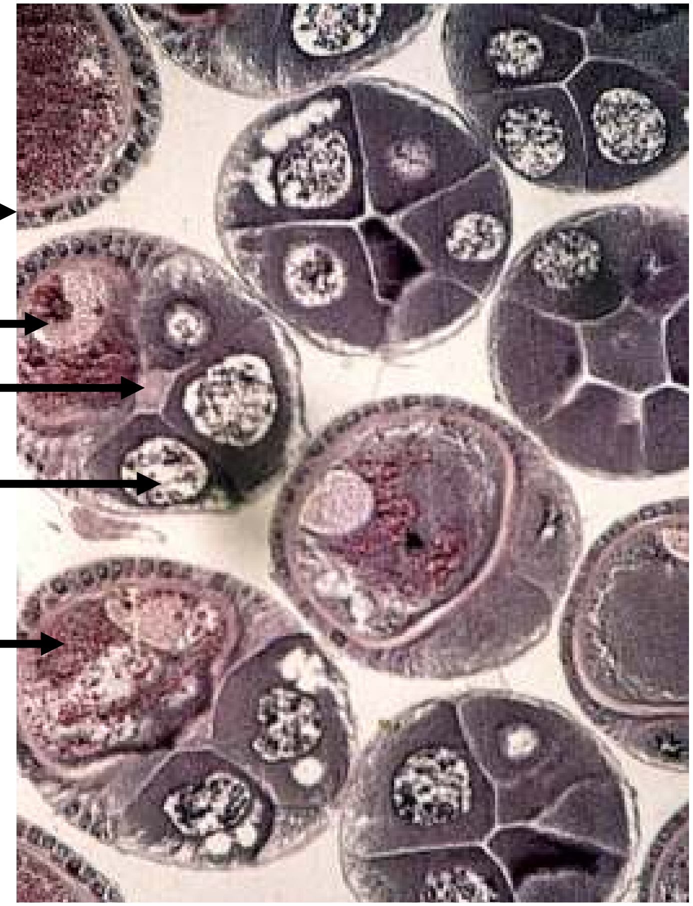
Follicle cells

Oocyte

Border cells

Nurse cells

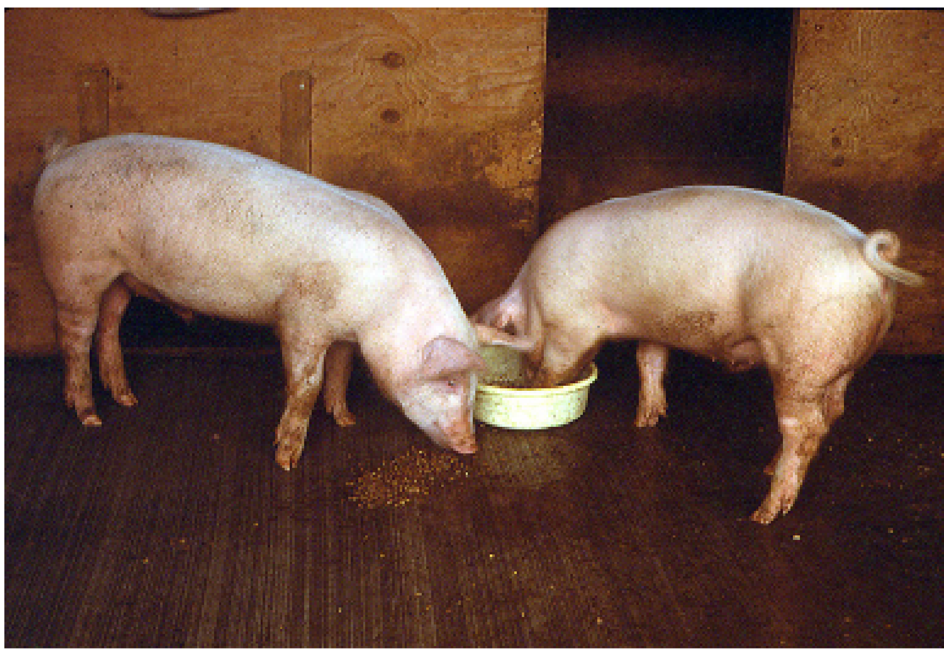
Vitellin



The follicle cells surround the follicle forming the follicular epithelium. In order for Vg to enter the oocyte they must separate, a process called patency, which is induced by JH. As the Vg enters it is converted to vitellin, which is the molecule of yolk used by the embryo and which surrounds the oocyte nucleus. The nurse cells provide the developing egg with maternal RNA while the border cells assist in producing the micropyle.

B. NUTRIENT CONTROL OVER OOGENESIS

It is well known in the literature that most insects need protein to produce eggs. How they get it varies. Some get it from the immature stages while others must obtain it as adults. In fact, in the case of *Phormia regina*, a high protein diet is the trigger for the neuroendocrine cascade leading to egg development to proceed.



EFFECT OF DIFFERENT DIETS ON FOLLICLE DEVELOPMENT IN *P. REGINA* ALSO FED 10% SUCROSE SOLUTION

	Stage of follicle development (%)		
Diet	3	4-9	10
sugar	100.0	0	0
liver	0	0	100
dog faeces	80.5	12.1	7.4
chicken faeces	90.7	7.0	2.3
cat faeces	92.0	8.0	0
sheep faeces	93.6	6.4	0
pig faeces	98.0	0	2.0

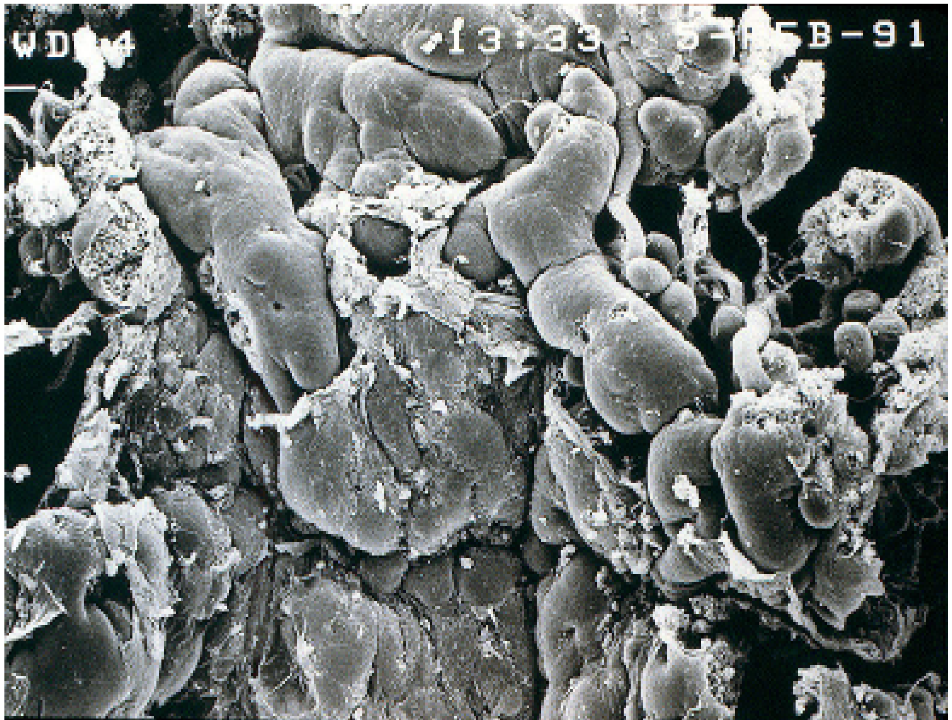
WHERE IS VITELLOGENIN (Vg) PRODUCED AND FOUND?

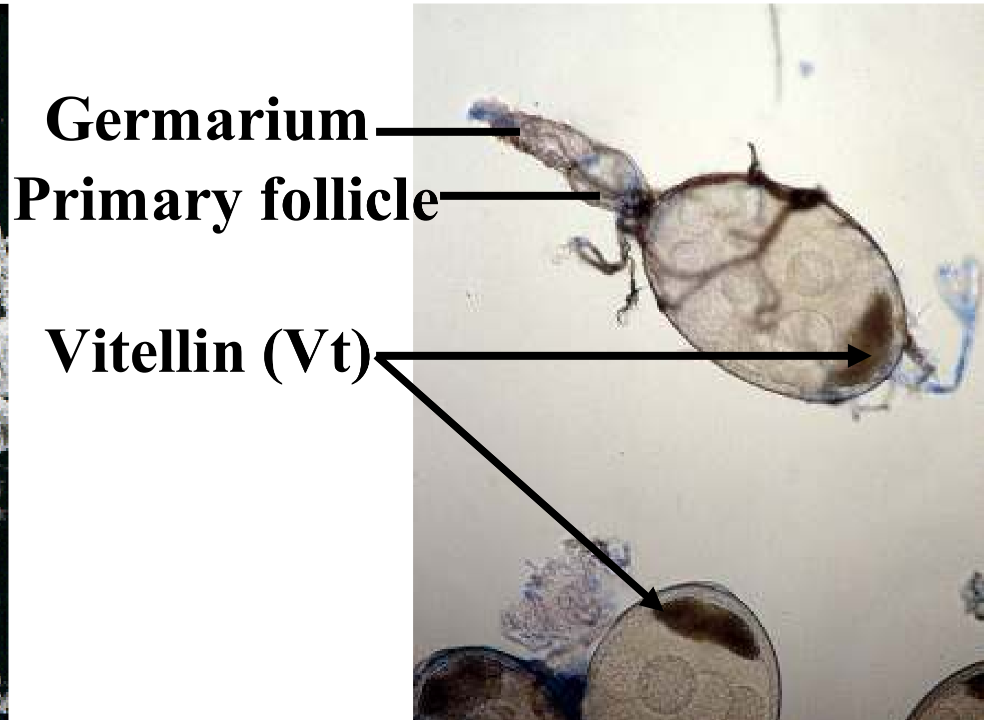
WHERE IS VITELLIN (Vt) PRODUCED AND FOUND?

UNDER WHAT CONDITIONS ARE EITHER PRODUCED?

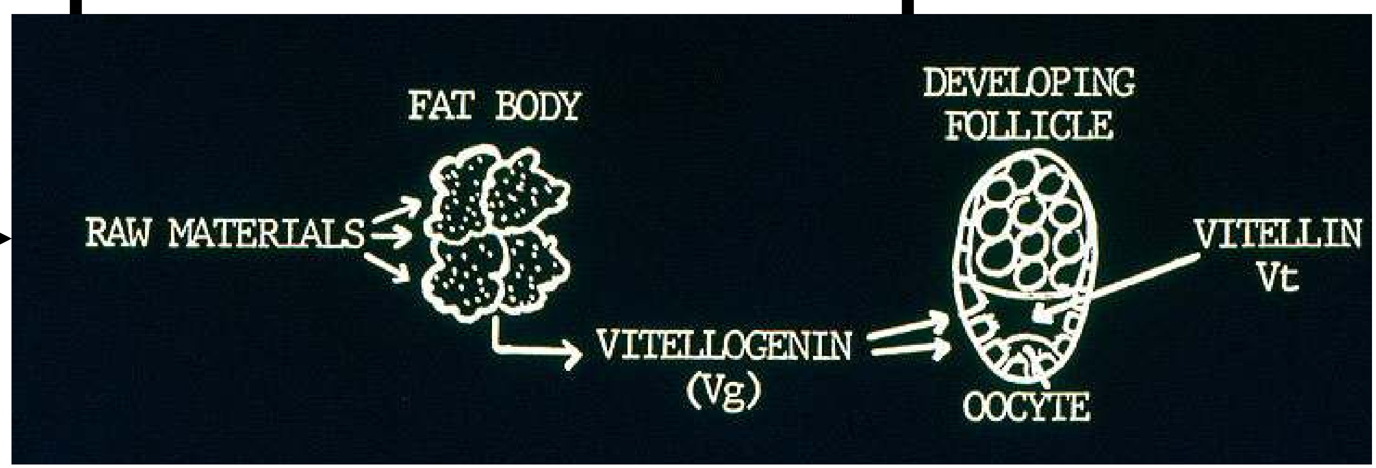
TABLE 1. ANALYSIS OF VARIOUS TISSUES AND HEMOLYMPH OF 48 HR ADULT PHORMIA REGINA FED SUGAR AND WATER, USING THE BRAMHALL AND ELISA TECHNIQUES FOR TOTAL PROTEIN AND VITELLIN RESPECTIVELY

TISSUE	TOTAL PROTEIN	VITELLIN/Vg
M-HEMO.	2.1 UG/UL	ND
M-HEMO. (24 HR-LF)	24.0 UG/UL	ND
F-BRAIN	7.1 UG/INSECT	ND
F-MIDGUT	41.0 UG/INSECT	ND
F-MALPIGHIAN TUBULES	10.0 UG/INSECT	ND
F-HEMO.	1.7 UG/UL	ND
F-HEMO. (28 HR-LF)	40.0 UG/UL	17 UG/UL
F-FAT BODY	5.3 UG/MG	ND
F-FAT BODY (24 HR-LF)	17.4 UG/MG	.89 UG/MG F.B.
F-OVARY	2.8 UG/OVARY	ND
F-OVARY (24 HR-LF)	175.0 UG/OVARY	7.5 UG/OVARY

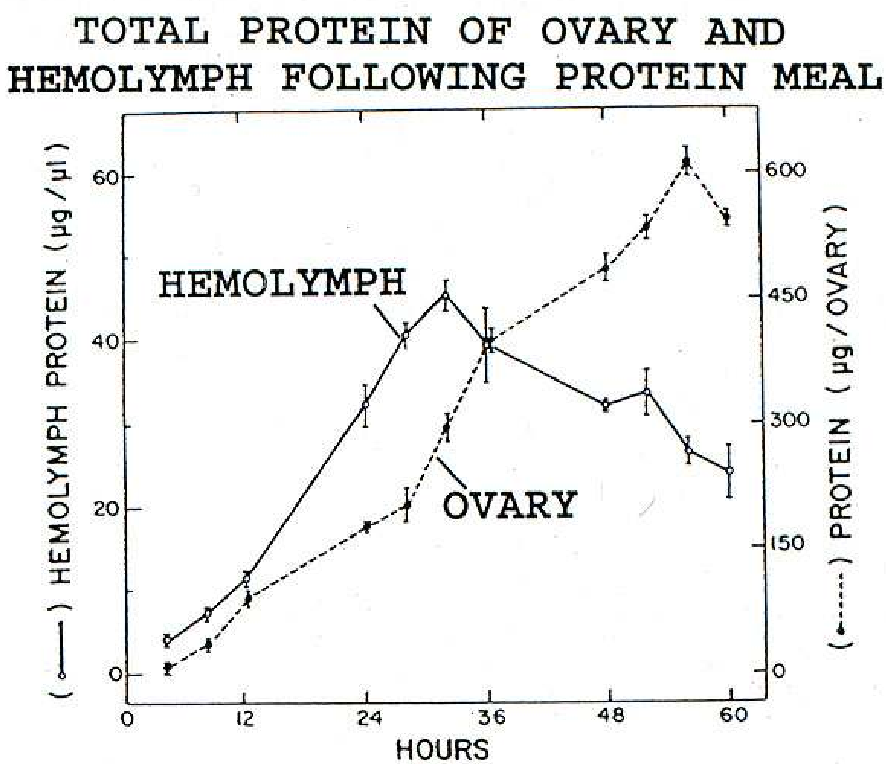


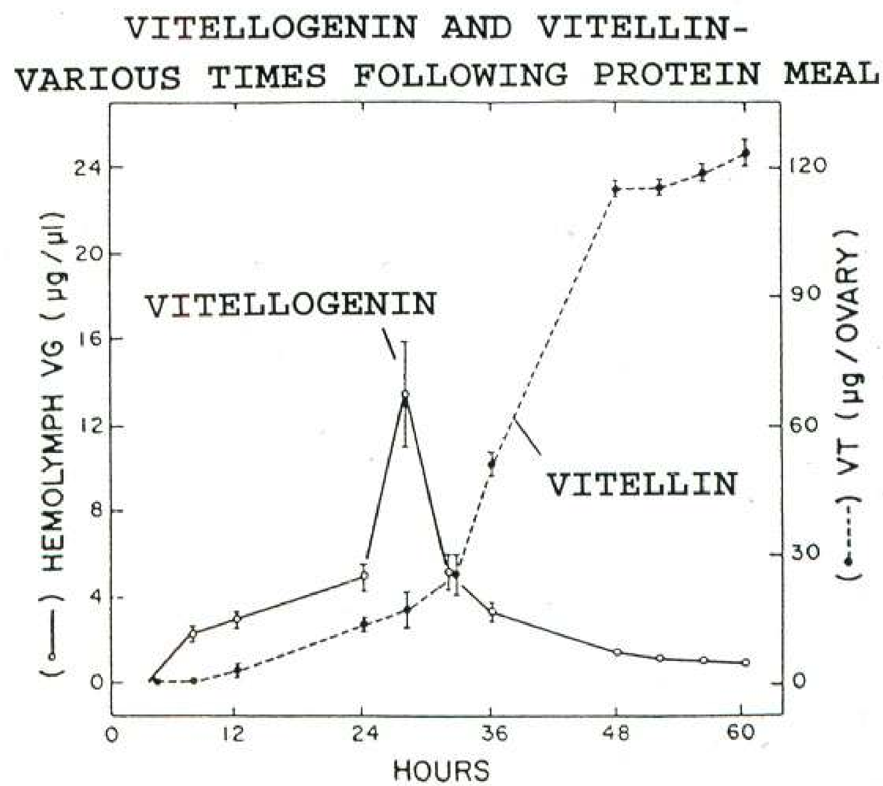


In *P. regina,* ecdysteroids from the ovary act on fat body to produce Vg



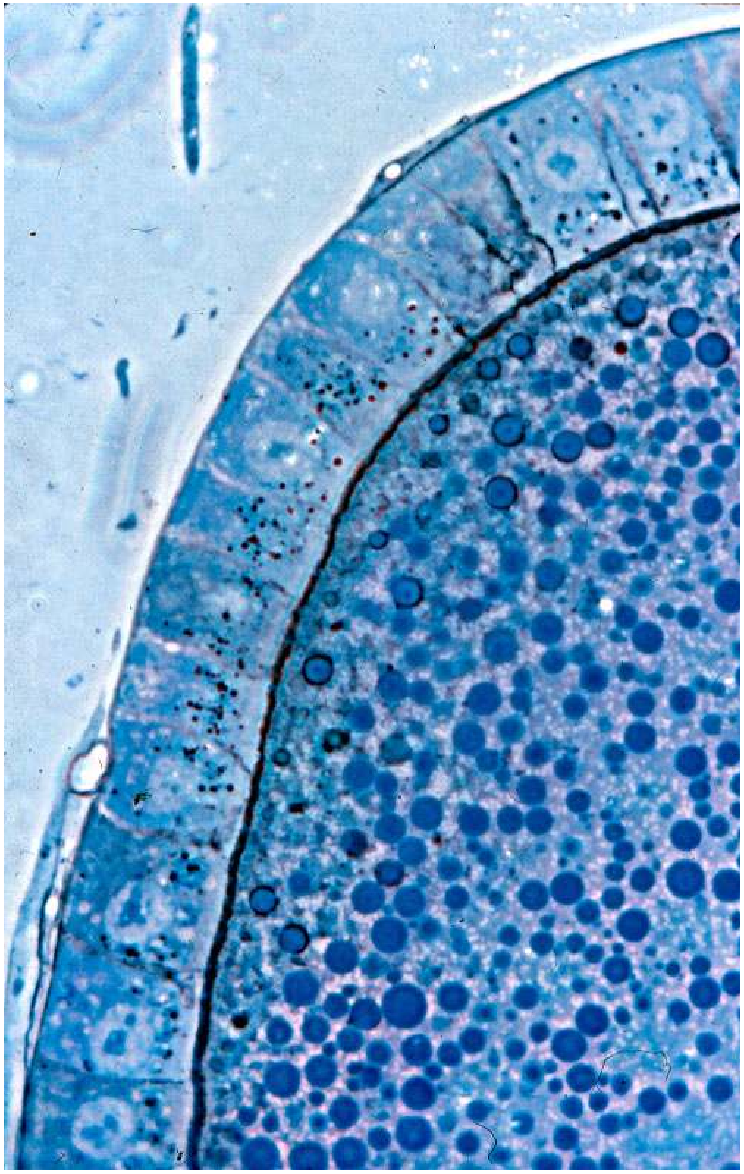
Concentration profiles of protein and Vg/Vt in the hemolymph and ovaries of *Phormia regina* at various times following a full protein meal.





C. HORMONAL CONTROL & INVOLVEMENT IN OOGENESIS

1. THE ROLE OF JH
   a. Induces patency in the follicles and uptake of Vg by the follicle cells and oocyte
2. THE ROLE OF ECDYSTEROIDS
   a. Turn on fat body to produce Vg or regulates Vg biosynthesis



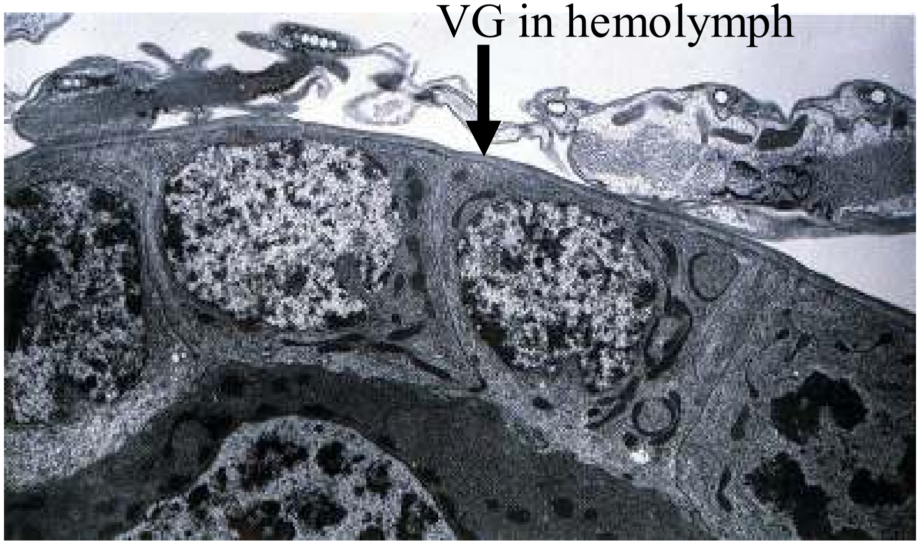
Hagedorn's story on finding ecdysteroids in the ovary of the mosquito

HOW DOES VITELLOGENIN (VG) GET INTO AN EGG THAT IS SURROUNDED BY A CONTINUOUS LAYER OF FOLLICLE CELLS, KNOWN AS THE FOLLICULAR EPITHELIUM?

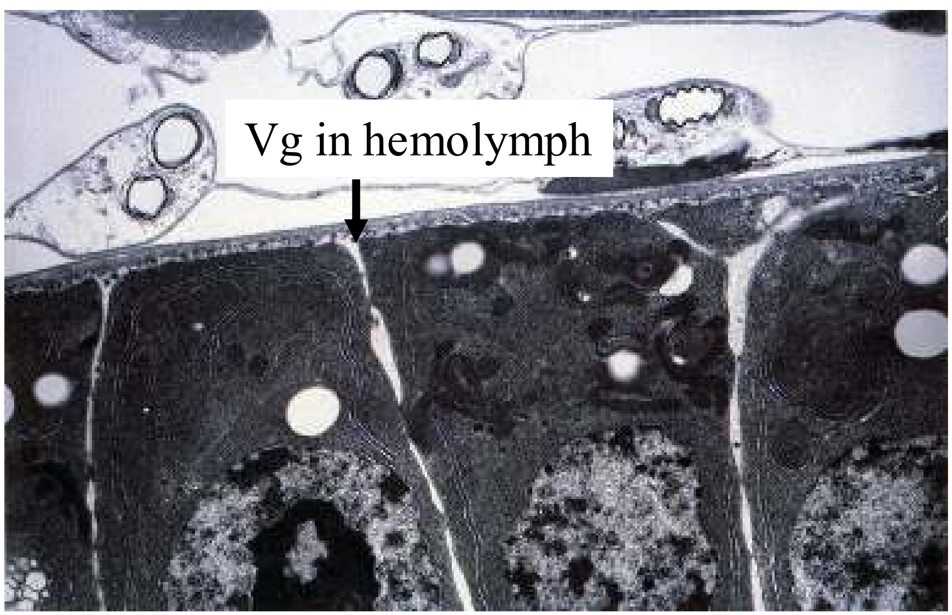
AND

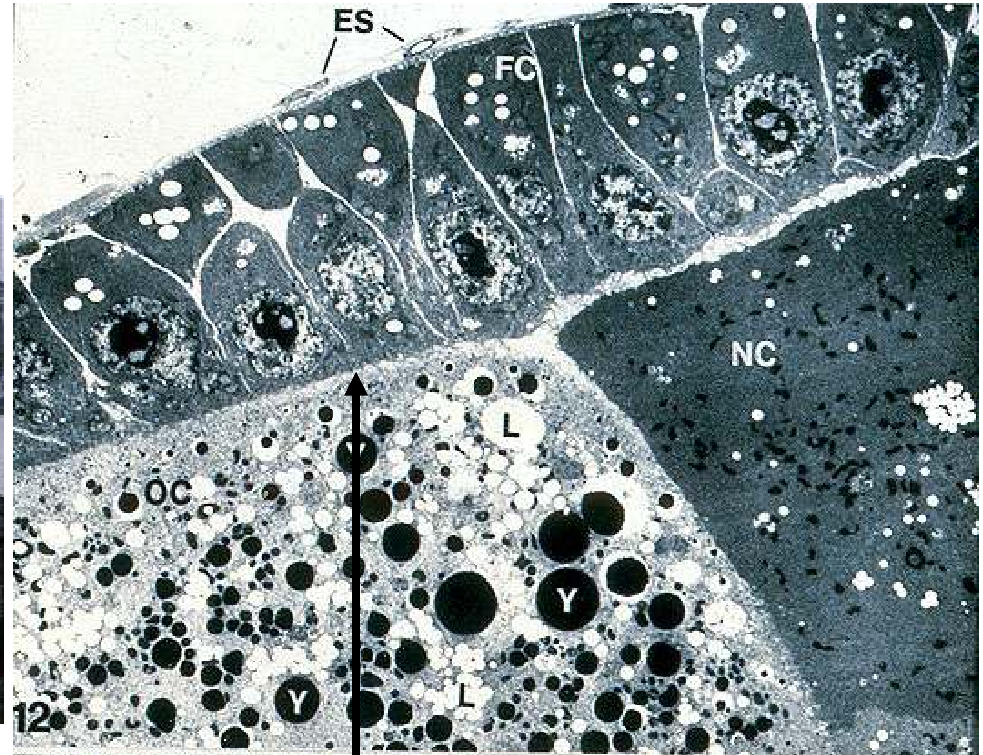
WHAT IS PATENCY?

Follicle cells with no JH



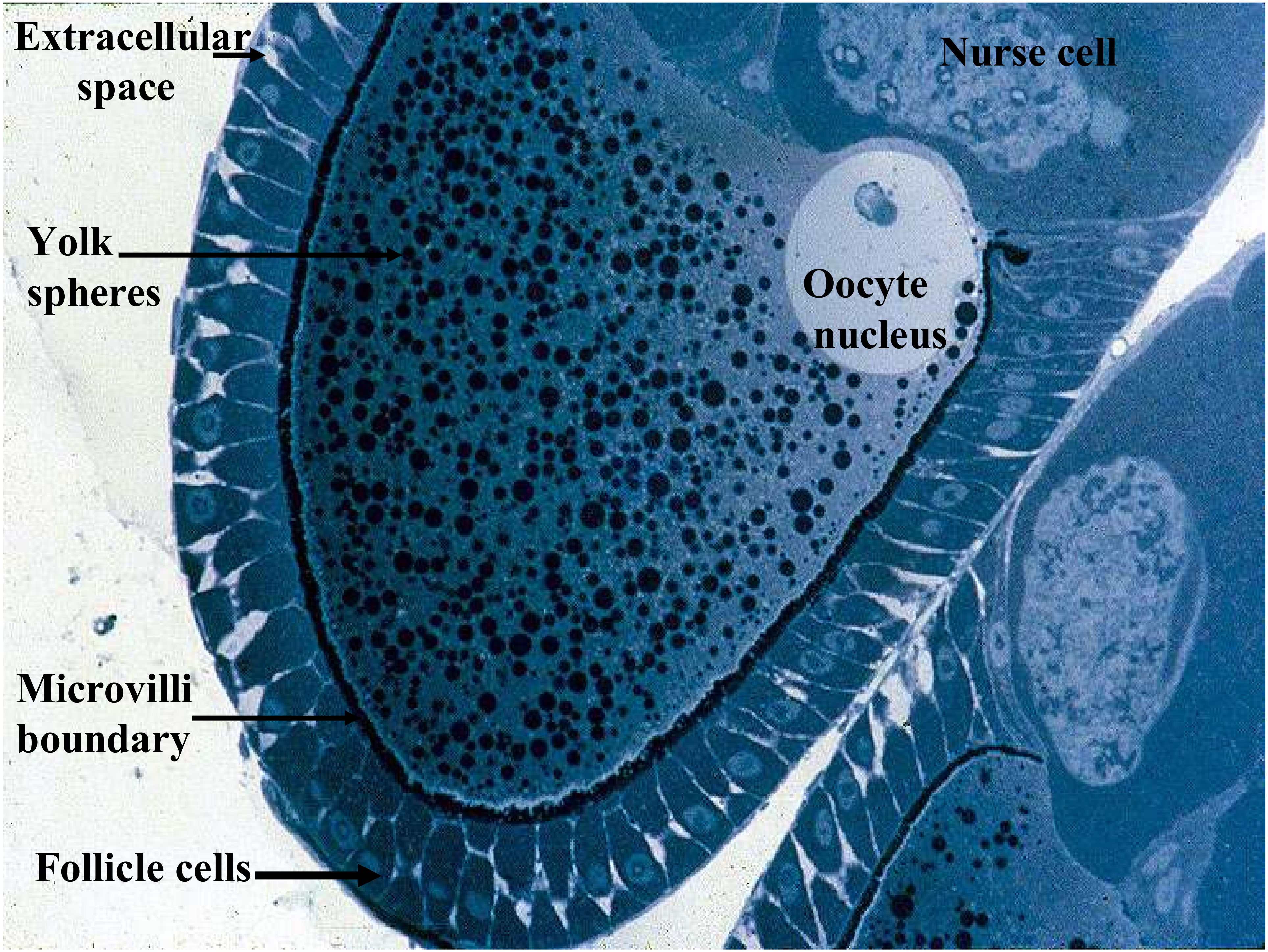
Follicle cells with JH

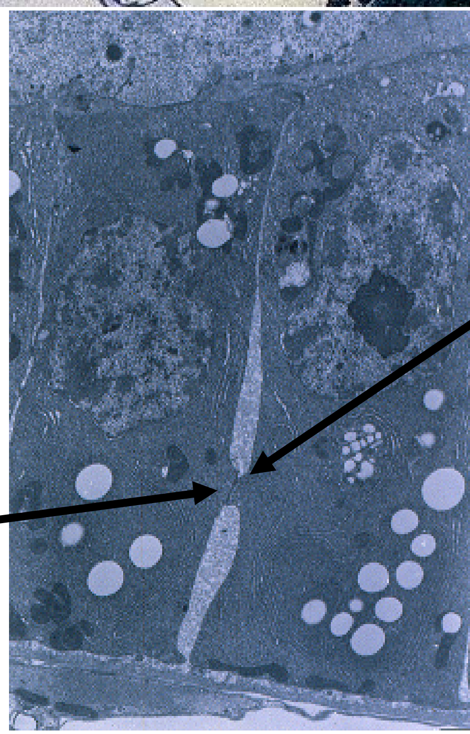


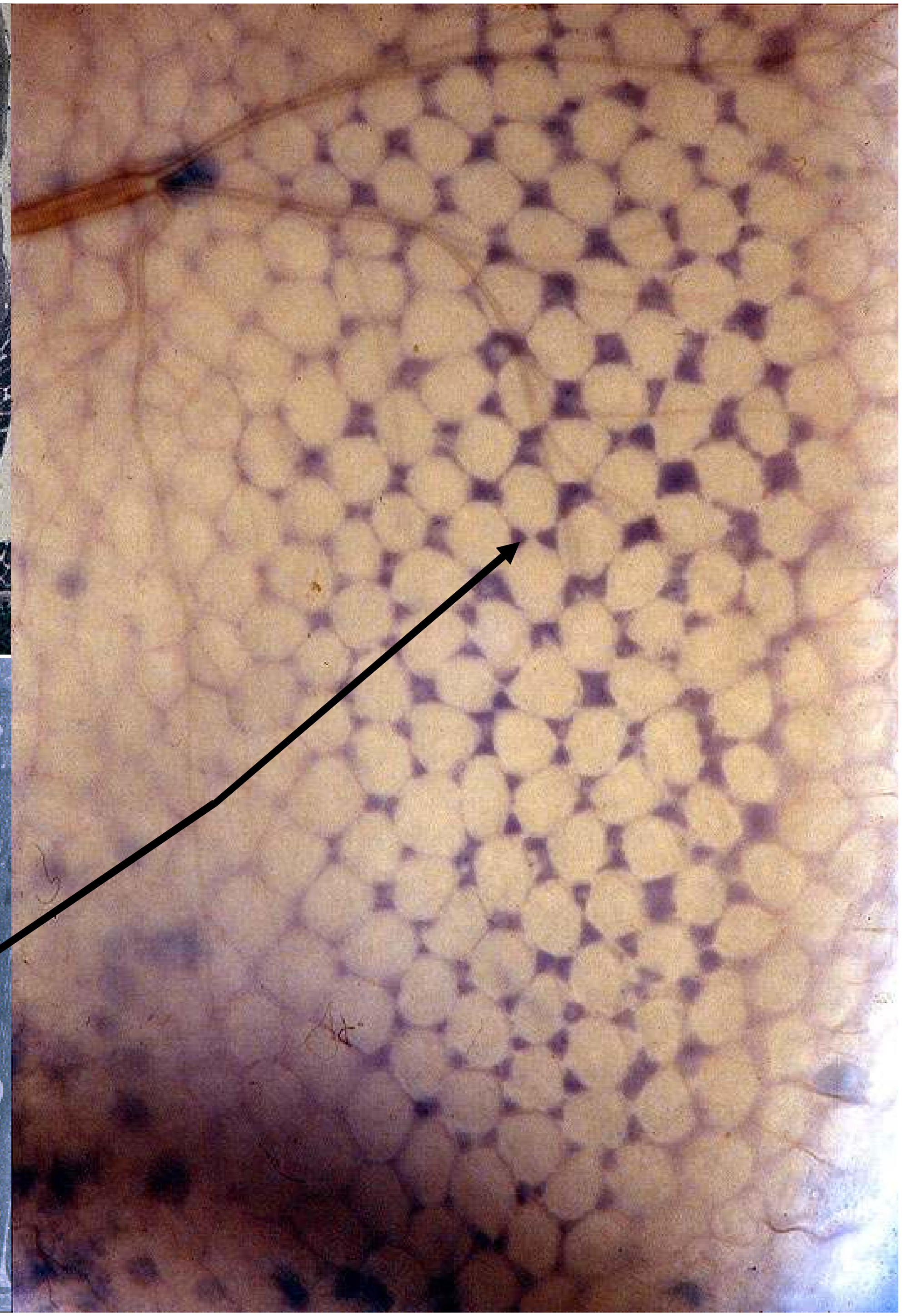


Another membrane to cross. How does the VG do it?

Once the VG molecules cross the barrier of the follicle cells they must get into the oocyte by crossing the oocyte membrane.

