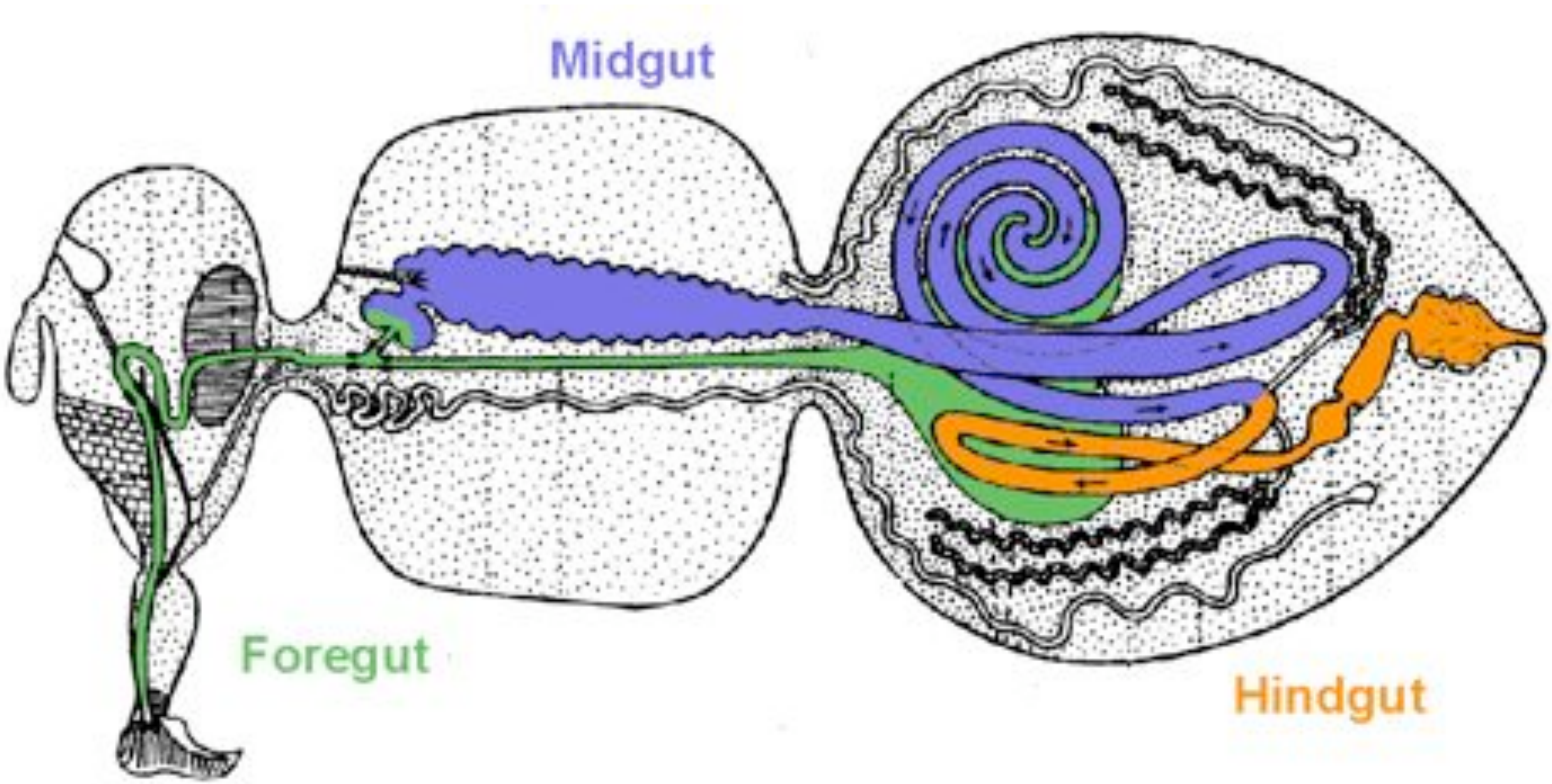
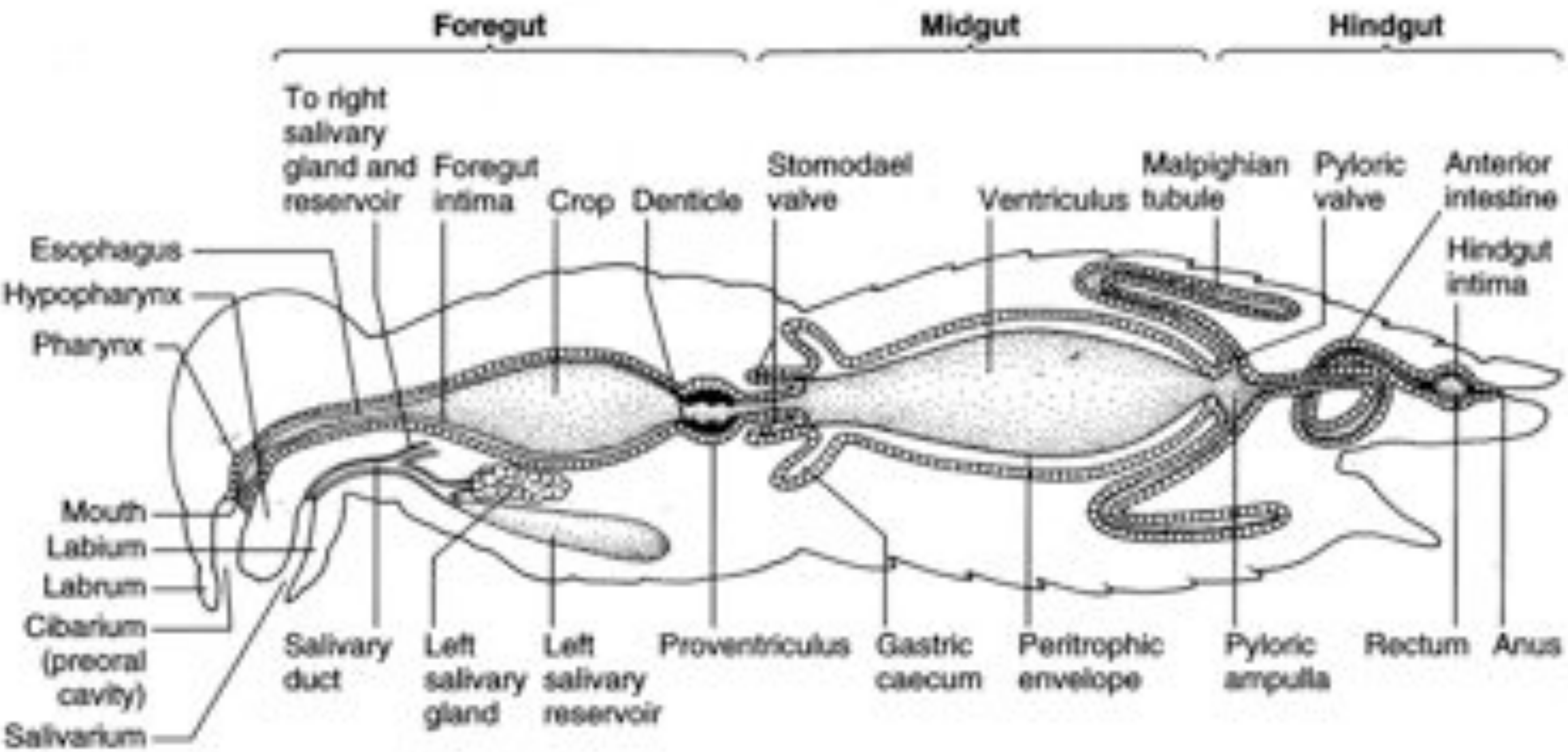
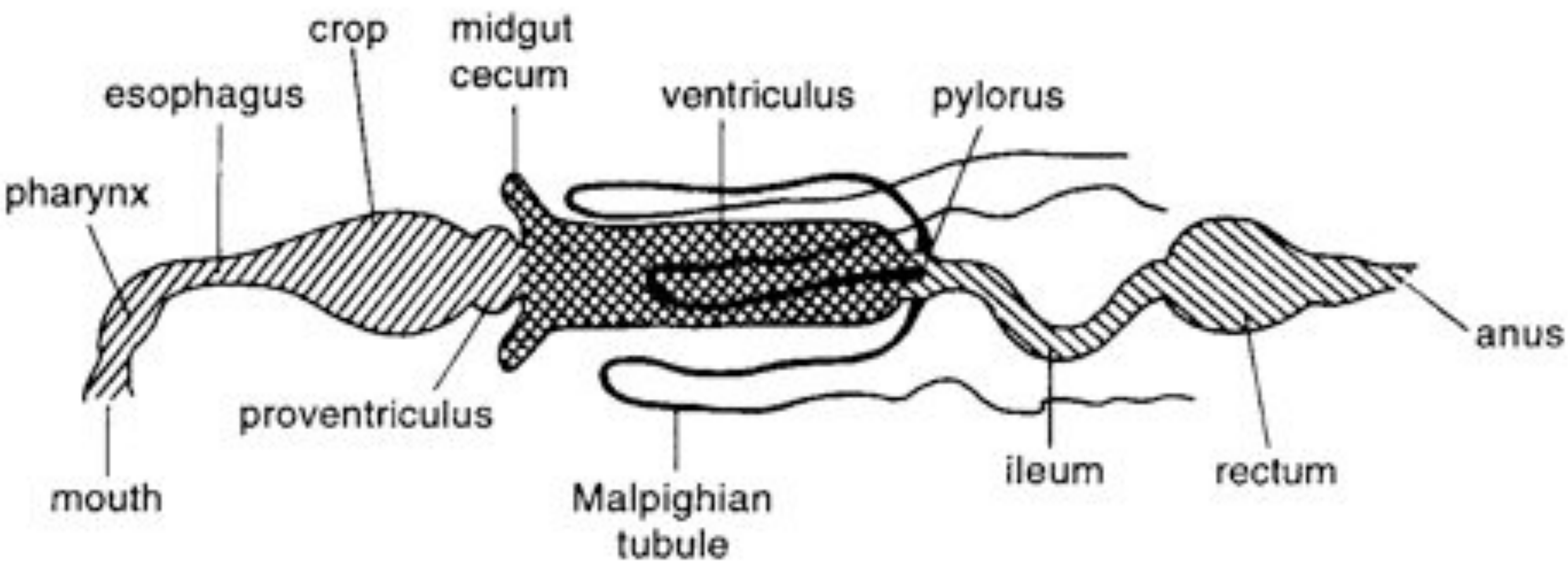
DIGESTIVE SYSTEM

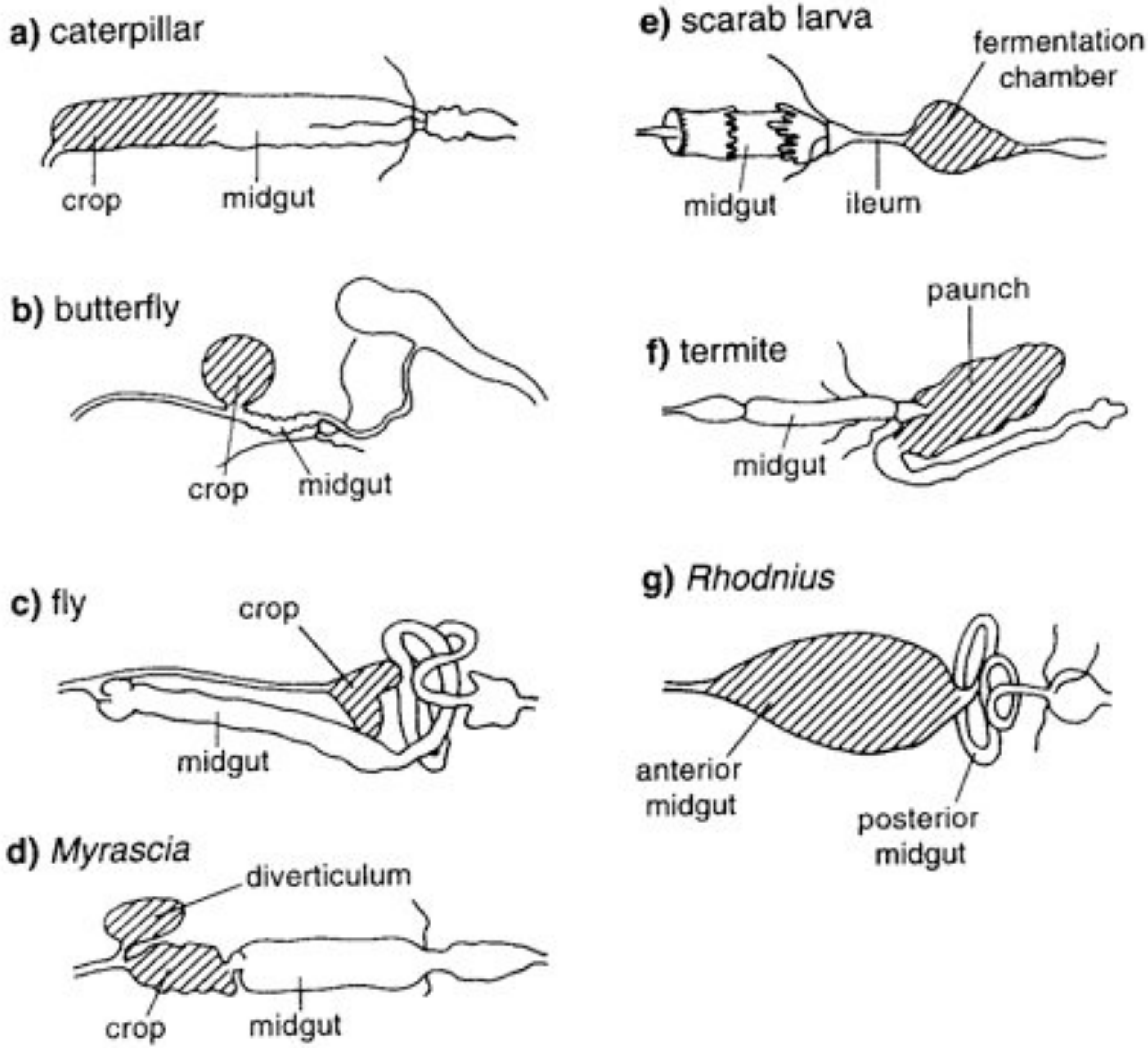


Generalized insect alimentary tract

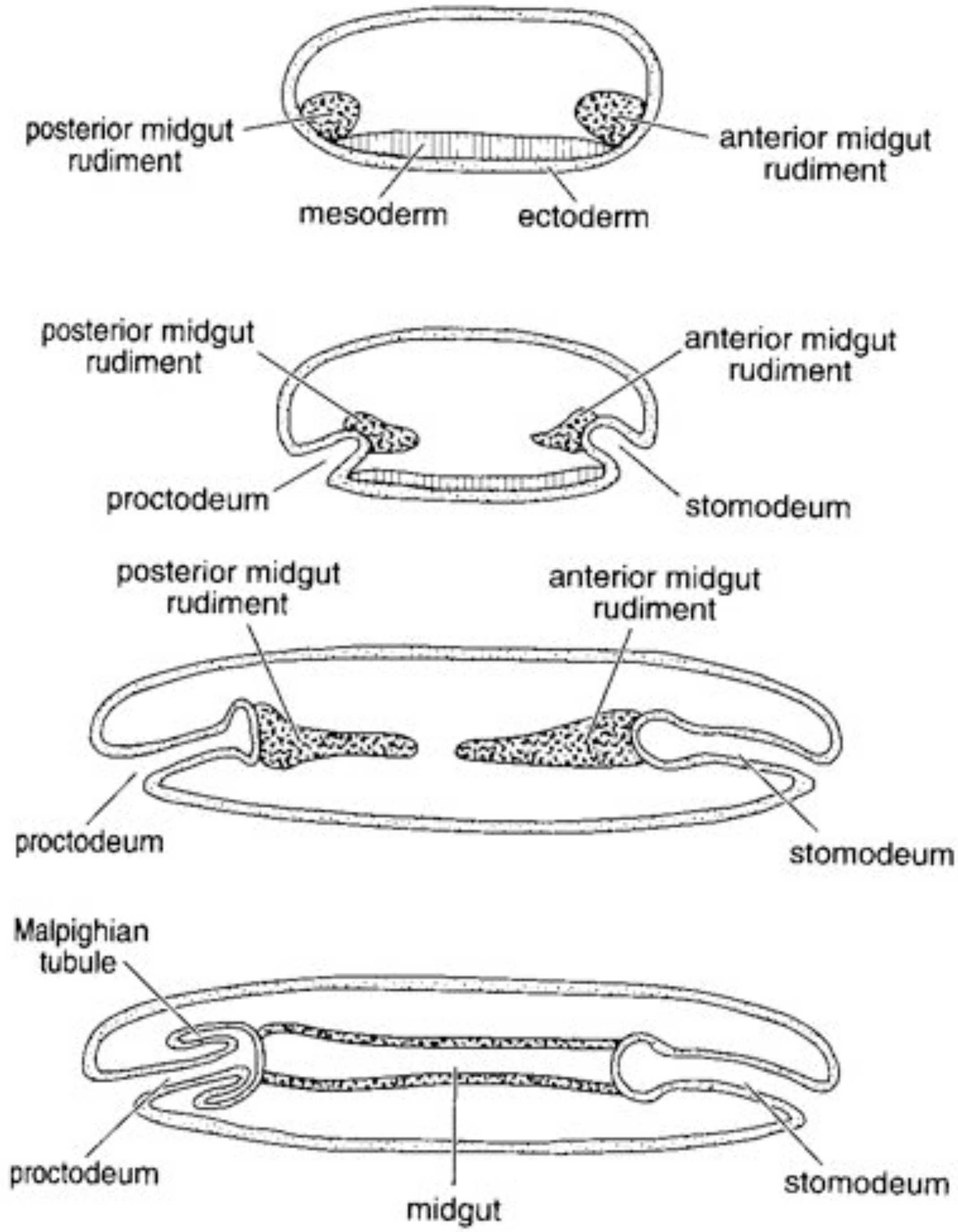


The digestive system is just a tube within a surrounding tube called the body. It starts with a mouth and ends with the anus. What goes on in between depends on the insect, its life stage and what it eats.