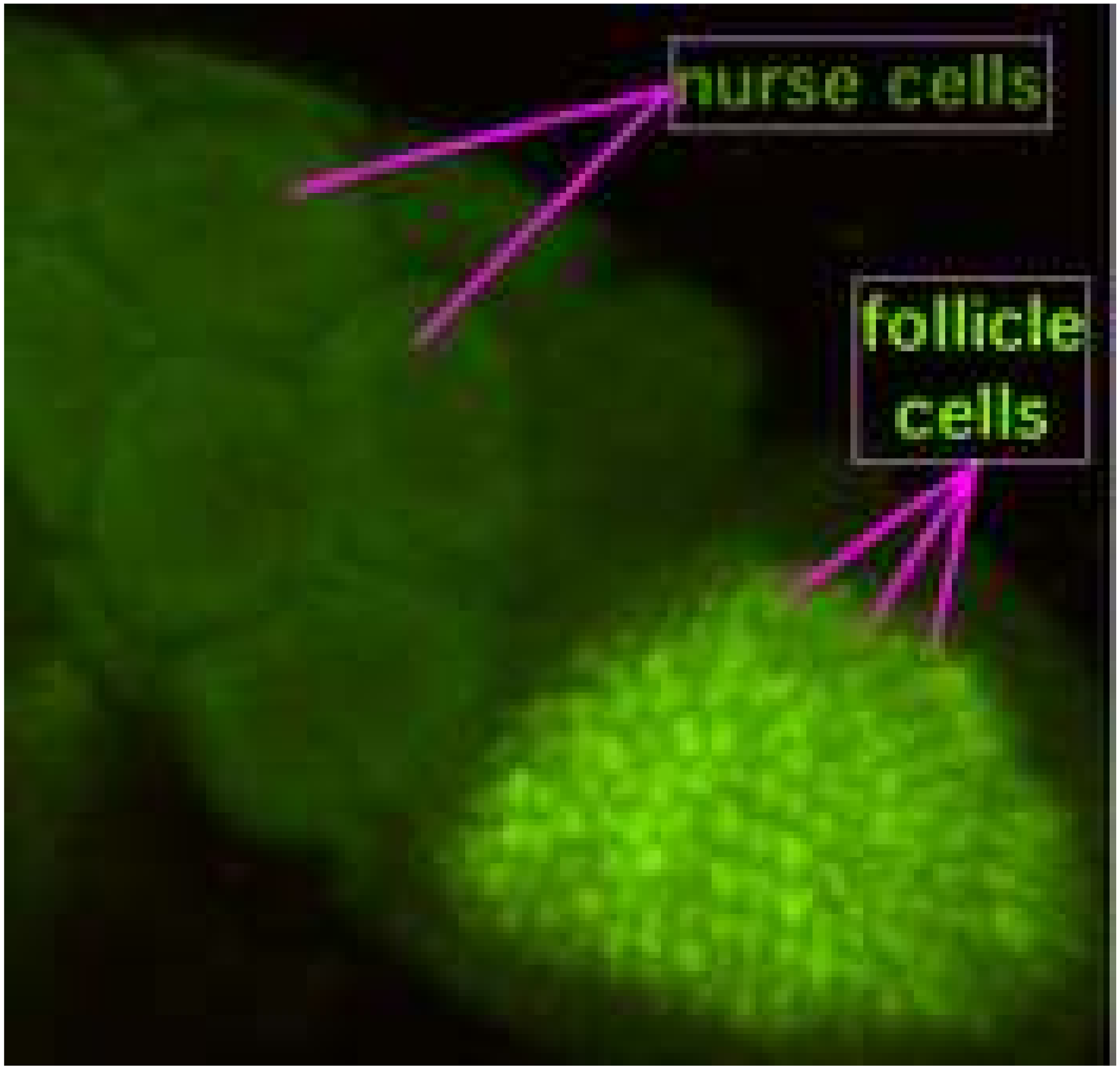

Extracellular
space
Nurse cell
Yolk
spheres
Oocyte
nucleus
Microvilli
boundary
Follicle cells





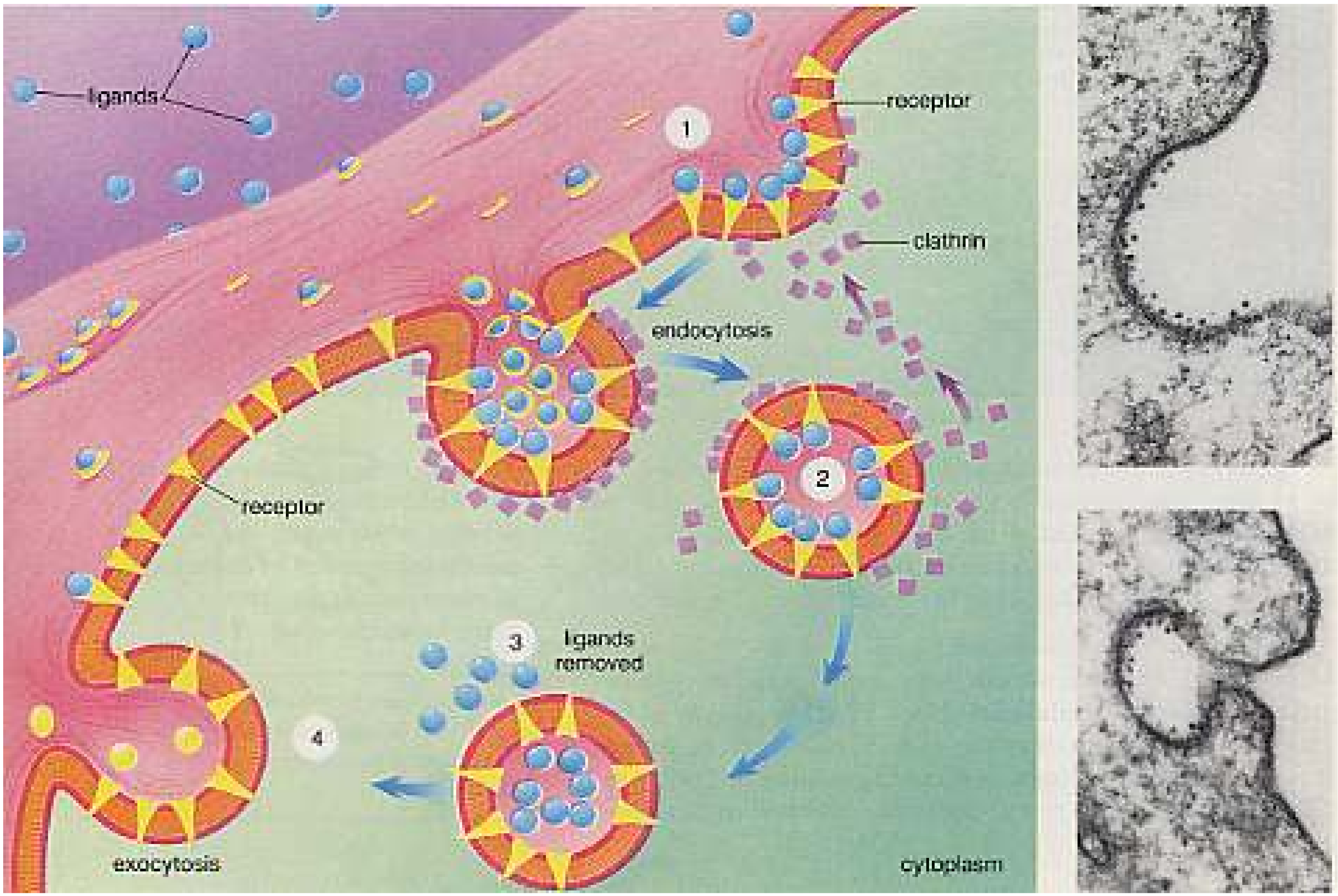
How is this layer of follicular epithelia held together and how do the cells communicate with one another, especially once patency occurs?

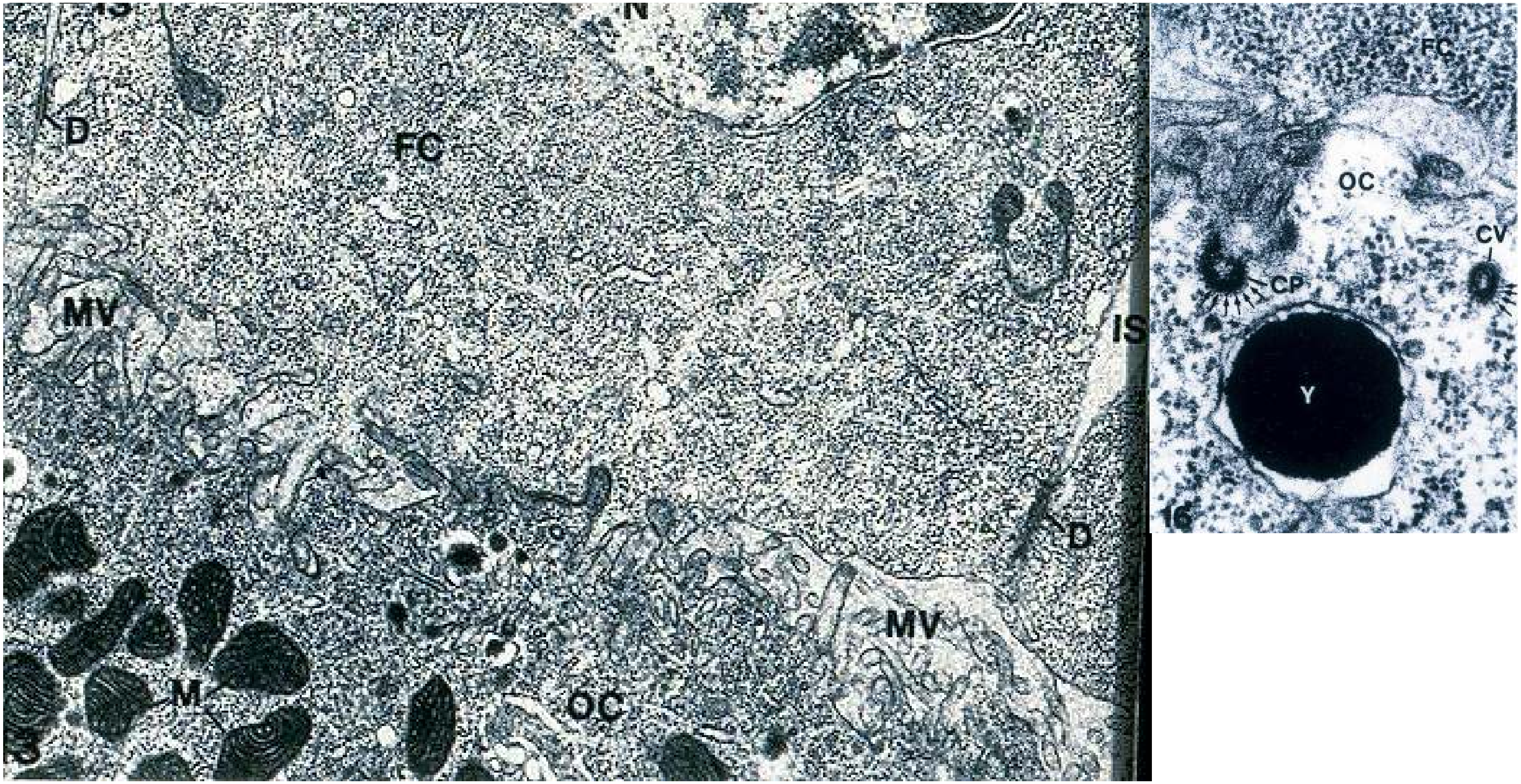
Interfollicular bridges



http://www.biocrawler.com/encyclopedia/Drosophila_melanogaster

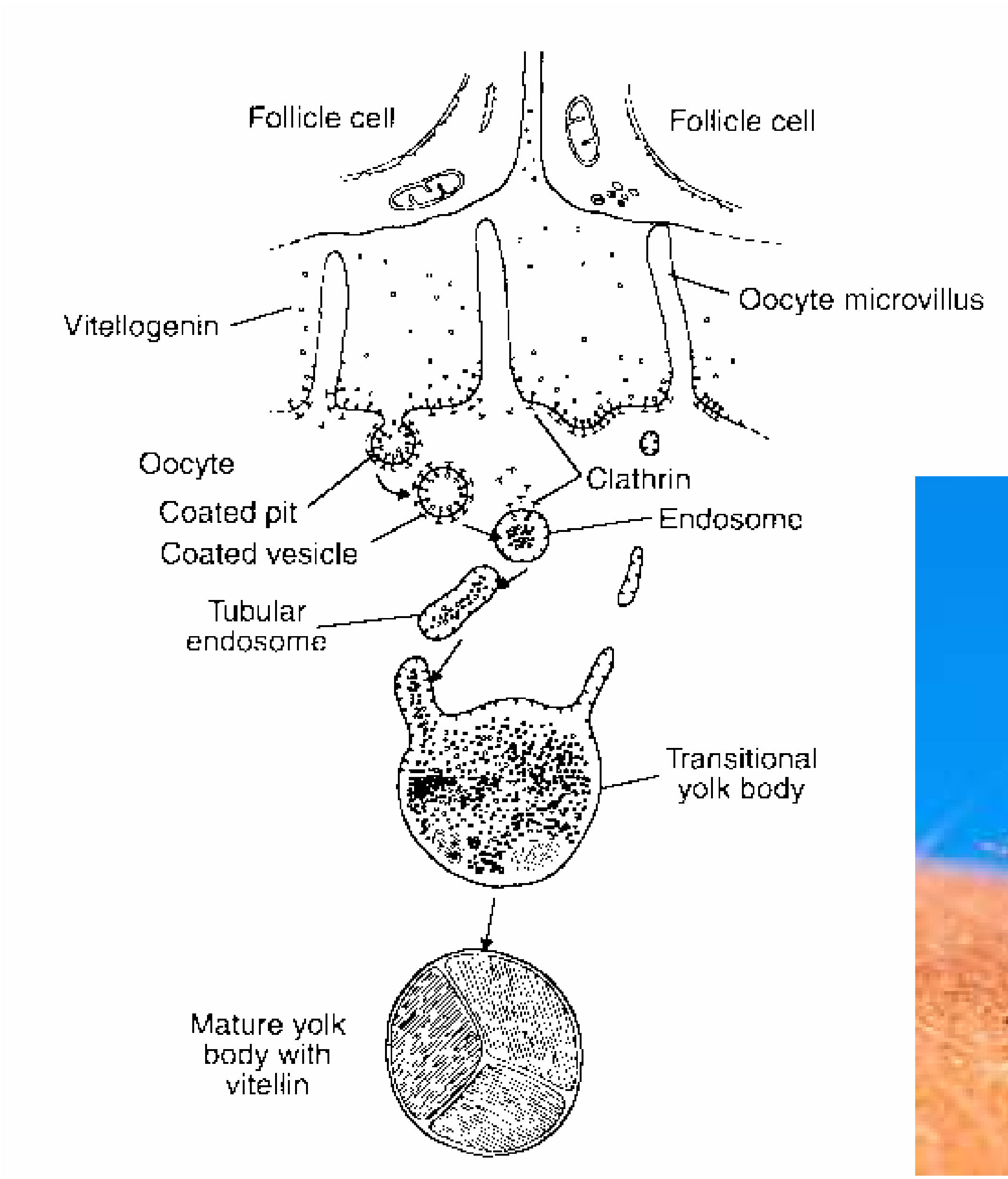
Receptor mediated endocytosis. A process involving clathrin, a molecule that surrounds the molecules being taken in and forms what are called coated pits. Eventually these pits pinch off and the molecules are internalized.



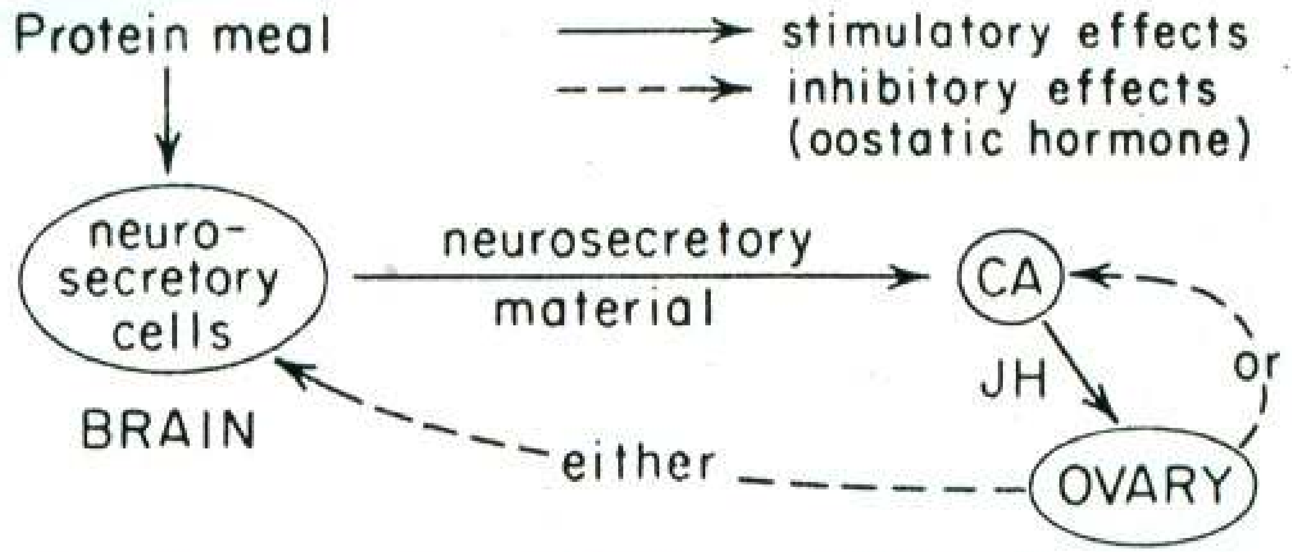


TEM showing how VG in a developing follicle of *Phormia regina* is taken up by receptor mediated endocytosis. Microvilli of the follicle cells and oocyte membrane form and intermingle. The formation of coated pits is essential for Vg uptake, which later fuse to form larger yolk spheres (now called Vt)

Receptor mediated endocytosis in the mosquito oocyte



HORMONAL REGULATION OF OOGENESIS IN *Phormia*



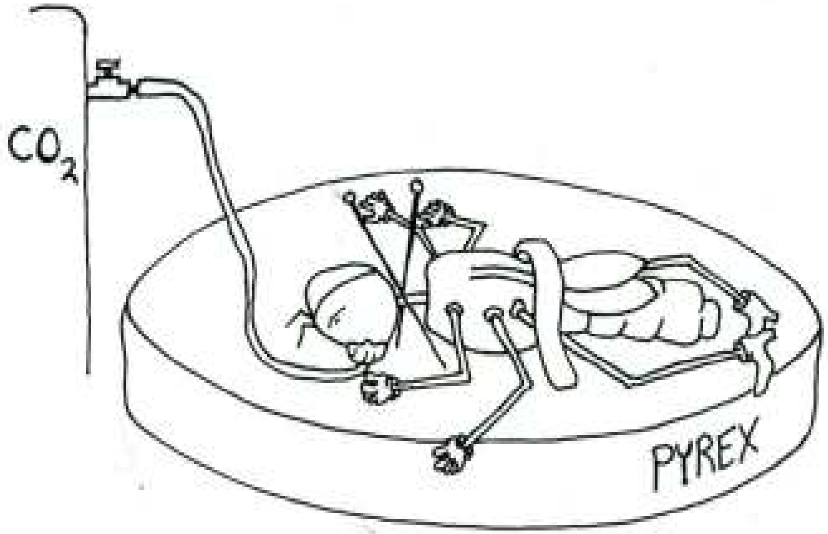
JH compensates for inhibition of oogenesis by early NSC⁻ or CA⁻

JH overrides effect of oostatic hormone

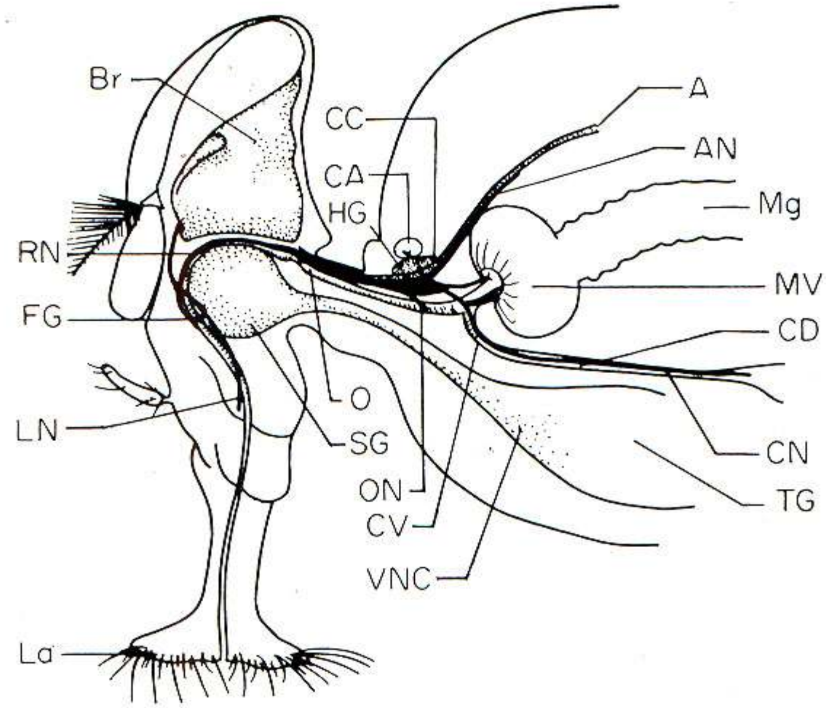
JH stimulates oogenesis in absence of protein meal

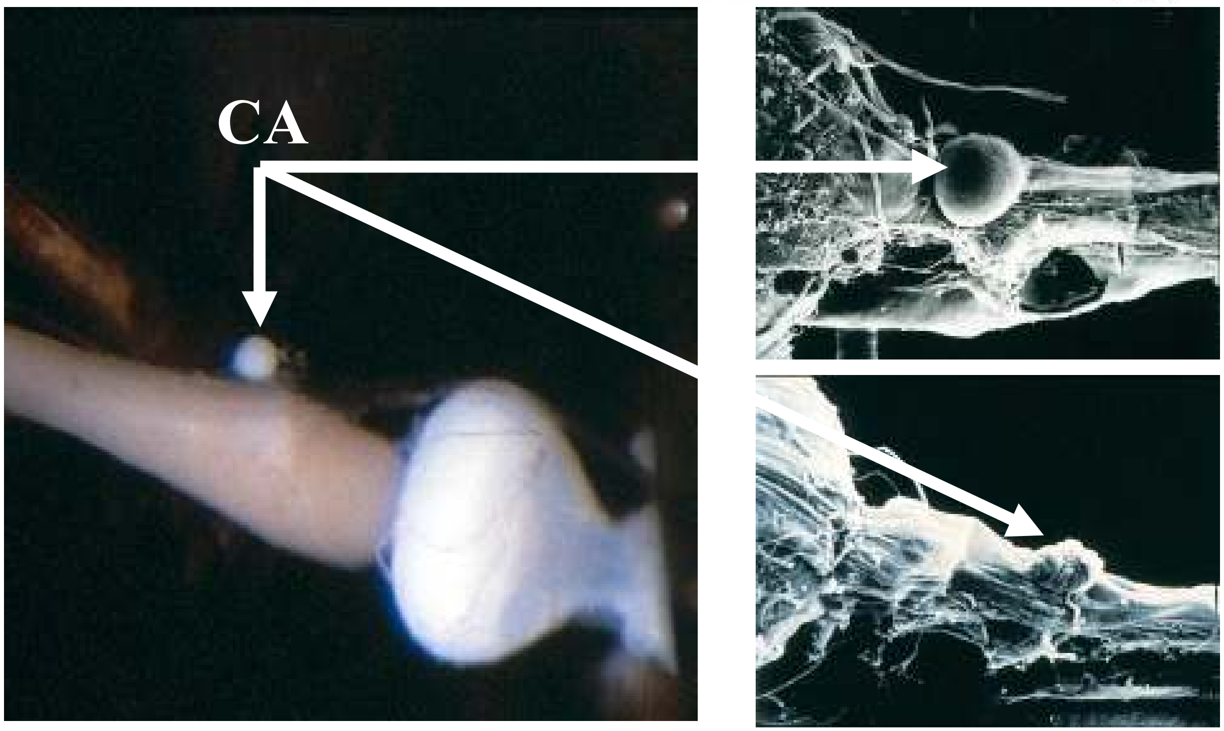
Fig. 7. Scheme of the effects and interactions of a protein meal, neurosecretory hormone, JH, and oöstatic hormone in the regulation of oögenesis in *Phormia regina*.

SCHEME PROPOSED BY G. FRAENKEL (1940)



CORPORA ALLATA AND OVARY REMOVAL





The CA in *Phormia* can be shut down or removed in two ways:

1. Surgical removal
2. Treatment with precocene (see bottom left photo)

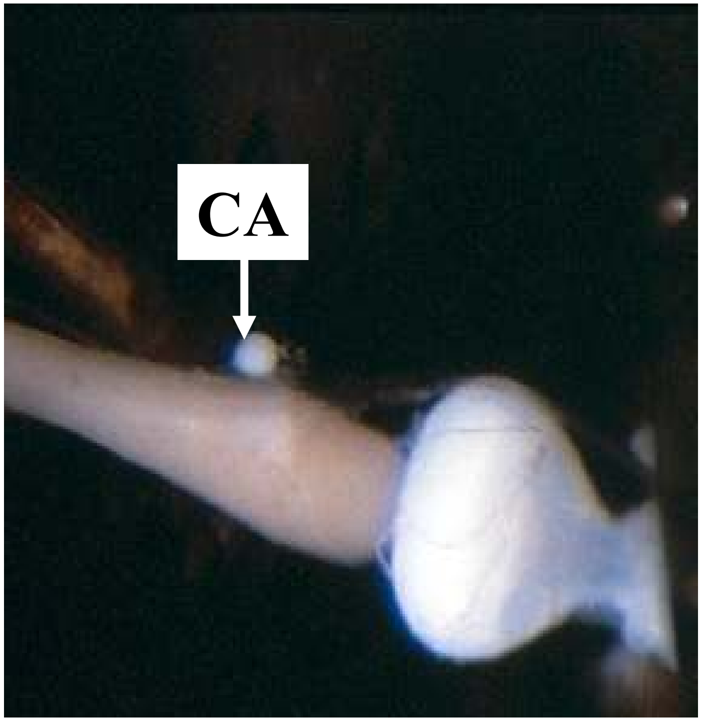


TABLE 4. The effect of diet on the incorporation (mean ±SE) *in vitro* of [^{3}H]methionine by CC–CA from 3-day-old females of *P.regina* under different feeding conditions.*

Dietary condition	Incorporation† (pmol/gland/h)
Sugar–water (SW)	0.07±0.01[b]‡
SW+liver§	0.86±0.09[a]

* Two CC–CA complexes were incubated in 50 μl MEM containing 0.05 mM methionine (pH 7.0) at 26°C for 4 h.

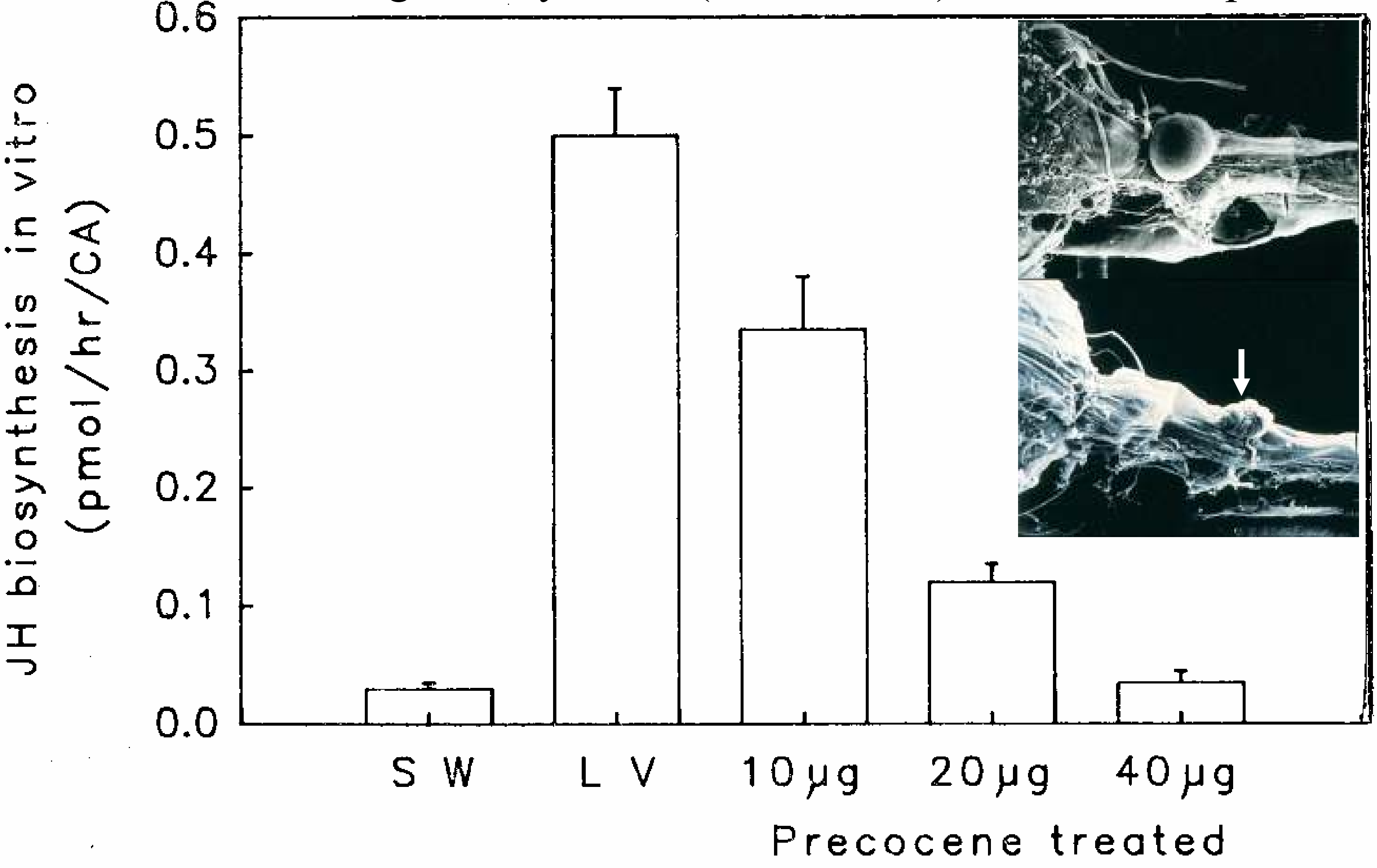
† Each mean represents twenty-two replicates. See Table 2, third footnote.

‡ Means followed by different letters are significantly different ($P<0.05$).

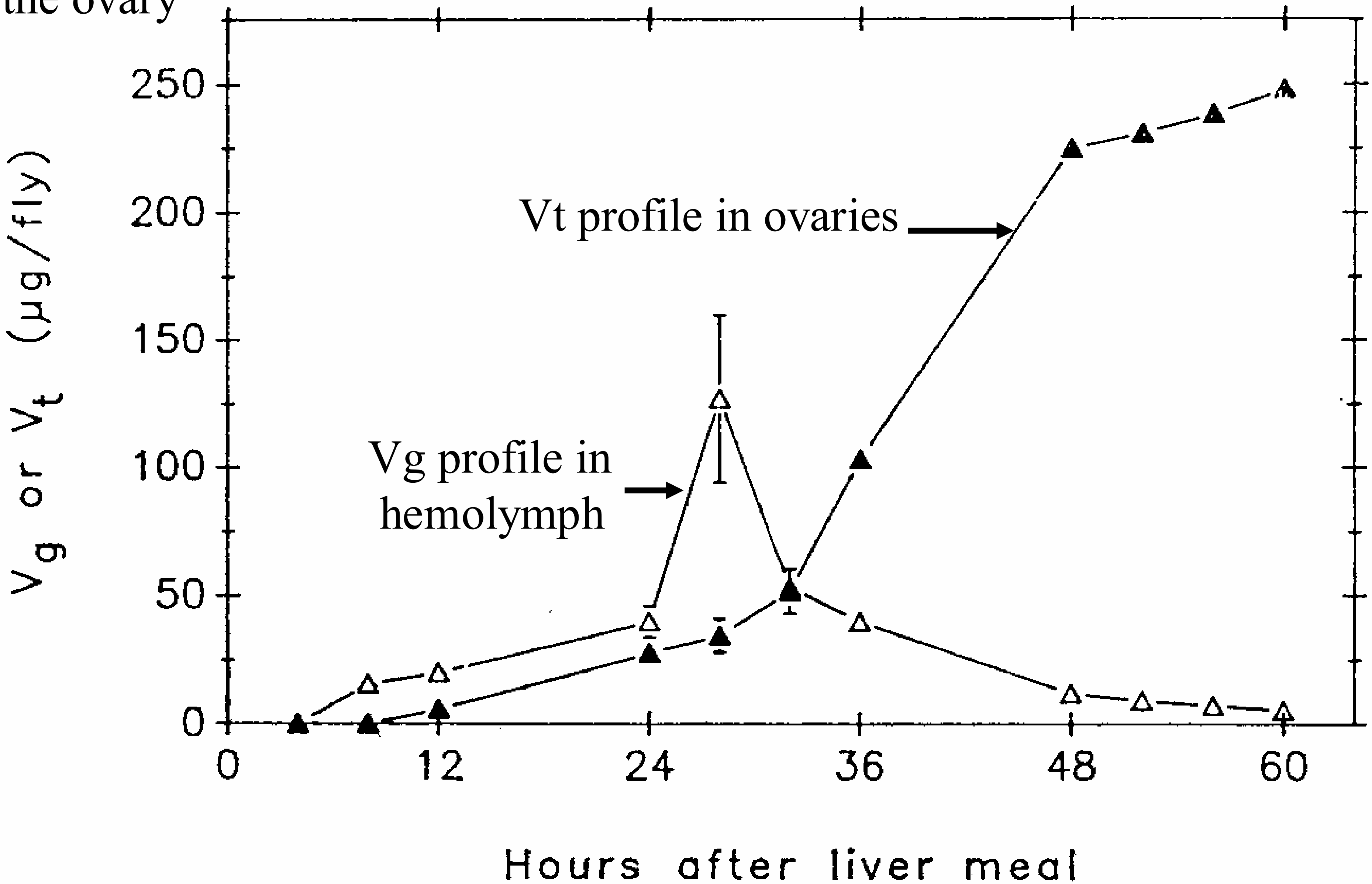
§ Flies were fed liver for 24 h starting on the second day.

Removal of the CA/CC complex and putting it into a medium containing radiolabelled methionine as the methyl donor to the JH and incubating it. Following incubation, one is able to evaporate and concentrate the medium and count what remains in a scintillation counter. Using this procedure, it is possible to determine the rate of incorporation or biosynthesis of the CA under different conditions. Note above that liver fed flies were significantly different from sugar fed flies.

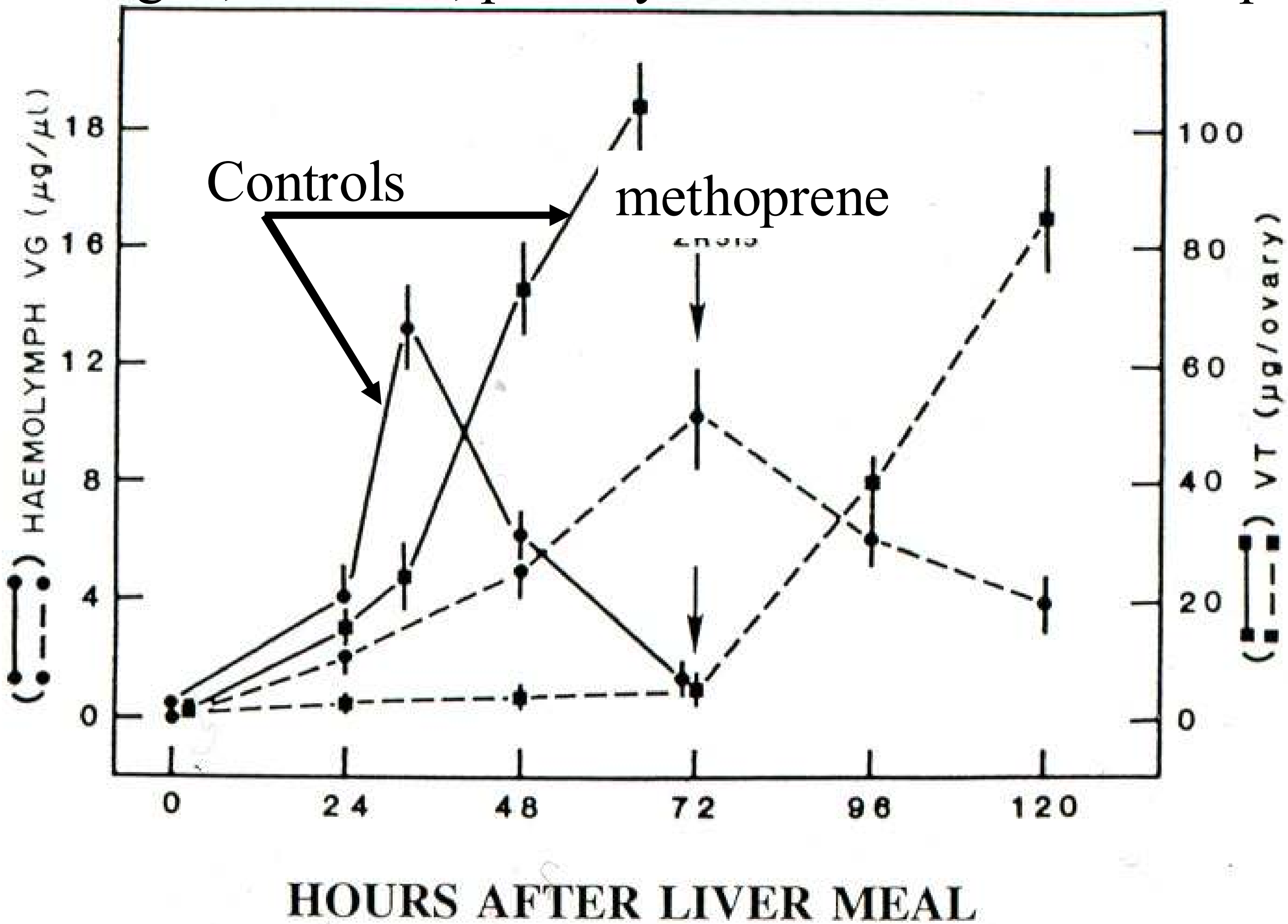
Precocene treatment of liver-fed *Phormia* prevents the production of JH biosynthesis. It does this by selectively killing the CA cells. See bottom slide showing destroyed CA (white arrow). Normal on top.



Profiles of Vg in the hemolymph and Vt in the ovaries of *Phormia regina*. Notice as the Vg in the hemolymph drops its conc. increases in the ovary

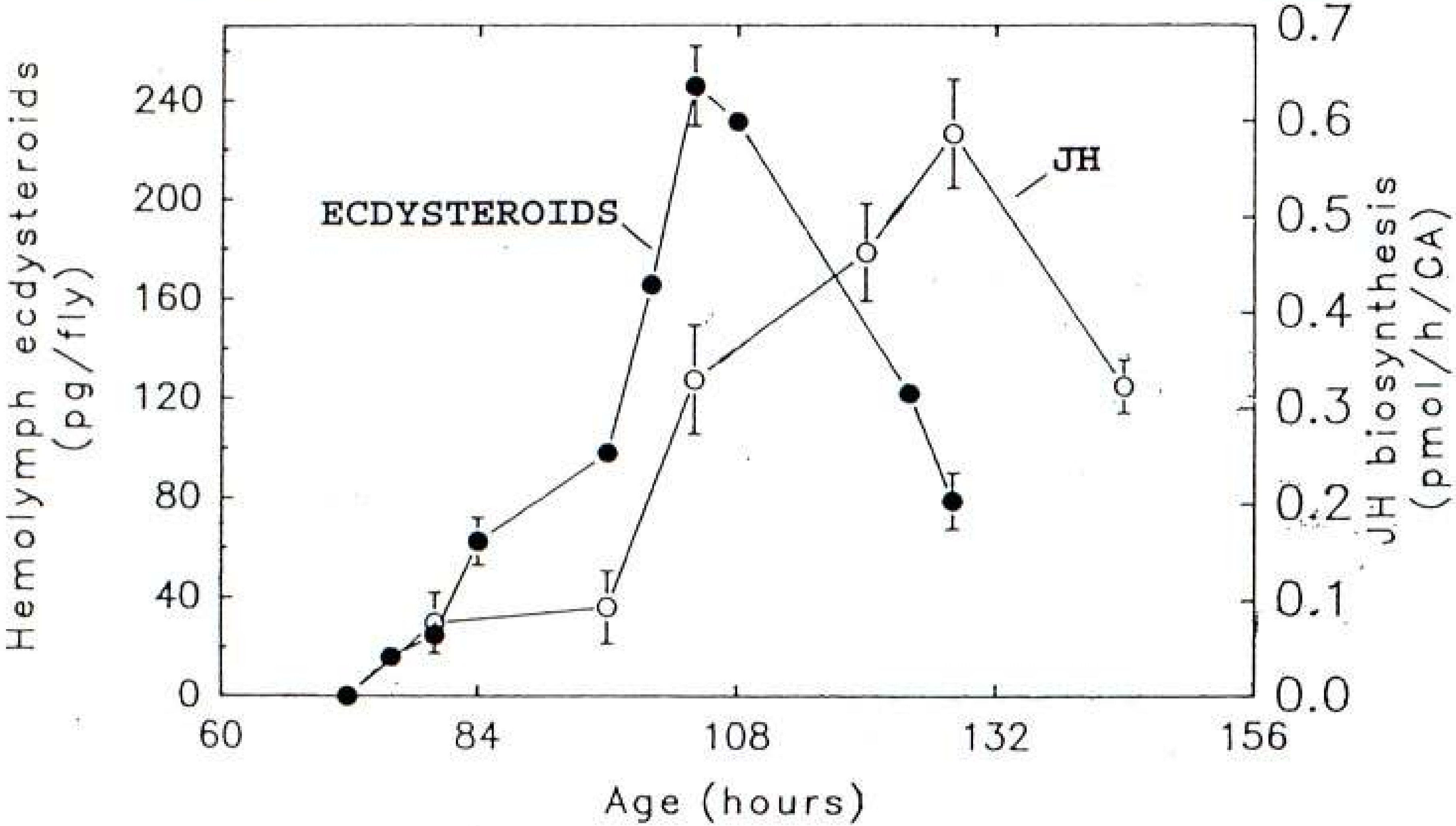


Effects of removing the CA on egg development in *Phormia regina* and rescuing the process by topical application of methoprene. Notice in the precocene treated (hatched lines) that the Vg conc. increases in the hemolmph but Vt in the ovaries does not. Once treated with methoprene, JH analogue, however, patency occurs and Vt is taken up.



Using a RIA for ecdysteroids and the radiolabelled methione, CA incubation bioassay for JH production, the graphs below were produced for *Phormia regina* following a full protein meal

TOTAL HEMOLYMPH TITER (ECDYSTEROIDS) AND JH BIOSYNTHESIS



D. HOW IS THE NUTRIENT CONTENT OF THE DIET TRANSLATED INTO HORMONAL MESSAGES THAT CONTROL OOGENESIS?

IS IT NEURAL OR HORMONAL? HOW CAN YOU PROVE THIS?

NERVE TRANSECTIONS BEFORE OR FOLLOWING A PROTEIN MEAL (Yin et al. 1993) REVEALED IT WAS NOT NEURAL

? ? ? ? ? ? ? ? ? ? ?

DIET → EGGS PRODUCED

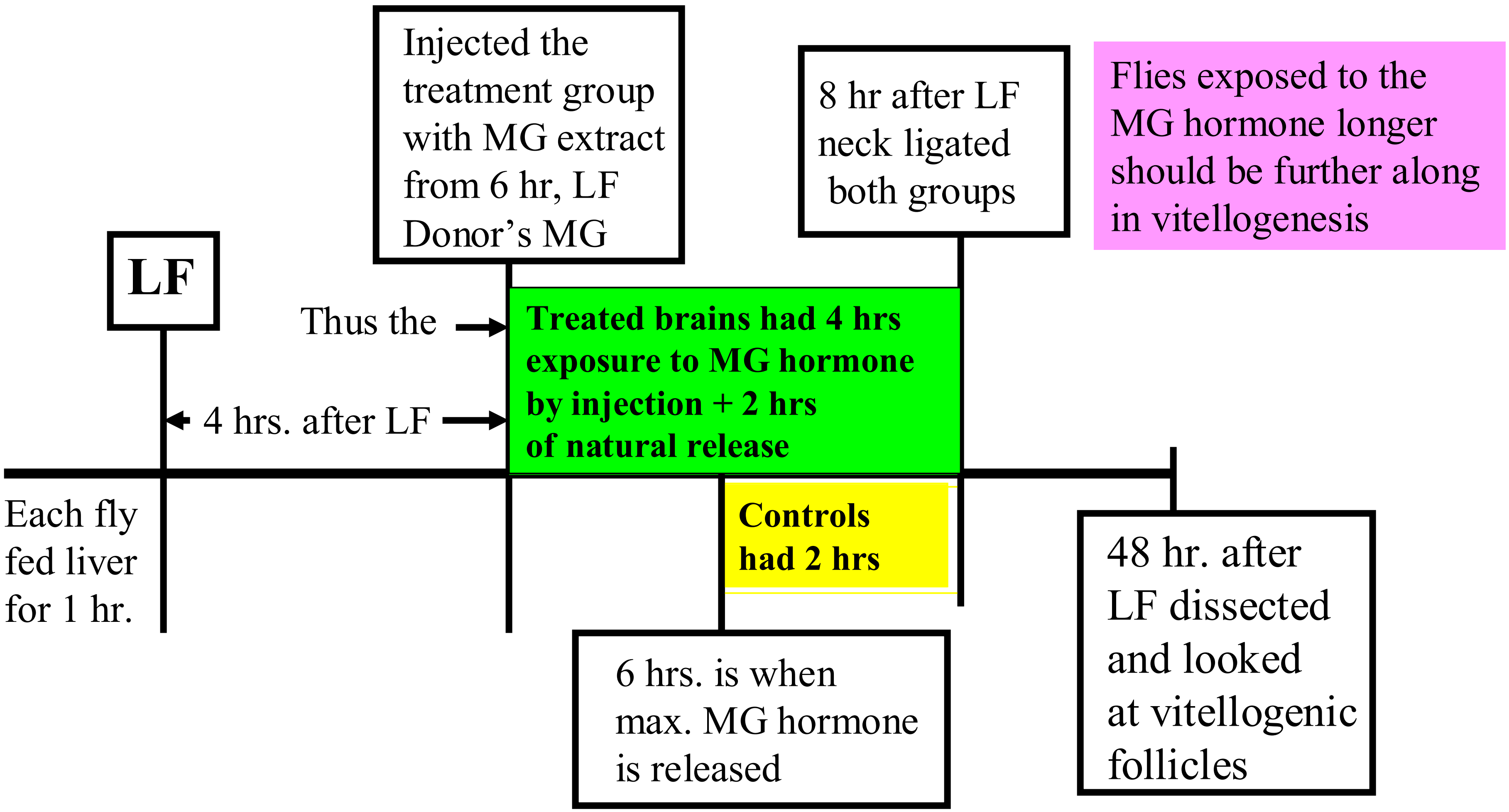
WHY DO INSECTS NEED PROTEIN IN THEIR DIET?

ANSWER-TO PRODUCE EGGS

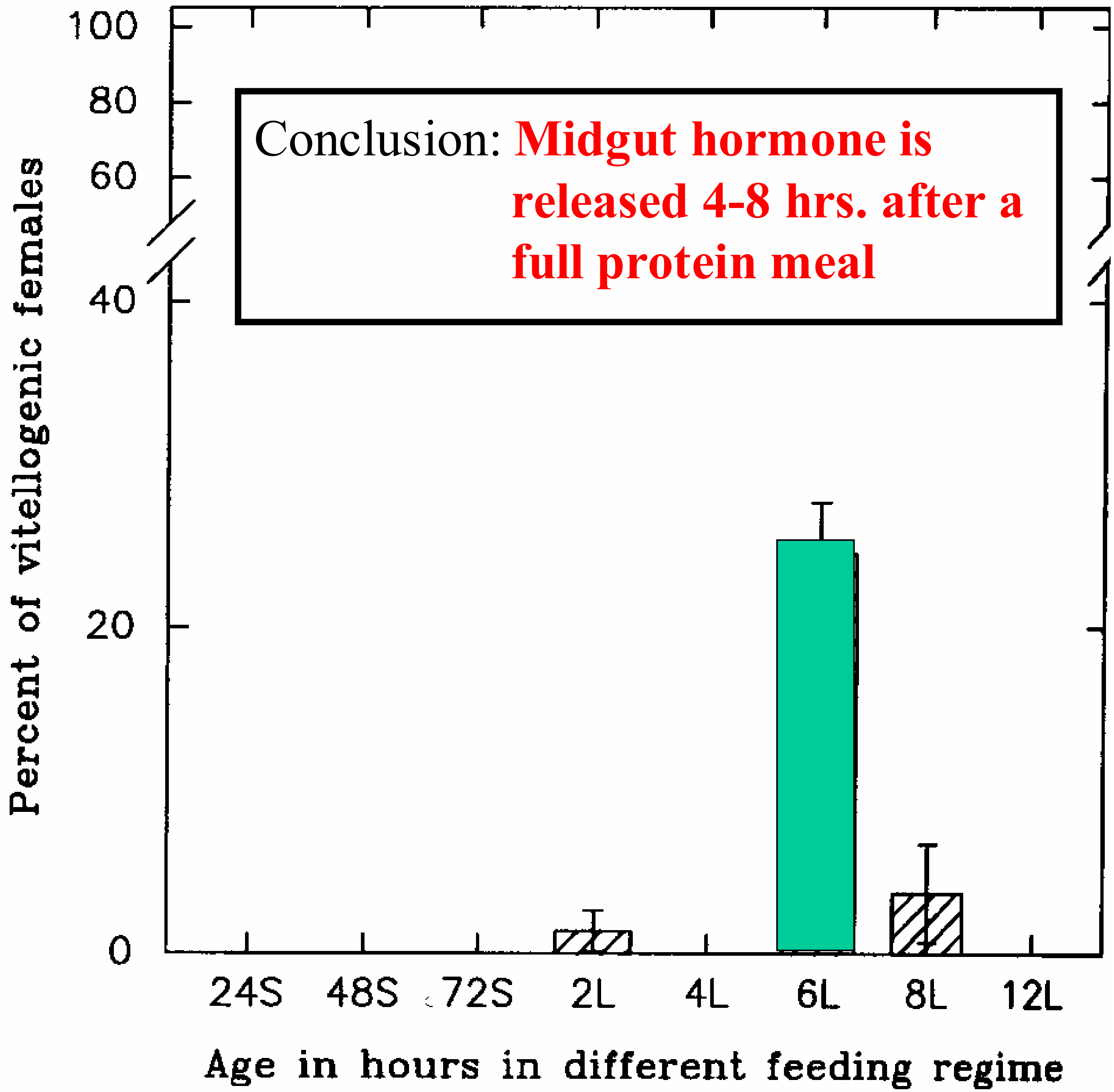
HOW DOES THE DIET AFFECT EGG PRODUCTION?

ANSWER-Probably multiple unknown mechanisms but, what seems the most probable area for nutrients to act?

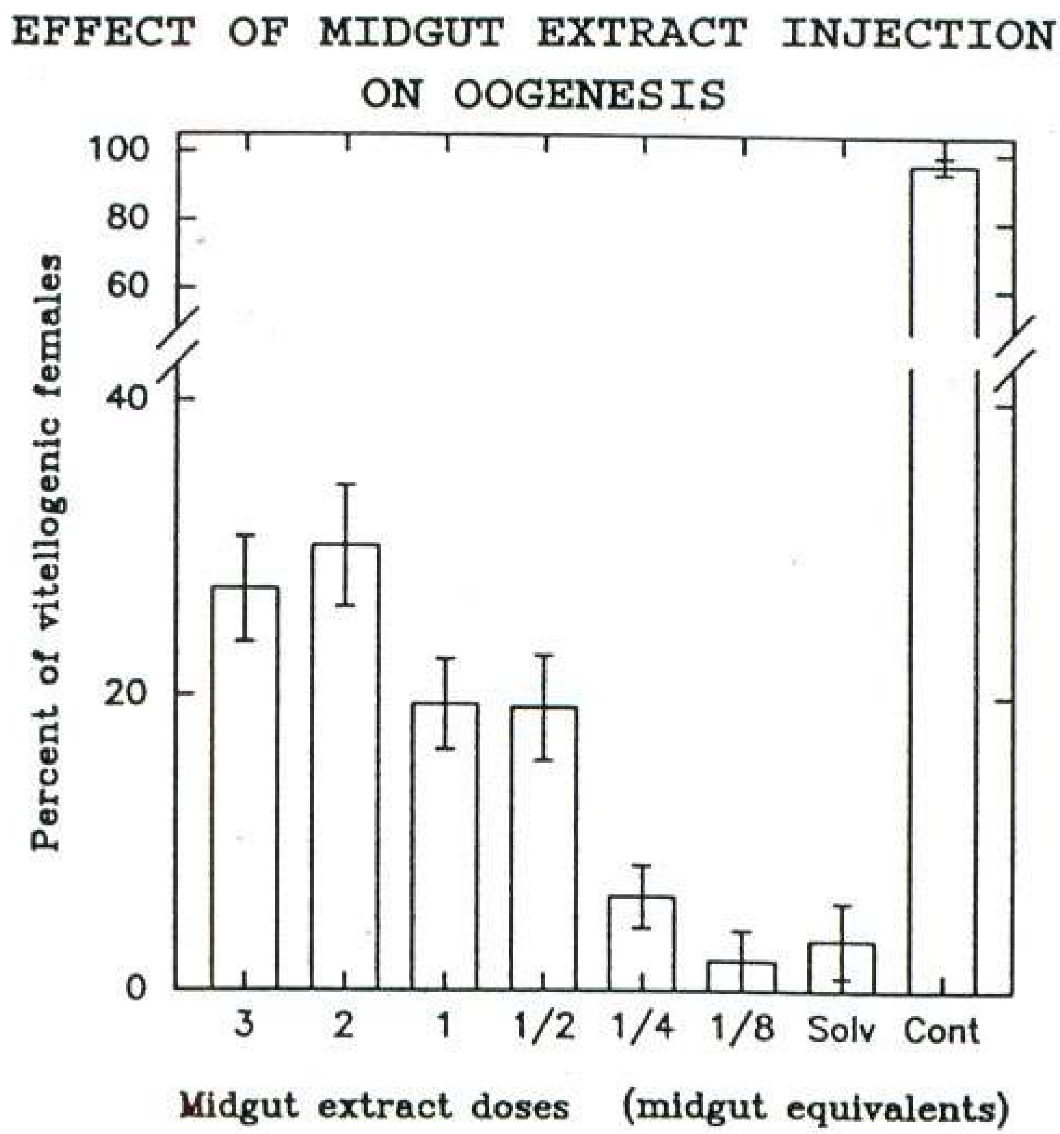
Experimental protocol for demonstrating the bioassay showing the presence of a midgut hormone that is activated by the protein in the gut and having its effect on the brain in *Phormia regina*.



Effect of midgut extracts injections from flies fed sugar or liver and extracted at different times following the liver meal. Note that gut extracts from sugar-fed MG did not assist in producing vitell-ogenic females but liver fed flies did, especially at 6 hrs. post-liver feeding.



Experiments to determine what dose or midgut equilavents were essential for inducing females of *Phormia regina* to produce vitellogenic follicles



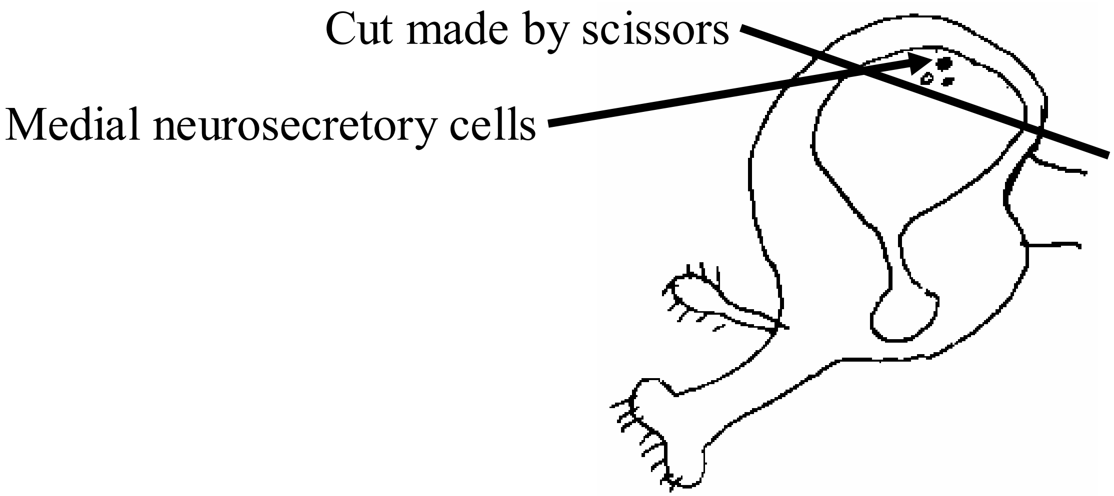
Hsiao and Fraenkel (1966) showed that following a protein meal the median neurosecretory cells in the protocerebrum significantly increased in size suggesting they are involved in the protein meal trigger for starting the neuroendocrine cascade.

Using the PAF (paraldehyde fuschin stain) one does not know the specific nature of the secretion. Need to use specific antibodies to show this.

Photo to the right is the CNR of *P. regina*

Effect of brain hormones + midgut hormone on egg development in *Phormia regina*

In order to test the influence of the brain neurosecretory cells and the midgut factor on oogenesis the following experiment was done.



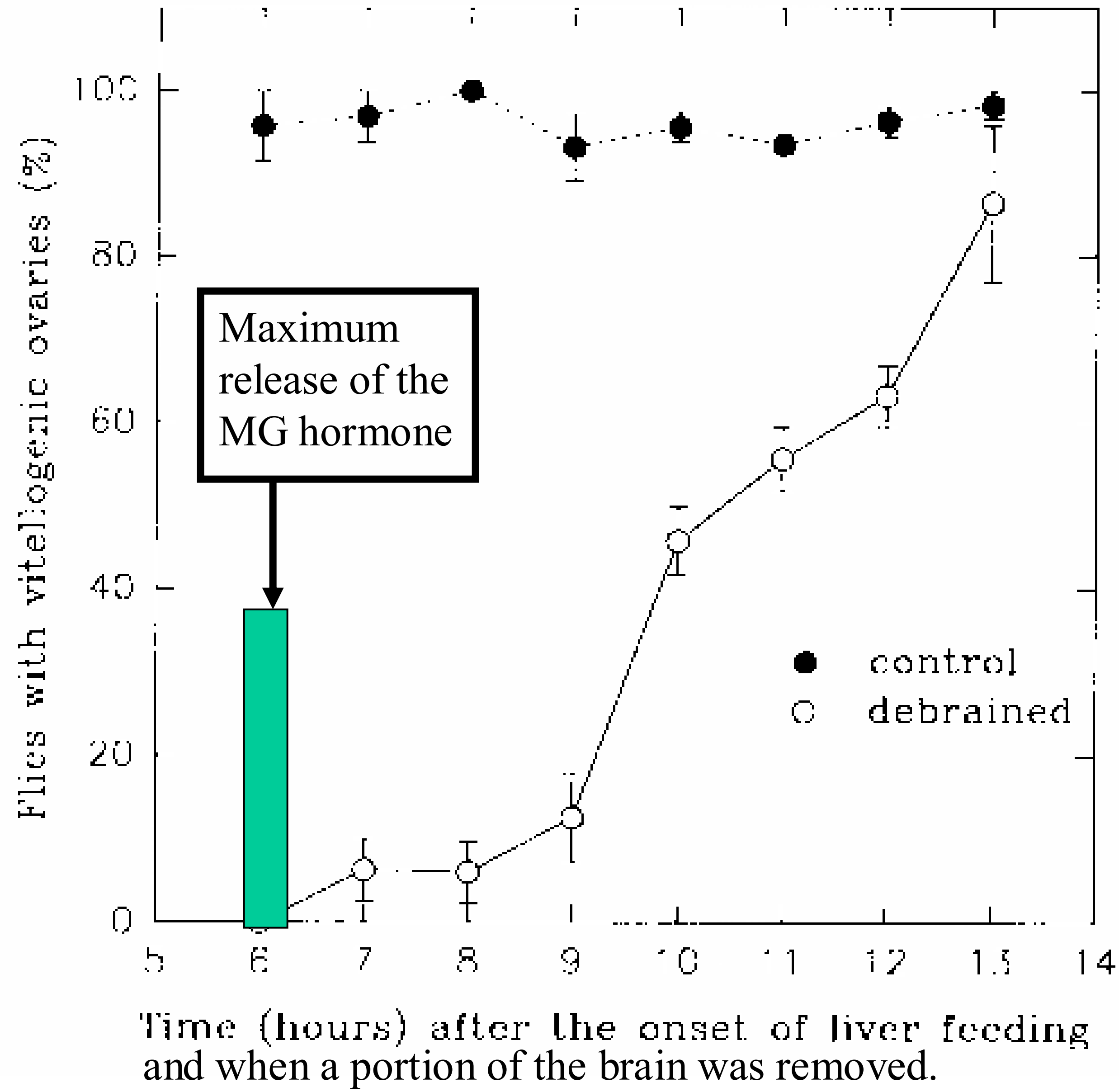
Dorsal posterior part of brain was cut off using scissors. Streptomycin and 1-phenyl-2-thiourea was added to the wound. It was then sealed with paraffin wax of low melting point. This removed the MNSC's

1. The flies were all protein fed for 1 hr.
2. At various times after the liver meal, part of the brains of some flies were removed, while in the controls the cut was made in another area (optic lobe area) but did not remove the MNSC.
3. Finally, 48 hrs after the liver meal, flies were dissected to check for oogenesis (see next slide for results)

Effect of brain hormones + midgut hormone on egg development in *Phormia regina*.

To do this, flies were liver fed and at various hours after liver feeding the protocerebrum was removed.

What does this graph show?



Effects of brain removal varied with the timing of removing the brain at different hours after the liver meal.

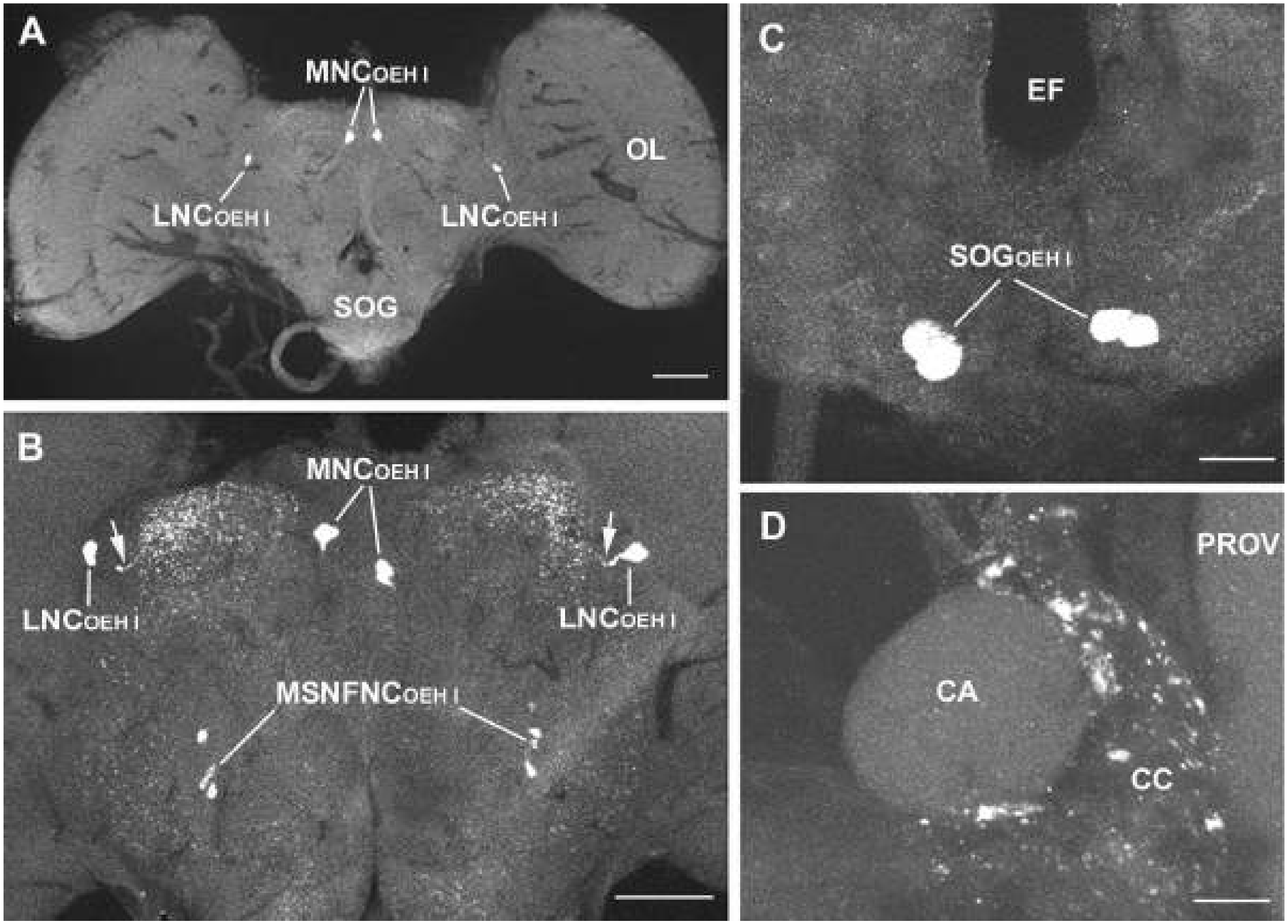
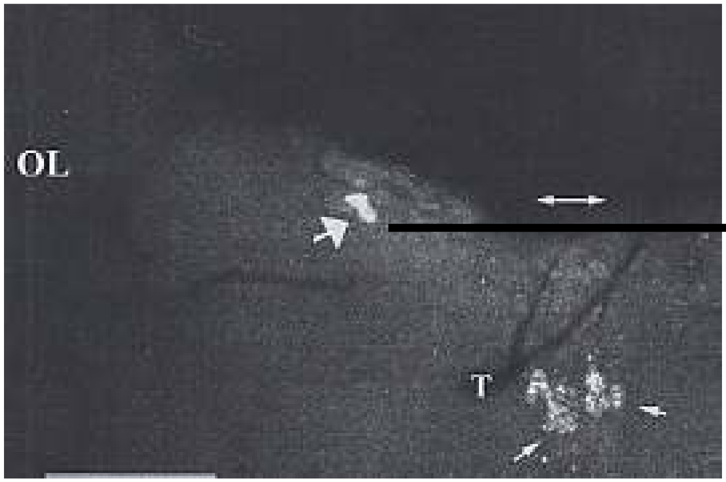


Fig. 1. Localization of the *Aedes aegypti* ovary ecdysteroidogenic hormone I (OEH I-like) (**A**, **B** and **C**) and the *Drosophila melanogaster* insulin receptor-like substances (**D**) in the neuroendocrine system of *Phormia regina*.



Presence of an allatotropin-like peptide in the brain of *Phormia regina*. The immunopositive cells shown in the fig. on the left show the positive response of these cells to antisera developed against Mas AT (*Mandica sexta* allatotropin. Based on this we can infer that *P. regina* has an allatotropin neurosecretion that is found in the brain.

If this is so, can we use Mas AT to stimulate the CA of *Phormia regina* to produce JH?

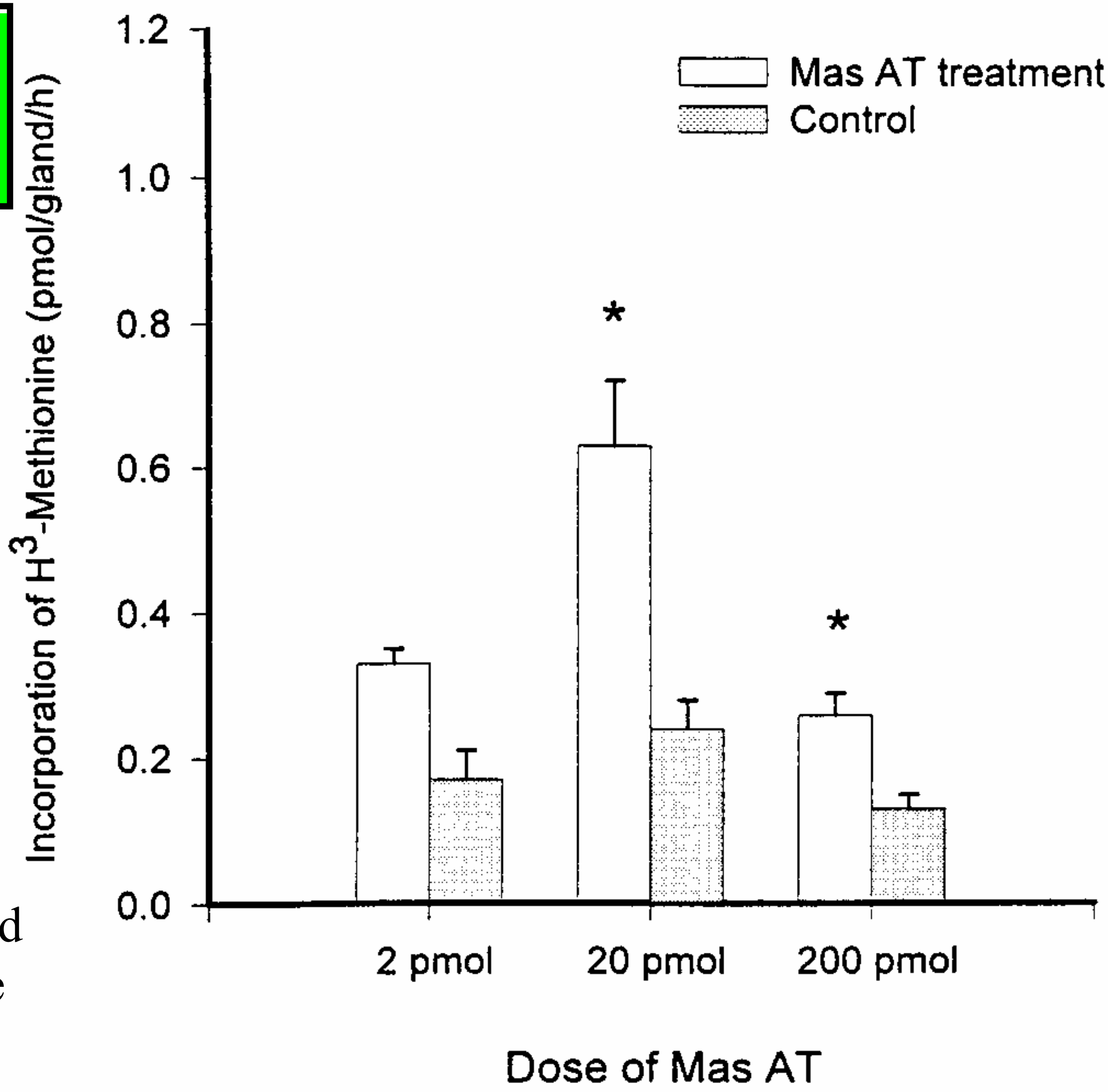
Photo to the right is the CNR of *P. regina*

Can Mas AT stimulate the CA of *P. regina* to produce JH?