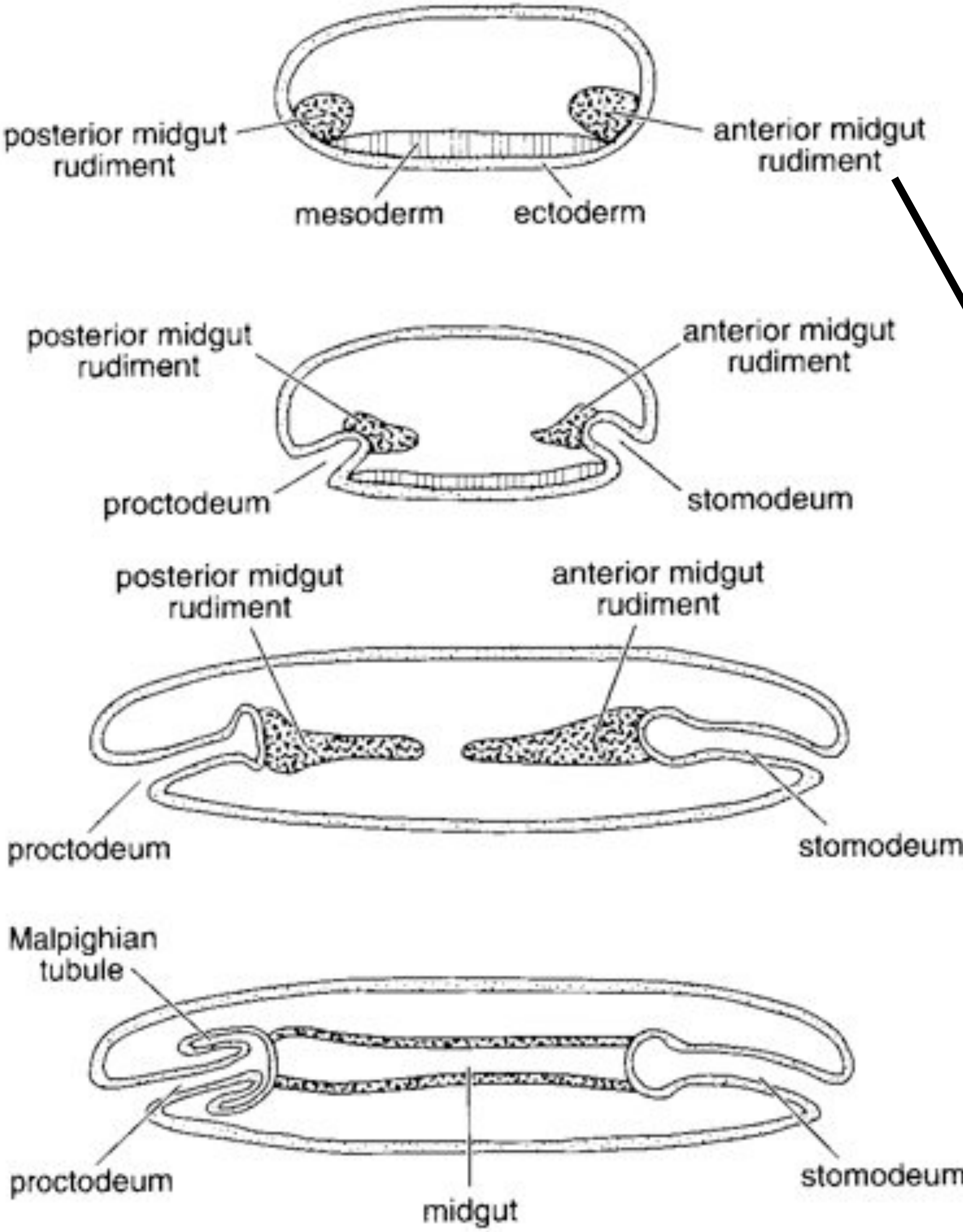


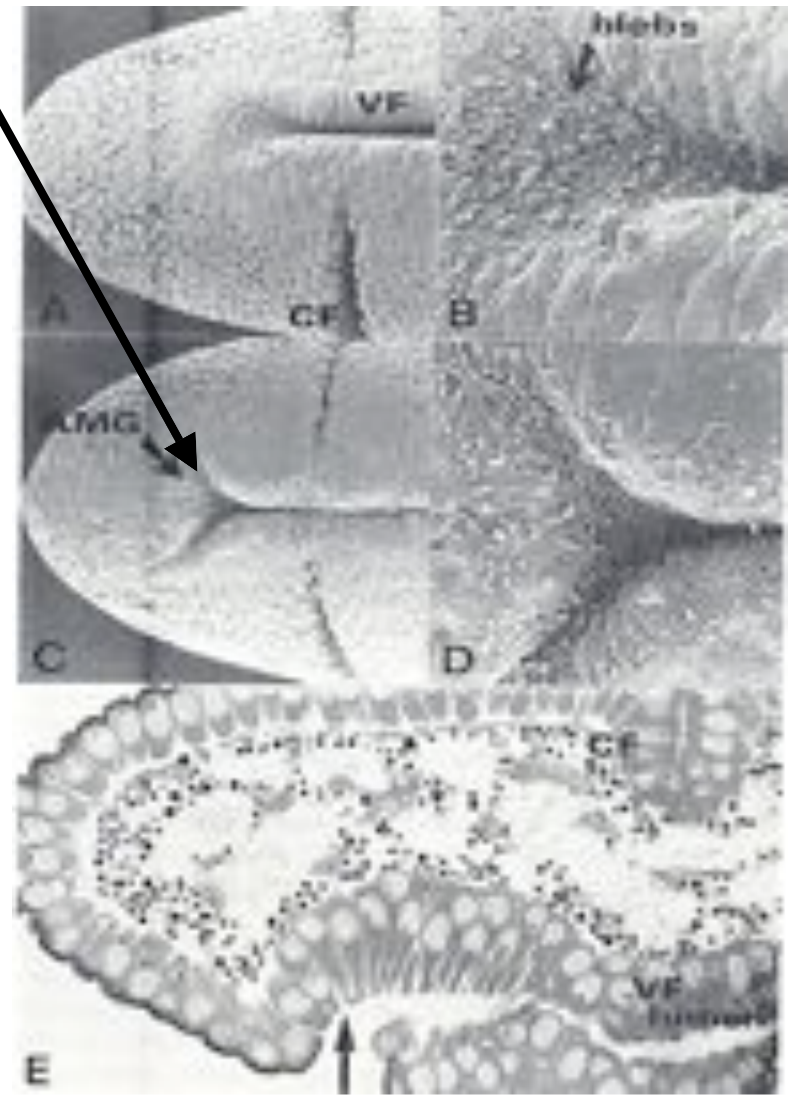

a) caterpillar
crop
midgut
b) butterfly
crop
midgut
c) fly
crop
midgut
d) *Myrascia*
diverticulum
crop
midgut
e) scarab larva
fermentation chamber
midgut
ileum
f) termite
paunch
midgut
g) *Rhodnius*
anterior midgut
posterior midgut

The origin of the digestive tract. At the anterior pole of the embryo an indentation forms that will be the foregut or stomodeum. At the other end a similar thing occurs and the proctodeum or hindgut is formed. Both are lined by cuticle. They both are of ectodermal origin while the midgut is of mesodermal origin and is also called the mesenteron. This different origin of the midgut from the endoderm and not the ecotoderm probably explains why it is not lined with cuticle

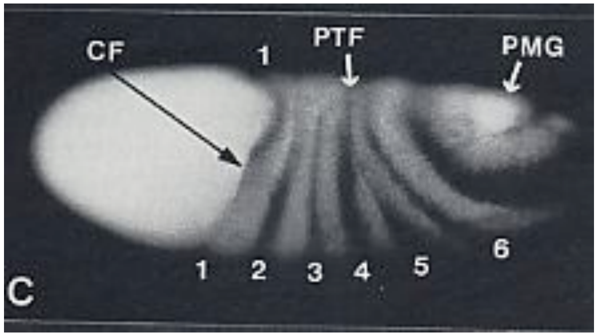


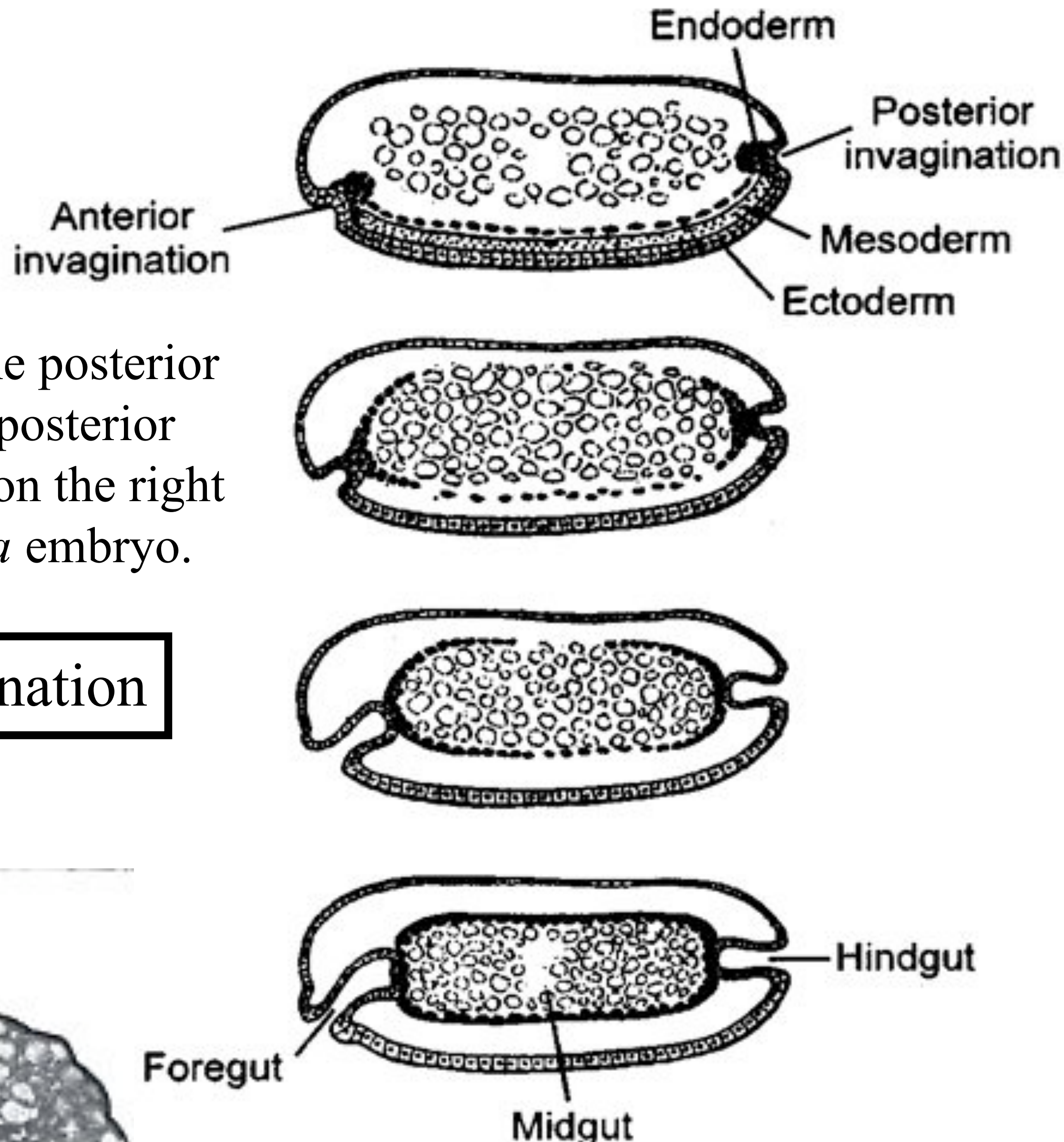
Anterior midgut invagination. In the bottom photo note the invagination starting forming the ventral furrow lumen (VF)





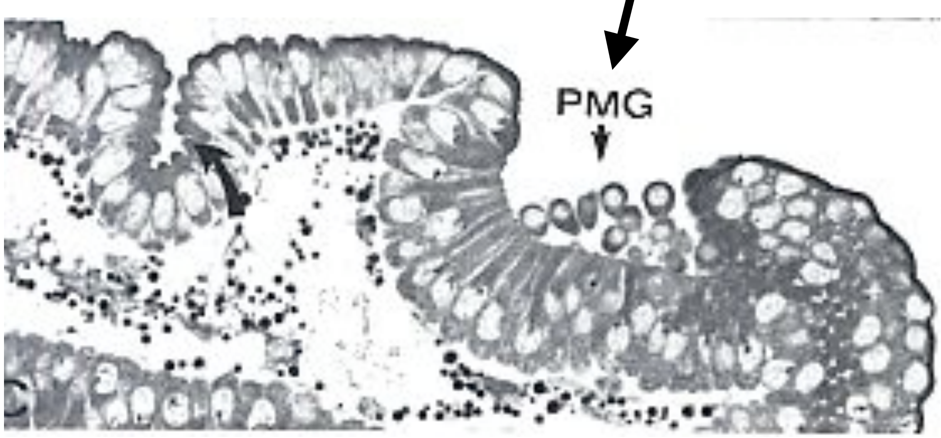
MIDGUT FORMATION IN THE EMBRYO





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.

Hindgut invagination



DIGESTIVE SYSTEM

The digestive tract not only aids in obtaining, processing and digesting food molecules - **It is the largest endocrine tissue in both humans and insects.**

The digestive system is involved in:

1. **Obtaining** food
2. **Mechanically** breaking it down into smaller particles that facilitate digestive enzymes acting on them
3. **Enzymatic** breakdown of larger food molecules into molecules that can pass through the digestive tract (midgut) and enter the hemolymph
4. **Produces molecules** or MESSENGERS (eg. Endocrines), that coordinate feeding and other activities of the digestive tract.