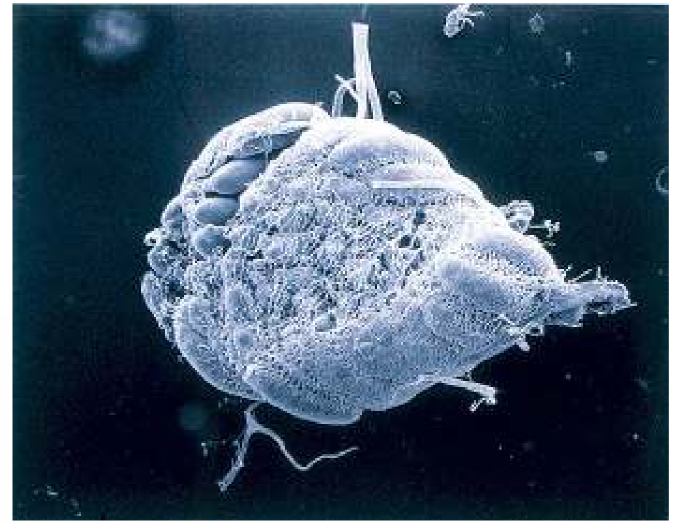
The radiolabelled methionine technique for measuring JH biosynthesis was used. Different conc. Of Mas AT were added to the incubation medium.

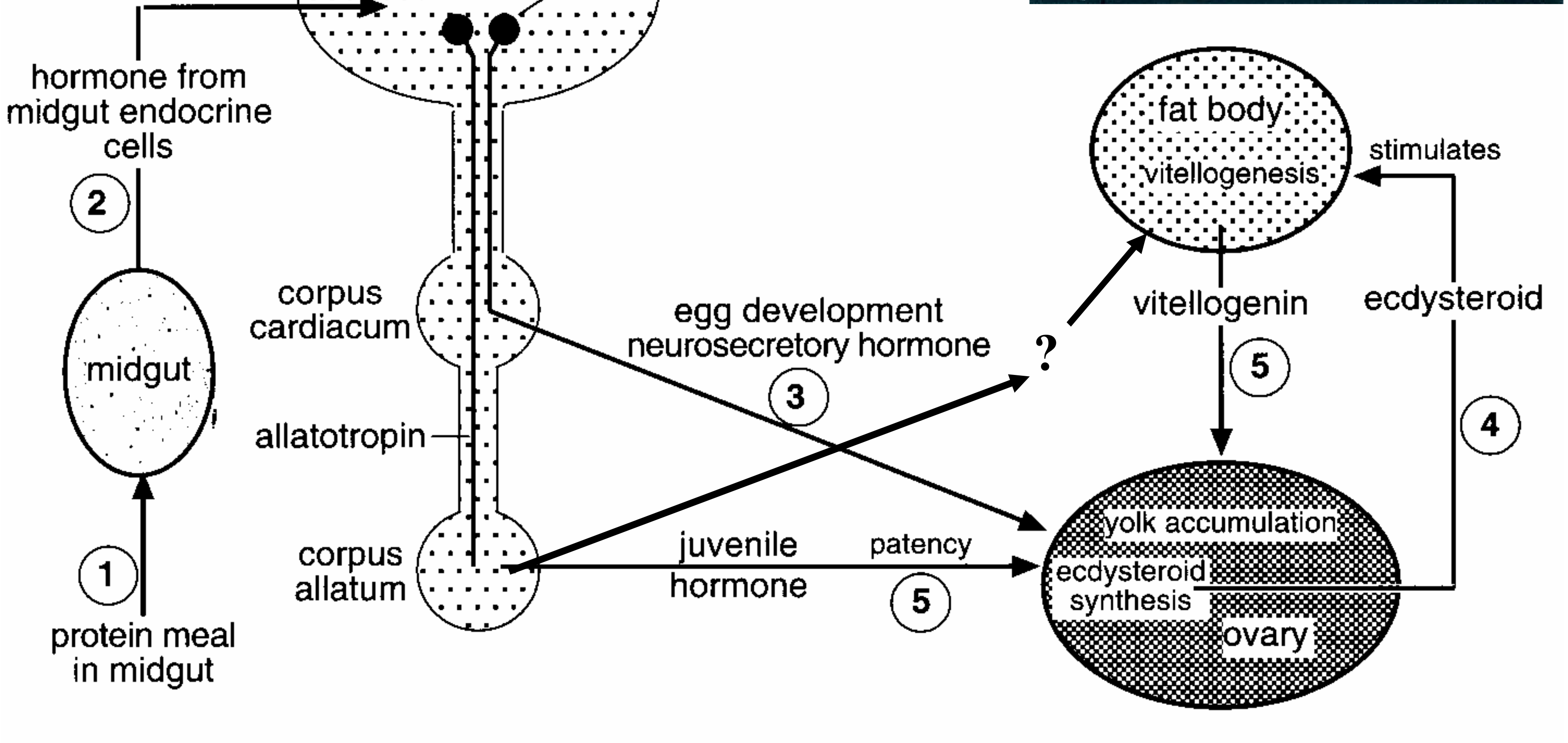
CONCLUSION-

Mas AT stimulated the CA to produce JH

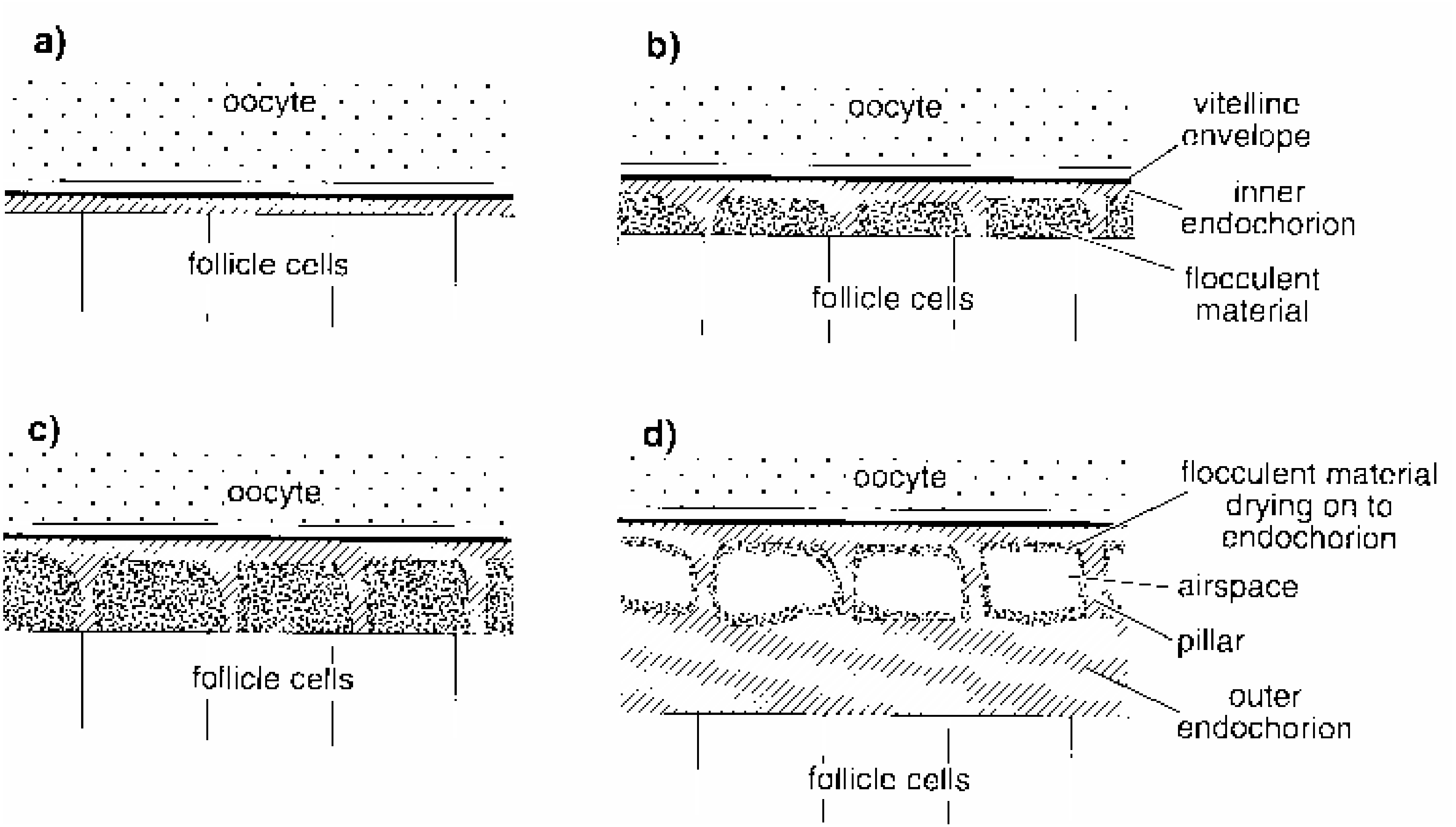


Oogenesis in *Phormia regina*

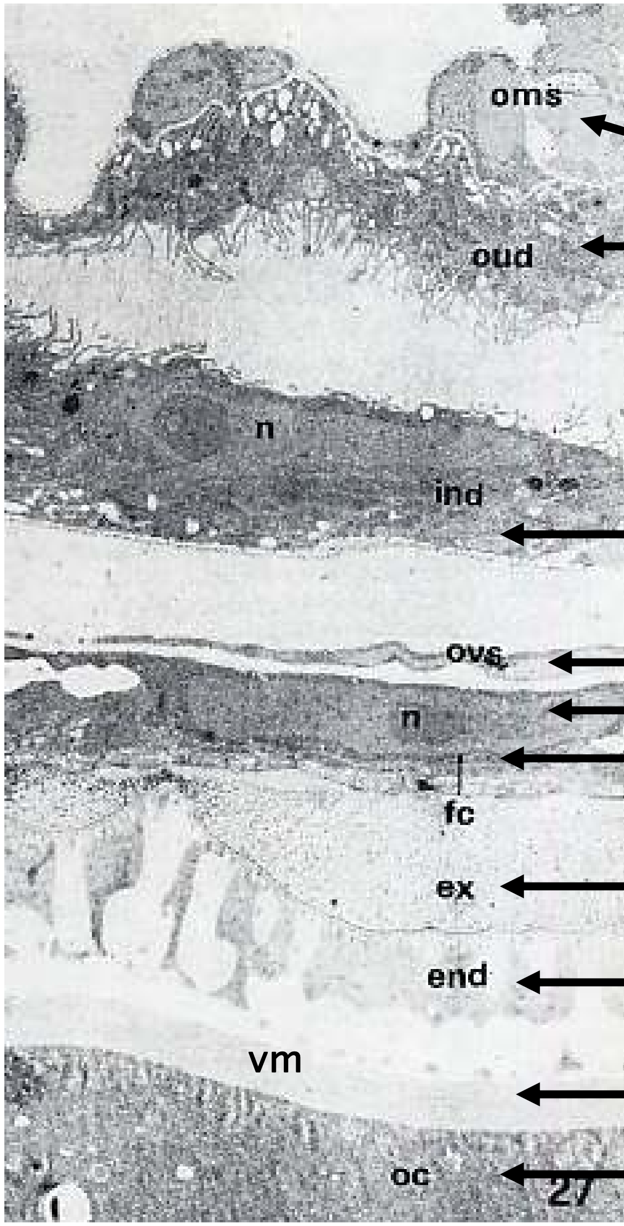




What cells of the follicle produce the chorion or egg shell and the vitelline membrane?



CHORIONOGENESIS-1.PRODUCTION OF THE EGG SHELL IN INSECTS
2. PRODUCTION OF VITELLINE MEMBRANE

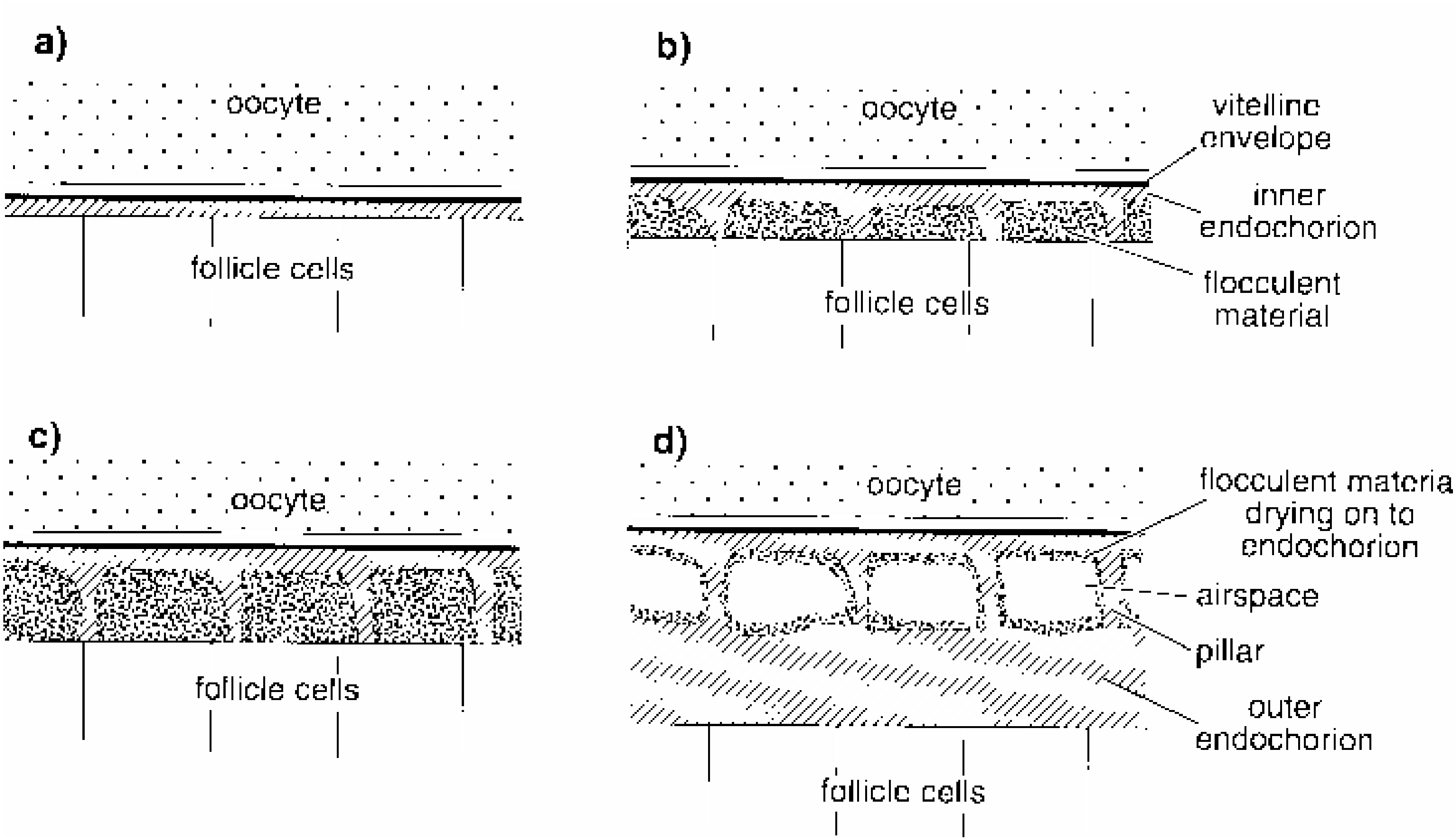


Ovarian muscular sheath
Outer ovarian duct layer
Inner ovarian duct layer
ovariolar sheath
Follicle cell nucleus
Degenerating follicle cell
Exochorion
Endochorion
Vitelline envelope (=membrane but not a real membrane
Oocyte

Production of the egg shell in *Phormia regina* by the follicle cells

In insects with polytrophic ovarioles, the vitelline envelope is only put around the oocyte and not the nurse cell area of the developing follicle

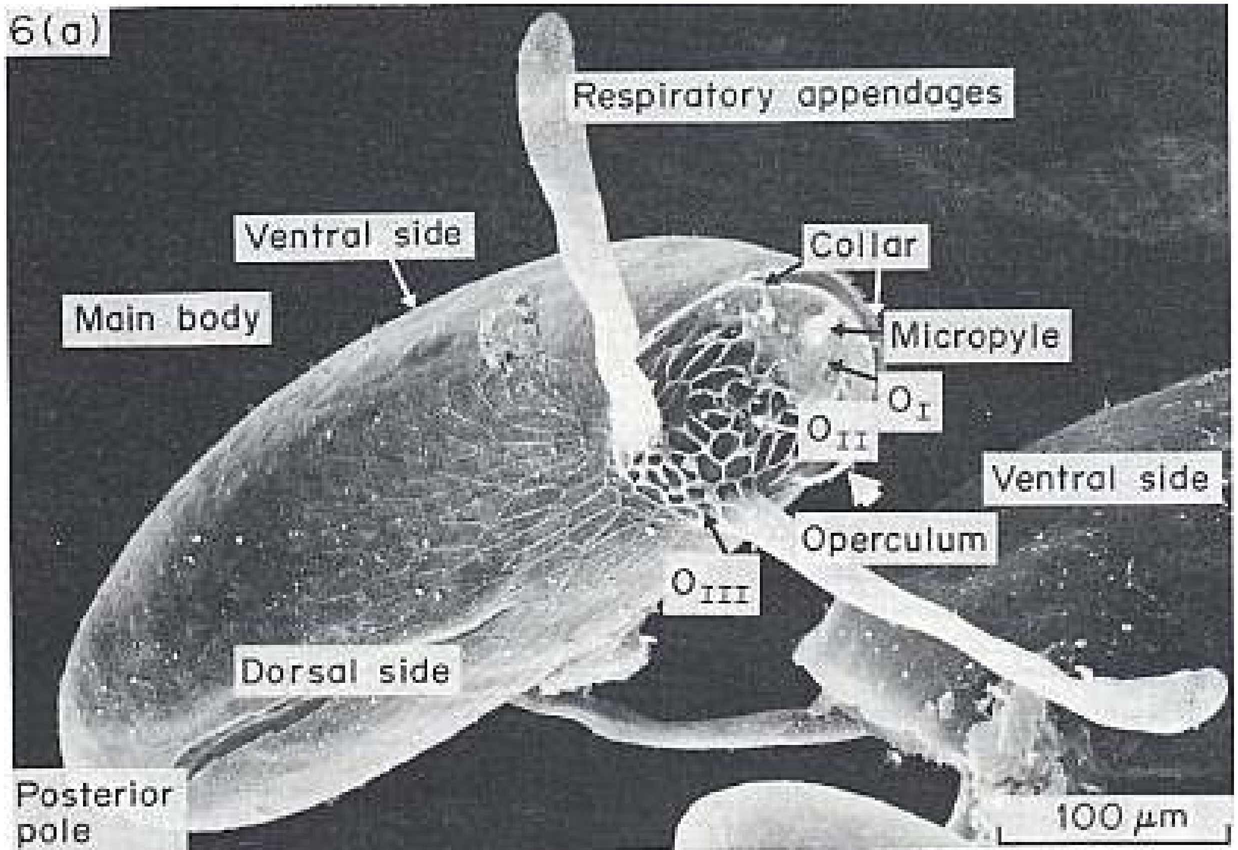
The follicle cells produce the vitelline envelope and the chorion in insect eggs. In *Aedes aegypti*, 20-hydroxyecdysone stimulates the follicle cells to produce the vitelline envelope

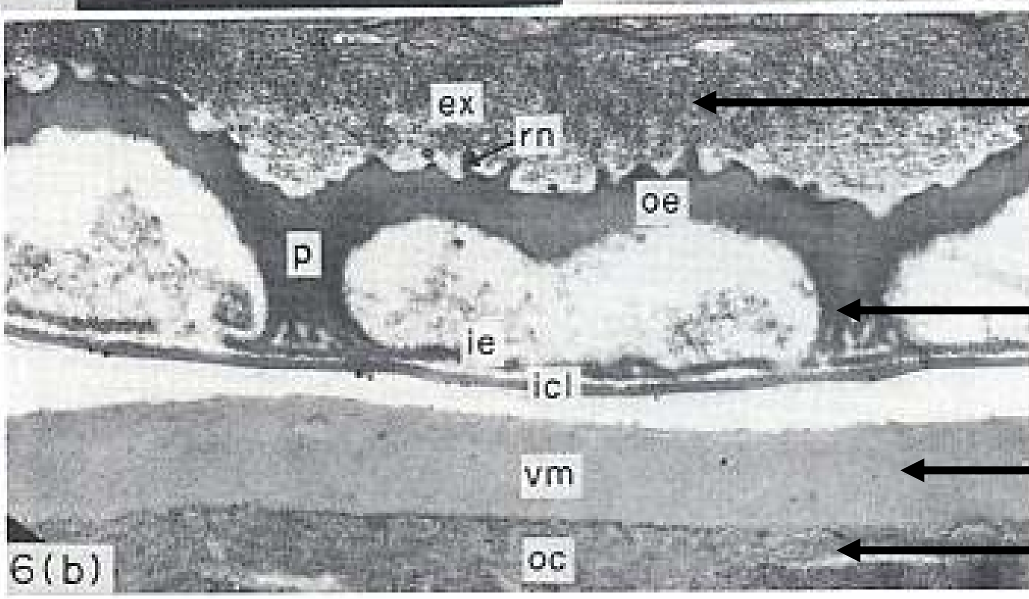


Egg shell development in *Drosophila* by the follicle cells

In addition to the egg shell many insects have accessory structures that need to be produced. Some of these are:

1. Aeropyles
2. Respiratory appendages
3. Hatching lines
4. Micropyle





Exochorion

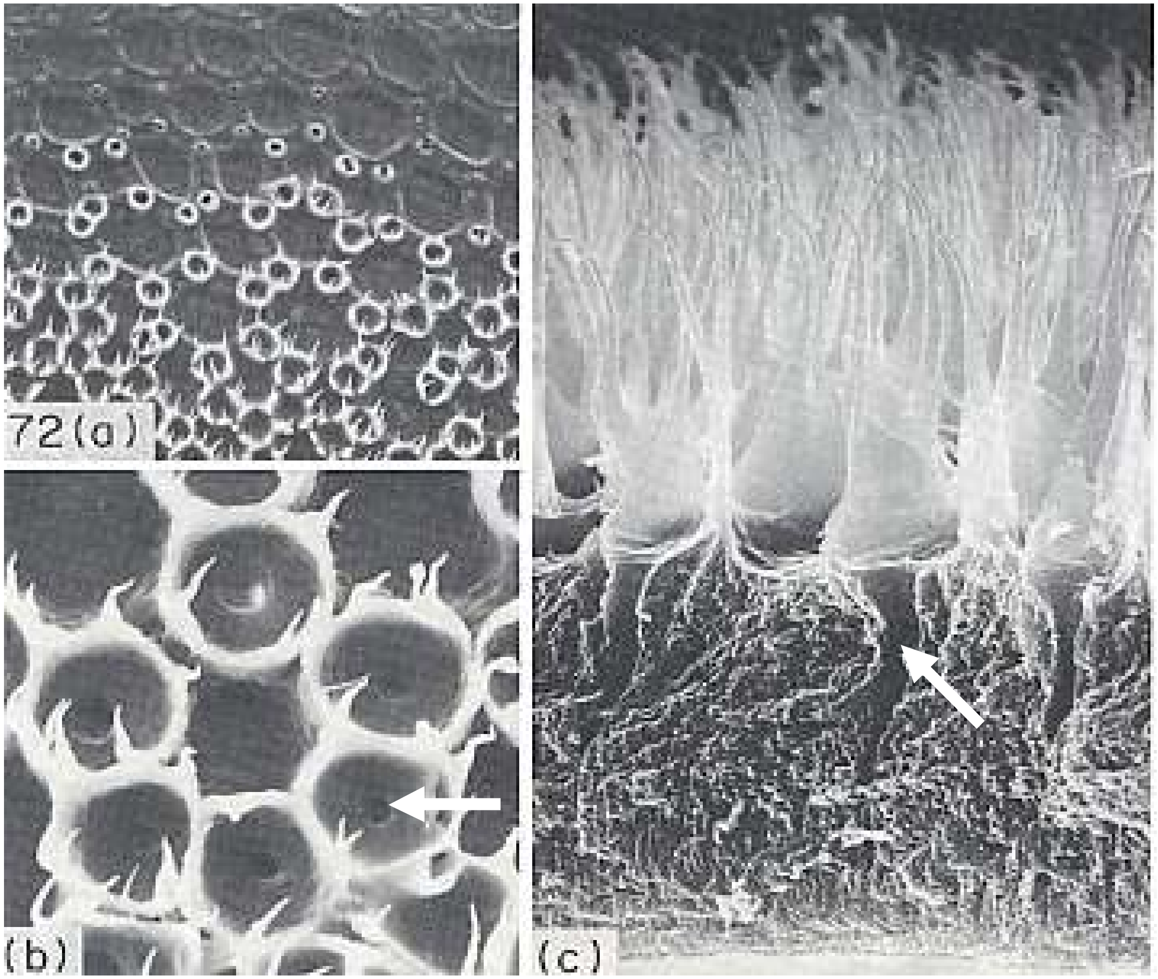
Endochorion

Vitelline envelope (membrane)

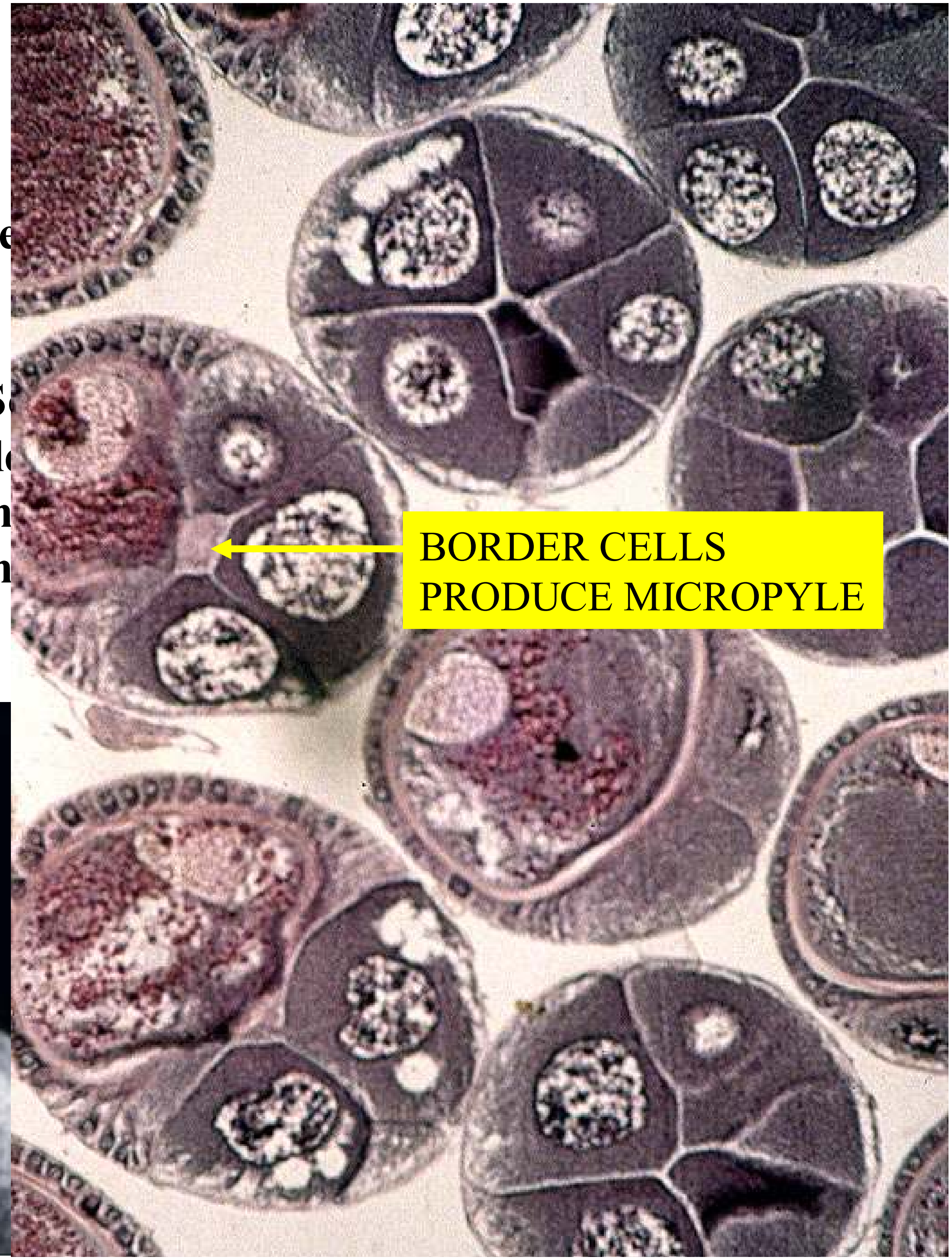
Oocyte

AEROPYLES OF EGGS-outgrowths of the chorion that have a pore to the environment that connects to the egg below and provides the developing oocyte and embryo with gaseous exchange but doesn't permit water to enter or leave.

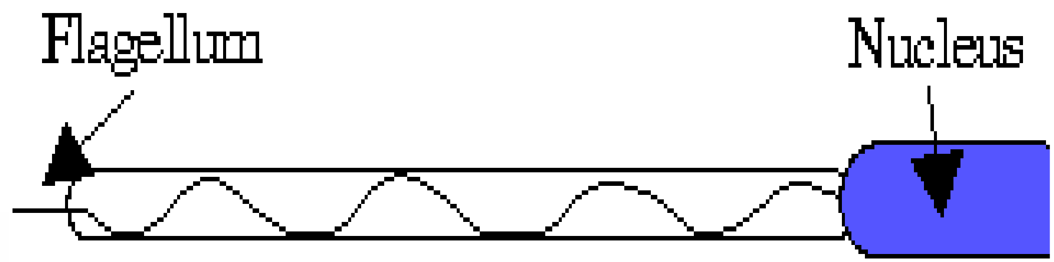
Egg shell of *Antheraea polyphemus* showing low magn. SEM in (a) and higher in (b) of the crown-like structure surrounding each pore of the aeropyle (arrow in b). To the right one can see the space below the channel (arrow) that allows gaseous exchange with the embryo or oocyte.

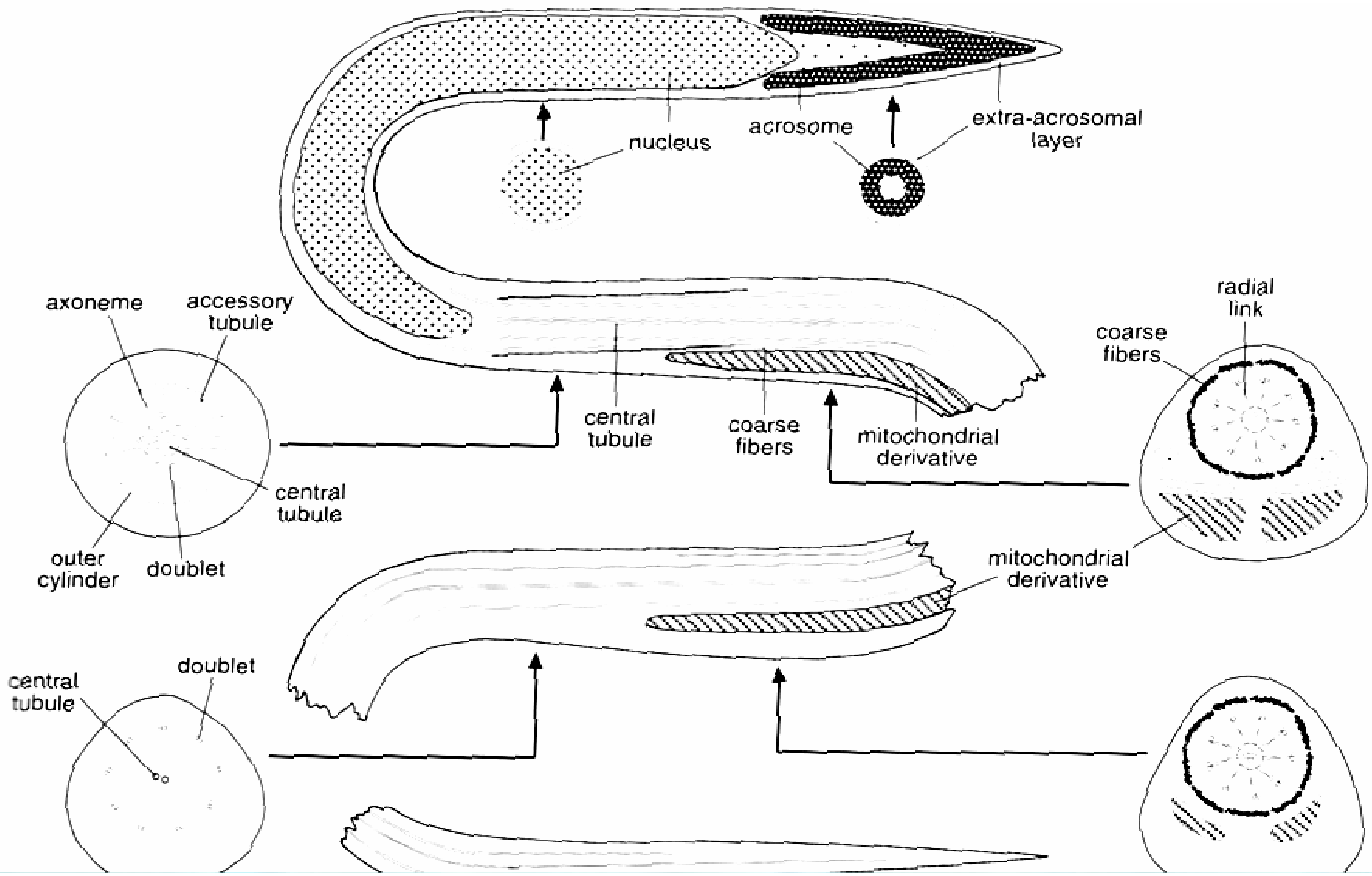


MICROPYLES-openings in the chorion or egg shell of insects that permits the entrance of sperm. Sinc the egg is laid with the egg shell completed, the sperm need some mechanism of getting into the egg. S insects have several micropyles whil others have only one. Note sperm on egg below and the sperm entering th micropyle.



If small organisms such as insects are ‘concerned’ with water loss or conservation, what evolutionary constraints might the size of the micropyle have on water loss and also, what constraints might the size have on insect sperm.



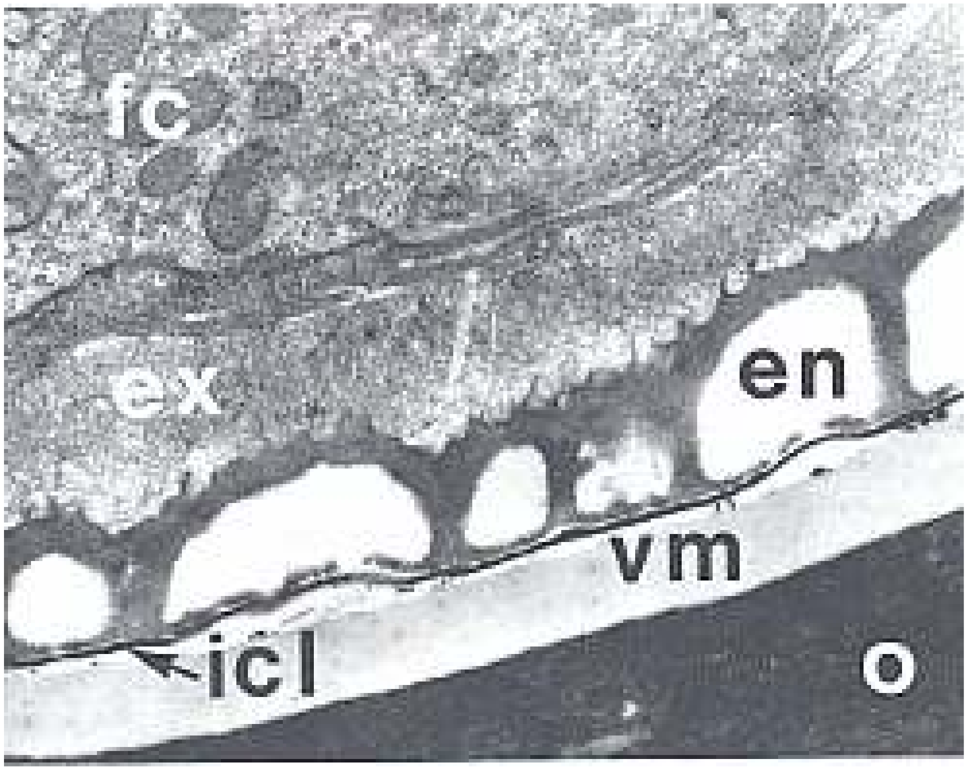


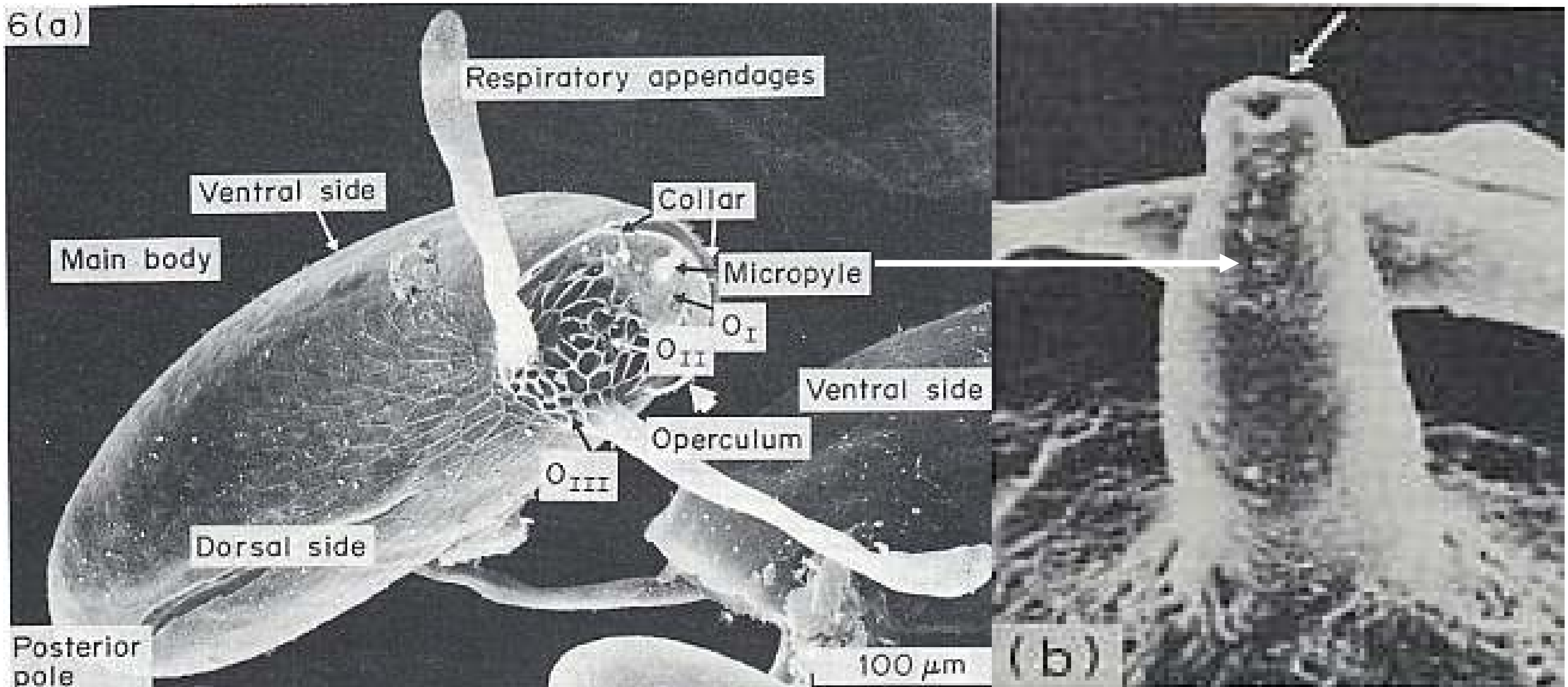
FUNCTION OF THE VITELLINE ENVELOPE?

This envelope is essential for the species specific binding of the sperm in vertebrates.

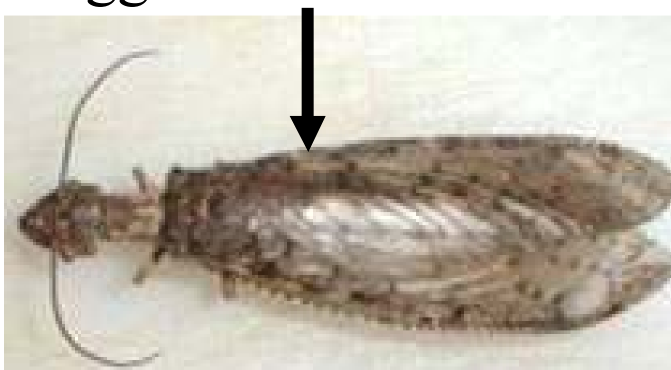
Klowden states that in the area directly underneath the micropyle, the vitelline envelope is lacking and is the site of sperm penetration. No reference for this statement.

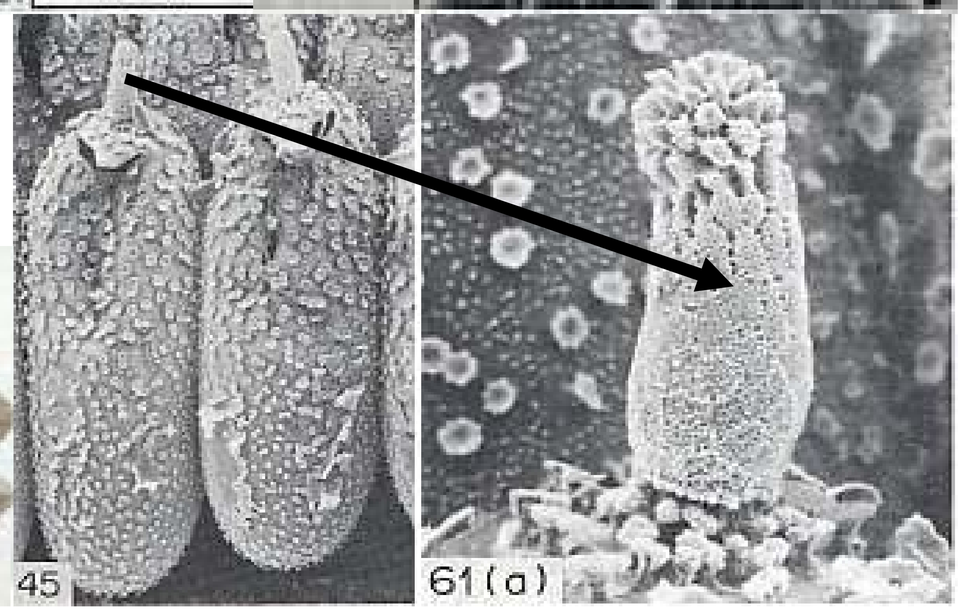
TEM of *Drosophila* egg
O=oocyte
Vm=vitelline envelope
En=endochorion
Ex=exochorion
Fc=follicle cell



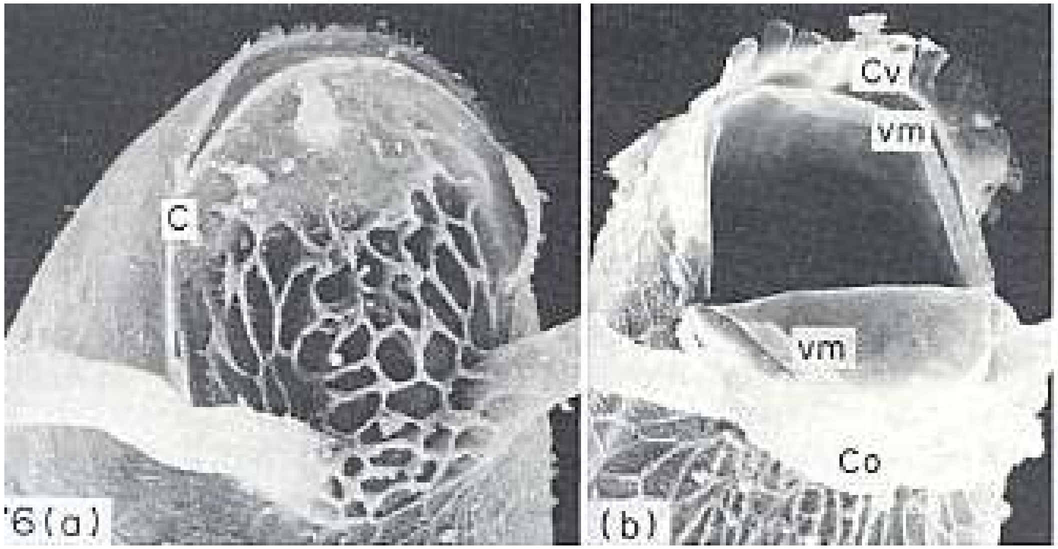


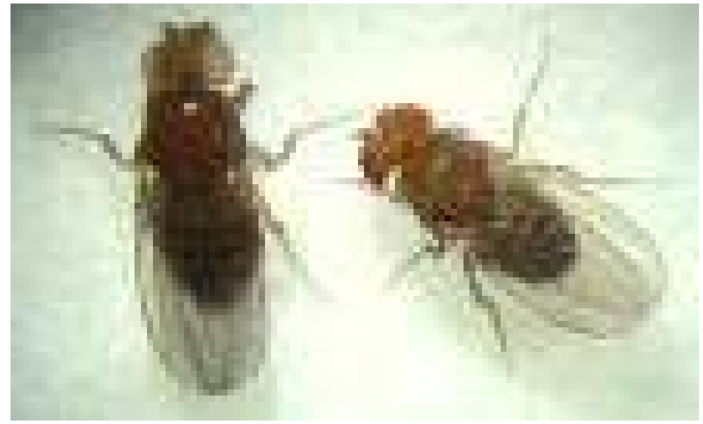
Some micropyles are elevated on stalks as seen in the *Drosophila* egg and the egg of *Sialis hasta*.





OPERCULUM AND HATCHING LINES IN INSECT EGGS

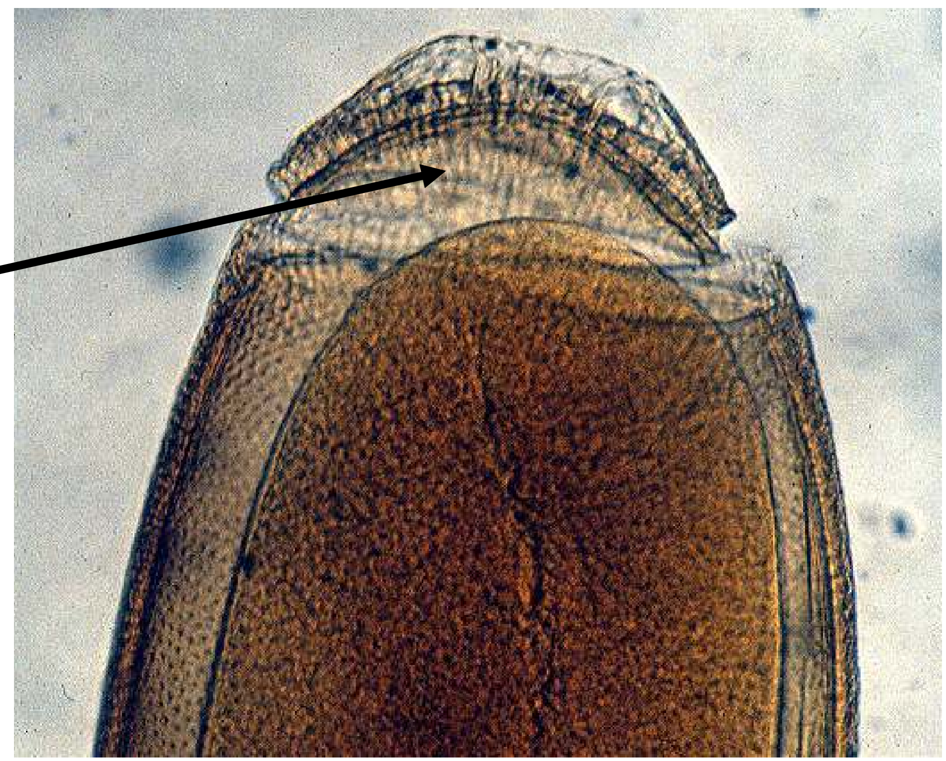




Drosophila eggs to the left. Bed bug egg below showing operculum

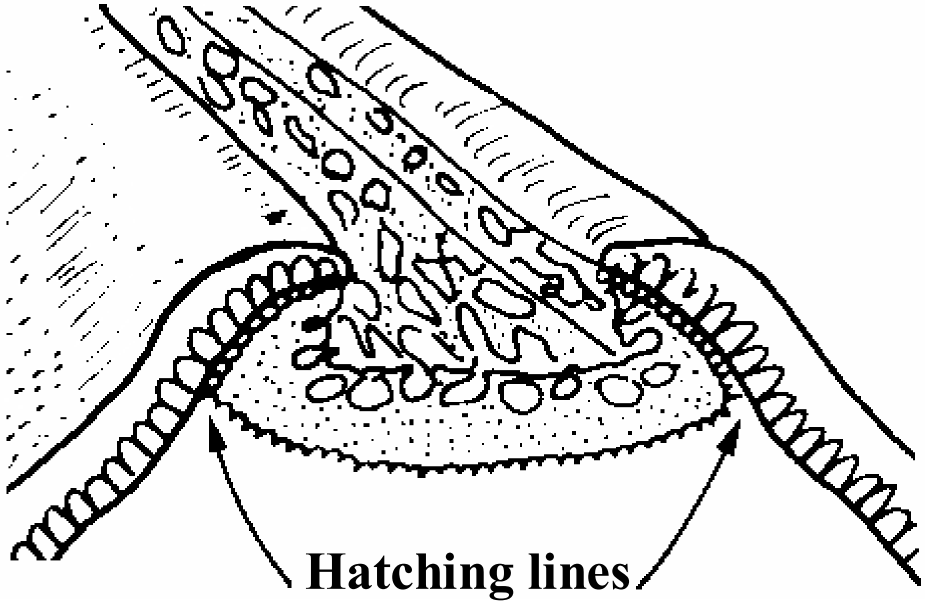
Insect larva and nymphs must have a mechanisms for getting out of the stiff and hardened egg shell. Two structures permit this:

1. **Operculum** is like a cap that pops off or opens
2. **Hatching lines** are areas of weakness in the chorion that are easily torn to allow the larva to escape.



Hatching lines are areas of weakness in the chorion that are easily torn to allow the larva to escape.

Calliphora scheme below is similar to other dipterans



Resorption or oosorption of oocyte-the ability to destroy an oocyte and reabsorb the yolk in various eggs at different stages but usually in the terminal egg. Widespread amongst Orthoptera, Blattodea, Dermaptera, Heteroptera, Coleoptera, Diptera and Hymenoptera.

Is a response to adverse conditions but usually occurs to a limited extent anyway. Is probably a way of assuring that eggs to be laid have maximum amount of yolk (vitellin)

FACTORS AFFECTING OOSORTION

1. Lack of nutrients in the adult. If you can't produce a full complement of eggs then the ones produced should have maximum amount of yolk for survival.
2. In Locusta the number of eggs reabsorbed is inversely proportional to the quantity of food eaten.
3. Inability to produce fertile eggs. Unable to mate.
4. Lack of suitable oviposition sites.

FACTORS INFLUENCING FURTHER MATING & EGG LAYING

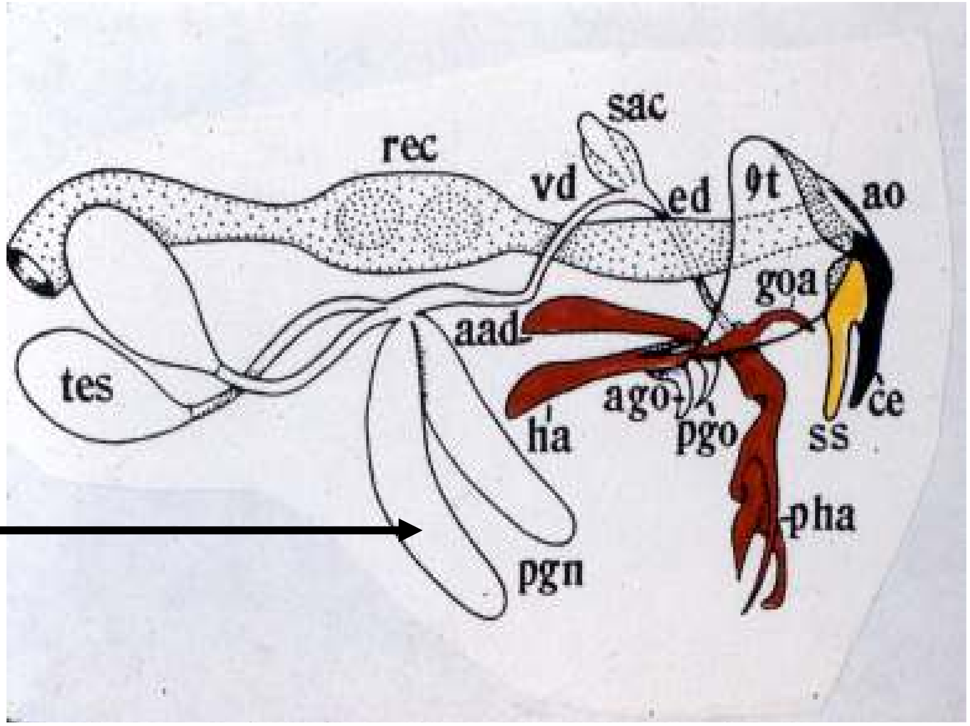
SEX PEPTIDE PHEROMONES- IN MALES ARG FLUID

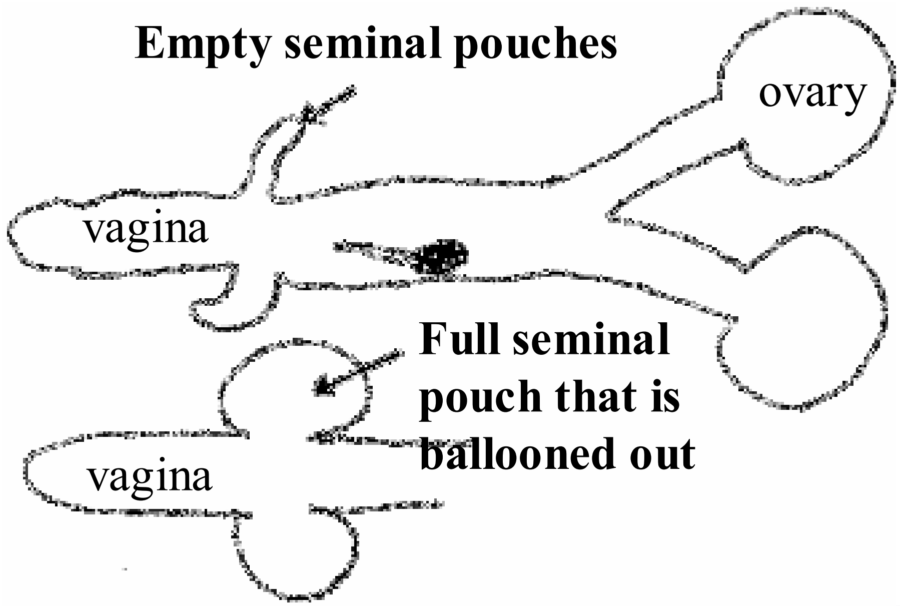
Why are they called pheromones and not hormones?

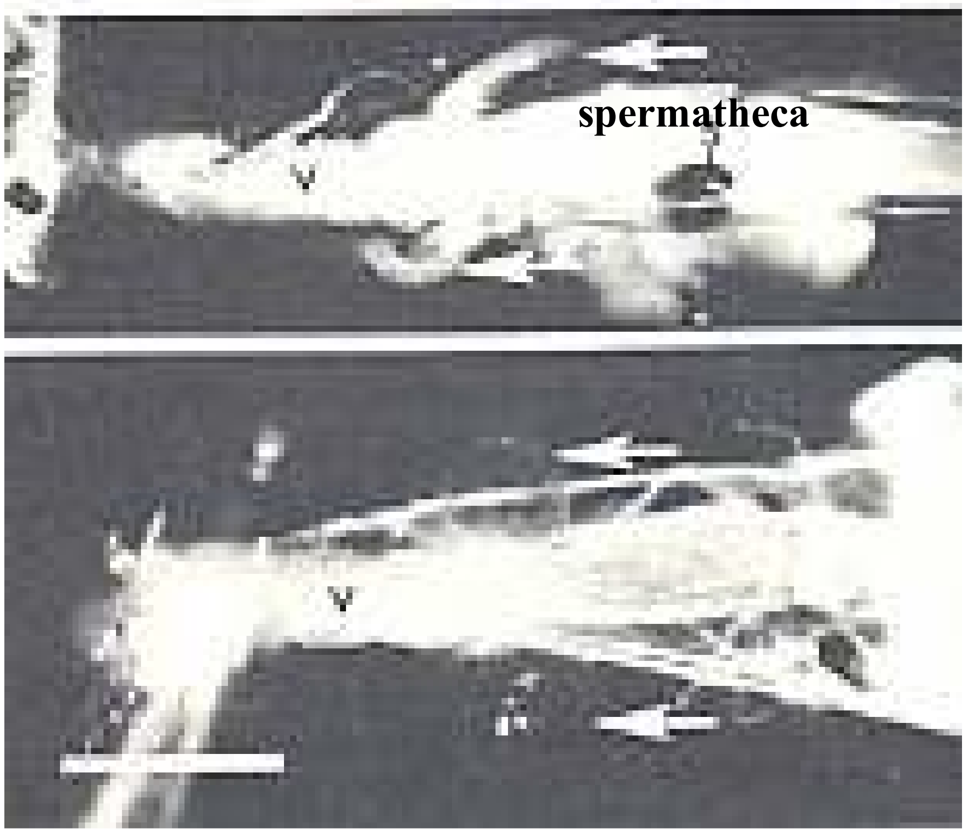
1. MALE ACCESSORY GLAND SECRETIONS

   Accessory reproductive glands in males are often called paragonia

ARG's in male *Phormia regina*. During copulation, ARG fluid is transferred to the females bursa where it remains for a while but by 2 days it has entered her bloodstream







Musca domestica male/female lack, as do other members of the genus, accessory reproductive glands. The mating refusal peptide is produced by the ejaculatory duct. Females have a very interesting structures known as seminal pouches. In unmated females these structures are not expanded as shown in the fig. on the left and below in the top photo. When mated, however, these structures blowup like a balloon as seen in fig. on the left and in bottom photo. What they are full of is fluid from the males. In a short time after transfer of this material, however, these structures go back to the not expanded shape because they are now empty.

Where did this mating refusal fluid go? + What is its function?

OBSERVATION-What does the table below tell you?

TABLE 1

Duration of copulation and frequency of remating by male and female house flies

Number of consecutive matings	Minutes *in copulo* [1]	Males remating [2]	Females remating [3]
		%	%
1	72.4 [a]	92.2	3.3 [a]
2	84.7 [b]	72.8	5.5 [a]
3	90.6 [b]	70.2	12.2 [b]
4	144.2 [c]	44.6	20.9 [c]

[1] Average of four replications beginning with ten pairs/sample.
[2] Average of three replications beginning with 100 pairs/sample.
[3] Observed 24 hours after initially mating with the males in column 3.
[a,b,c] Figures in columns with different superscripts differ significantly at $P < 0.05$.

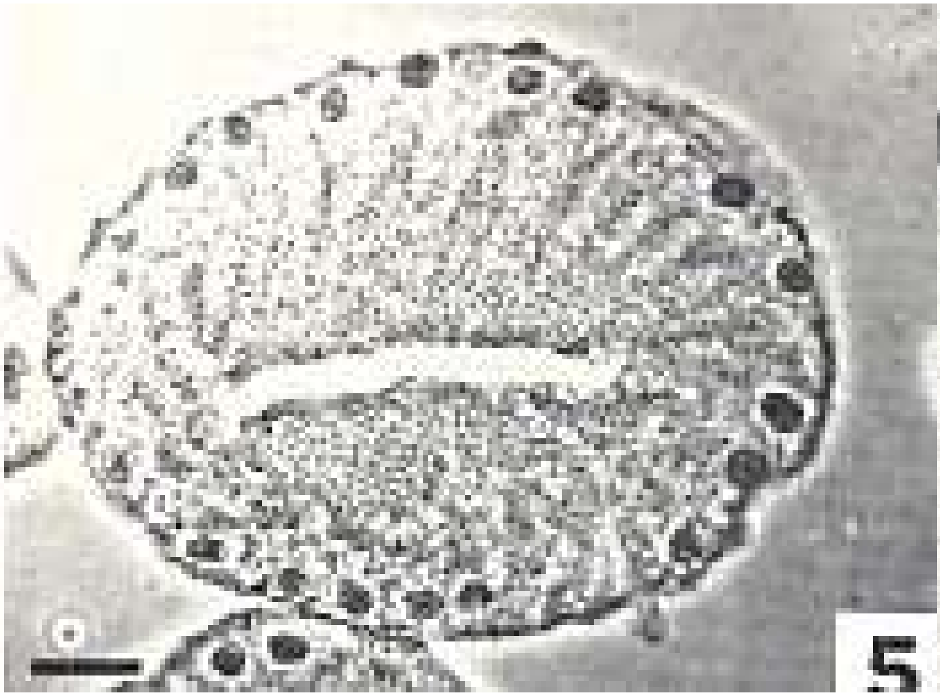
1. The more consecutive matings by the male, the longer they remain in coplula and the less they will remate
2. The more consecutive matings by the male the greater the percentage of females that will remate
3. Female HF's tend to refuse mating after the initial mating. Mating can only take place if the female extends her ovipositor. Thus, if she fails to do this, she is refusing to mate.

HYPOTHESIS-Something from the male's ejaculate causes this.

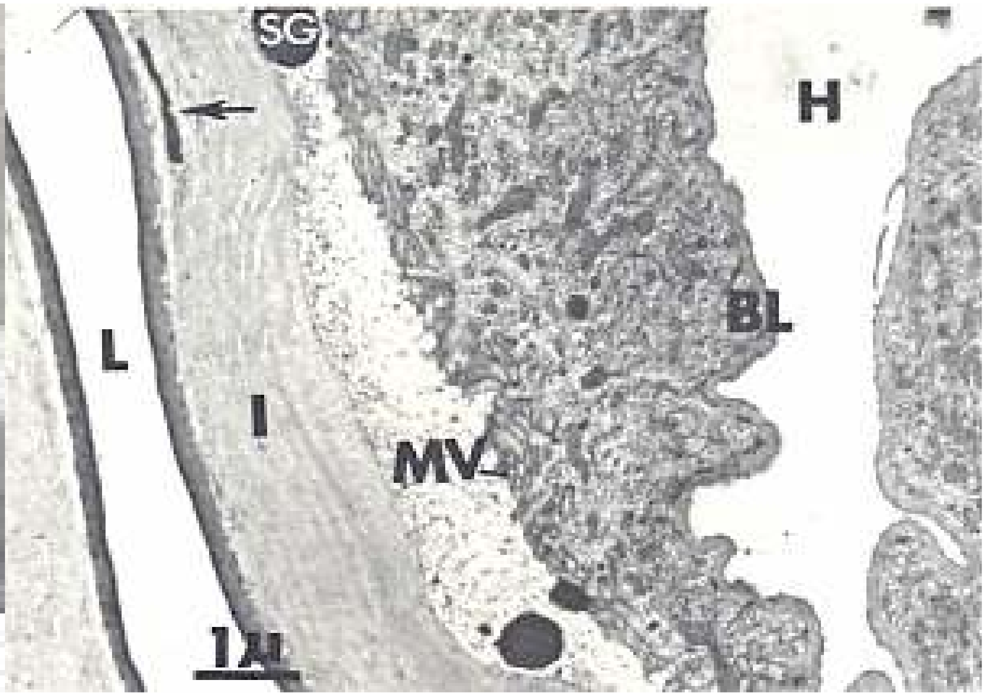
EXPERIMENTS:

1. Where is this substance located in the male? How do you test this?
2. How can we prove this substance is passed onto the female?

Autoradiogram-male's ejaculatory duct



TEM of female seminal pouch showing the lumen (L) and the intima of the pouch (I). Note the dark secretory granule (SG) + the arrow pointing to the fluid coming through the intima on its way to the hemolymph (H).



3. How can we show what part of the female responds to this substance?

a. Based on the previous slide, we know that it travels in the hemolymph but, where does it act?

How can one test this?

Leopold, R.A. et al. 1971. Mating refusal in ***Musca domestica***: Effects of repeated mating and decerebration upon frequency and duration of copulation. Jour. Exp. Zool. 176: 353-359.

Leopold, R.A. et al. 1971. The biosynthesis of the male housefly accessory secretion and its fate in the mated female. J. Insect Physiol. 17: 987-1003.

Table 2. Localization and assimilation of [^{3}H] arginine-labelled accessory secretion by female houseflies during and after mating

Time of observation after union	Dis/min per female*				
	Whole body	Head	Thorax	Abdomen	Haemolymph (μl)
10 min	4779	90	197	4492	218
20 min	6895	131	329	6436	336
30 min	8219	106	277	7836	402
40 min	12,333	239	540	11,554	428
Completion†	9811	252	703	8856	1218
8 hr	6082	335	1,138	4609	448
24 hr	4812	188	893	3731	396
48 hr	4940	195	863	3872	428
72 hr	3168	134	607	2427	204

* Average of three replicates; 5 females in a sample.
† 50–70 min.

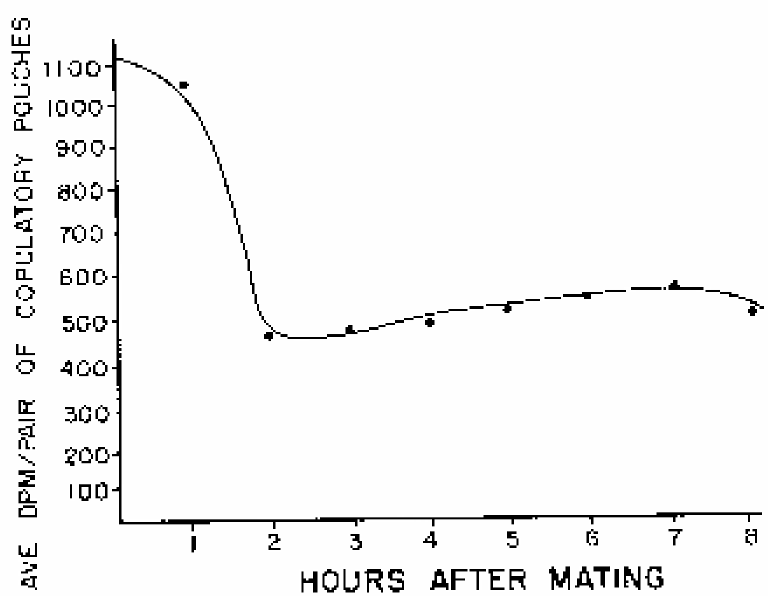


Fig. 15. Loss of ^{3}H-labelled male accessory secretion from the vaginal pouches after completion of mating. (Each time period represents three replicates, 5 females per sample.)

TABLE 4

The effects of decapitation or cephalocautery on the length of time spent in copulo

Treatment	Average length of mating (min)[1]				
	1st	2nd	3rd	4th	5th
Control	52.9	45.7	0	0	0
Decapitated virgin	94.6	62.0	60.0	59.6	65.0 [3]
Decapitated during mating	85.2	76.4	67.7	30.1	0
Decapitated after first mating	67.3	111.6	76.6	73.6	60.0
Decapitated two days after mating	69.1	78.7	75.6	68.6	63.8
Cephalocauterized virgin	108.6	98.3	63.0	87.0	0
Cephalocauterized after first mating	63.1	92.3	70.0	68.0	48.6
Cephalocauterized two days after mating	55.0	82.0	71.3	67.3	48.0
Control newly emerged [2]	71.6	0	0	0	0
Cephalocauterized newly emerged [2]	87.5	52.0	43.5	0	0

[1] Three replicates; 25 flies in original sample.
[2] In the presence of mature females.
[3] Data consists of only one observation.

TABLE 5

The effects of ligation on the frequency of female house fly mating

Treatment	Percentage mating [1]				
	1X	2X	3X	4X	5X
Ligated virgin	96.0	83.3	46.6	21.0	0
Ligated after first mating	100	44.7	0	0	0

[1] Three replicates; 25 flies in original sample.

WHY HAVEN'T THE ORIGINAL STUDIES ON HF BEEN CONTINUED LIKE THEY ARE IN *DROSOPHILA*?

1. Lack of ARG is HF, thus difficult to get large quantities of the mating refusal molecule
2. *Drosophila* has all of the genetics behind it

Drosophila mating →

Chen, P.S. 1984. The functional morphology and biochemistry of male accessory glands and their secretion. Annu. Rev. Entomol. 29: 233-255.

Chen, P.S. et al. 1988. A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. Cell 54: 291-298.

WHY DID CHEN'S PIONEERING WORK NOT PROCEED IN HIS LABORATORY?

T. Chapman et al., "The sex peptide of *Drosophila melanogaster*: investigation of post-mating responses of females using RNA interference," *PNAS*, DOI:10.1073/pnas.1631635100, July 21, 2003.
http://www.pnas.org

H. Liu, E. Kubli, "Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*," *PNAS*, DOI:10.1073/pnas.1631700100, July 21, 2003.
http://www.pnas.org

Y.S. Rong, K.G. Golic, "Gene targeting by homologous recombination in *Drosophila*," *Science*, 288:2013-2018, June 16, 2000.
[PubMed Abstract]

Kubli, E. 1996. The *Drosophila* sex-peptide: a peptide pheromone involved in reproduction. Adv. Dev. Biochem. 4: 99-128.

Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*

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Edited by Wendell Roelofs, Cornell University, Geneva, NY, and approved May 21, 2003 (received for review March 25, 2003)

Effects of the sex peptide from the male's ejaculate on the female

1. **Renders her unreceptive to another mating attempt**
2. **"Tells" female she has mated and to terminate the mating**

An ovulation pheromone (ovulin) is also present in the male's ejaculate

1. **The ovulation hormone (Acp26Aa)**
   Hormones transferred to females in seminal fluid mediate several important behavioral changes. The Wolfner lab at Cornell has a long-standing interest in Acp26Aa (ovulin), a prohormone-like protein that stimulates females to ovulate. Upon entering the female, Acp26Aa targets to the base of her ovaries and also enters her bloodstream. Acp26Aa is processed in females, exposing a region similar to mollusk egg-laying hormones. Our goals include defining Acp26Aa's active regions and receptor(s), and the signaling molecules through which Acp26Aa acts.