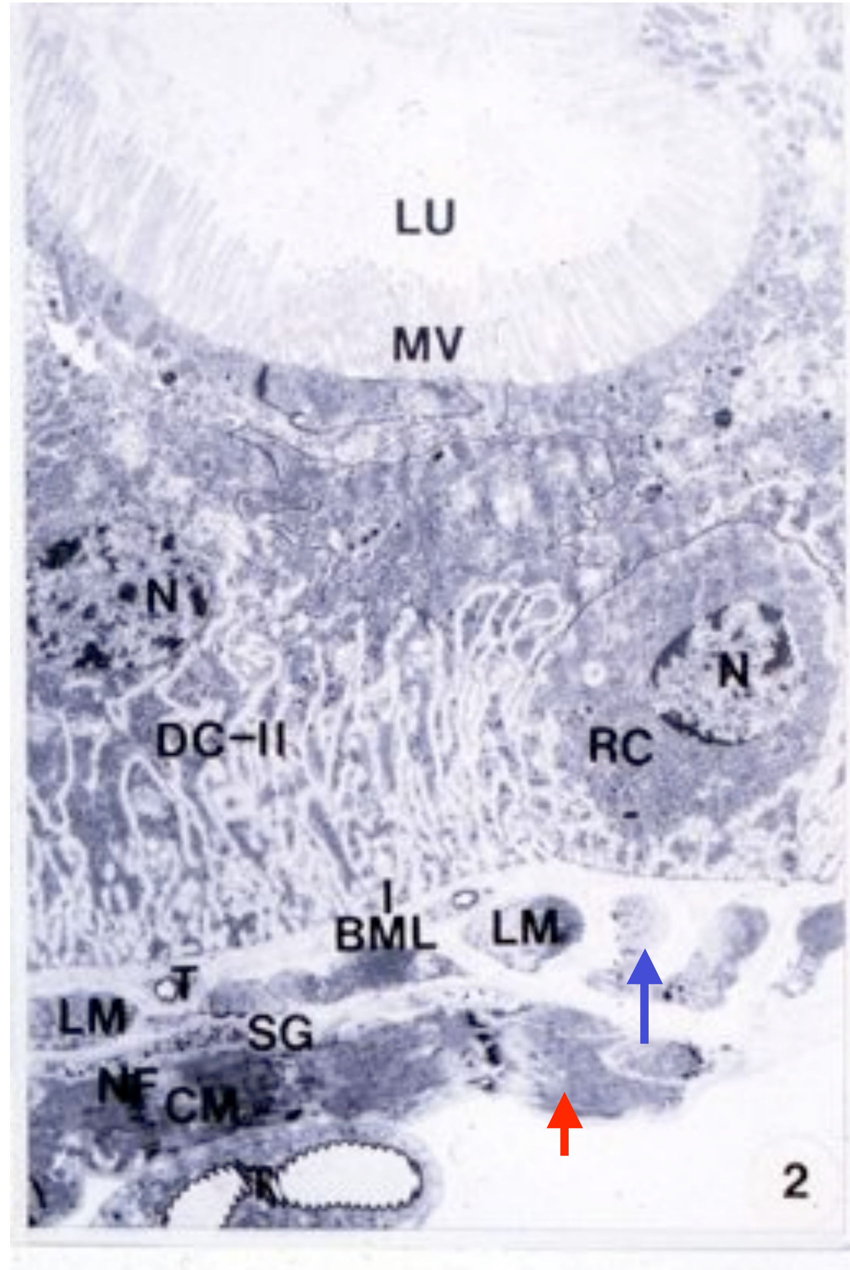
DIGESTIVE SYSTEM

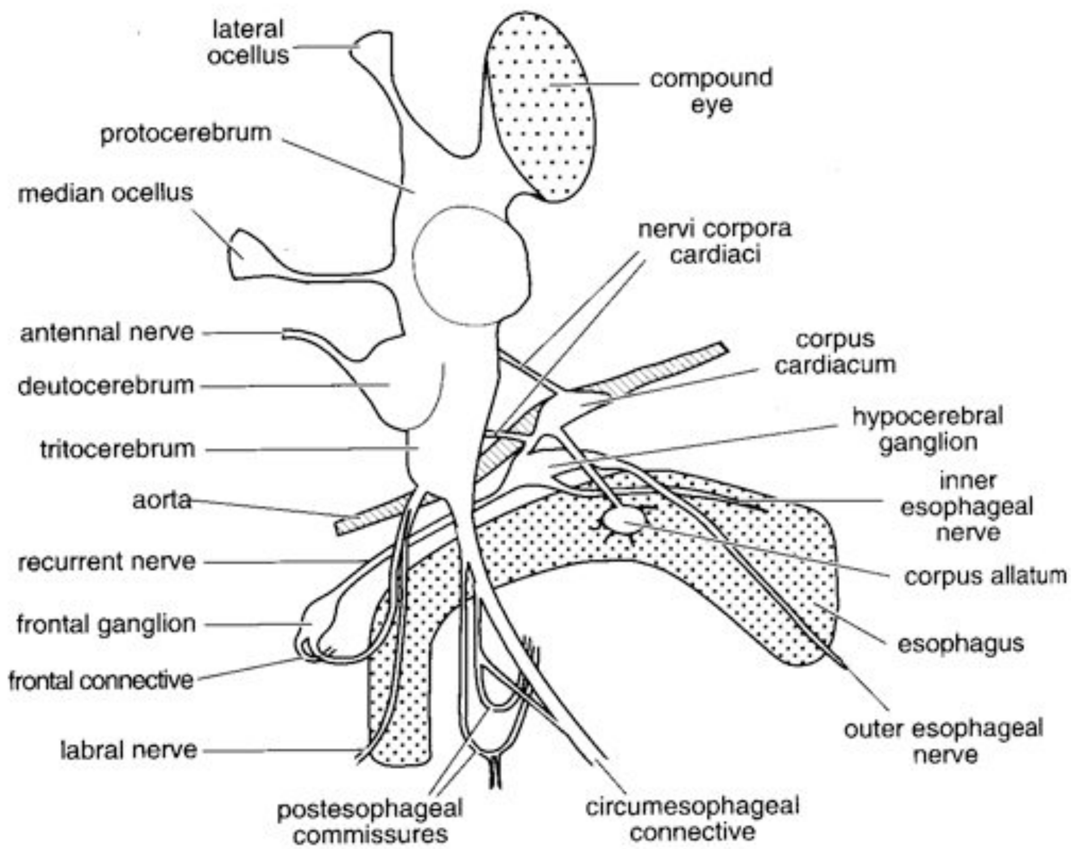
1. Generalized structure of digestive tract
2. Mechanisms of ingestion
3. Pharyngeal and cibarial pumps
4. Salivary glands
   a. Sexual dimorphism in mosquitoes
   b. Types of secretions
5. Cardiac and pyloric sphincters
6. Foregut
7. Midgut
8. Hindgut
9. Basic and applied aspects of the digestive system

GENERALIZED STRUCTURE

1. Tube starting at mouth and ending at anus
2. Tube surrounded by circular + longitudinal muscles

TEM of *Phorma* adult midgut (see on right, notice the LM, which is **longitudinal muscle** and the CM, which is **circular muscle** surrounding the gut. MV=microvilli, LU=lumen, BML= Basal membrane labyrinth, RC=regenerative cell).



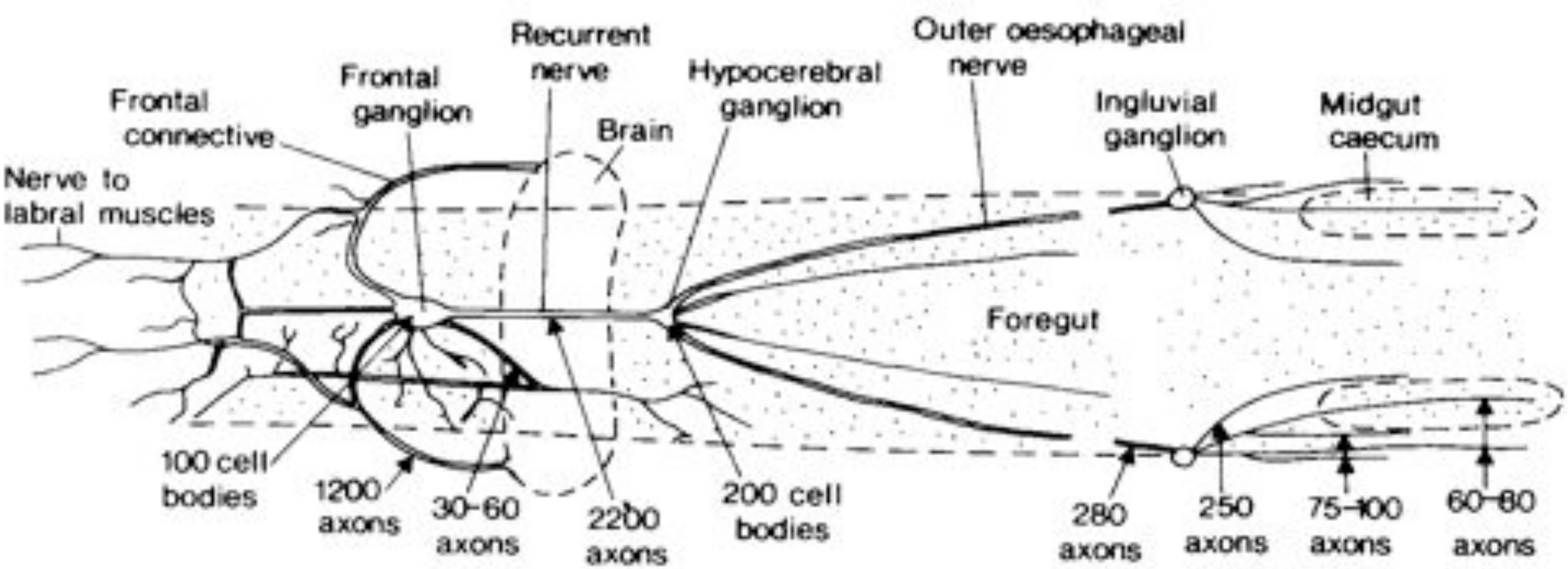


CNS of a locust

GENERALIZED STRUCTURE

3. Is innervated by the stomadeal or visceral nervous system

Stomadeal nervous system of Locust (below)



MECHANISMS OF FOOD INGESTION

Different types of mouthparts have added greatly to insect diversity and evolution.

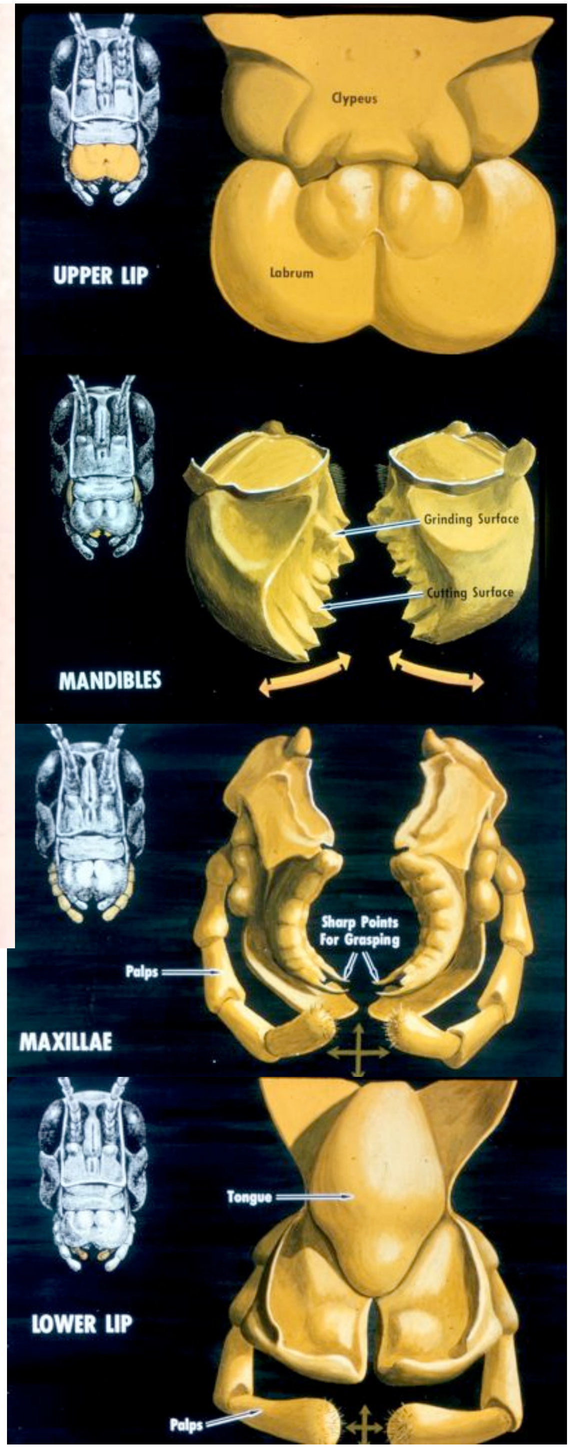
a. **Chewing or mandibulate**-primitive (ex. Grasshopper)

b. **Haustellate or sucking** (ex. Tabanid)

   a. Piercing/sucking-blood and plant feeders

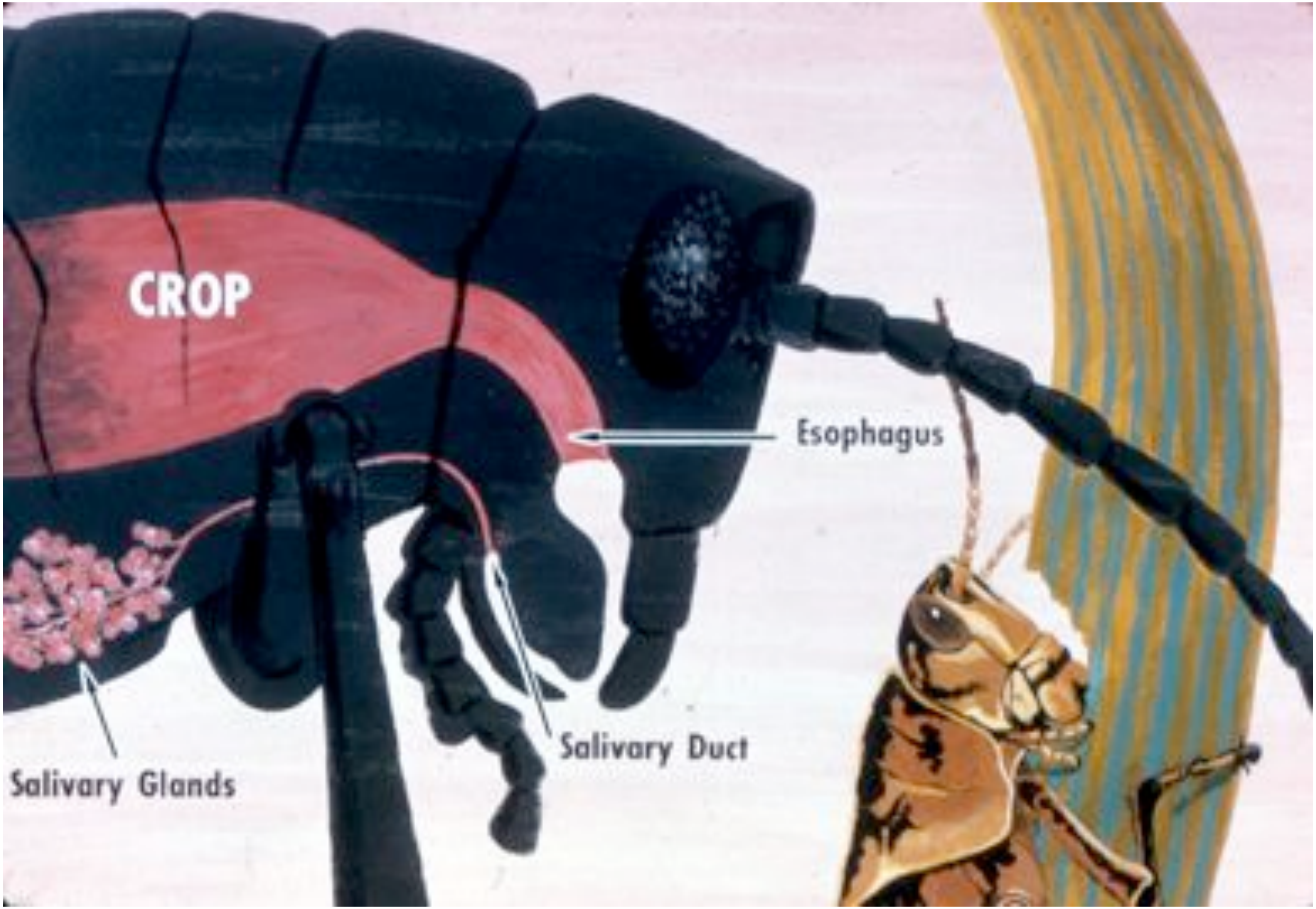
   b. Rasping/sucking-thrips

   c. Lapping/sucking-bees



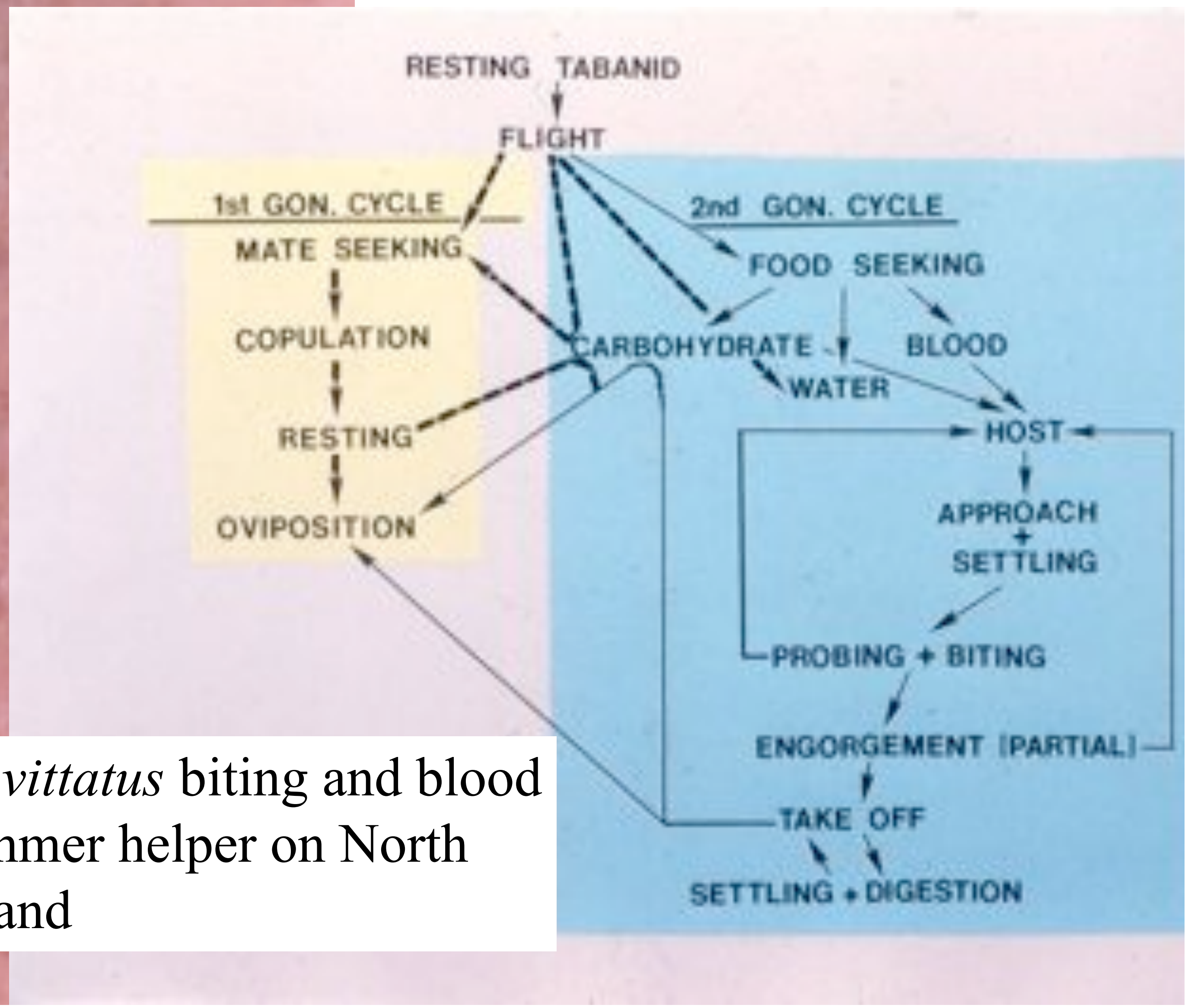
Mouthparts of lubber grasshopper and some associated sensilla