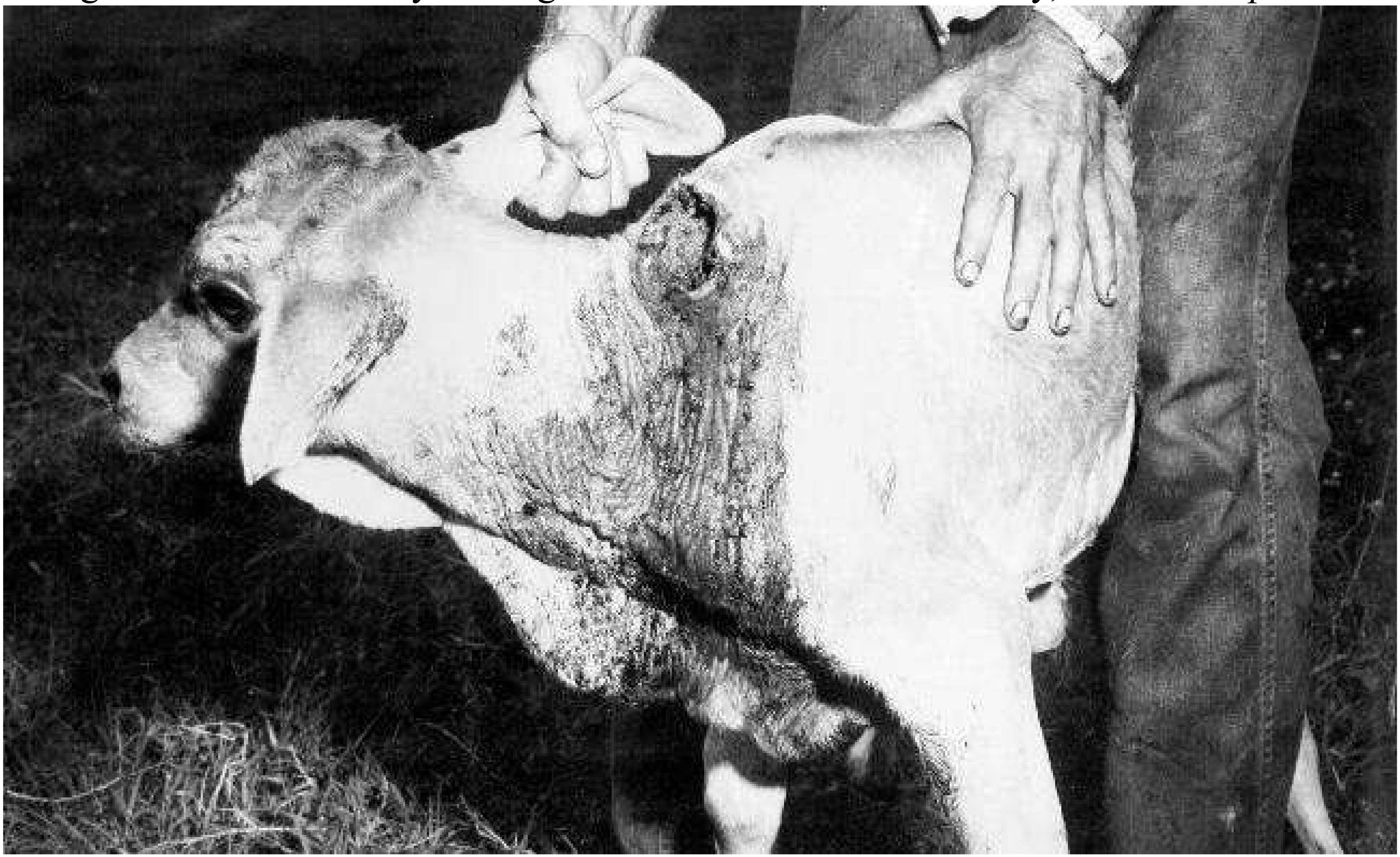
What major insect control strategy has its success based on the effects of the male's ARG fluid, what insect was it done with, and why was it important for the program to succeed.?

Sterile male release technique or **SIT** (sterile insect technique). Sterile males compete with wild males and when a sterile male mates with a female it is not good if she mates again because it could be with a wild male, thus either sperm mixing or sperm precedence.

Florida Entomologist: **Vol. 85, No. 4, pp. 666–673.**
A PERSONAL ACCOUNT OF DEVELOPING THE STERILE INSECT TECHNIQUE TO ERADICATE THE SCREWWORM FROM CURACAO, FLORIDA AND THE SOUTHEASTERN UNITED STATES. Alfred H. Baumhover[a] *[a]USDA-ARS Research Leader (Ret.), 4616 Nevada Ave. N., Minneapolis, MN 55428*
See pdf file http://www.fcla.edu/FlaEnt/fe85p666.pdf

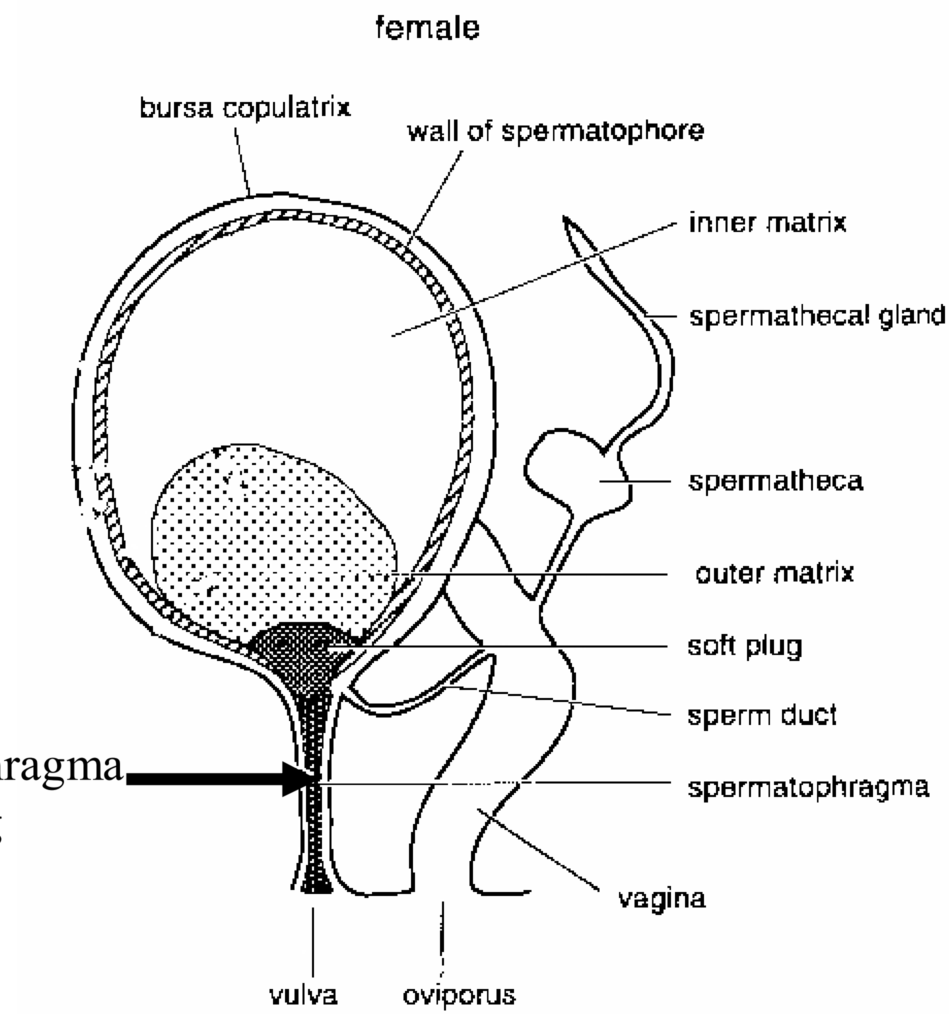
The Screwworm, *Cochliomyia hominovorax*, is a serious problem for livestock owners because the female lays her eggs on wounds. The larvae soon hatch and eat the living tissue. This is an example of myiasis.

Program also successfully used against the Mediterranean fruit fly, *Ceratitis capitata*.



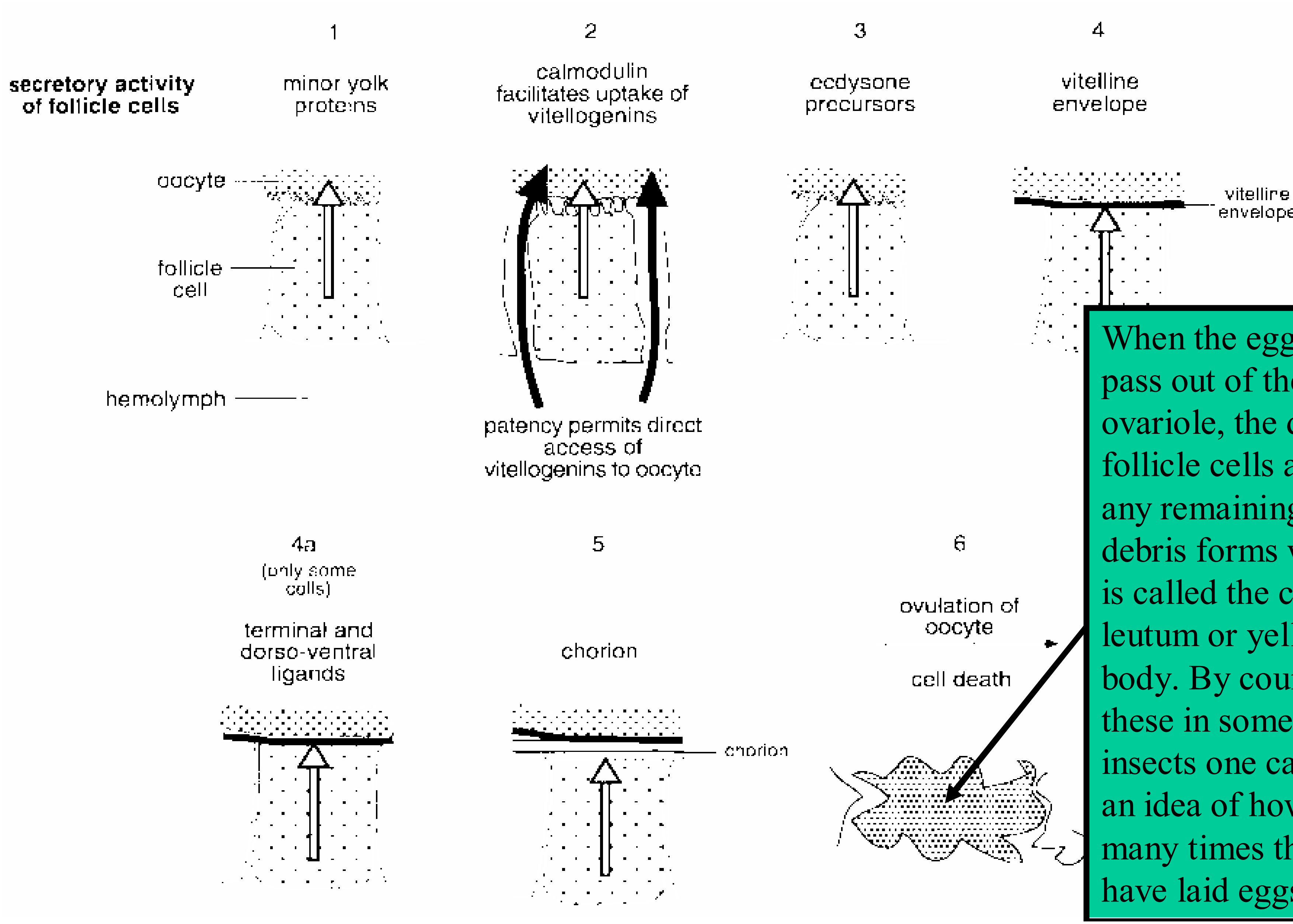
MALE STRATEGIES TO PREVENT FURTHER MATINGS OF FEMALES

1. Mate guarding- Odonata
2. Sex peptide pheromone – Diptera
3. Mating plugs – distinct from spermatophores, produced from the males ARG fluid, and found in several mosquito species, honeybee, and some Lepidoptera.

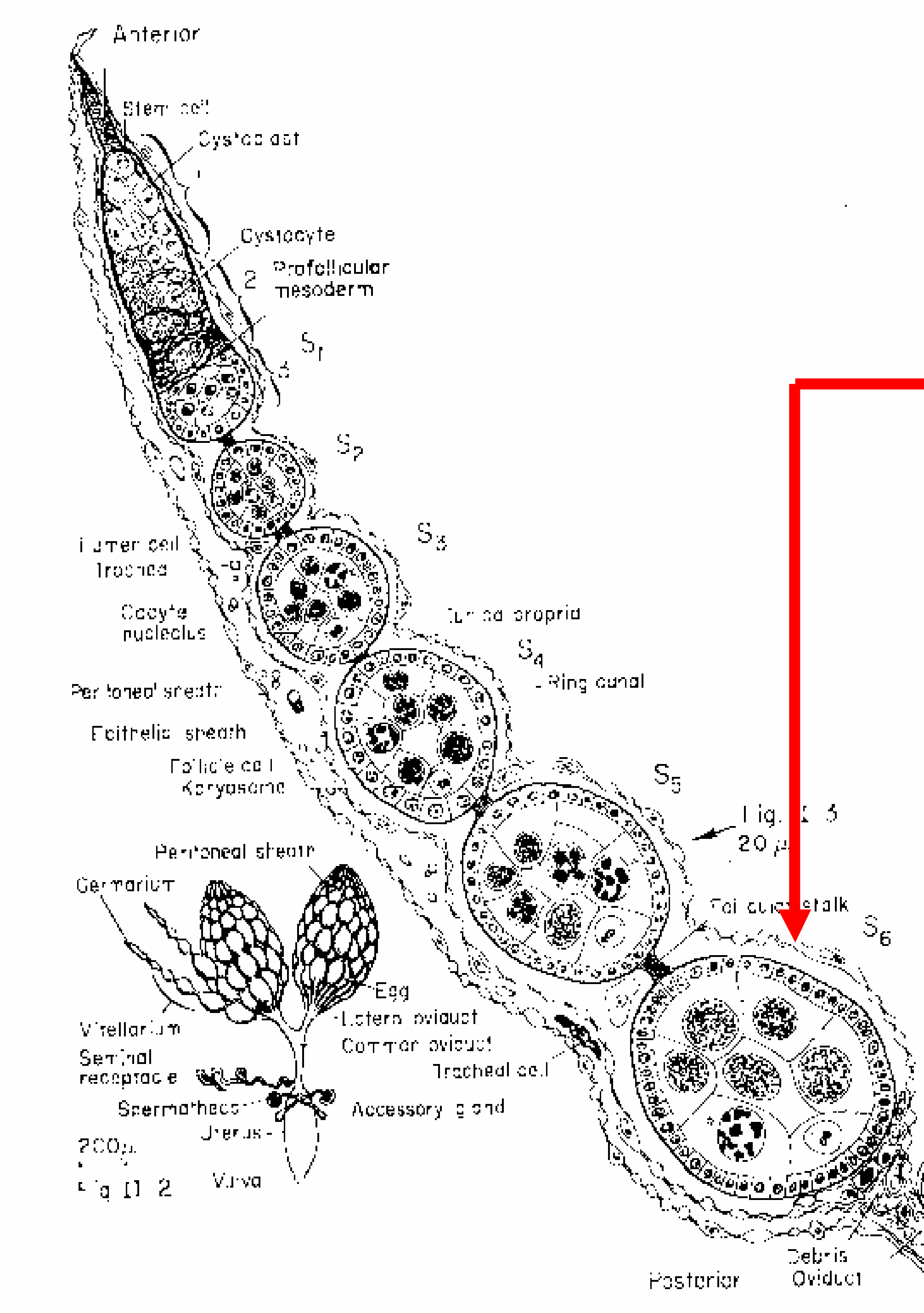


In Lepidoptera, the spermatophragma becomes the mating plug

OVULATION-The passage of the egg into the oviduct. IT IS NOT CLEAR HOW OVULATION IS CONTROLLED.



When the eggs pass out of the ovariole, the dead follicle cells and any remaining debris forms what is called the corpus leutum or yellow body. By counting these in some insects one can get an idea of how many times they have laid eggs.



This is the follicle where yolk deposition takes place. Looking at the structure of the ovariole and the linearly arranged follicles what might be a good question to ask with respect to follicle development?

Why don't the other follicles develop and take up VG?

Oostatic hormone????

In house fly and the mosquito, Adams (1976) and Lea (1975) proposed that a hormone, not ecdysone, from the ovaries and probably from the developing follicle was released that shut off the neurosecretory cells involved in the development of another follicle.

Trypsin-modulating oostatic factor: a potential new larvicide for mosquito control
D. Borovsky ***The Journal of Experimental Biology*** **206, 3869-3875 (2003)**

Oostatic peptide Neb-Collostatin

This peptide has a gonadoinhibitory effect and a cardiotropic effect

Trypsin-modulating oostatic factor: a potential new larvicide for mosquito control

D. Borovsky *The Journal of Experimental Biology* 206, 3869-3875 (2003)

Trypsin-modulating oostatic factor (TMOF), a mosquito decapeptide, terminates trypsin biosynthesis in the mosquito gut. The hormone is secreted from the ovary, starting 18 h after the blood meal, circulates in the hemolymph, binds to a gut receptor and stops trypsin biosynthesis by exerting a translational control on trypsin mRNA. Because of the unique primary amino acid sequence of the hormone (YDPAPPPPPP) and its stable three-dimensional conformation, TMOF is not degraded by gut proteolytic enzymes and can traverse the gut epithelial cells into the hemolymph of adults and larvae. Using this unique property, hormone fed to different species of mosquito larvae stops food digestion and causes larval mortality. To determine the shortest amino acid sequence that can bind to the gut receptor and still cause high larval mortality, 25 analogues of TMOF were synthesized and tested. The tetrapeptide (YDPA) was as effective as the decapeptide, indicating that the binding to the gut receptor is at the N-terminus of the molecule. Cloning and expressing the hormone on the coat protein of tobacco mosaic virus (TMV) in *Chlorella* sp. and *Saccharomyces cerevisiae* cells and feeding the recombinant cells to mosquito larvae caused larval mortality. These results indicate that TMOF can be used as a new biorational insecticide against mosquito larvae.

AGE GRADING MOSQUITOES AND OTHER INSECTS

It is often important to know which females in a population have not yet laid eggs in proportion to those that have. This will give you some idea as to the nutrition available during the season and some information about the insects physiological age.

Nulliparous females are those that have not yet laid a batch of eggs.

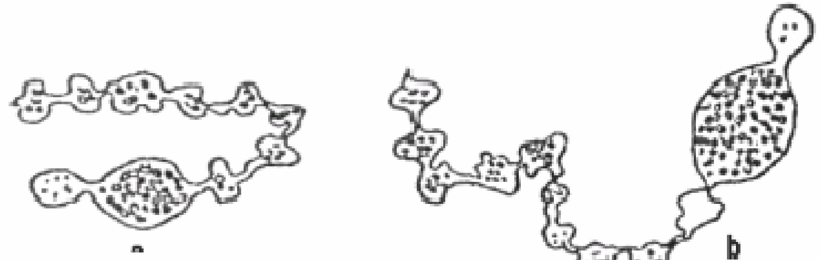
Parous females are those that have laid at least one batch of eggs. It is important sometime to know how many batches they actually have laid.

To determine parity, one must look at the ovarioles to look for what are called dilatations and follicular relics (often called yellow bodies). One can often use the stretching of the trachea (or skeins as they are called) to aid in this process.

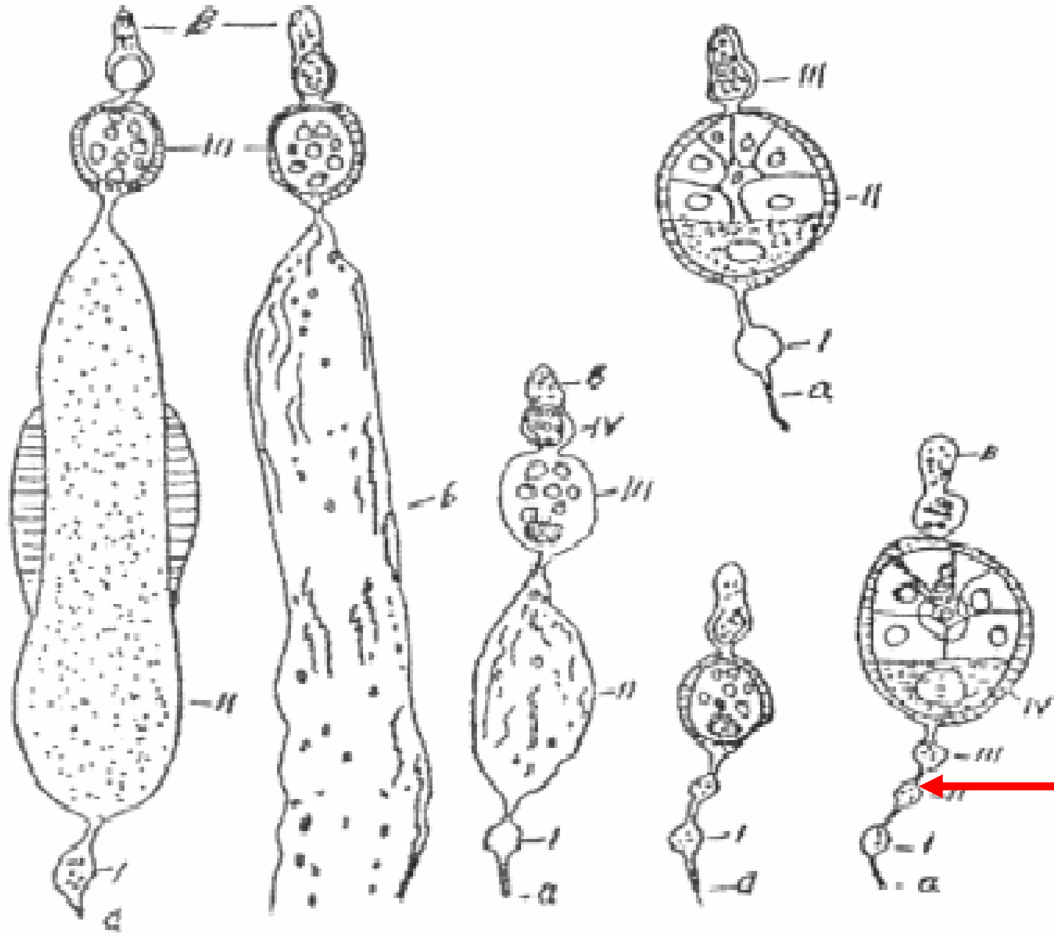
World Health Organization. 1958.

World Health Organization. 1958.
The age-grouping of anopheline mosquitoes by Polovodova's method. Published papers translated (1957) by Dr. M.T. Gillies, Malaria Institute, Amani, Tanganyika <Tanzania>.
Division of Malaria Eradication, WHO, Geneva. December 1958. MHO/AS/142.58.

Dilatations of ovariole can also be used to age grade mosquitoes



nis. a. 8-parous; b. 10-parous.
s in a degenerating condition.



A dilatation is where a developing egg was and after it is laid it is laid, a ‘stretch’ in the ovariole is left.

Figs. 1 and 1a. Scheme for illustrating thickenings of the ovarioles. I — 1st follicle to develop and its site after extrusion; II — 2nd follicle and its site, ditto; III — 3rd follicle and its site, ditto; IV — 4th follicle. a — terminal stalk of the ovariole; b — intima, stretched after passage of the ripe egg — “sac”; B — zone of growth.

FEMALE ACCESSORY GLAND SECRETIONS FUNCTION TO:

1. Glue eggs onto a substrate

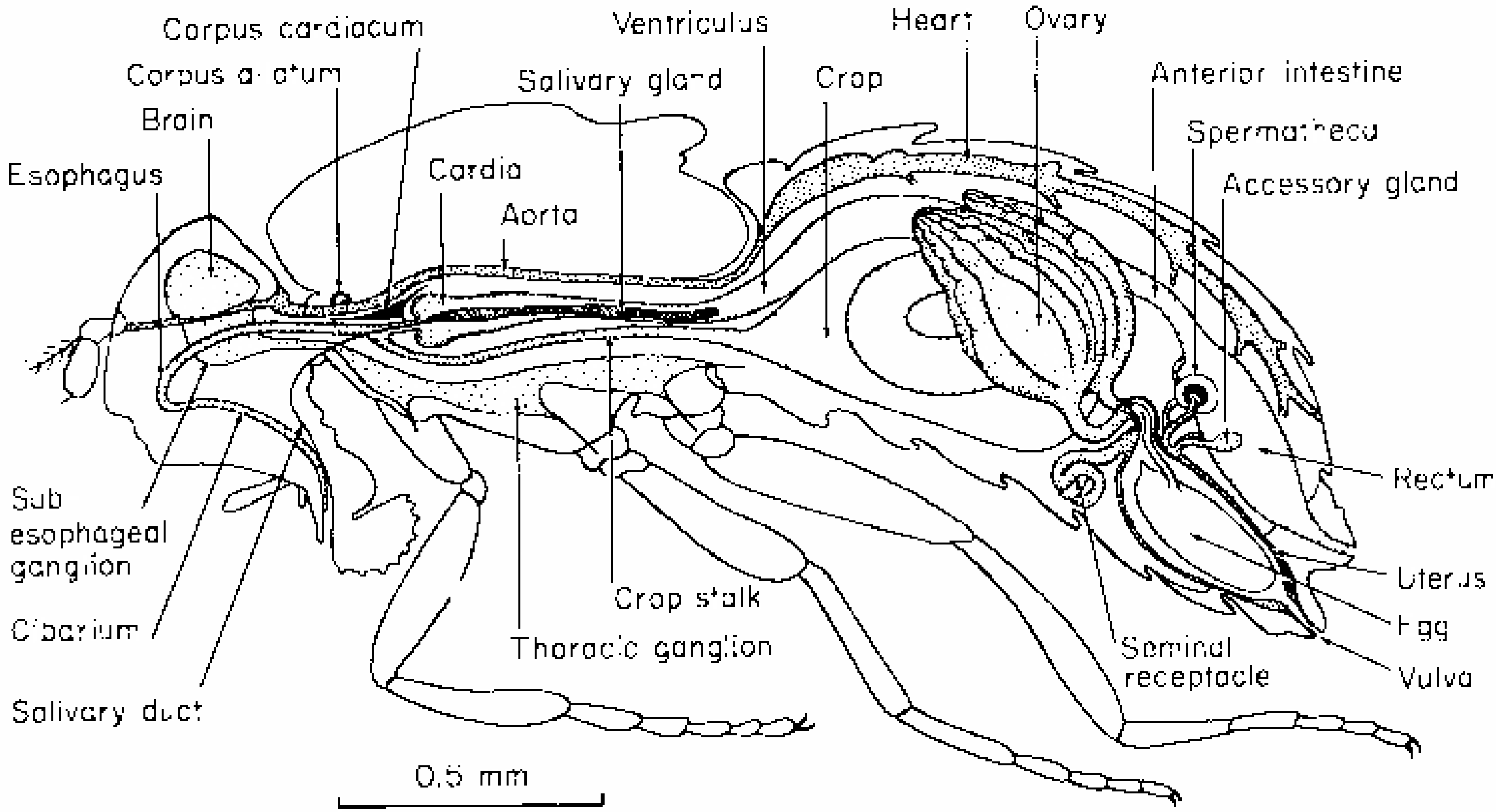
Females of several insect species use the ARG fluid to glue their eggs onto a substrate. Below and to the left is a female butterfly gluing her eggs on the underside of a leaf. In the two right photos one sees a female Tabanus nigrovittatus laying her eggs and gluing them onto the salt marsh grass (*Spartina alternaflora*). Notice the eggs she has already laid and the enlarged SEM showing these eggs glued onto the grass by the ARG fluid of the female.

FEMALE ACCESSORY GLAND SECRETIONS FUNCTION TO:

2. Produce the material of the ootheca in praying mantids & cockroach

The ootheca is an egg case that is houses the eggs and serves as a protective device for the developing embryos. Note first instar nymphs emerging from the praying mantid ootheca. Above the female is getting ready to deposit the cockroach ootheca.

In the diagram of *Drosophila melanogaster* below one should note the fully mature egg that has passed down the oviduct and is now in the correct position below the spermathecal duct to receive sperm. Once this has been completed, the egg will be laid.



MECHANISMS CONTROLLING SPERM PRODUCTION

1. **In many insects complete sperm maturation occurs prior to adult eclosion. In these insects it appears that 20-hydroxyecdysone stimulates spermatogenesis.**

2. **Insects with long adult lives probably also use 20-hydroxyecdysone to stimulate spermatogenesis.**

3. **In most insects, however, the factors affecting complete maturation of sperm are not well understood.**

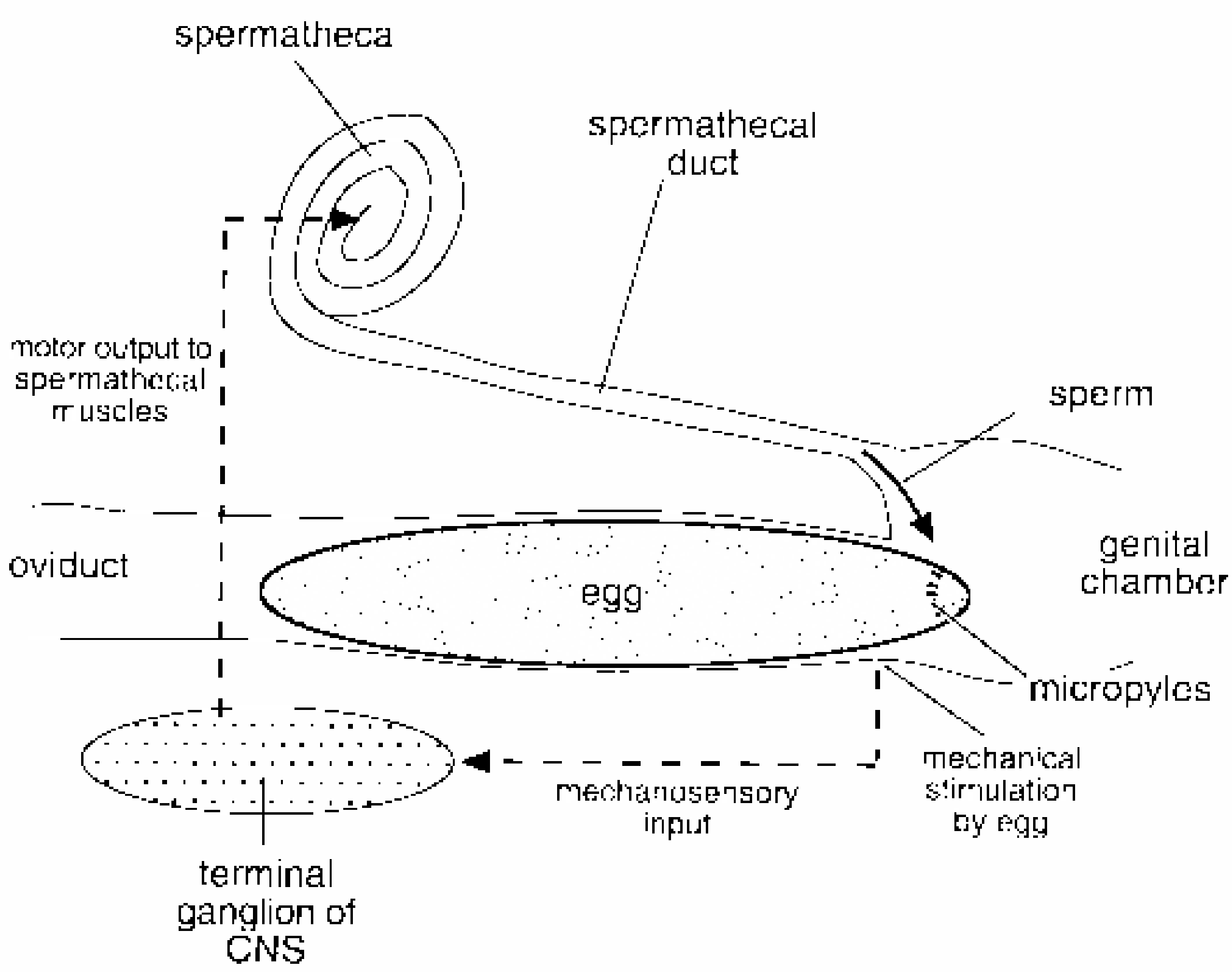
4. **The source of 20-hydroxyecdysone has been shown to be from the males testes.**

CONTROL OF FERTILIZATION OR RELEASE OF SPERM

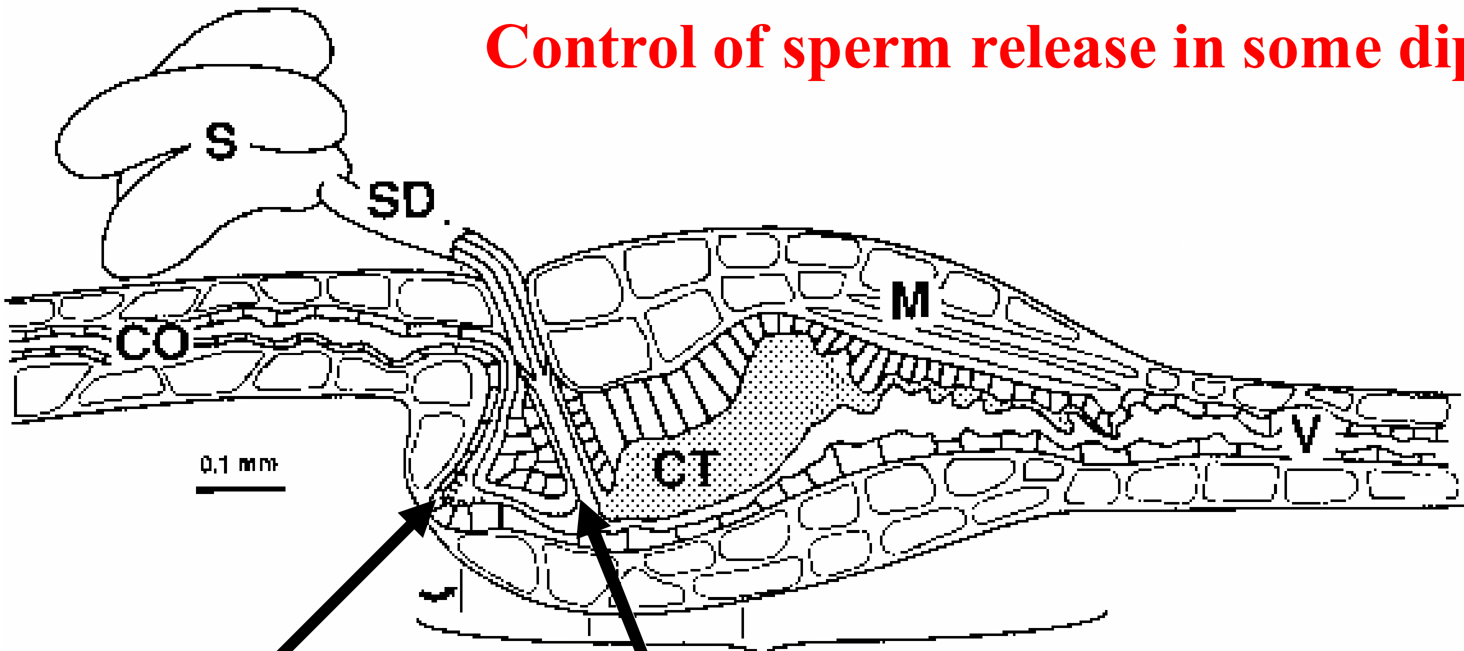
In many insects the terminal abdominal ganglion controls events associated with:

1. Mating
2. Release of sperm
3. Egg laying

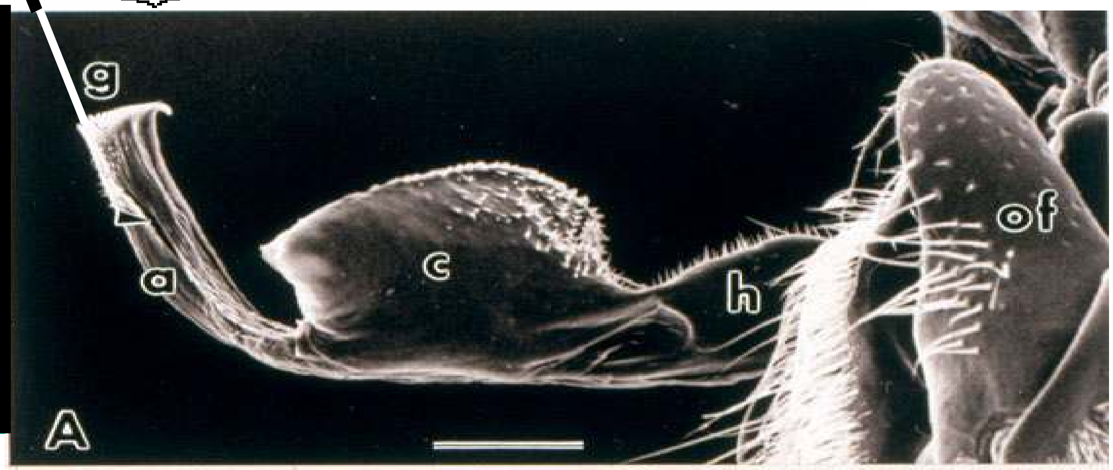
In grasshoppers when the egg enters the oviduct it causes mechanical stimulation because of the egg. A nervous impulses is sent to the terminal ganglion which provides direct motor output to the spermathecal muscles.