Chemosensilla on inner surface of labrum (top and arrow) and on tip of maxillary palp (below)

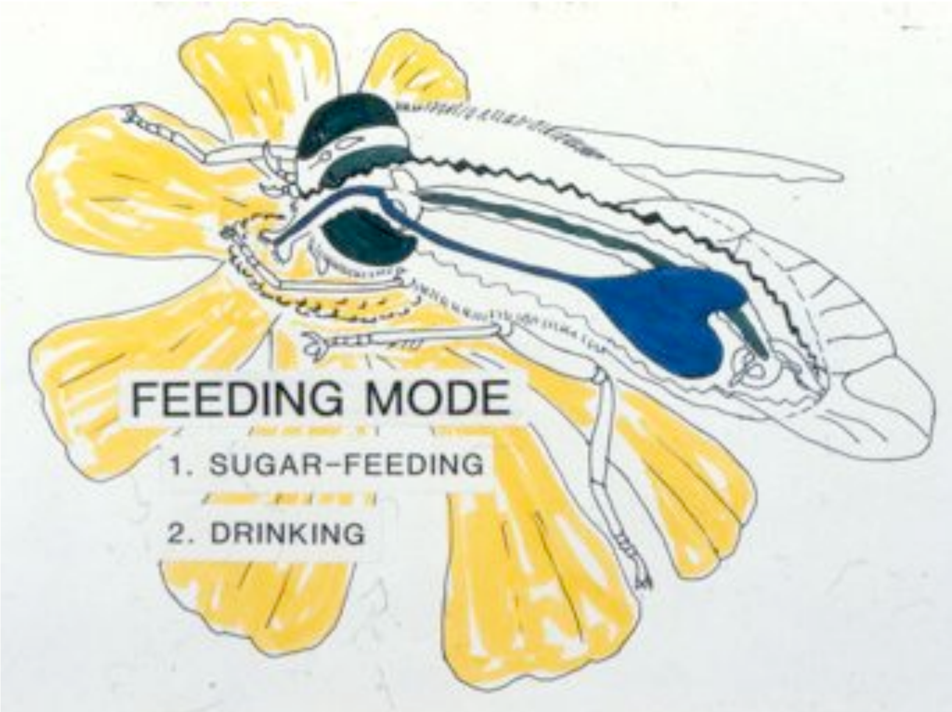

CROP
Esophagus
Salivary Duct
Salivary Glands


Virginia Tech
Virginia U.S.A

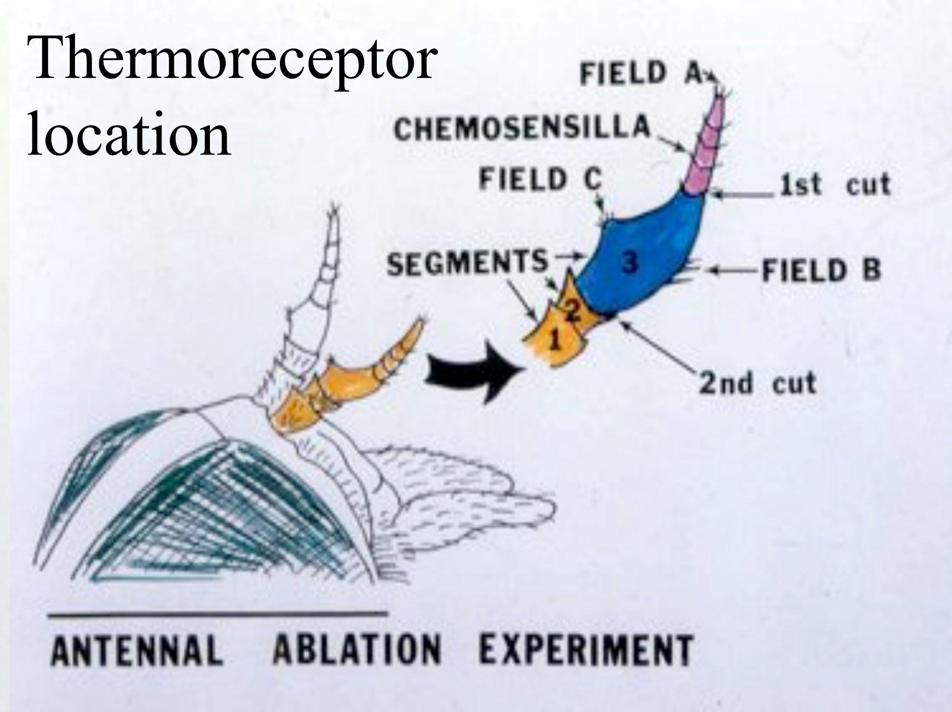
llate or piercing/sucking mouthparts of *Tabanus nigrovittatus*

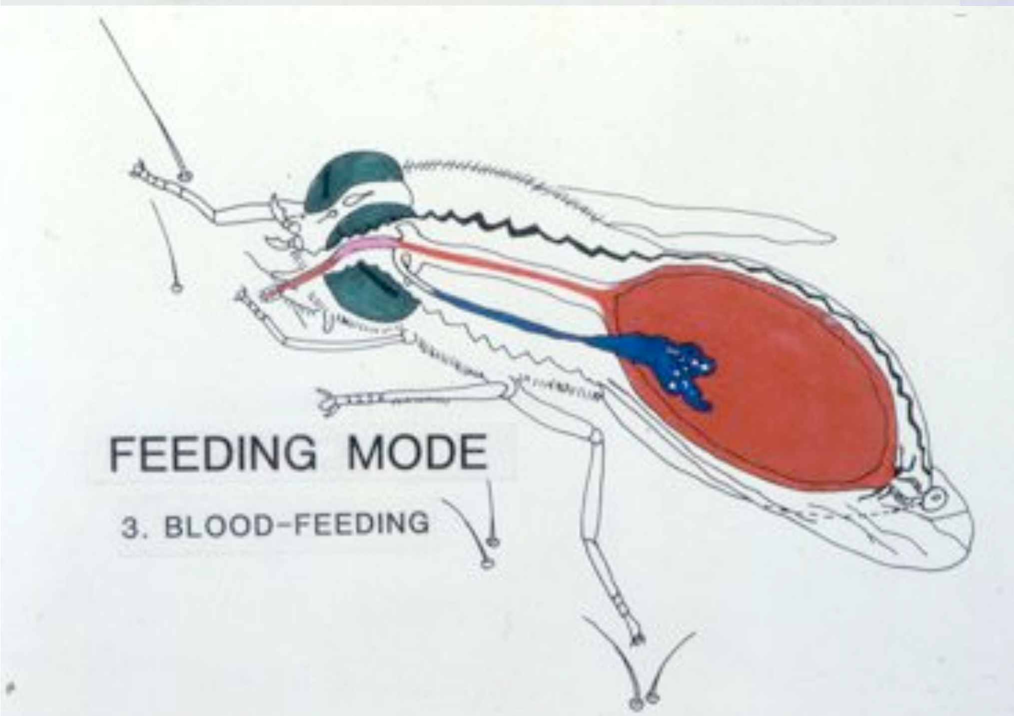


Tabanus nigrovittatus biting and blood feeding on summer helper on North Shore-Pine Island



Thermoreceptor location

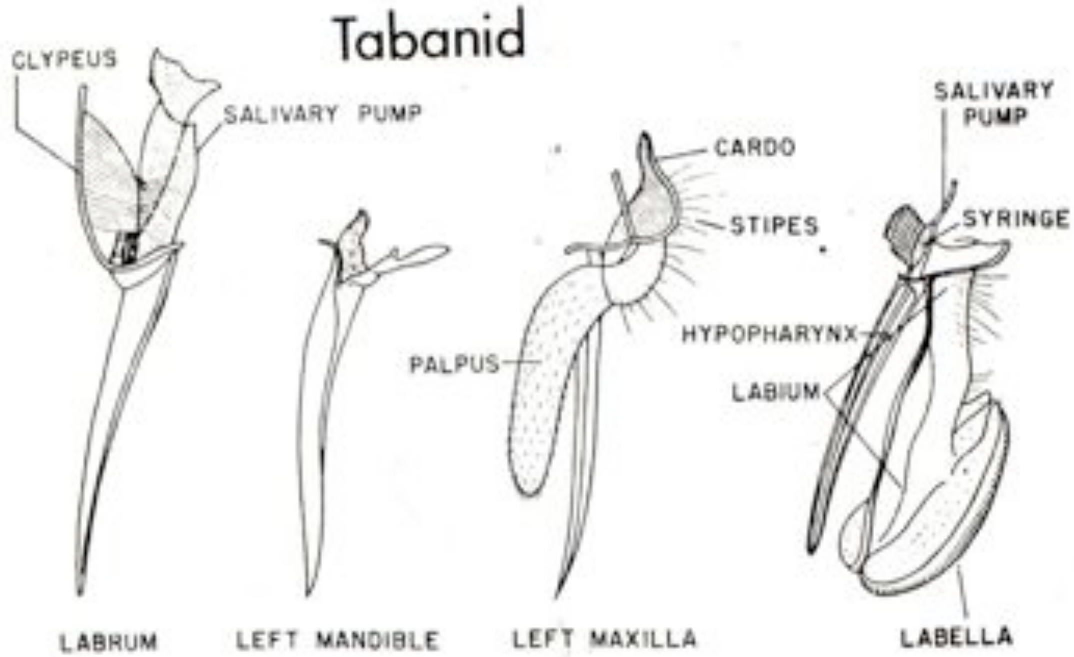


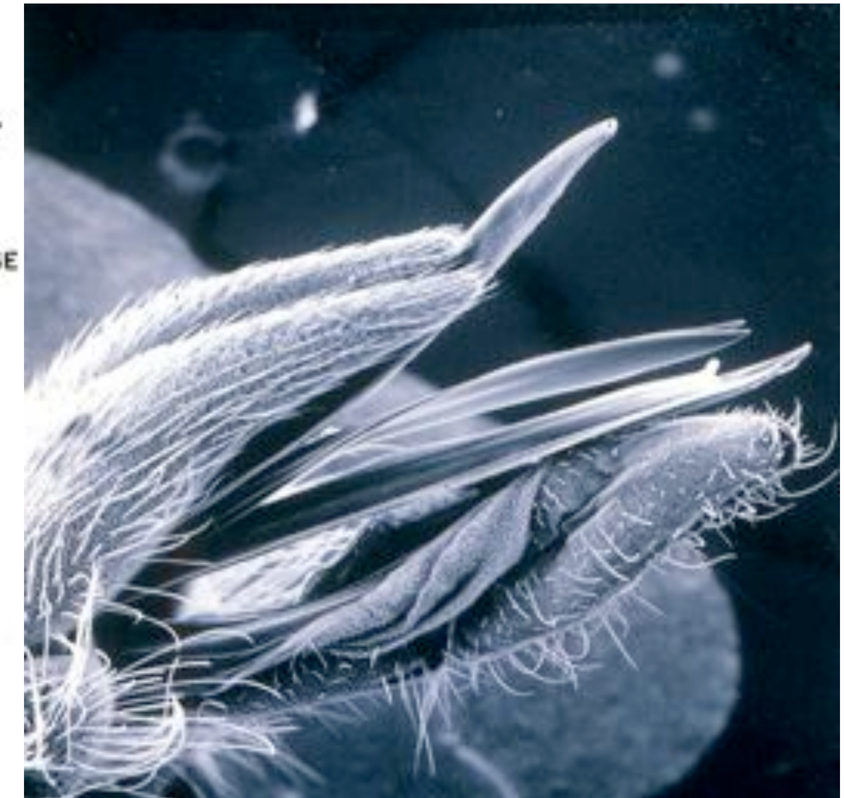


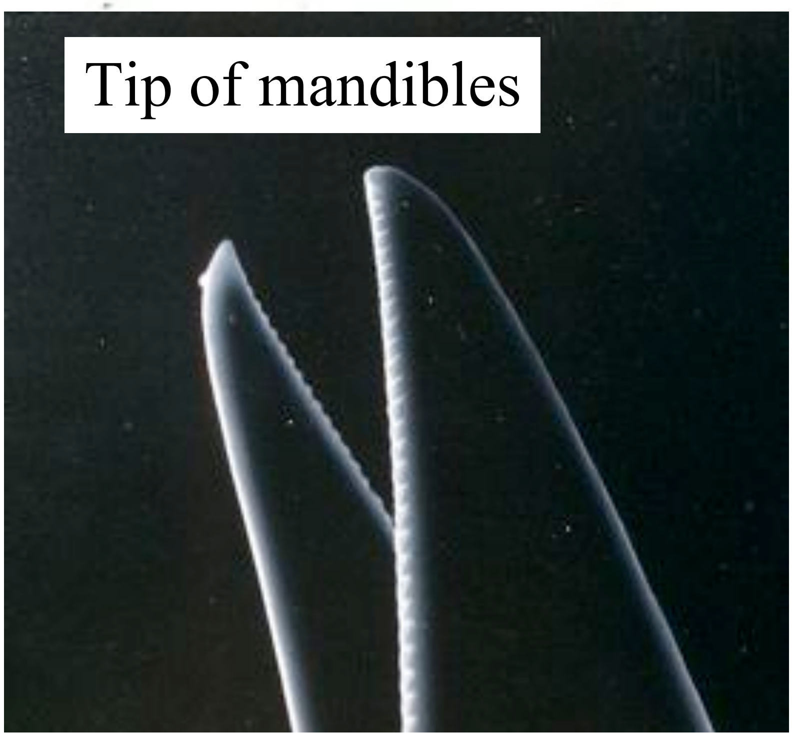
EFFECT OF REMOVING VARIOUS APPENDAGES ON BITING THE HUMAN HAND

REGION REMOVED	NO. TESTED	% BITING
PALPS	12	42
FORE TARSI	12	42
1 ANTENNA	7	0
2 ANTENNAE	12	0
TIP EPIPH.	4	25
CONTROL (N.S.)	12	42
BASAL 3rd +	12	25
BASAL 3rd -	12	0