Control of sperm release in some dipterans



In some dipterans there are mechanosensory hairs that monitor the presence and position of the egg. These are probably connected somehow to the muscles of the spermatheca



During copulation the male inserts his phallosome into the female's bursa. Note that the gonopore (g) lines up with the opening of the spermathecal ducts. Also note that during egg laying, the egg passes out of the common oviduct (CO) and as it passes down is in the correct position just under the spermathecal duct for sperm to be deposited.

Control of sperm release in some other insects

1. **Cricket (*Teleogryllus commodus*)-**
   In this cricket, the egg, on entering the genital chamber, presses on the cuticle and stimulates subcuticular mechanoreceptors. The input from these receptors inhibits the activity of the oviductal muscles so that the egg stops moving, and, at the same time, activates the muscles of the spermathecal duct, which squeeze the sperm, tail first, towards the egg (Sugawara, 1993)."

2. **In some other insects, such as the Hymenoptera, the female has the ability to control the release of sperm depending on the environmental conditions.**
   A. Queen lays male eggs, which are haploid, in large cells while female eggs are laid in smaller cells.

Control of sperm release in some other insects continues

2B. Some parasitic female wasps lay male eggs (also haploid) into small hosts and diploid eggs (females) into larger hosts. WHAT DOES THIS SENTENCE MEAN IN TERMS OF WHAT THE FEMALE WASP MUST KNOW?

THEY MUST BE ABLE TO EVALUATE THE SIZE OF THE HOST

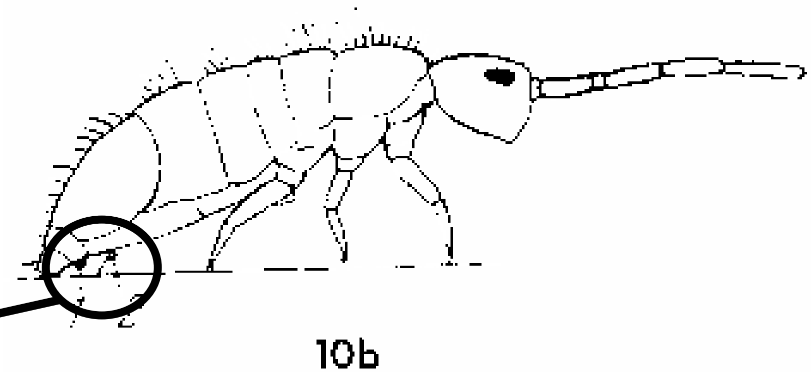
Schmidt, J.M. & J.J.B. Smith. 1989. Host examination walk and oviposition site selection of *Trichogramma minutum*: studies on spherical hosts. Jour. Insect Behavior 2: 143-171.

3. In some Aphelinidae parasites, female eggs are laid in host nymphs but male eggs are laid if the ovipositor enters a nymph already containing one of its own larval species.

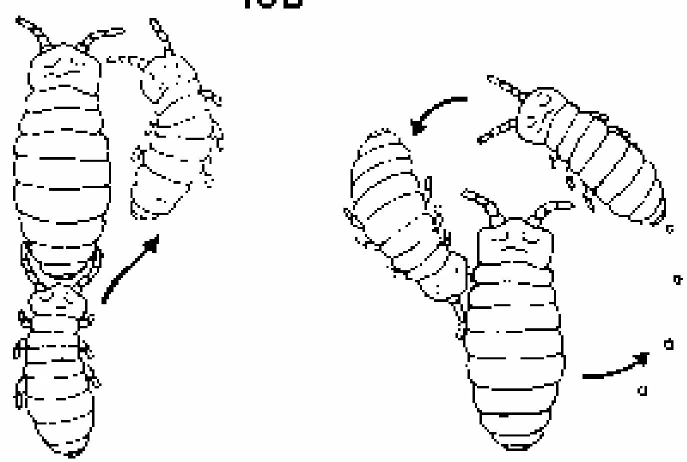
FEMALE PARASITES MUST OBTAIN CHEMICAL SIGNALS FROM THE HOST'S HEMOLYMPH AS TO WHETHER THEY ARE ALREADY PARASITIZED. THIS MEANS CHEMOSENSILLA MUST BE PRESENT ON THE OVIPOSITOR.

SPERMATOPHORES- **a gelatinous/proteinaceous capsule in which the sperm sacs are packaged and transferred to the female during copulation, or in some insects (Apterygota) it is deposited on a surface on which the female picks it up with her genital opening.**

Spermatophores are mainly found in the primitive groups: Apterygota, Orthoptera, Blattoidea, some Heteroptera, all the Neuroptera, some Trichoptera, Lepidoptera, some Hymenoptera and Coleoptera, and a few Diptera (Nematocera and *Glossina*).



Spermatophore of Collembolla



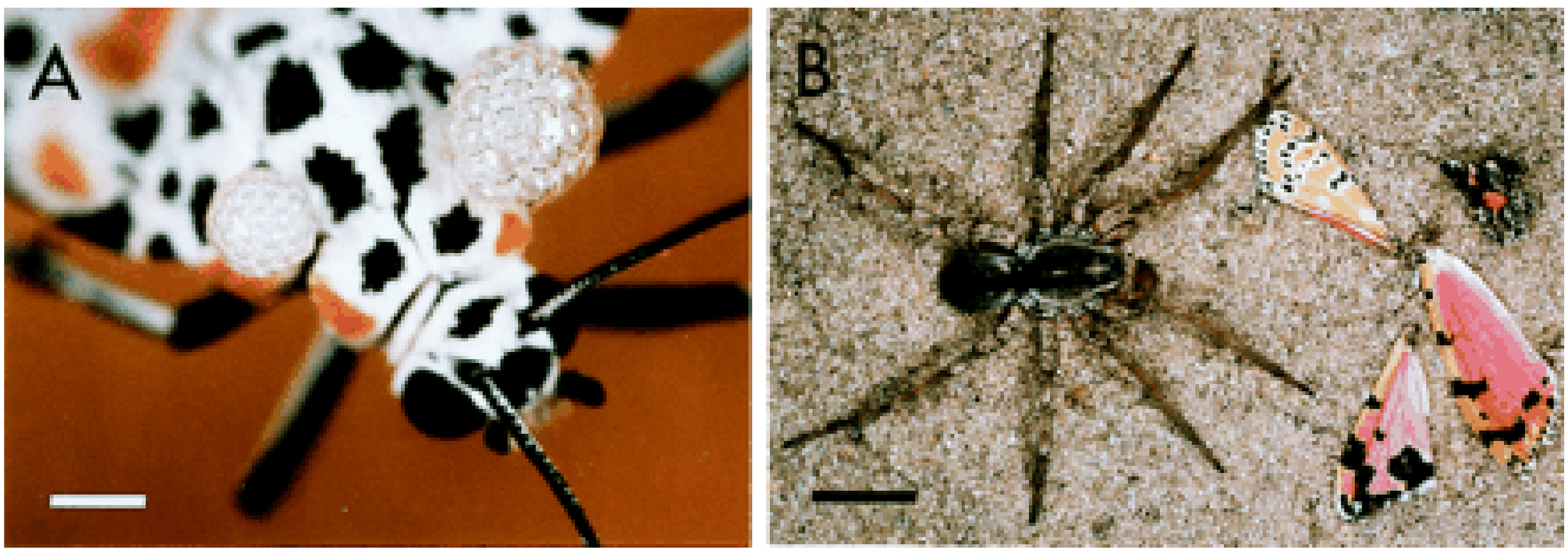
Importance of **Pyrrolizidine alkaloids in the female**

Female with PA in froth

WHERE DOES THIS PA MATERIAL COME FROM?

← These females are not eaten

Female with PA absent - note the fate of this female



THE MALE'S SPERMATOPHORE

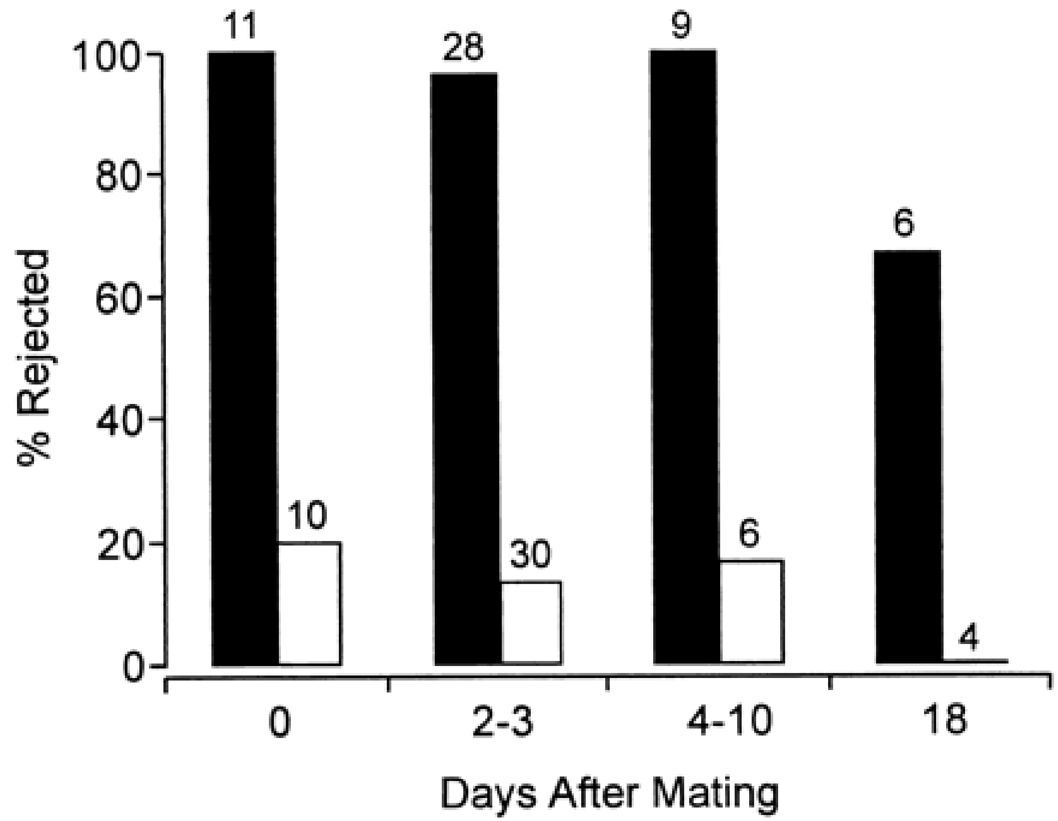


Fig. 3. Fate of ***Utetheisa*** females in laboratory tests with the spider ***L. ceratiola***.
All females were raised to be **Pyrrolizidine alkaloids**/PA-free and were mated either with PA-laden males [(+); solid bars] or with PA-free males [( ); open bars]. Numbers above bars give sample sizes. The discrimination against the [(+)] females is significant (*G* test on pooled data; $P < 0.0001$).

Weighing more than 10 percent of the male moth's body mass, the **spermatophore** contains enough sperm to fertilize hundreds of ***U. ornatrix*** eggs, nutrients to help the female produce numerous healthy eggs and PAs, now known to protect the female as well as her eggs, González says. "It makes sense that the male should contribute to the female's defense. She is, after all, the recipient of the sperm."

Pyrrolizidine alkaloids from plants are extruded as a froth when the rattlebox moth is disturbed. *Photo by Thomas Eisner*

Defensive chemicals from the rattlebox plant make *Utetheisa ornatrix* moths distasteful to spiders. *Photo by Thomas Eisner.*

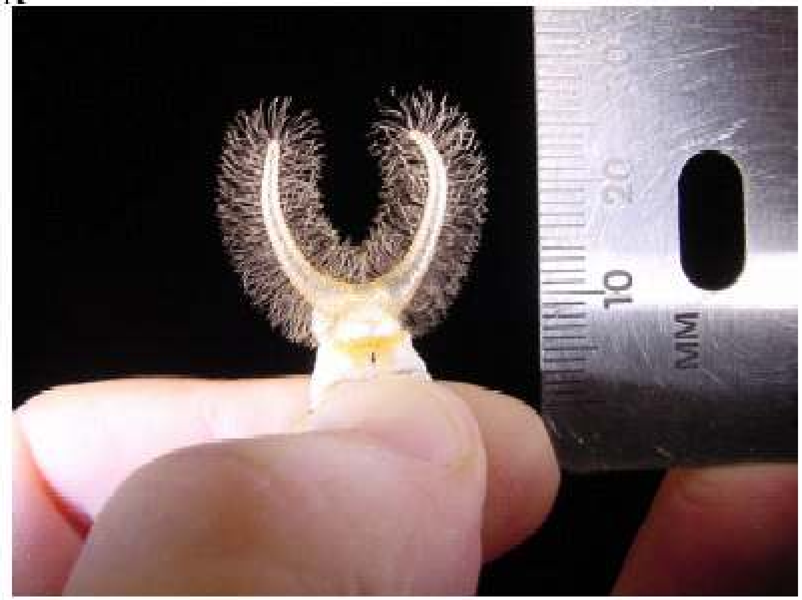
One of the few moths that flies during the day.

An enduring nuptial gift is included in every sperm package from a male rattlebox moth (*Utetheisa ornatrix*)to his freshly mated female: a potent, plant-derived chemical that protects her for life against predatory spiders, biologists at Cornell University have discovered.
The first (but almost certainly, not the only) example of a sexually transmitted chemical defense to benefit a female animal is reported in the current (May 11, 1999) *Proceedings of the National Academy of Sciences* by Cornell biologists Andrés González, Carmen Rossini, Maria Eisner and Thomas Eisner. The protective chemical, pyrrolizidine alkaloids (PAs) that the adult male obtains by eating rattlebox plants (*Crotalaria mucronata*) while in the larval stage, reaches every part of the female's body within minutes after mating, the biologists say. It also protects her eggs.

Males of many Lepidoptera have scent-organs located on various parts of their bodies. These go by the names androconial scales, scent fans, hair-pencils, brushes or coremata. Many of the 10,000 arctid species have eversible sacs or tubes called coremata. They emerge from pockets between the 7th & 8th abdominal sternites or in the genital valves. Air enters a large tracheal sac connected to the lateral tracheal trunk at the base of the tubes. The sac inflates and everts the coremata. Many of these males selectively feed on plants high in pyrrolizidine alkaloids (PAs) (as seen in the fig. on the left and below it). Both the size of the Coremata and pheromone content depends on the larvae ingesting PAs. There is a direct correlation between the size of the coremata and the amount of PAs the larva has ingested (see figure below). Whether the size aids in female choice is still a question.

Effects of larval ingested monocrotaline N-oxide on corematal development in *Estigmene acrea* (A, PA5+ male), PA1 +male (B) and PA-male (C).

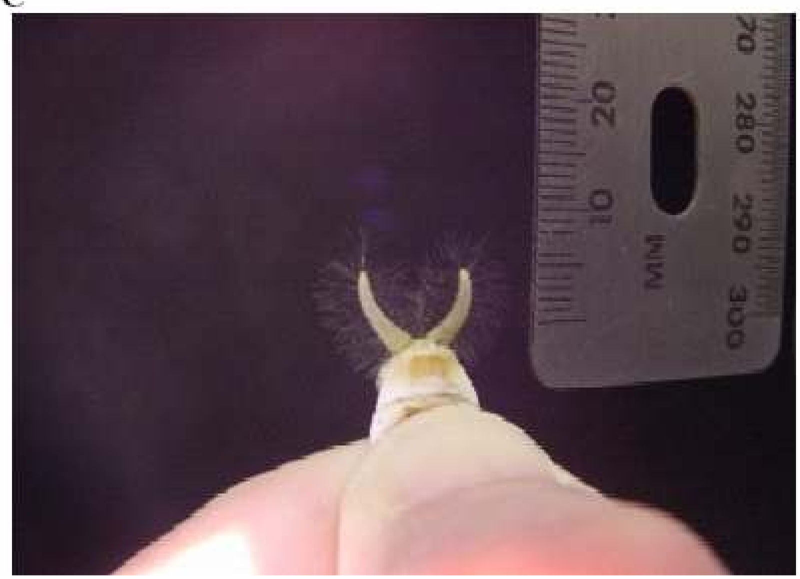
A



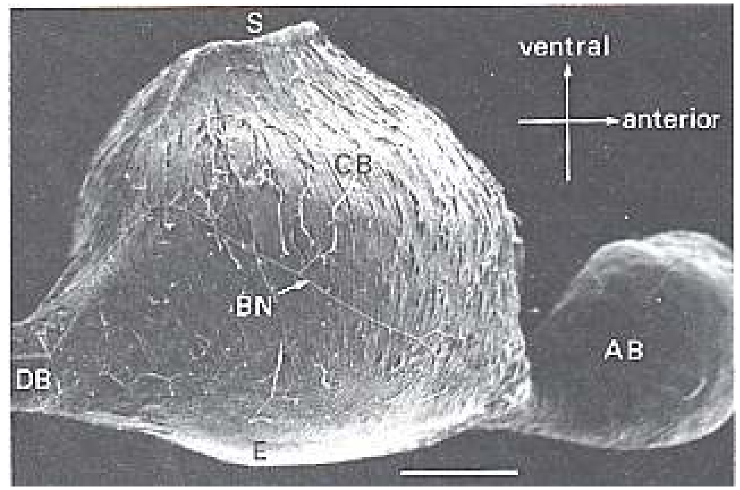
PAs somehow affects size of the coremata. Possibly ecdysone on the imaginal disc for the development of that structure

B

C



FORMATION AND STRUCTURE OF THE SPERMATOPHORE

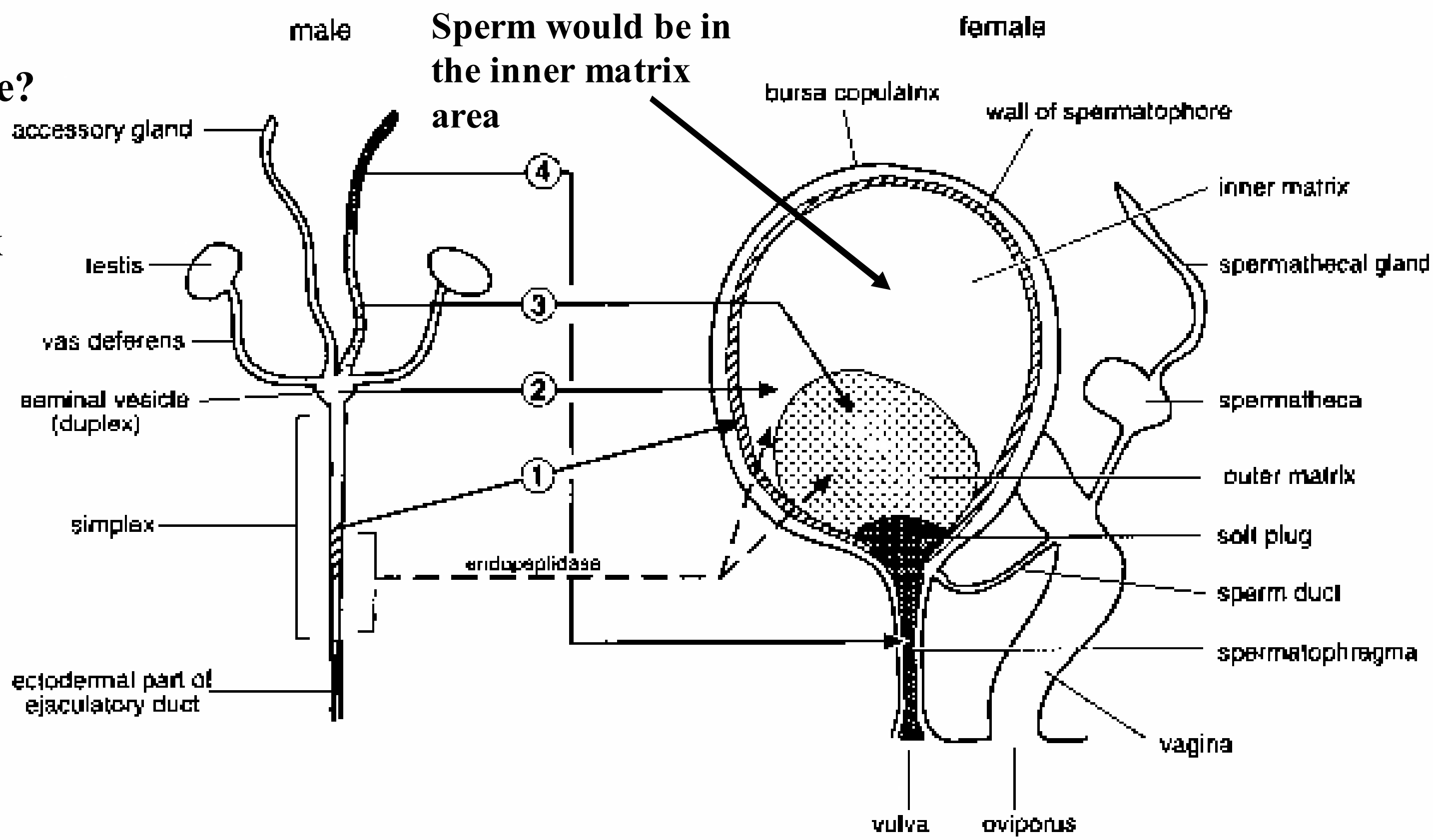


In the Lepidoptera the spermatophore is formed inside the females bursa copulatrix, using this as the mold for the shape of the spermatophore. Remember from the topic on the nervous system that the bursa has a stretch receptor (BN) that informs the female that she has mated.

In some species, one can count the number of sperm to know if the female multiply mates and how many times.

How does the sperm get out of the spermatophore?

In some Lepidotera, the females bursa copulatrix is lined with spines that, through muscle action, tear open the spermatophore permitting the sperm to then gain access to the spermatheca.

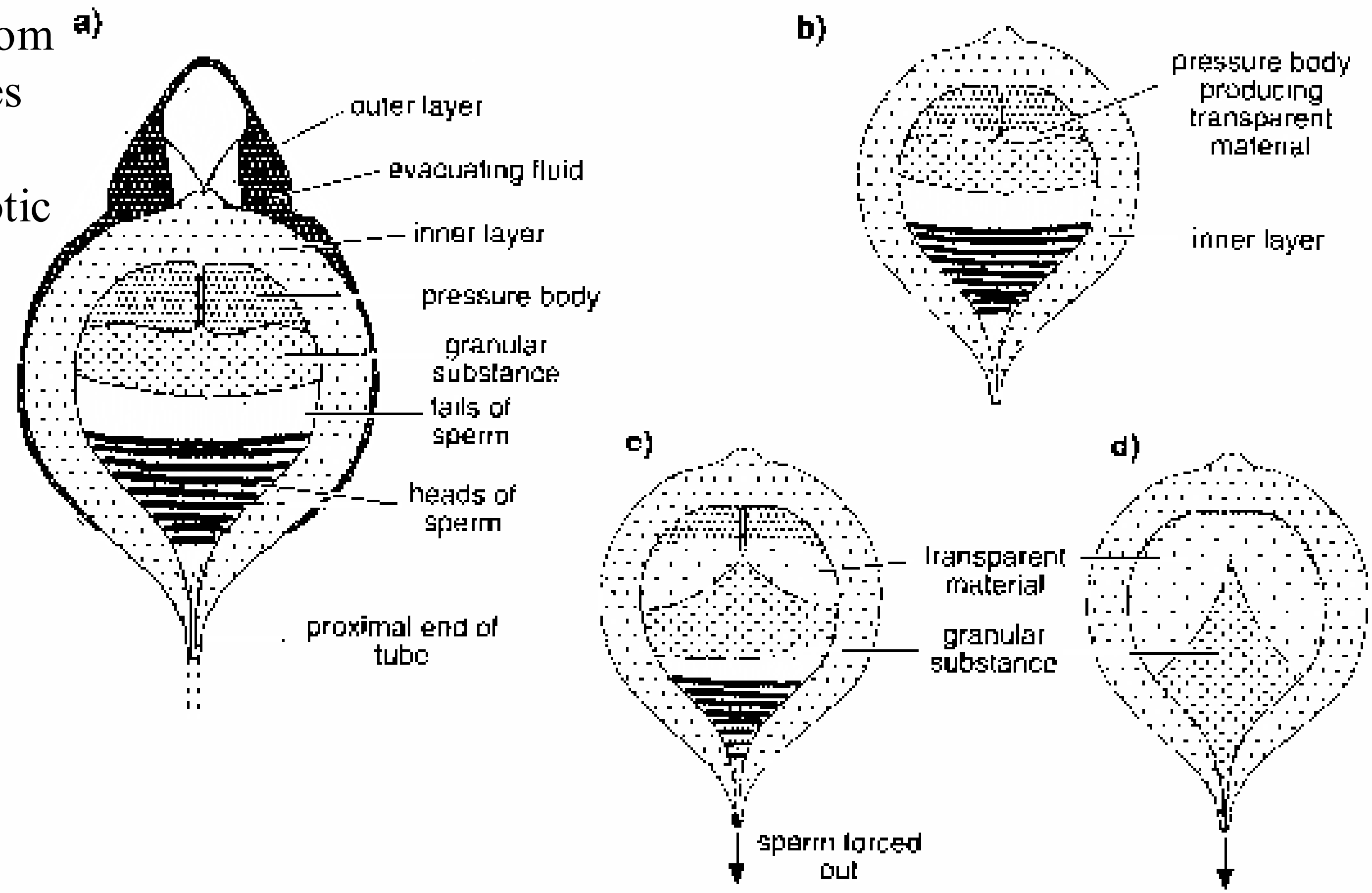


Sperm would be in the inner matrix area

How does the sperm that leaves the spermatophore get into the spermathecae?

1. **Passive movement with the aid of the females oviduct**
2. **Actively and under pressure pushed by fluids from the spermatophore**

In the cricket, *Acheta.* When deposited, water from the evacuating fluid passes into the pressure body because of different osmotic pressure. The pressure body produces a transparent material and swells, thus forcing the sperm out of the ampulla and into the spermatheca.



TRANSFER OF OTHER CHEMICALS THAT HAVE FUNCTIONS OTHER THAN REPRODUCTIVE

1. **Spanish fly (*Lytta*, Coleoptera)-here the males synthesize cantharadin while the females do not and it is probably transferred to the females during copulation and from the males ARG. Function not well known.**

2. **Large quantities of JH produced and stored in the ARG of *Hyalophora cecropia* and is transferred to female during copulation. Function not understood.**

3. **Pyrrolizidine alkaloids-Produced and passed onto female in spermatophore. Renders her and her eggs unreceptive to predators. Phagodeterrent.**

4. **Cardiac glycosides-In some milkweed butterflies the males obtain and sequester the compound from the plant surfaces and transfer large amounts to the female during copulation. Deterrence against predation.**

5. **Some species of male cockroaches store uric acid in their ARG and transfer this to the female during copulation. Some of this uric acid is even passed onto the egg, thus a nuptual gift and a paternal gift. The nitrogen in the uric acid is believed to aid the female in egg maturation and the embryo in development. The idea is that this 'gift' saves the female from predation and finding protein.**

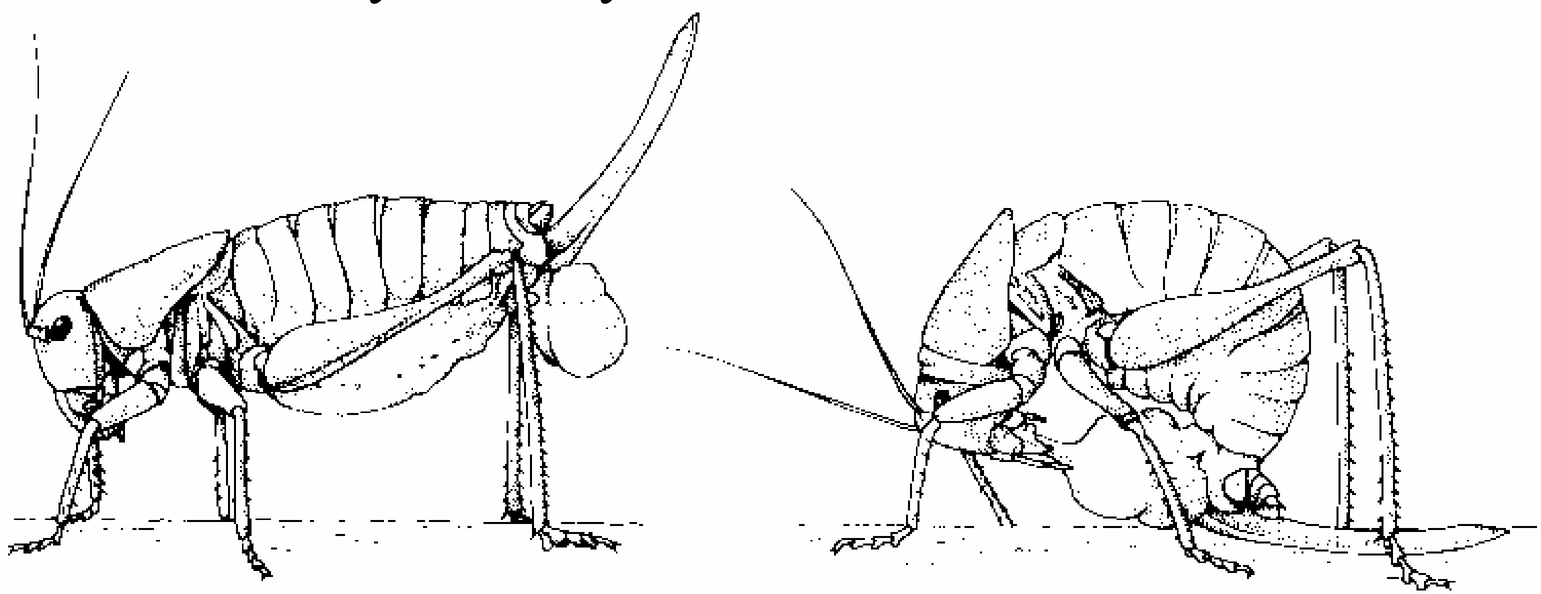
AN OVERVIEW:
FUNCTIONS OF THE MALE'S SPERMATOPHORE

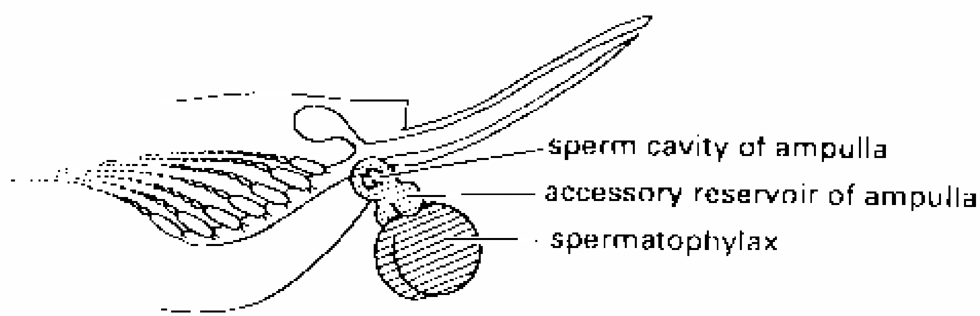
1. Housing and carrying the sperm
2. May provide some nutrients to the female (nuptual gifts)
3. May contain other substances that protect the female and the eggs (e.g., pyrrolizidine alkaloids) from predators

Other facts about spermatophores:

1. May be formed inside the males body or inside females body
2. Sperm may passively get into the spermatheca or they may be pushed there by pressure from within the spermatheca itself

In some species, the spermatophore itself is not inserted into the females genitalia but remains outside and is connected to the female by a tube. Instead, the whole structure itself is like an intromittent organ with a long tube that enters the female. The sperm is forced into the female and the remaining gelatinous/proteinaceous mass, known as a spermatophylax, remains outside and is usually eaten by the female. Is considered a nuptual gift.





Hymenopteran parasites can regulate the sex of the egg by whether they are fertilized or not

In a few insects, fertilization occurs while the eggs are in the ovary.

Cimicoidea=practice hemocoelic insemination

Aspidiotus=a coccid in which the sperm become attached to cells which proliferate in the common oviduct and then migrate to the pedicels

Shutting down egg development in insects

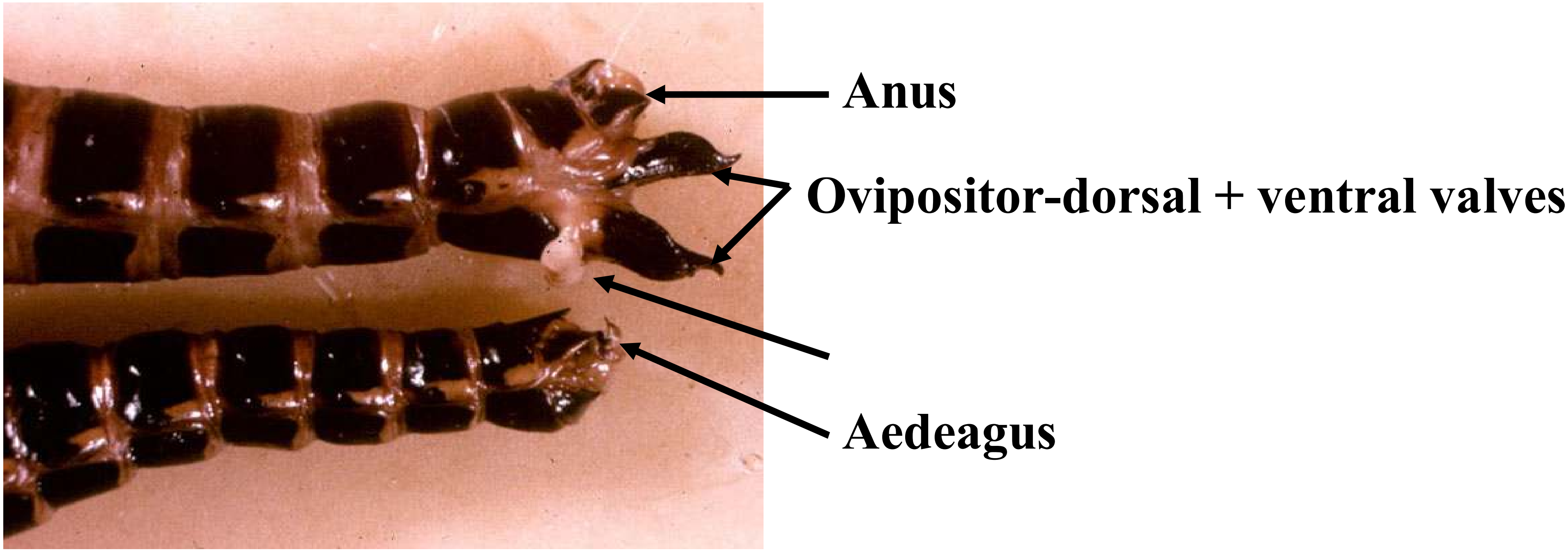
1. Presence of an ootheca in the brood chamber neurally shuts down JH production, thus egg development.