N.S. = NOT SHAM CONTROL



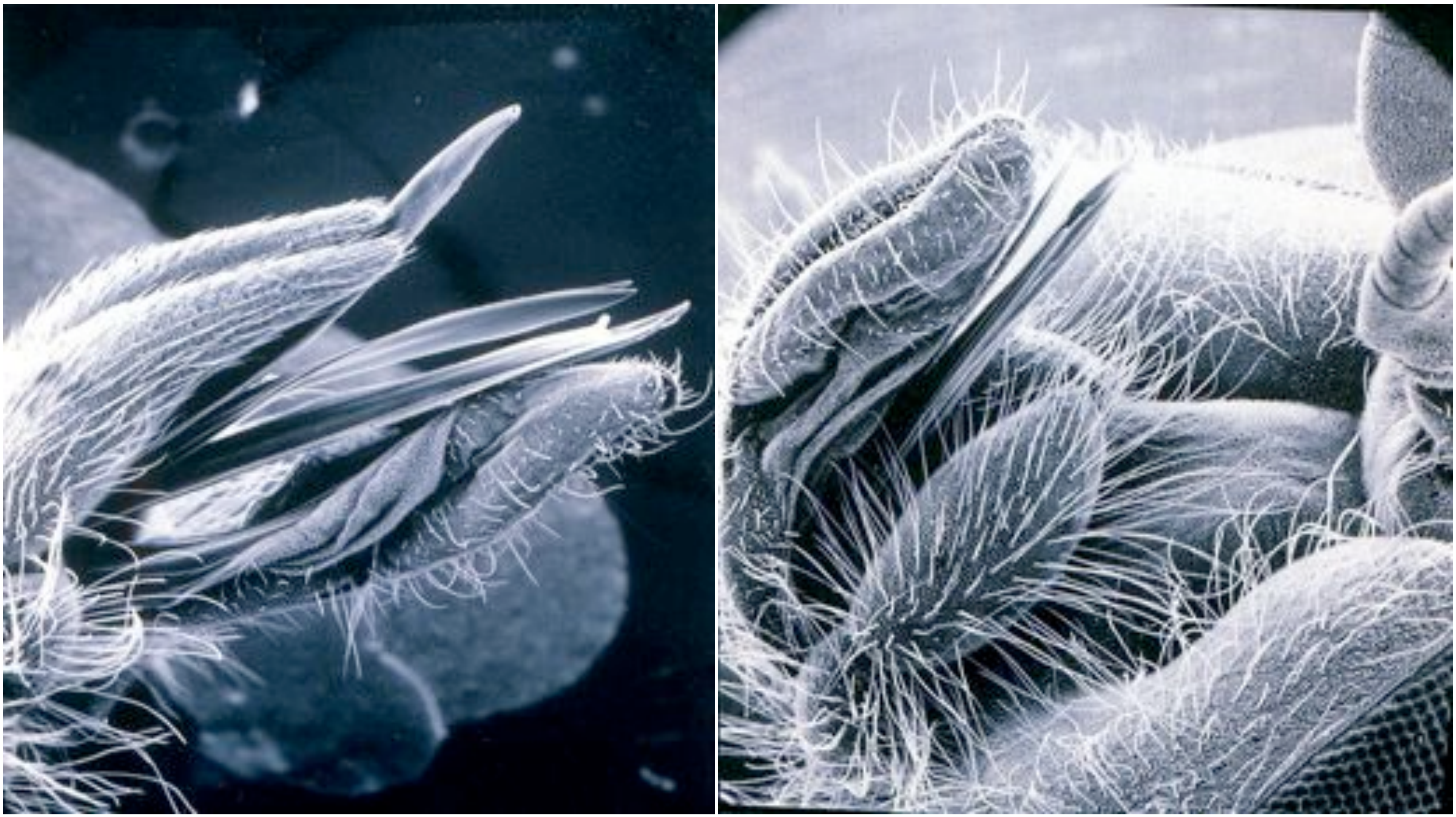




Tip of mandibles

Mechanosensilla on mandible

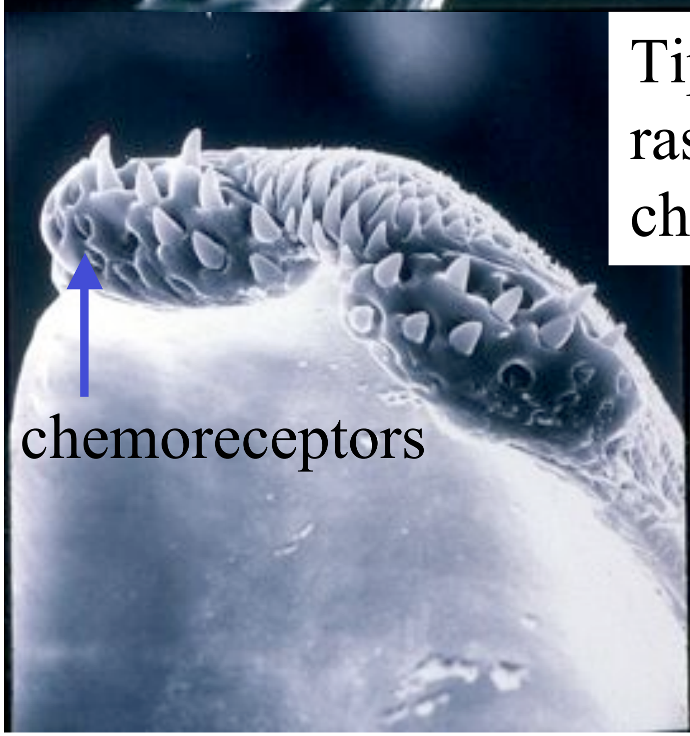
Sexual dimorphism is shown in the mouthparts of many bloodfeeders. On the right is the female with a full complement of mouthparts including mandibles and maxillary galae. On the right is the male which lacks mandibles and has a modified galae. MP of *Tabanus nigrovittatus*



Female on left
Male on right

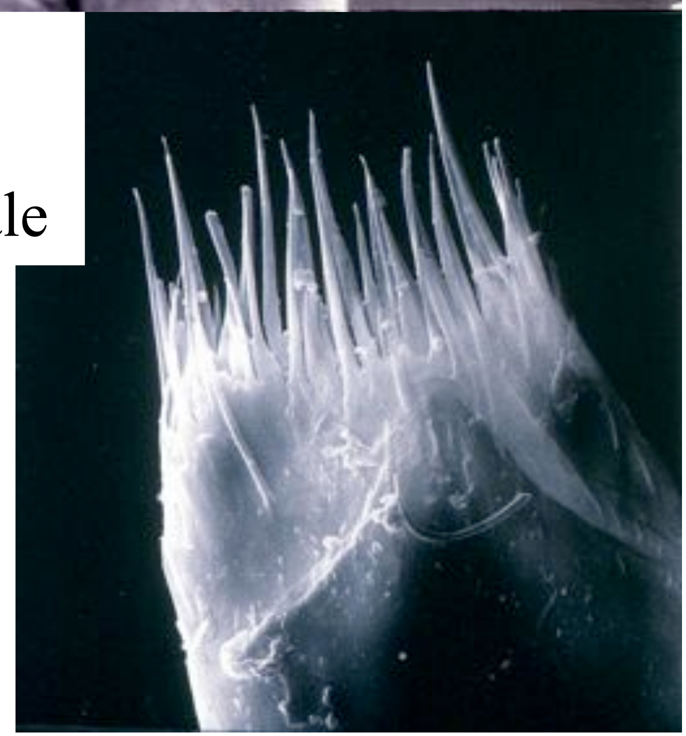
Tip of maxillary galae.
Note retrose teeth on
female but not on male.

SEXUAL DIMORPHISM IN MOUTHPARTS

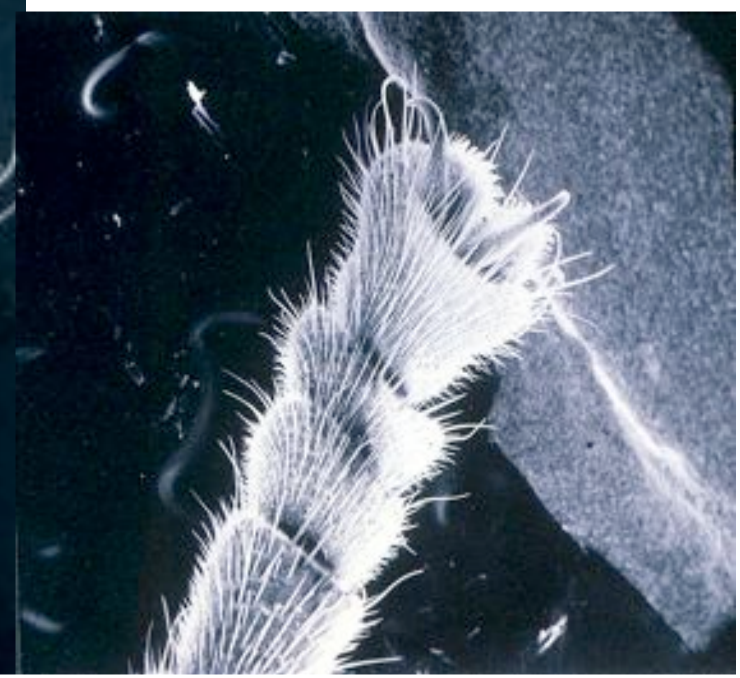


Tip of labrum-Note
rasping teeth and
chemoreceptors in female

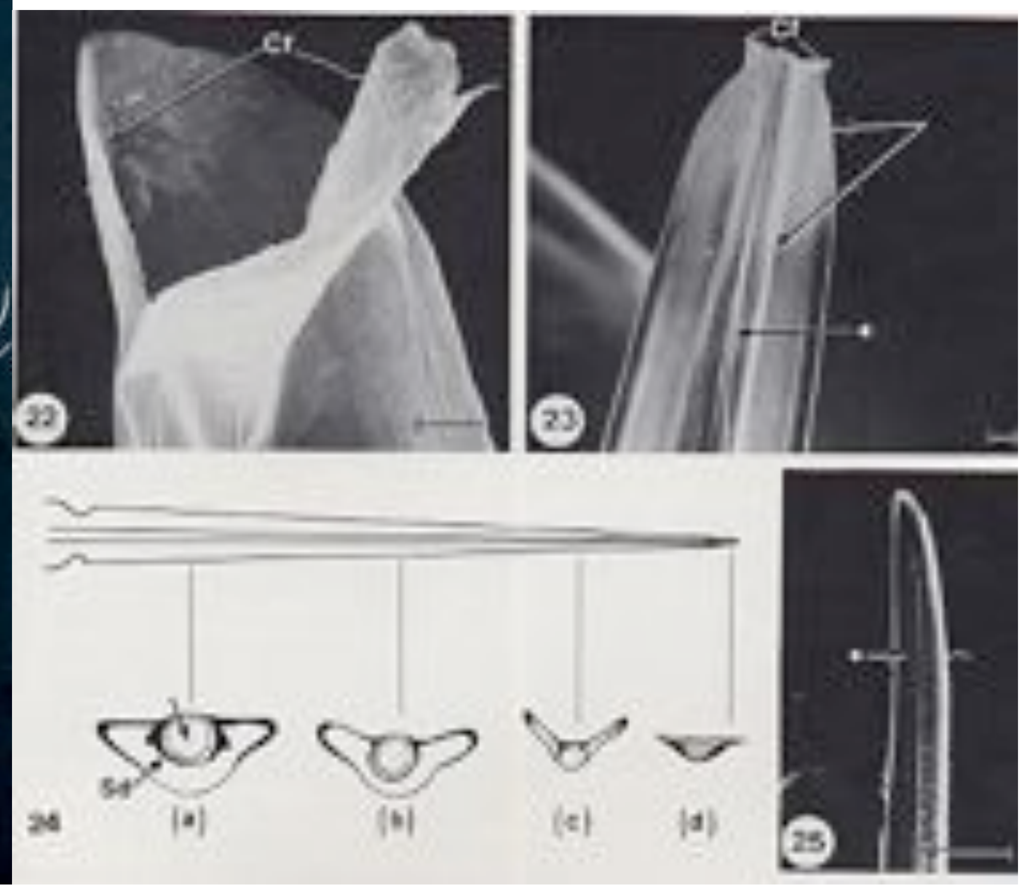
Females on right
and males on the
left showing
sexual dimorphism
in mouthpart stylets.



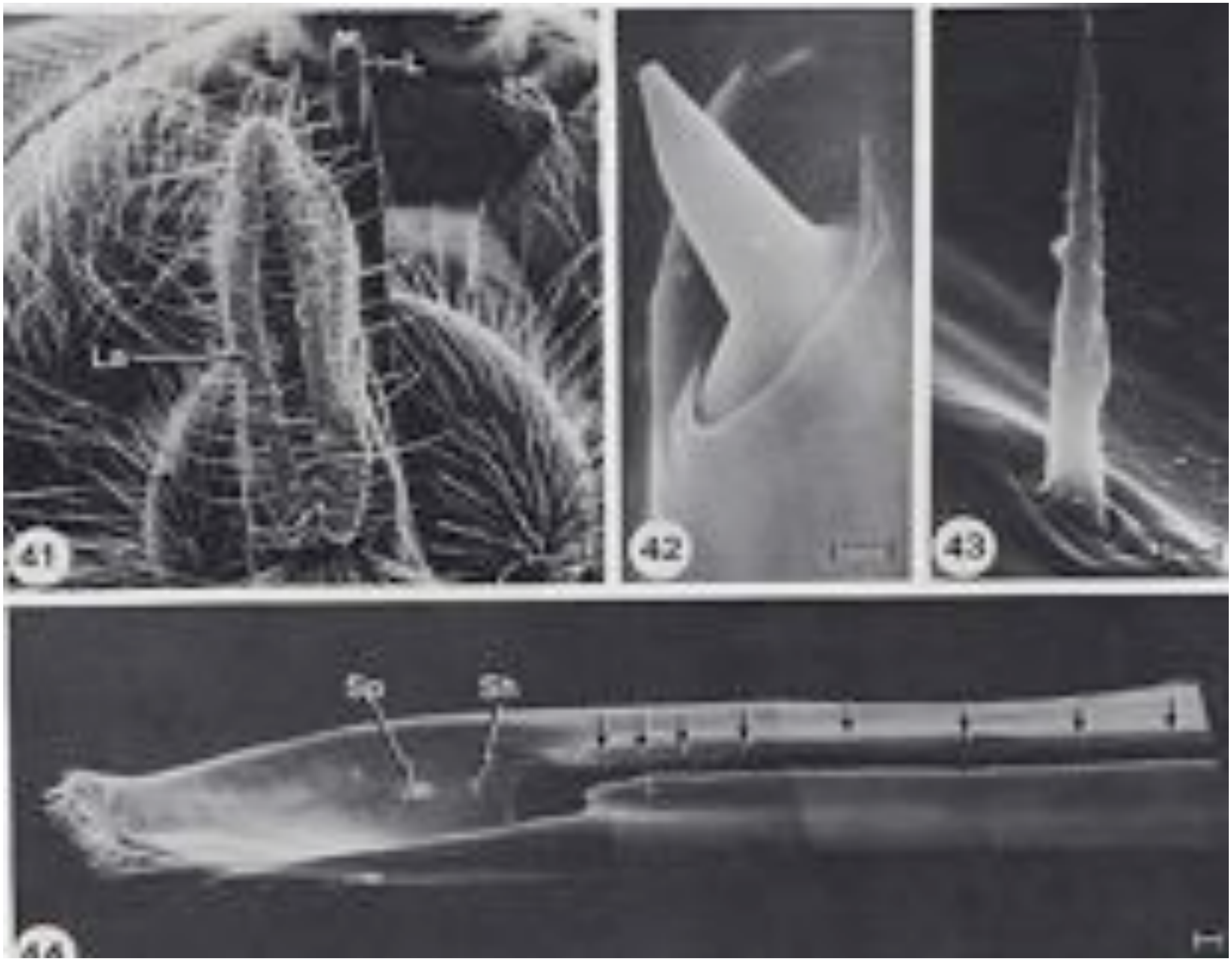
Information must be obtained by the insect in order to feed. Chemoreceptors on labium and also on the tarsi. *Tabanus nigrovittatus*



Once the stylets are inside the wound, the tabanid secretes salivary secretions that come out of the hypopharynx (see white arrow on the left and the openings of it in figs. 22 and 23). Note the midrib and flanging that occurs to give the hypopharynx rigidity.

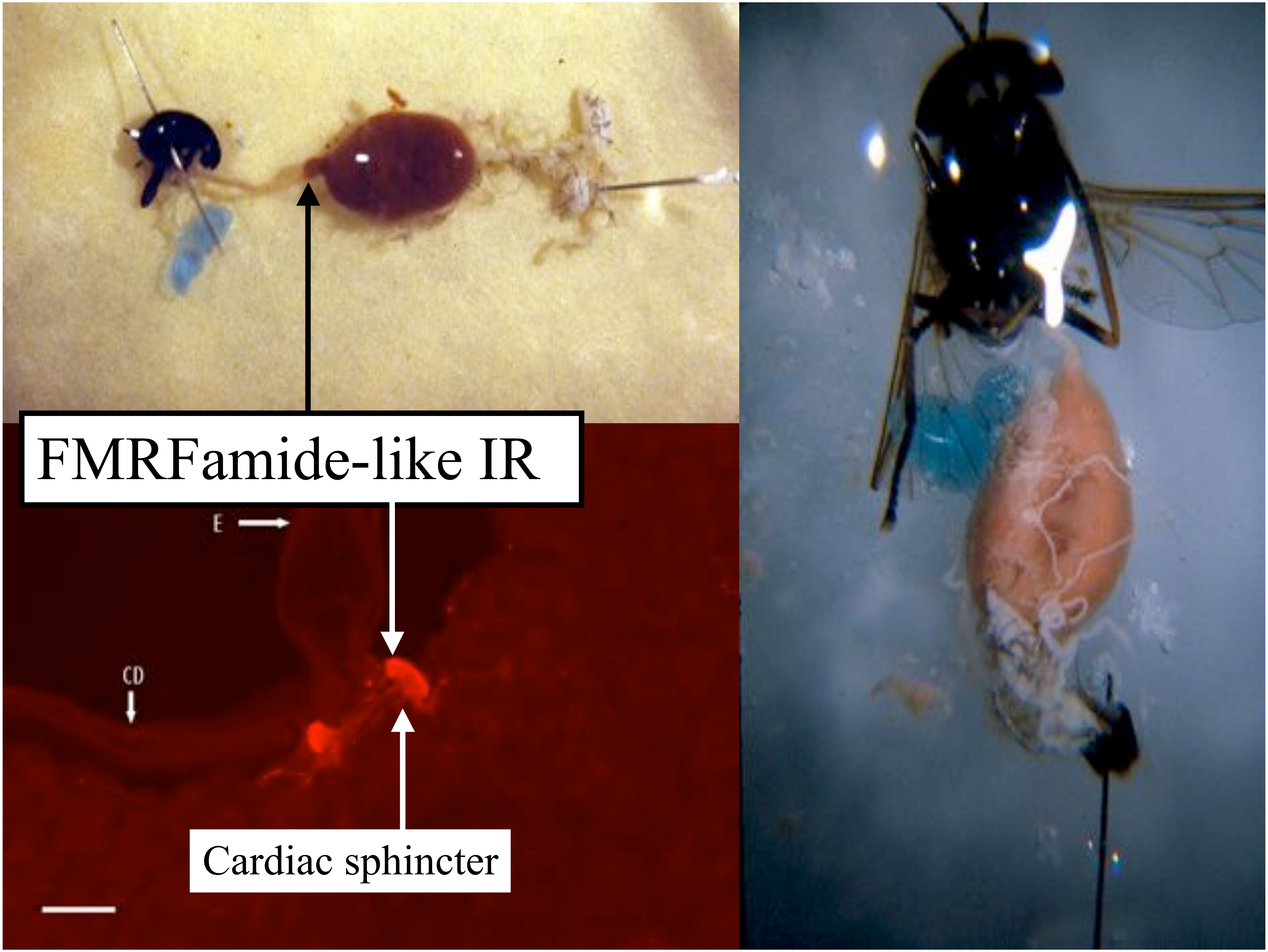


The labrum is the main structure that aids in sucking up the blood. See in fig. 44 it is shaped like a straw and also has chemo and mechano-receptors to monitor blood flow and composition (ATP).



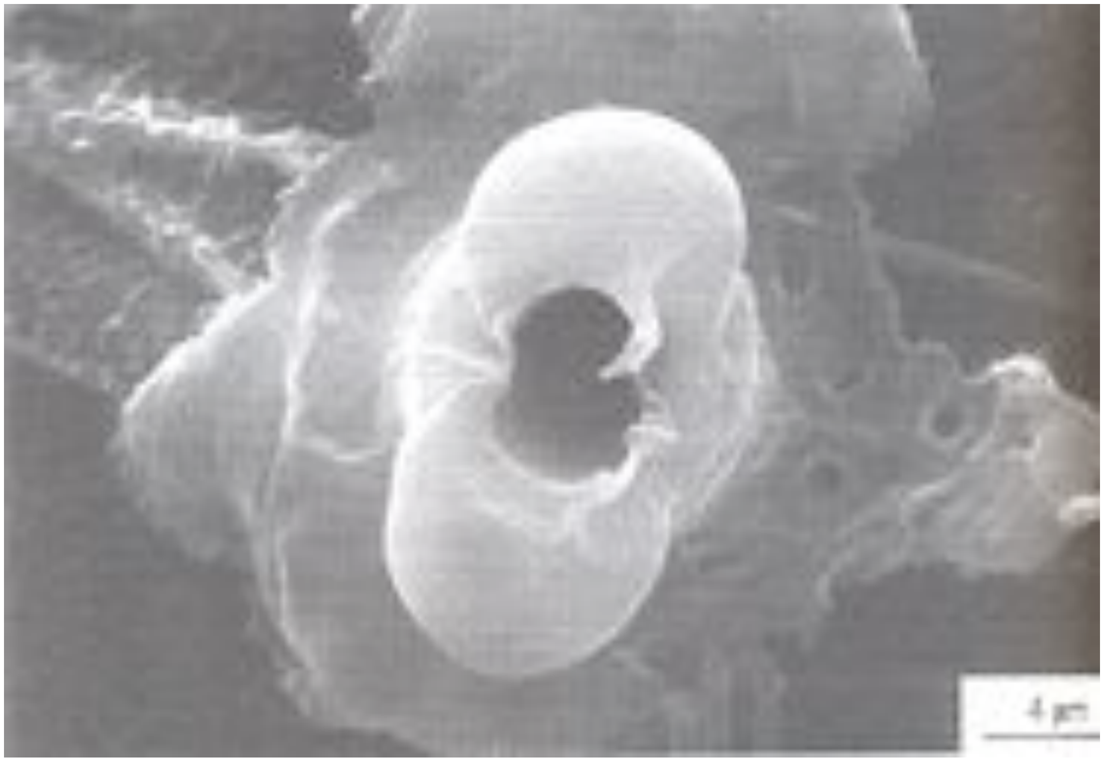
Peptides with a RFamide C terminus but with unique N-terminal extensions comprise a large family of peptides.
FMRF-amide-related peptides (FaRPs) are small peptides of 4–18 amino acids with **RFamide** (arg-phe-NH2) at the C terminus. FMRFamide was the first RFamide-containing peptide to be discovered in mollusk (cardioexcitatory peptide). Since then a lot of different FaRPs (FMRFamide-related peptides) have been discovered.

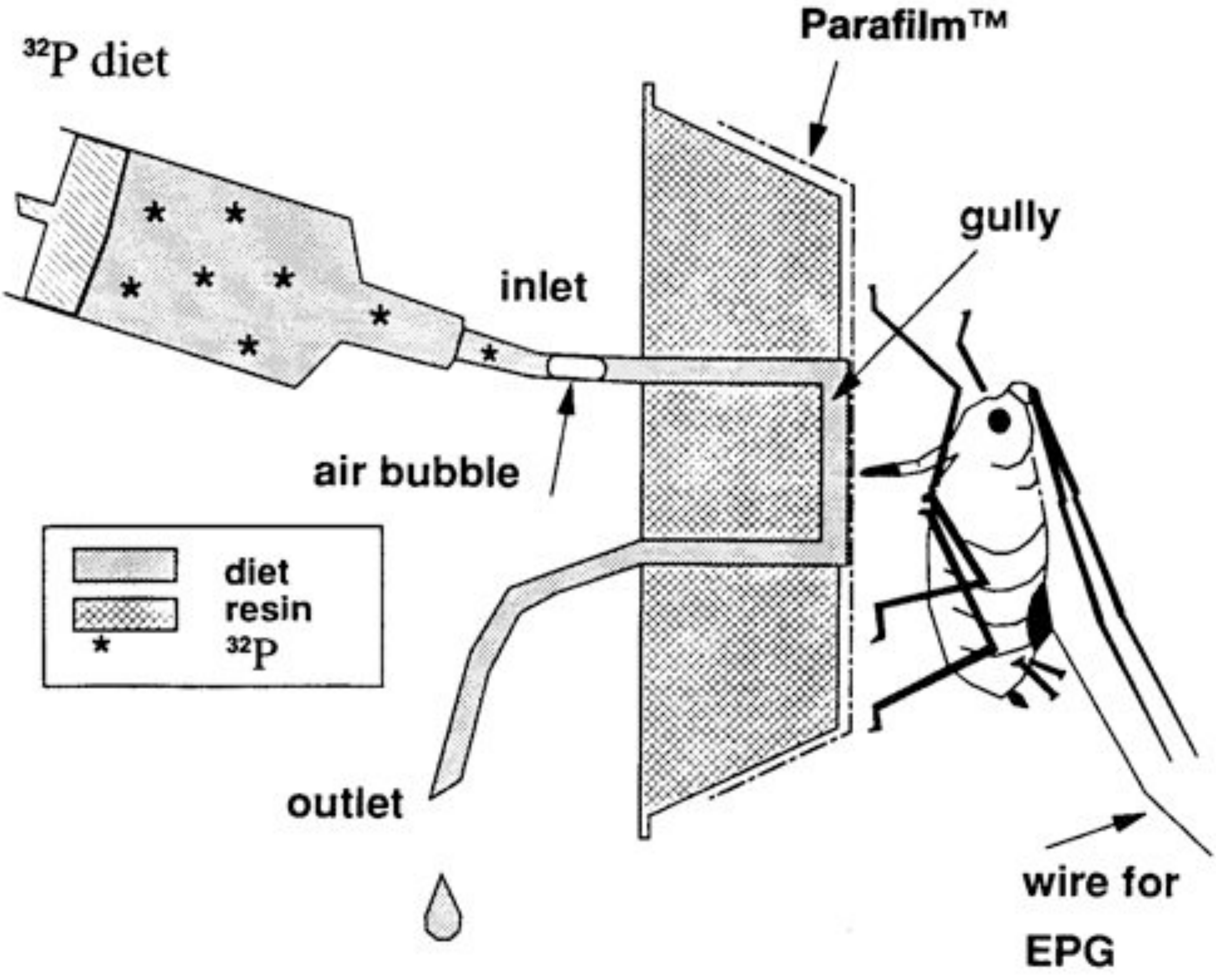
Affect behavior to physiology in both invertebrates and vertebrates


FMRFamide-like IR
E
CD
Cardiac sphincter

Feeding on pressure systems for long periods of time could lead to the fluids leaking from between the mouthparts and the system the insect is feeding on. Several groups produce a **sieve tube** from saliva that forms a secure tube and also seals the feeding apparatus. Aphids, plant-hoppers,
and ticks (produce a cement-like material that firmly attaches them to the host) have such a mechanism. This causes difficulty in pulling ticks off.

Salivary flange produced by plant-hopper feeding on sorghum. The hole is where the stylets were removed from the plant while the small holes to the right of the larger hole were made by chemoreceptors on the tip of the labium.

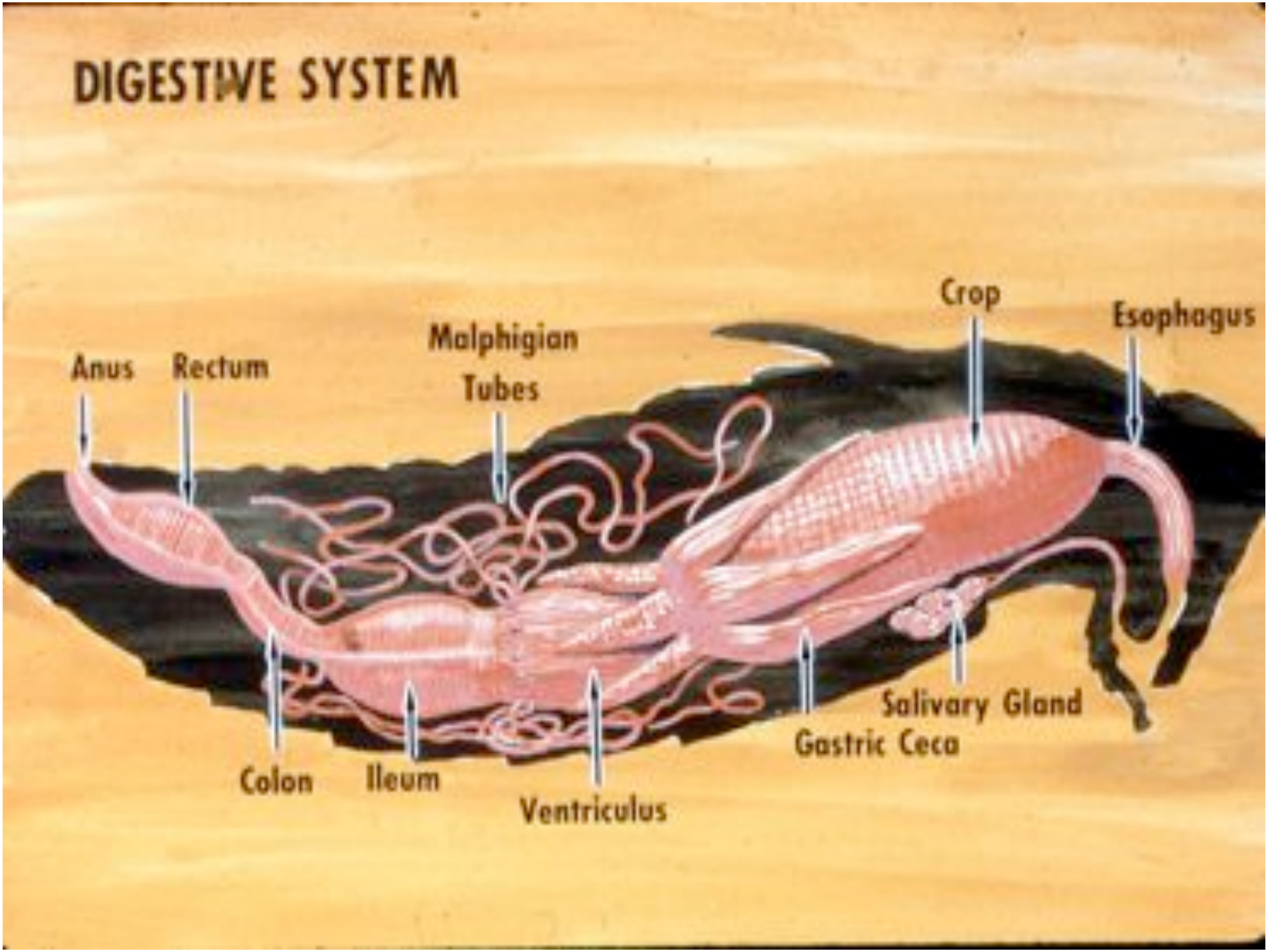




^{32}P diet
Parafilm™
gully
inlet
air bubble
diet
resin
^{32}P
outlet
wire for
EPG


Department of Entomology, Virginia Tech
Blacksburg, Virginia U.S.A

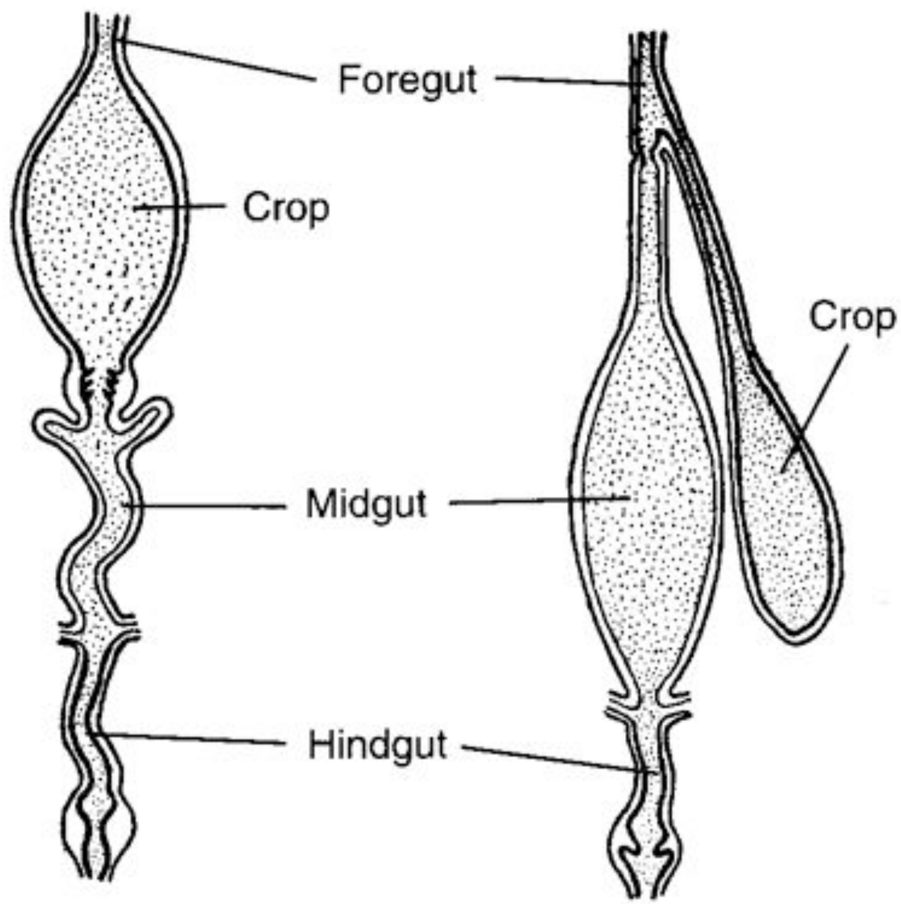

Department of Entomology, Virginia Tech
Blacksburg, Virginia U.S.A.

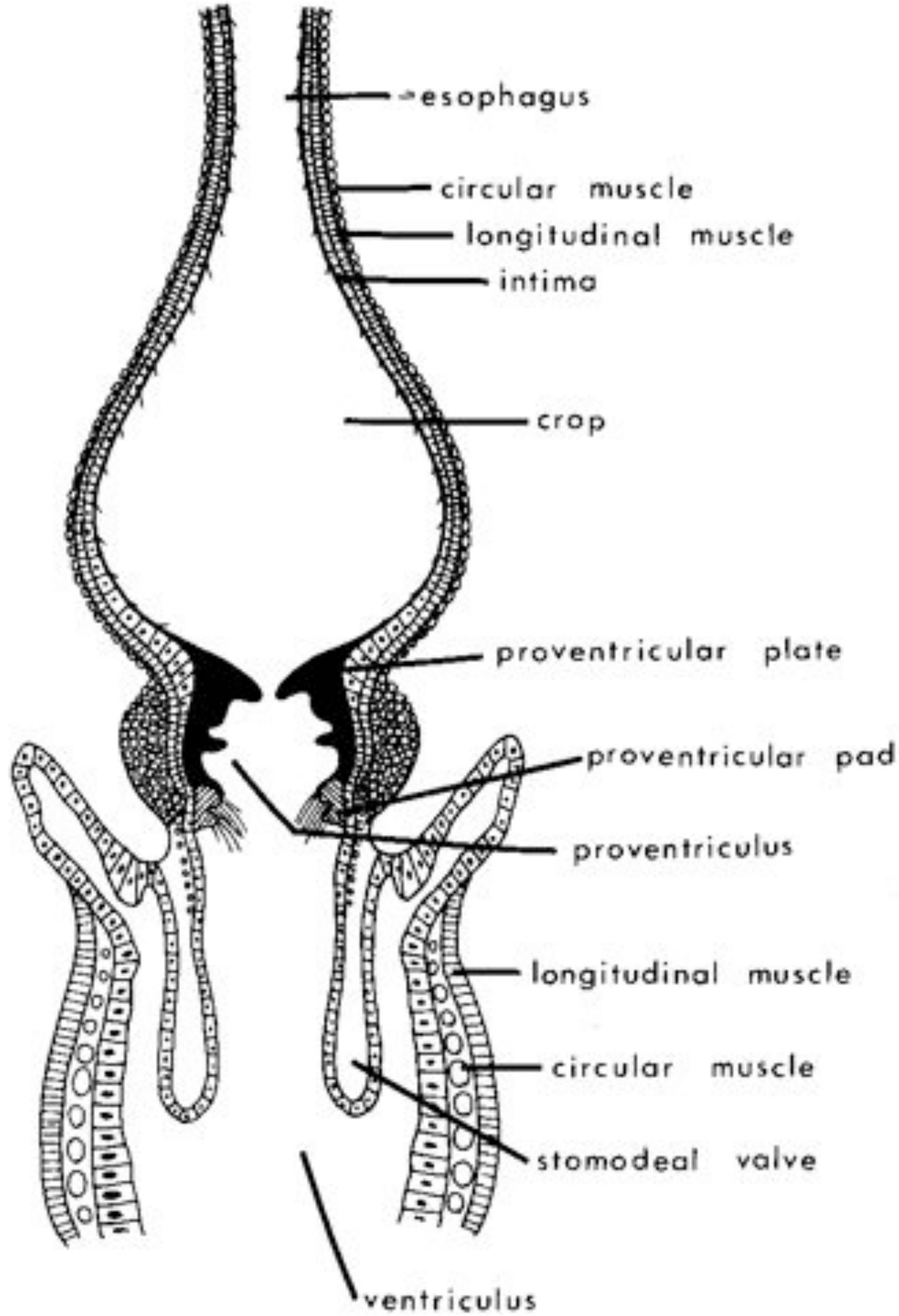

DIGESTIVE SYSTEM
Crop
Esophagus
Malphigian Tubes
Anus
Rectum
Salivary Gland
Gastric Ceca
Colon
Ileum
Ventriculus