Shutting down egg development in insects

2. In locusts adipokinetic hormone acts on fat body

3. Lack of sufficient proteinaceous supply either internally or from diet.

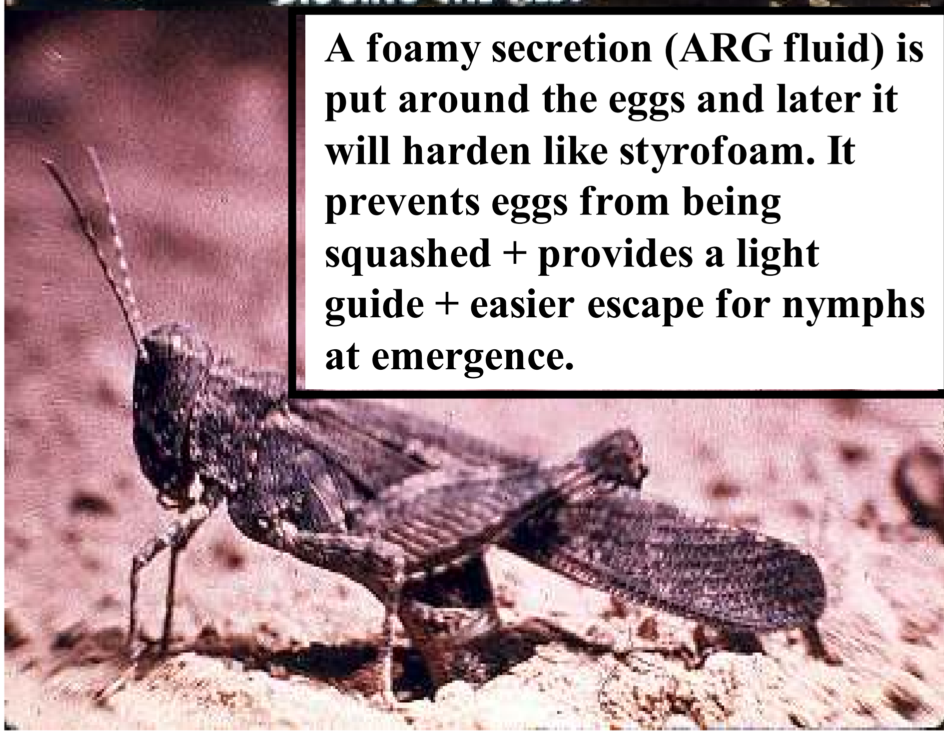
GENITALIA OF LUBBER GRASSHOPPER (MALE+FEMALE)



LAYING EGGS - OVIPOSITIONING

OVIPOSITION-the deposition of the eggs by the female

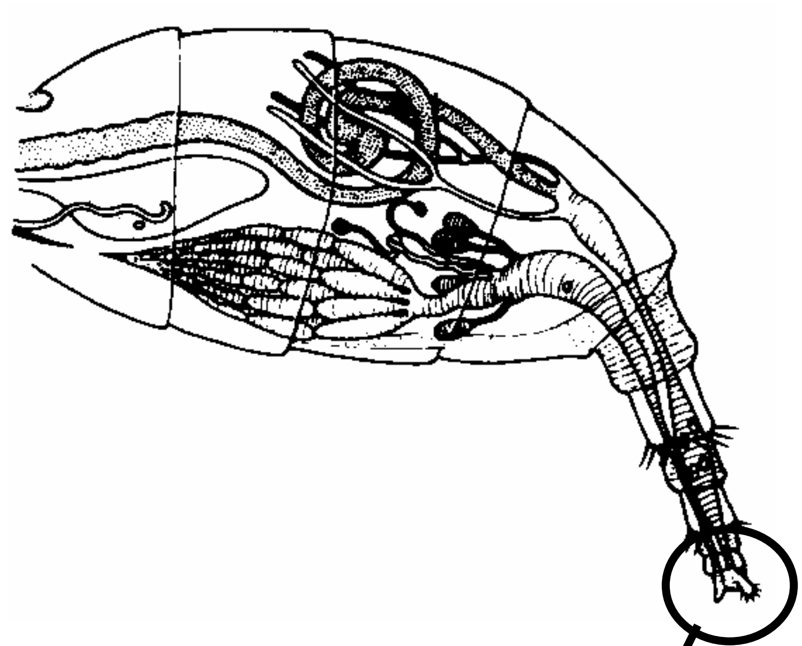
A foamy secretion (ARG fluid) is put around the eggs and later it will harden like styrofoam. It prevents eggs from being squashed + provides a light guide + easier escape for nymphs at emergence.



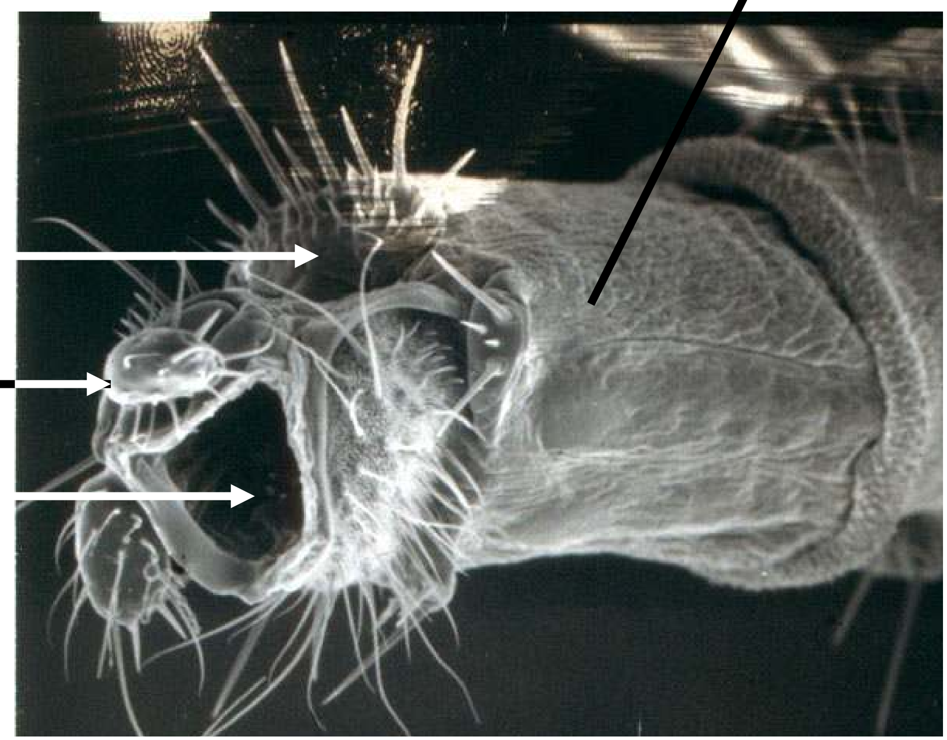
Lubber grass-hopper laying eggs. Note the extension of the abdomen into the hole. This is possible because of plasticization of the cuticle.

OVIPOSITORS

Most insect ovipositors lack chemoreceptors. They are present, however, in the following groups: Orthoptera, Diptera, parasitic Hymenoptera and Lepidoptera. Females lacking chemoreceptors on the ovipositor obtain information about where to lay their eggs from chemoreceptors on either the tarsi or antenna or both. Ovipositors may also contain mechanosensilla.



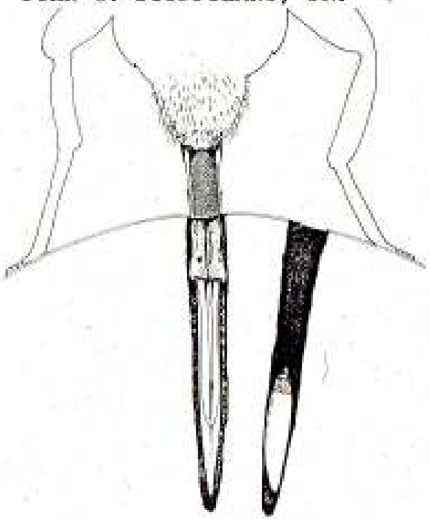
Ovipositor of ***Phormia regina*** showing gonopore and chemoreceptors

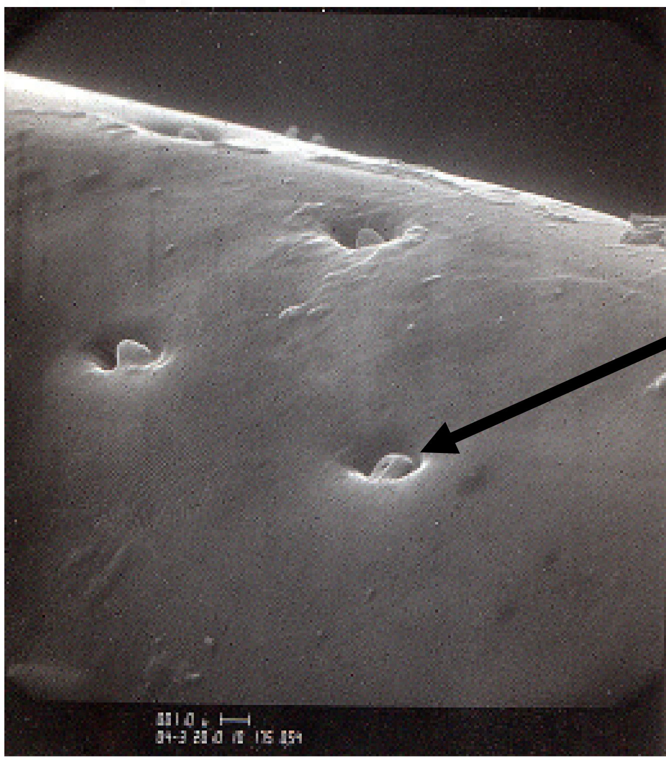


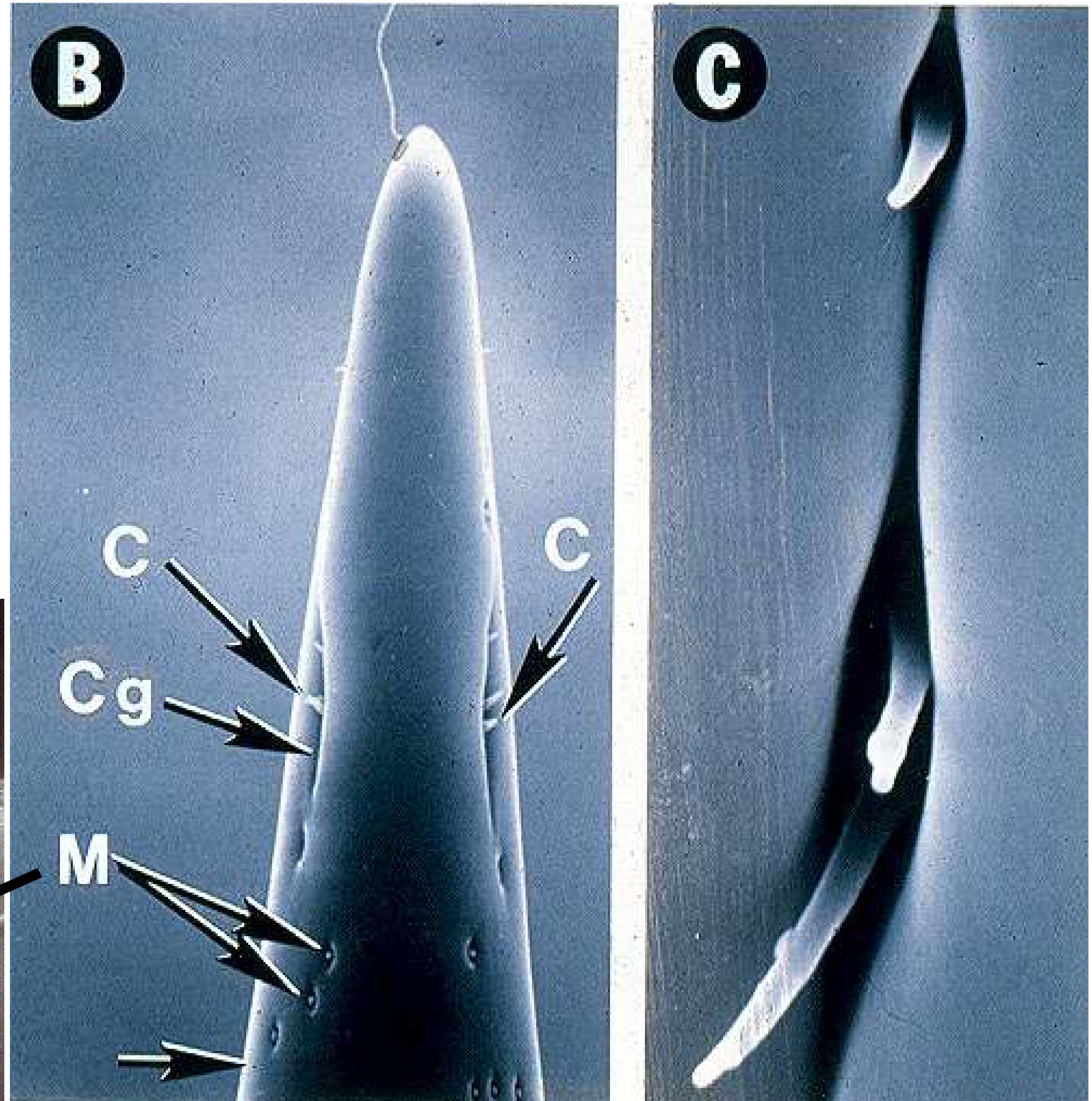
OVIPOSITION BEHAVIOR IN THE TEPHRITIDS

BY

JOHN G. STOFFOLANO, JR.







M=campaniform sensilla + chemosensilla (C)

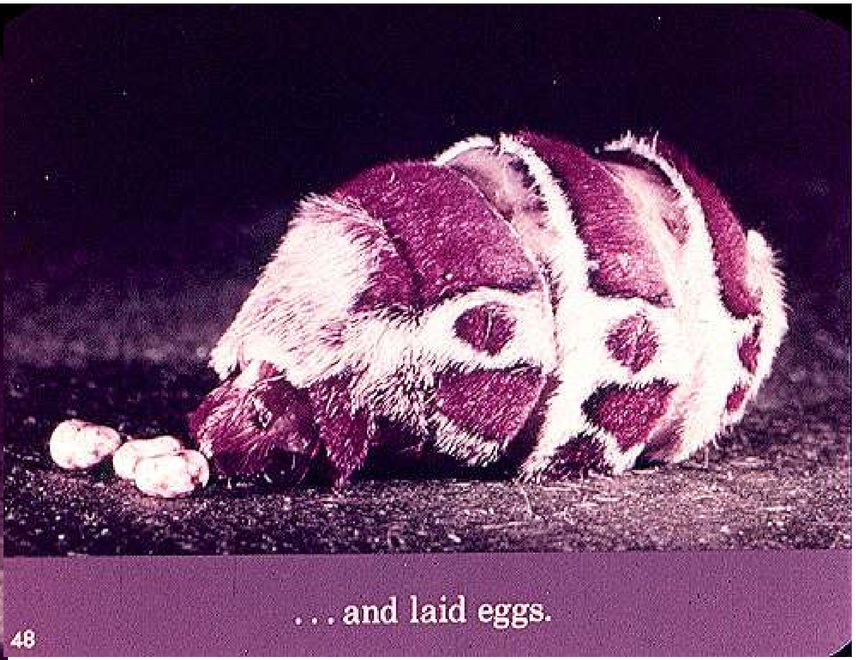
CONTROL OVER OVIPOSITION BEHAVIOR-In most insects, the readiness to oviposit is influenced by mating.

1. **Mated females seek out and lay eggs as soon as possible. If they can't find an oviposition site they may abort and reabsorb the eggs.**

2. **Virgin females will retain eggs as long as possible. If no mate is found they may also abort and reabsorb the eggs.**

Generalized control involves:
1. Input from the ovulation peptide in the male's ARG-ovulin peptide pheromone
2. Central pattern generator in last abdominal ganglion
3. Neural input from the ovipositor
4. Feedback from the CNS
5. Myotropic hormones and/or biogenic amines in the blood

Control of oviposition in silkmoths



How can this isolated abdomen mate and then lay eggs?
Separation of 'state's rights' expressed in insect ganglia
Many behavioral events are controlled by prepackaged neural circuits already present in the ganglia of insects. One of these is egg laying.

Neuronal circuits for fixed motor behavior programs or fixed action patterns

Command neuron-a neuron (interneuron) whose excitation is essential to activate a central motor program

Preprogrammed motor circuits or Central programmes-a neural circuit that produces a constant (stereotyped) and reliable set of neural outputs that once activated continue with little or no sensory input.

1. These programs continue to function even when the CNS is experimentally isolated from peripheral sensory input.
2. The circuitry is usually made up of an array of interneurons.
3. Neurons involved are usually dedicated lines
4. Usually found in ventral nerve cord ganglia

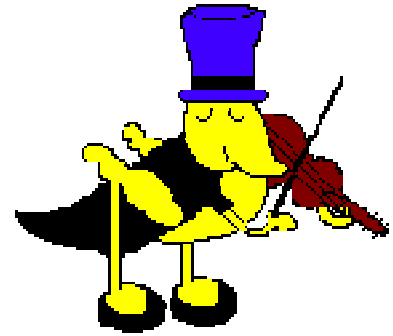
EXAMPLES OF CENTRAL PROGRAMMES

1. **Oviposition**
2. **Stridulation**
3. **Flight**
4. **Walking**
5. **Ventilation or breathing**

In the intact insect, however, these basic patterns are adjusted by input from the peripheral sensilla or receptors to adjust movements, etc.

Stridulatory central programme in crickets for courtship

1. Important for this song to be stereotyped for species separation + female recogn.



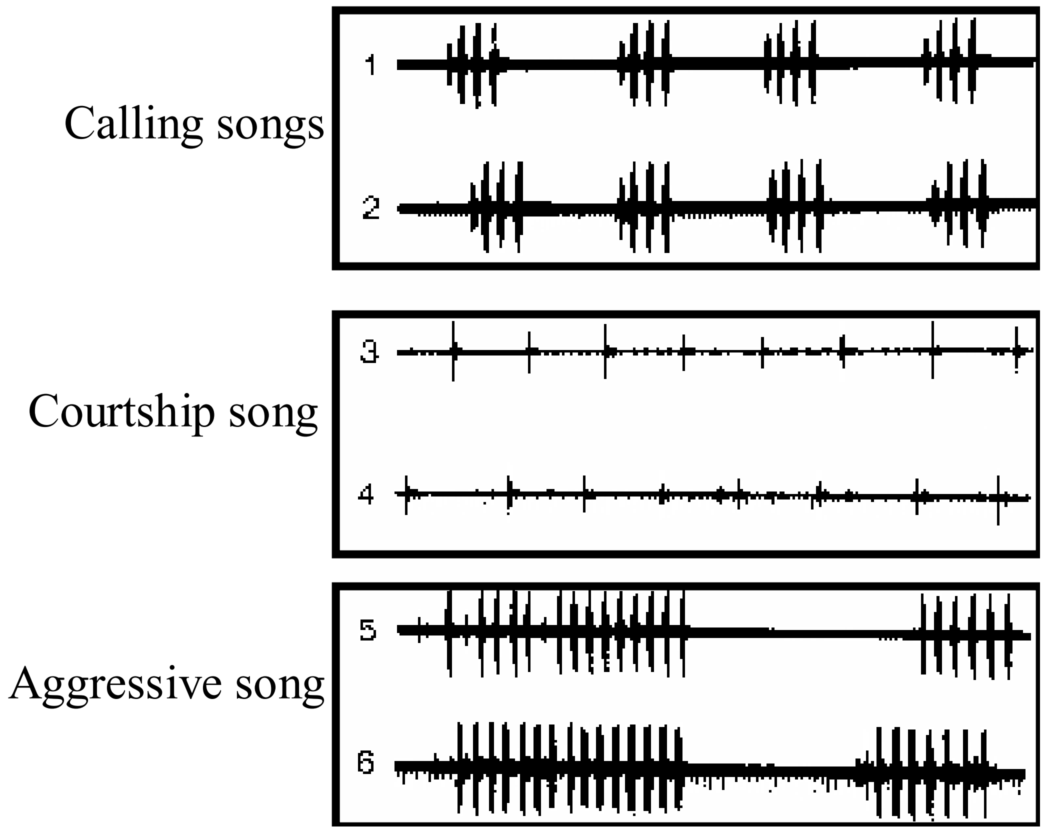
a. Removal of the legs, tegmina, or cutting their motor nerves doesn't change the motor output when it is recorded from

b. Hooking the wings together so they can't produce sound still results in the same motor output

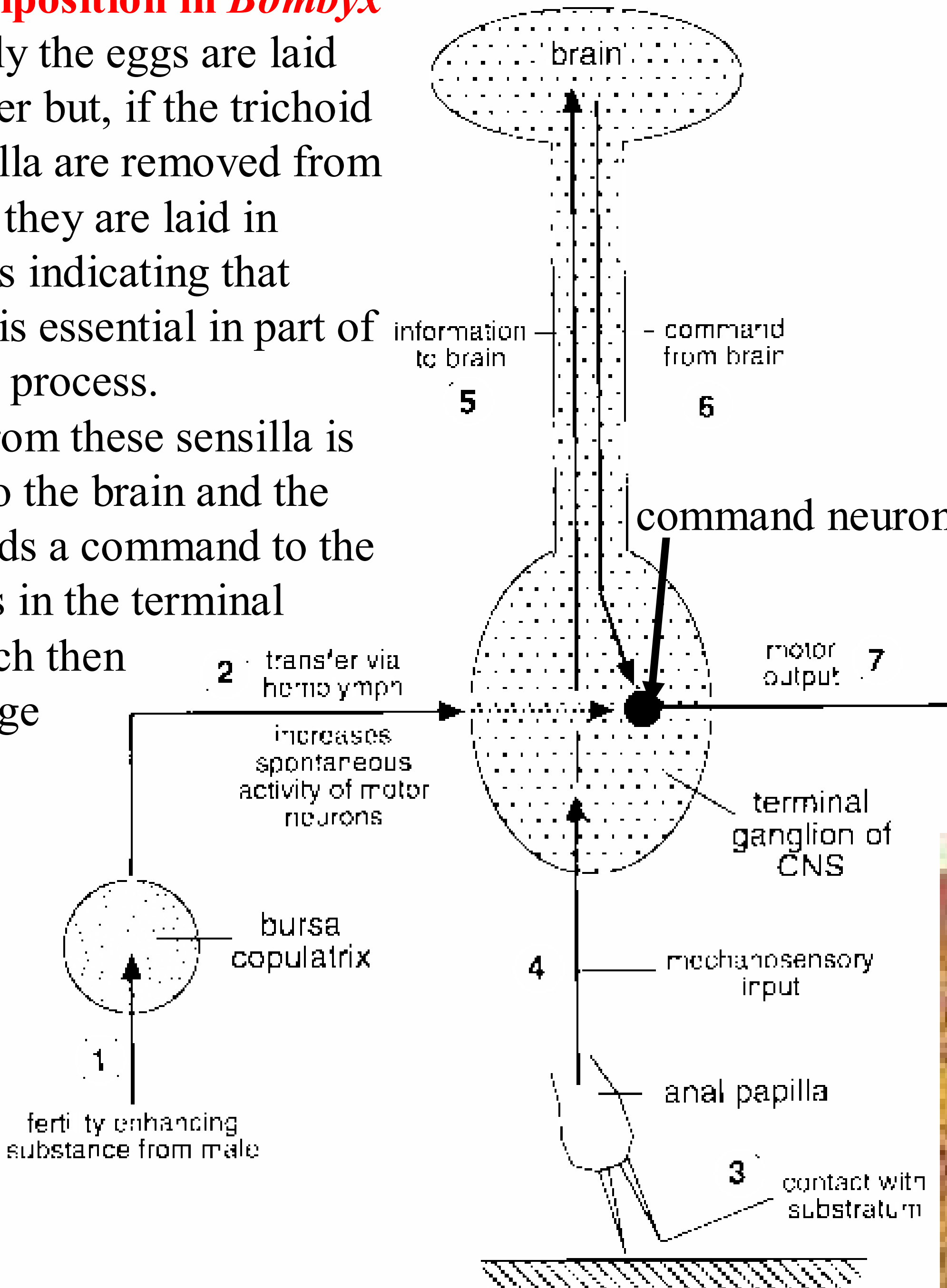
c. Can stimulate the program by just electrically stimulating the brain

d. Decapitated males can still produce all 3 types of songs

Chirp rate of *Gryllus campestris*



Control of oviposition in *Bombyx mori*. Normally the eggs are laid in a single layer but, if the trichoid mechanosensilla are removed from the ovipositor they are laid in uneven clumps indicating that sensory input is essential in part of the egg laying process. Information from these sensilla is sent directly to the brain and the brain then sends a command to the motor neurons in the terminal ganglion, which then sends a message to the oviduct muscles.



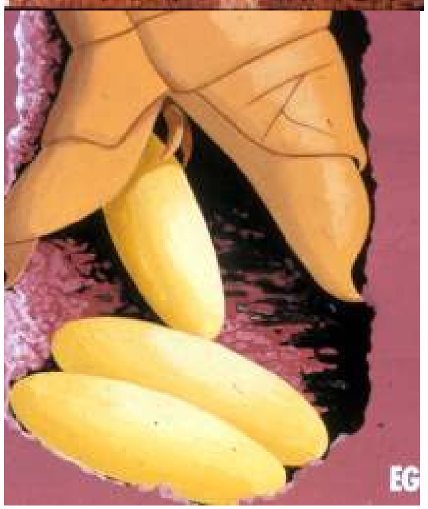
HOW ARE INSECT EGGS PROTECTED FROM PREDATORS/PARASITES?

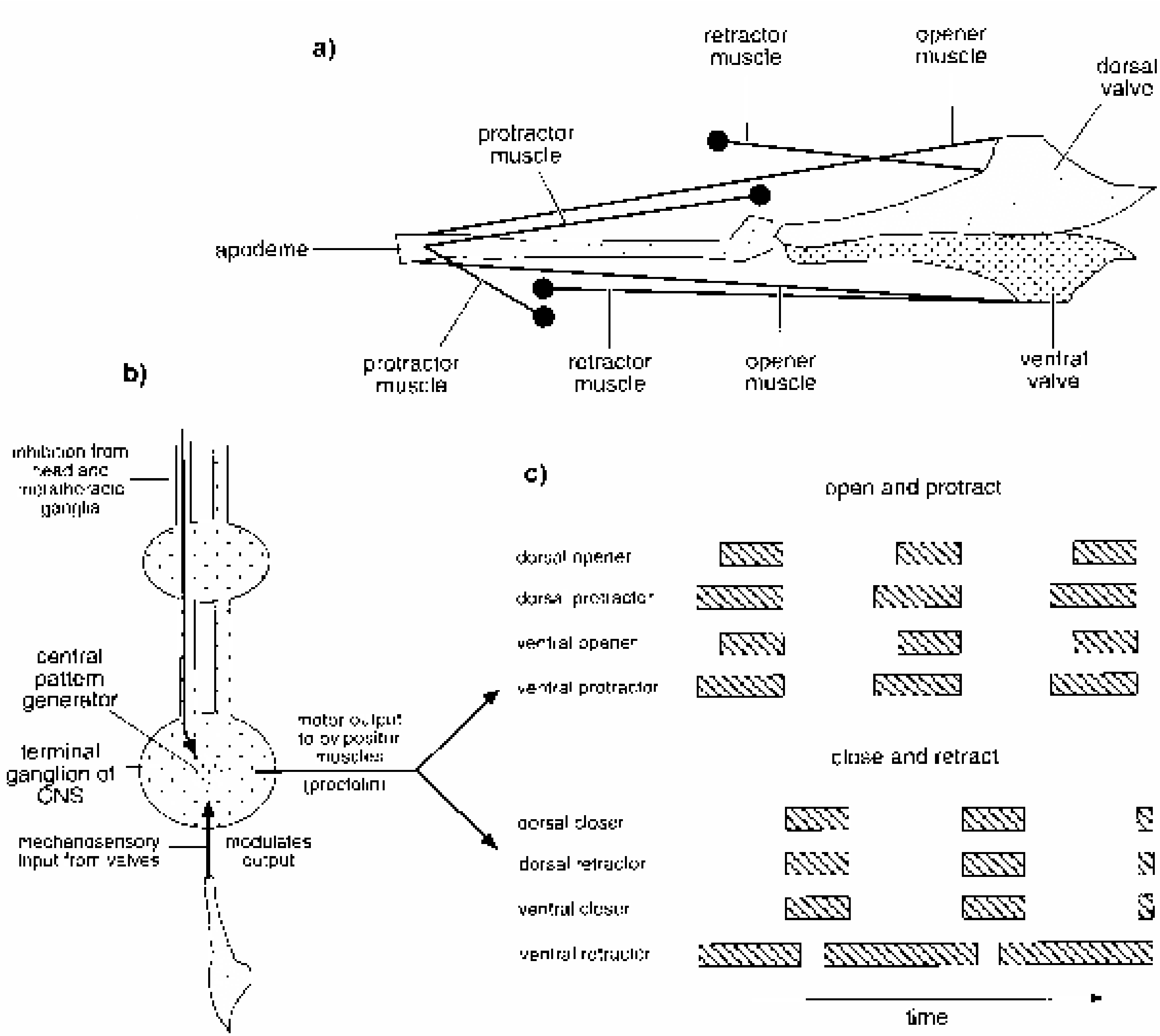
This is a poorly understood topic.

1. Dermaptera female protects eggs
2. Social insects take care of their eggs
3. Gypsy moth female covers eggs with her abdominal hairs
4. Hiding eggs on the underside of plants or inside their tissue
5. Chemical defense (see below)

T. Eisner, M. Eisner, C. Rossini, V. K. Iyengar, B. L. Roach, E. Benedikt, and J. Meinwald
Chemical defense against predation in an insect egg
PNAS, February 15, 2000; 97(4): 1634 - 1639.

A. Bezzerides, T.-H. Yong, J. Bezzerides, J. Husseini, J. Ladau, M. Eisner, and T. Eisner
Plant-derived pyrrolizidine alkaloid protects eggs of a moth (Utetheisa ornatrix) against a parasitoid wasp (Trichogramma ostriniae)
PNAS, June 15, 2004; 101(24): 9029 - 9032.





Control of egg laying in the locust

SYMBIONTS AND PATHOGENS IN EGGS

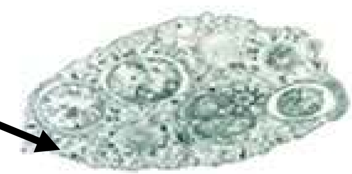
Many insects rely on intracellular symbionts for some aspect of their survival. Yet we know very little about how they are transferred from the female to the egg, where they reside in the pre-embryonic stage of the egg, and where they are located in the embryo. Their functions during these periods remains unknown.

Arthropods of vector borne disease agents of both plants & animals.

Horizontal transmission-between two unrelated individuals

Vertical transmission-between the female, via the egg and into the next generation. Called transovarial transmission.

UNDESIRABLE SEX PARTNERS-bacteria (rickettsiae) manipulate reproduction of insects and other species. *Wolbachia* infect around 20 of all arthropod species.



1. Are found in the testes and ovaries of host
2. Alter the reproduction of their hosts
   a. Infected males can generate offspring only if they mate with infected females.
   b. Infected females produce offspring without the need of the opposite sex.
   c. *Wolbachia* can transform female embryos into males
   d. Infected females only produce females-male embryos die
3. In some parthenogenetic groups, treatment with antibiotics brings back the male contribution
4. *Wolbachia* are transovarially transmitted.

Cytoplasmic incompatibility in mosquitoes. When infected males mate with uninfected females: In such matings, no offspring are produced. This reproductive barrier can be eliminated with antibiotics.

http://www.wolbachia.sols.uq.edu.au/

Wolbachia infection influences the development of Culex pipiens embryo in incompatible crosses

Duron O, Weill M

Abstract: Wolbachia are maternally inherited endosymbiotic bacteria that infect many arthropod species and have evolved several different ways for manipulating their host, the most frequent being cytoplasmic incompatibility (CI). CI leads to embryo death in crosses between infected males and uninfected females, as well as in crosses between individuals infected by incompatible Wolbachia strains. In the mosquito Culex pipiens, previous studies suggested developmental variation in embryos stemming from different incompatible crosses. We have investigated this variation in different incompatible crosses. Unhatched eggs were separated into three classes based upon the developmental stage reached by the embryos. We found that incompatible crosses involving uninfected females produced only embryos whose development was arrested at a very early stage, irrespective of the Wolbachia variant infecting the male. These results differ from other host species where a developmental gradient that could reach late stages of embryogenesis or even living larvae was observed, and indicate a novel peculiarity of CI mechanism in C. pipiens. By contrast, all incompatible crosses with infected C. pipiens females produced embryos of all three classes. The proportion of embryo classes appeared to be associated with the strains involved, suggesting specific CI properties in different incompatible crosses. In addition, the contribution of parental genome was characterized in embryo classes using molecular markers for each chromosome. Embryo phenotypes appeared linked to the paternal chromosomes' contribution, as described in Drosophila simulans. However, this contribution varied according to maternal infection and independently of male factors.

PSR (paternal sex ratio)(right bottom, arrow) is a killer chromosome found in the developing sperm cells of the Jewel wasp (top right). After fertilization, PSR destroys all of the chromosomes. Is a piece of parasitic DNA.

BASIC AND APPLIED ASPECTS OF STUDIES ON THE REPRODUCTIVE SYSTEM

APPLIED-

1. **Chemosterilants including plant products such as precocene and neem (azadarachtin)**
2. **SIT-Sterile insect technique**
3. **Pest management strategies-Knowing how long it takes a female to develop her eggs can influence period prior to spraying**
4. **Pheromones and traps**
5. **Nutrition and poisonous baits**

BASIC-

1. **Understanding the role of nutrition in reproduction**
2. **Understanding the role of hormones on reproductive events**

Azadarachtin from the neem seed affects reproductive events in the insect. It inhibits insect molting and is structurally similar to ecdysone. It appears to inhibit the production of ecdysone by the ecdysial glands

Azadirachtin clearly affects both male and female reproductive systems and does so probably through action on ecdysteroids.

Linton, Y. M., A. J. Nisbet, and A. J. Mordue (Luntz). 1997, The effects of azadirachtin on the **testes** of the desert locust, *Schistocerca gregaria*: J. Ins. Physiol. 43 1077–1084.

Garcia ES, Azambuja P, Forster H, Rembold H 1984. Feeding and molt inhibition by azadirachtins A, B and 7-acetyl-azadirachtin A in *Rhodnius prolixus* nymphs. *Zeitschrift für Naturforschung 39c*: 1155-1158.

Garcia ES, Luz N, Azambuja P, Rembold H 1990. Azadirachtin depresses release of **prothoracicotropic hormone** in *Rhodnius prolixus* nymphs: evidence from head transplantation. *J Insect Physiol 36*: 679-682.

Clearly, azadirachtin decreases the ecdysteroid levels in the hemolymph (Garcia et al. 1986b, 1987) and blocks synthesis of the new cuticle, which is dependent on the ecdysteroid hormones (Garcia et al. 1986a).

We have now covered all of the systems:

How would you define what an insect physiologists does or studies?

or

What is insect physiology?

It is the study of how insects solve their problems.

The mechanisms involved and how these mechanisms work.

How these mechanisms play out in a particular scheme or pathway and

How one system interacts with another to produce a result for the integrated unit we call the insect.

Can we make any generalizations about what we have learned?

1. There are usually generalized schemes that operate in solving a particular problem (eg. Retention of water and ions)
2. There are usually subtle differences in the way these schemes operate (eg. Dipterans use rectal papillae to solve these problems whereas another group may use the rectum without the papillae). These subtle differences truly reflect the great diversity of insects.
3. Insects in most cases are not that different from vertebrates in the way they solve the problems facing them.