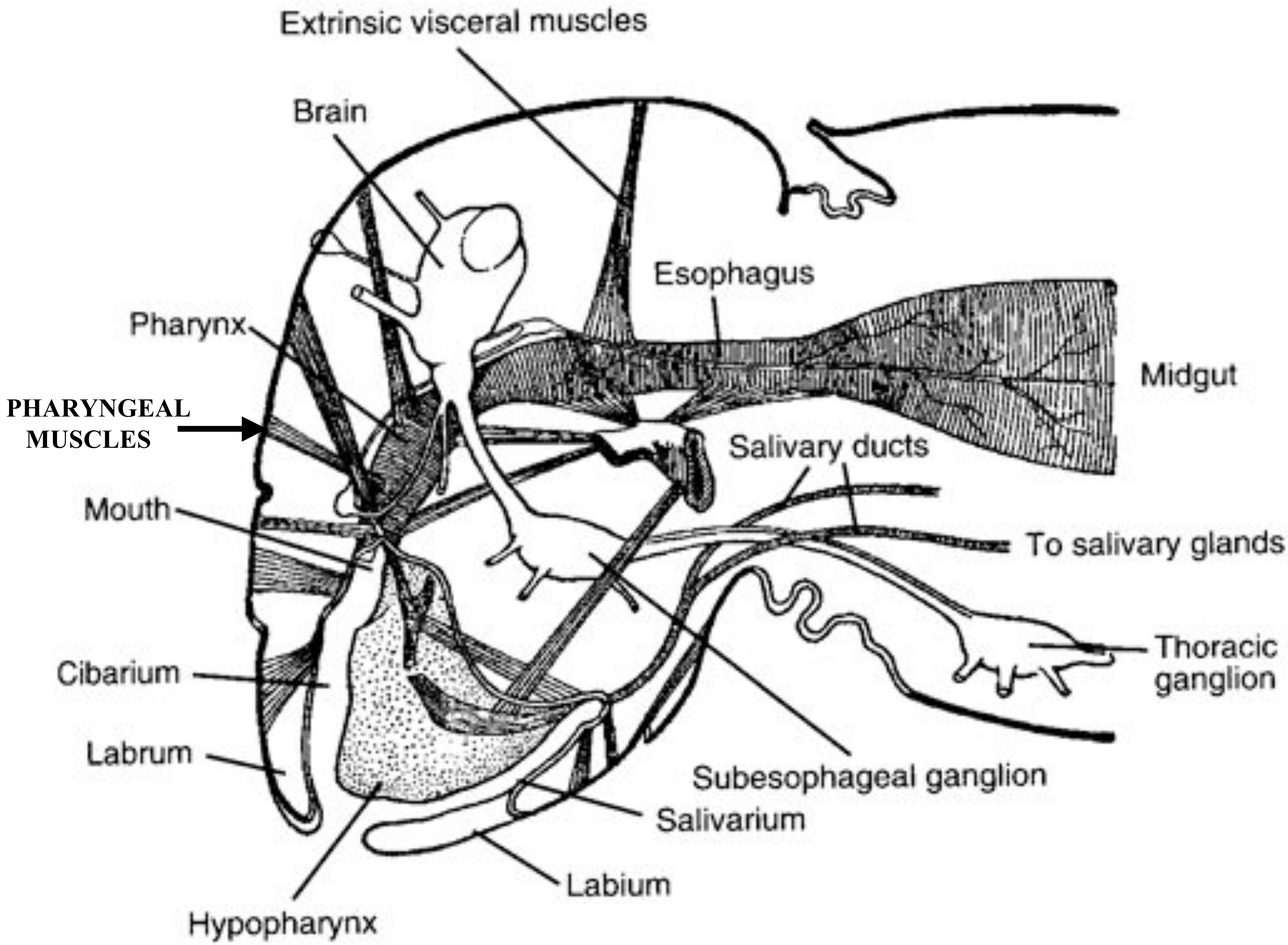
FOREGUT

1. Foregut starts at mouth and ends just after proventriculus but before the cardiac sphincter
2. Little or no digestion occurs in the foregut
3. Foregut is mainly involved in ingestion of food and the mechanical softening of it with salivary secretions and breakdown with special ‘teeth’ as in the cockroach or muscle-sets in other insects
4. In some insects it has a dilated structure called the crop while in other insects this crop may be diverticulated and connected by a crop duct
5. The salivary glands empty into the foregut or mouth area depending on the insect

Normal Diverticulated







Extrinsic visceral muscles
Brain
Esophagus
Pharynx
Midgut
PHARYNGEAL
MUSCLES
Salivary ducts
Mouth
To salivary glands
Thoracic
ganglion
Cibarium
Labrum
Subesophageal ganglion
Salivarium
Labium
Hypopharynx

FUNCTIONS OF SALIVARY GLANDS

1. Moisten food
2. Lubricate the mouthparts
3. Contains digestive enzymes
   a. May contain enzyme amylase, which breaks down complex sugars into simpler sugars
   b. Digestive enzymes that are used by both predatory insects that inject the saliva or preoral digestion that occurs in some insects

FUNCTIONS OF SALIVARY GLANDS

Formation of stylet sheath in some hemipterans

4. Non-digestive processes
   a. Contains toxins of predators that act on nervous system of prey food, such as asilids and giant water bugs
   b. In some plant feeders contains substances that counter the action of plant allelochemicals (chemicals associated with interspecific interactions – allomones and kairomones)
   c. Anticoagulants
   d. Formation of silk-Lepidopteran larvae
   e. Contain bacteria in myiasis producing flies

REGULATION OR CONTROL OF SALIVATION IN INSECTS

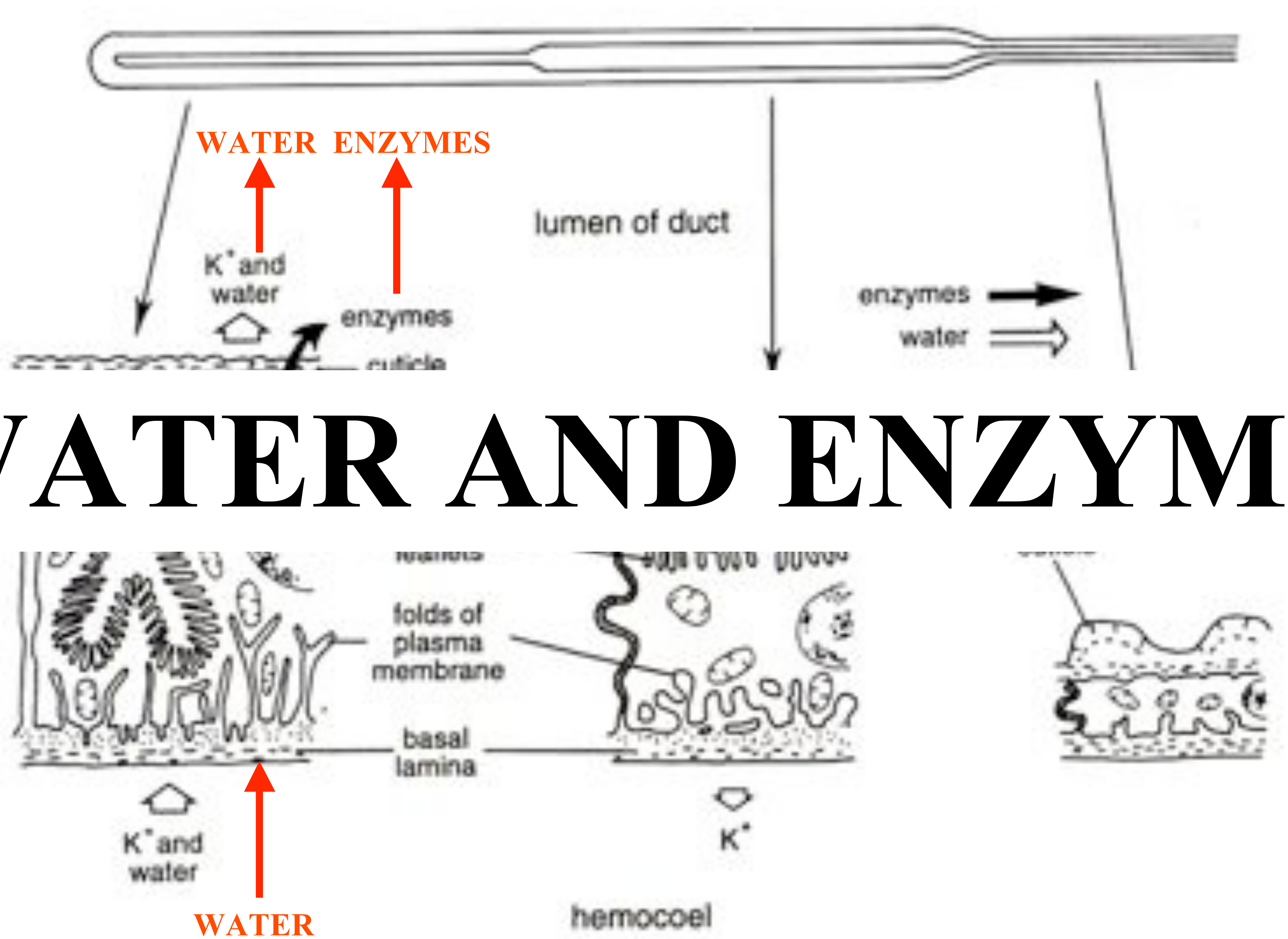


Fig. 2.17. Tubular salivary gland of *Calliphora*. At the top is a representation of the gland showing the positions of the cells depicted below. Left: secretory cell; center: cuboid cell; right: duct cell. Open arrows show the movement of potassium and water; black arrows show the movements of enzymes (after Oschman & Berridge, 1970).

REGULATION OR CONTROL OF SALIVATION IN INSECTS

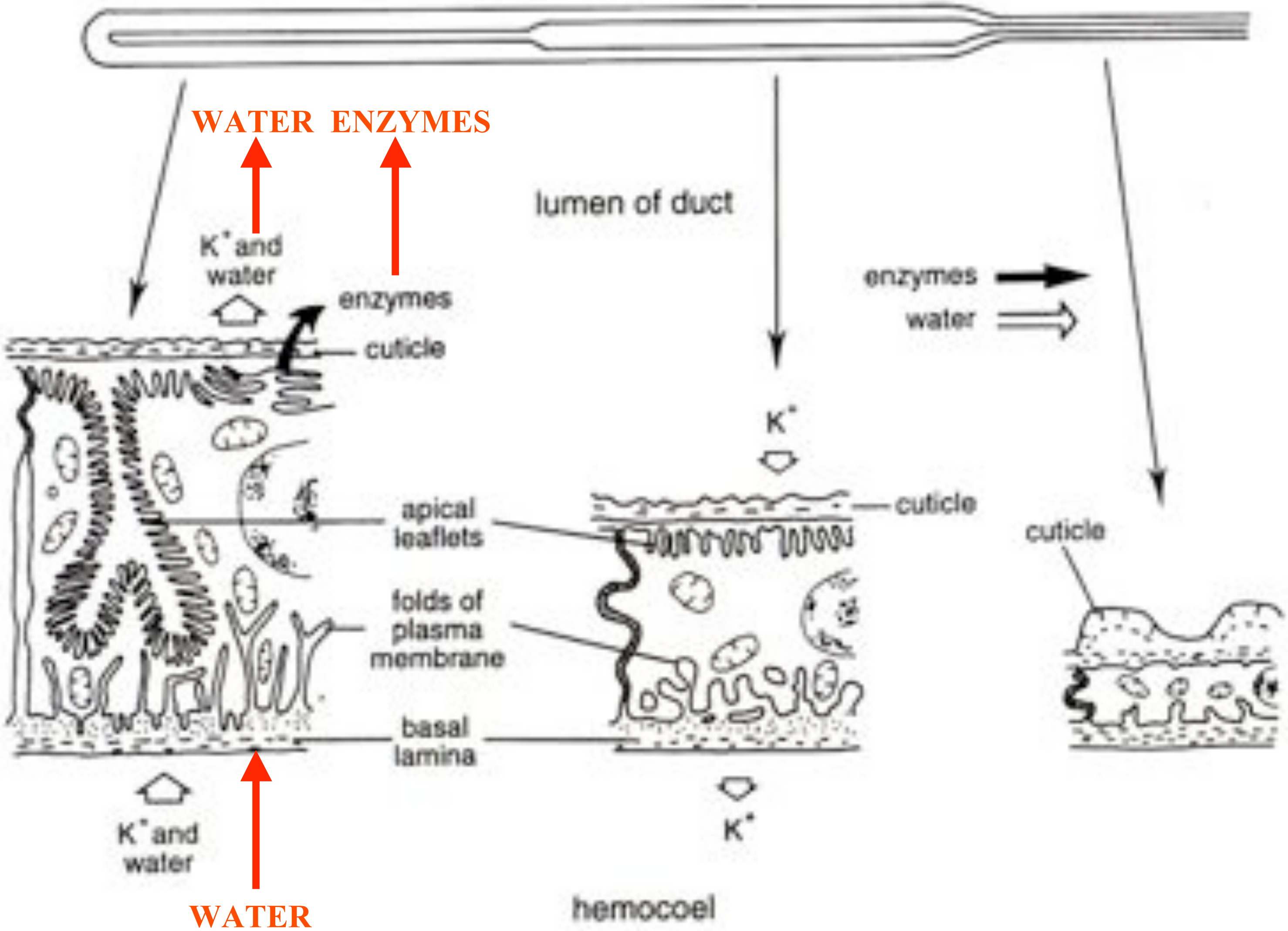
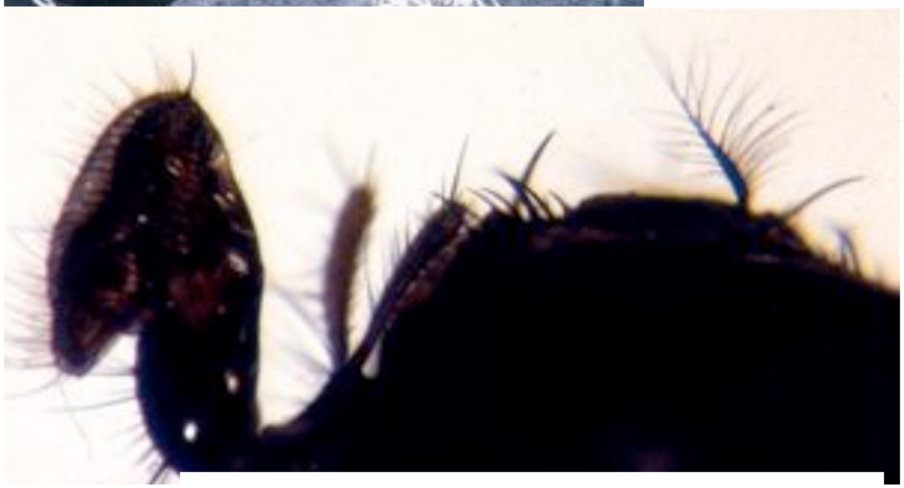


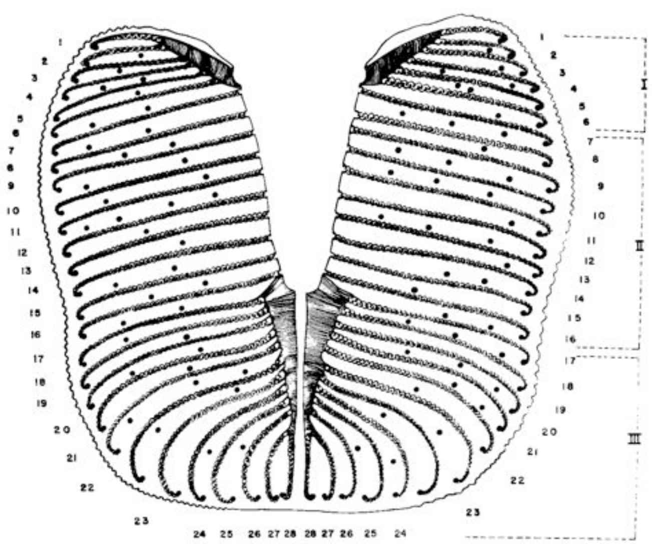
Fig. 2.17. Tubular salivary gland of *Calliphora*. At the top is a representation of the gland showing the positions of the cells depicted below. Left: secretory cell; center: cuboid cell; right: duct cell. Open arrows show the movement of potassium and water; black arrows show the movements of enzymes (after Oschman & Berridge, 1970).

2 MAJOR TYPES OF SALIVARY GLANDS

1. Acinous - acinous gland (ʹas·əʹnəs ʹgland) (*anatomy*) A multicellular gland with sac-shaped secreting units. Also known as alveolar gland. **They are usually innervated** by neurons from suboesophageal ganglion and stomatogastric system. Cockroach and locust.
   a. One neuron produces dopamine which stimulates fluid secretion
   b. Another neuron produces serotonin which causes cells to produce the enzymes.

2. Tubular glands – **Not directly innervated**
   a. *Aedes* and *Calliphora* serotonin is released into hemolymph and regulates the production and release of saliva.





Steps involved in contact with food

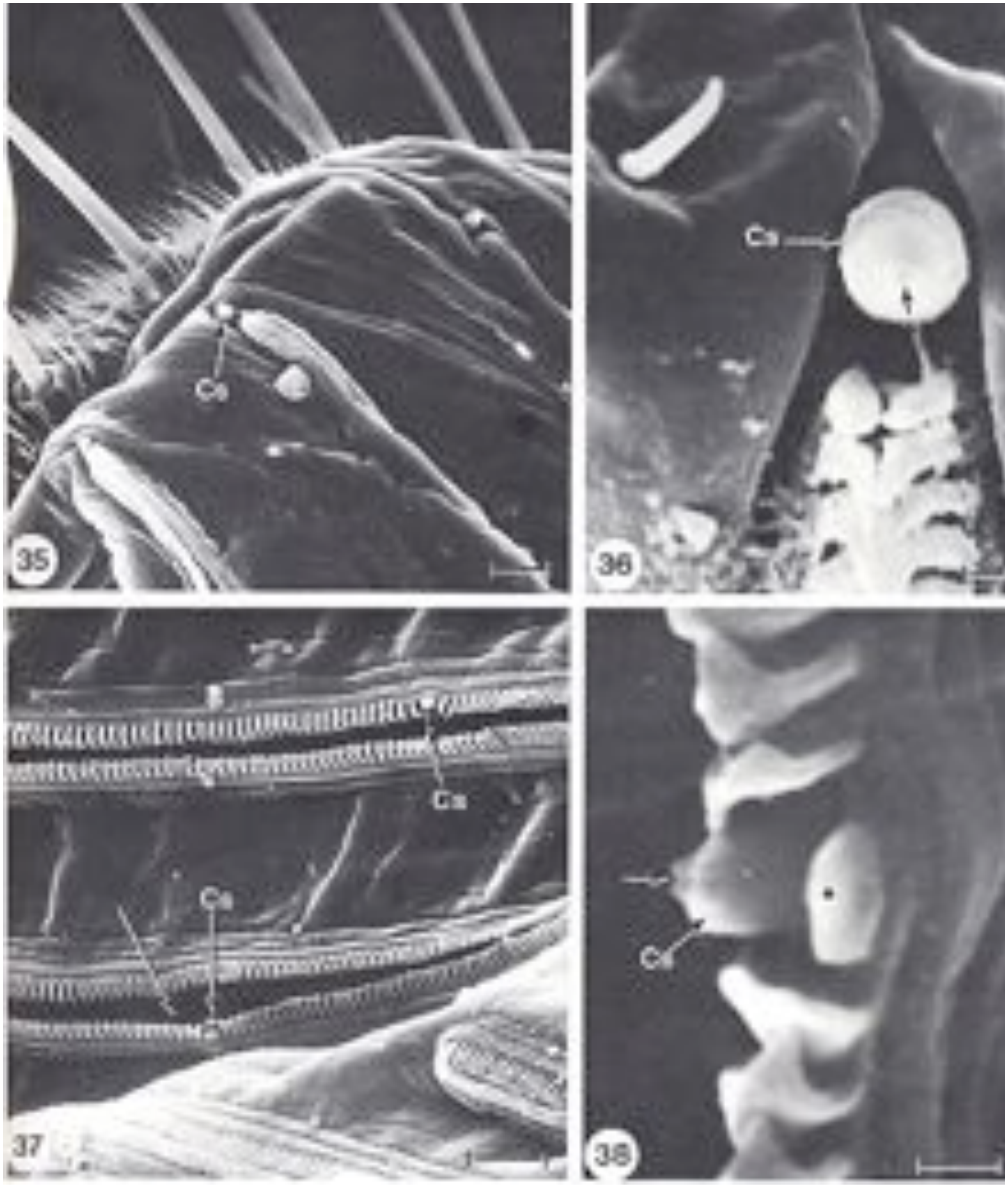
1. Contacts tarsal chemosensillum
2. Proboscis extension
3. Contacts labellar chemosensillum
4. Contacts interpseudotracheal or pseudotracheal chemosensillum
5. Cibarial pump activated
6. Contacts cibarial receptors

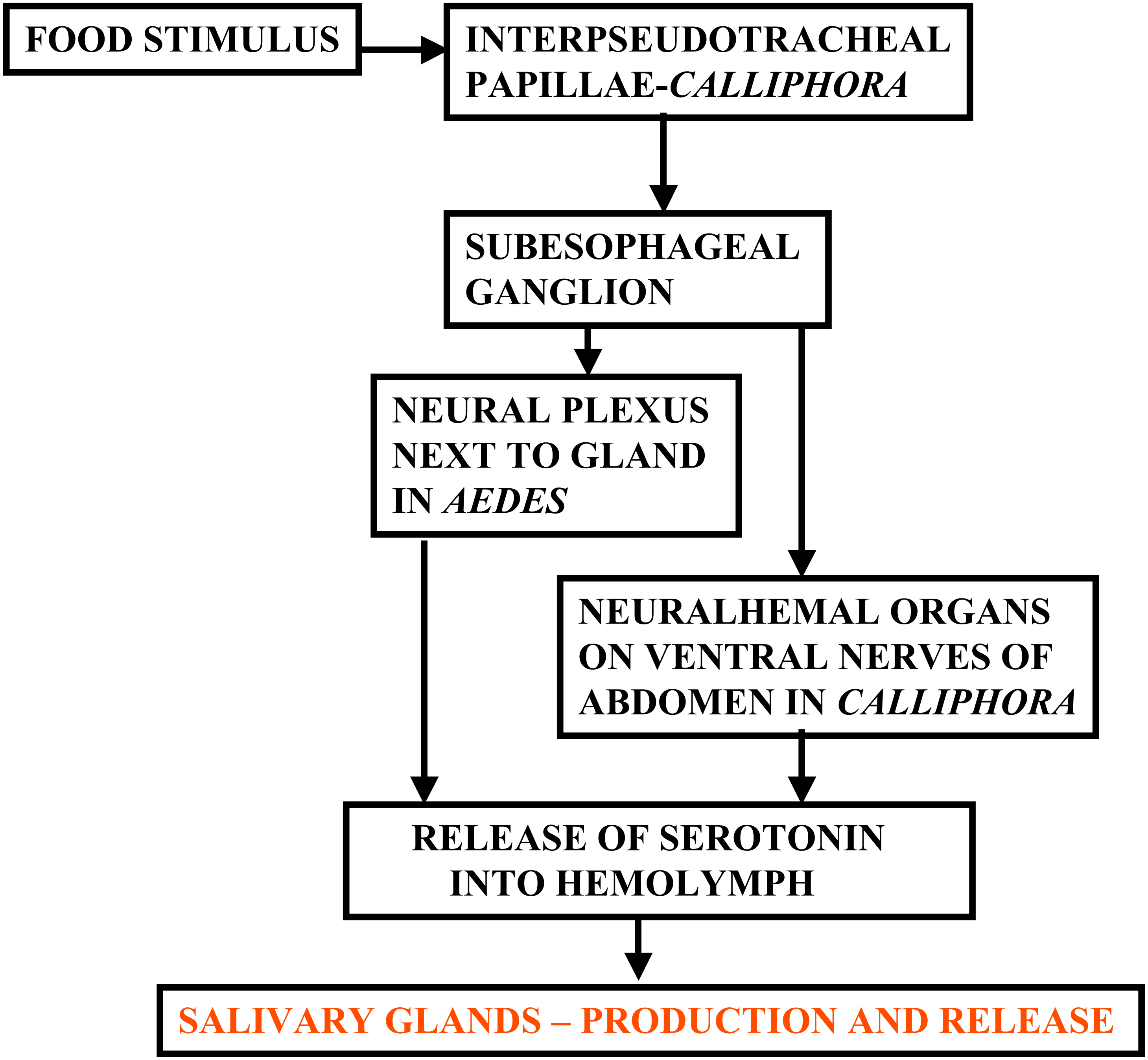
Threshold levels for these sensilla

1. Tarsal chemosensilla have the highest and then it decreases in a decending order

INTERPSEUDOTRACHEAL PAPILLAE - CHEMORECEPTORS

Photos to the right are from *Tabanus nigrovittaus*. Note in fig. 35 the long labellar chemosensilla and the small, peg-like chemosensillum at the end of the pseudotrachea. Fig. 36 shows the pore in the peg-like chemosensillum while figs.. 37-38 show the pseudotracheal groove (arrow in fig. 37) that directs the liquid to the mouth opening. Also note the **pseudotracheal chemosensilla (Cs)** and in fig. 38 the pore in the sensillum.




FOOD STIMULUS
INTERPSEUDOTRACHEAL PAPILLAE-*CALLIPHORA*
SUBESOPHAGEAL GANGLION
NEURAL PLEXUS NEXT TO GLAND IN *AEDES*
NEURALHEMAL ORGANS ON VENTRAL NERVES OF ABDOMEN IN *CALLIPHORA*
RELEASE OF SEROTONIN INTO HEMOLYMPH
SALIVARY GLANDS – PRODUCTION AND RELEASE

SALIVARY TOXINS

Below you can see that a giant water bug has paralyzed or killed a water snake. Strong salivary toxin.

The asilids have large salivary glands that produce a toxin that can kill an 85 gm. mouse. Above you can see the fly has taken down a bigger and better flyer, a dragonfly adult.

Involvement of salivary glands in pathogen or parasite transmission

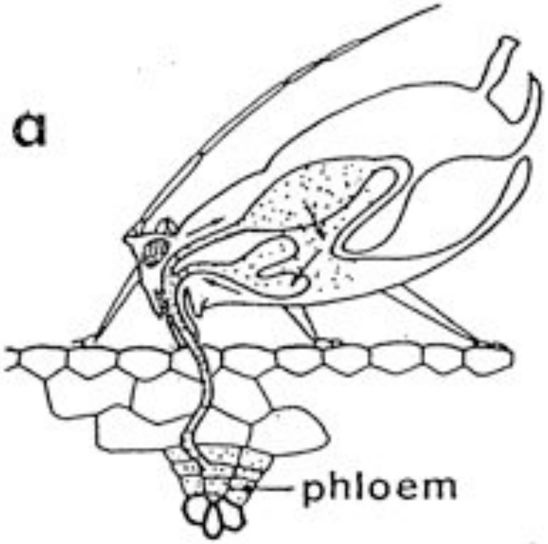
Plant feeders

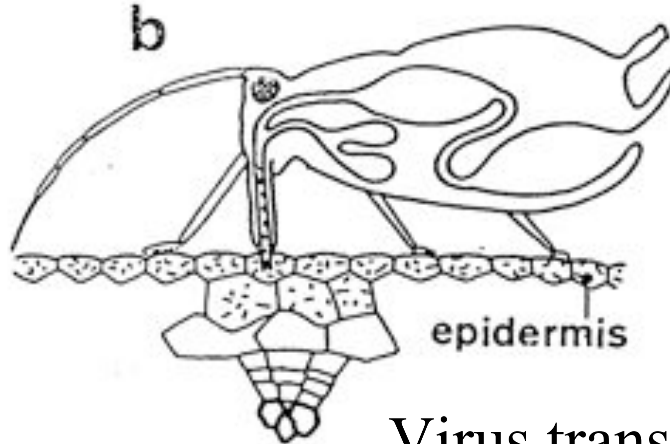
a. Aphids and viruses

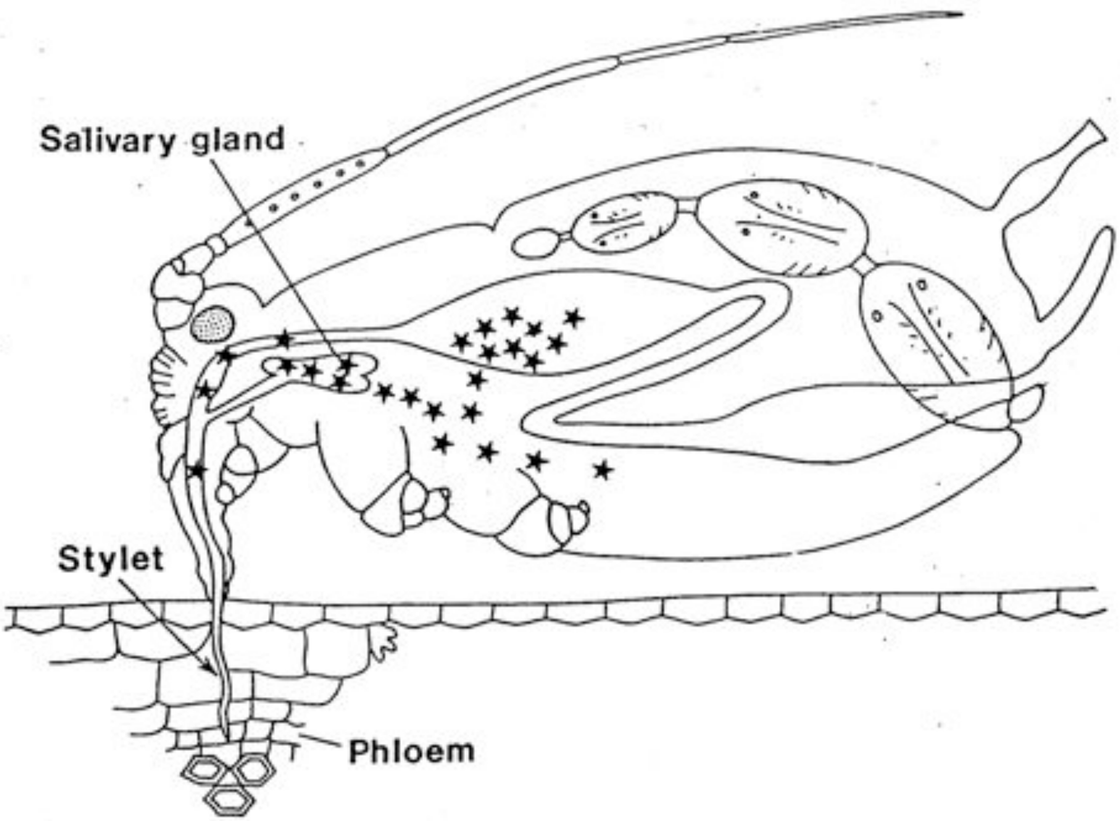
Blood feeders

a. Mosquito and plasmodium

PLANT FEEDERS

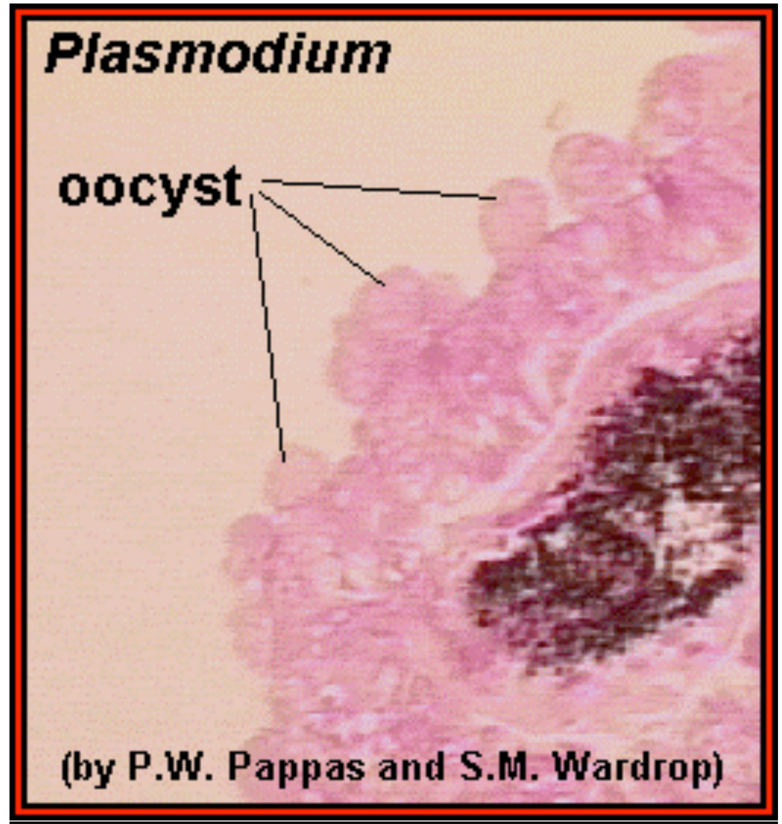






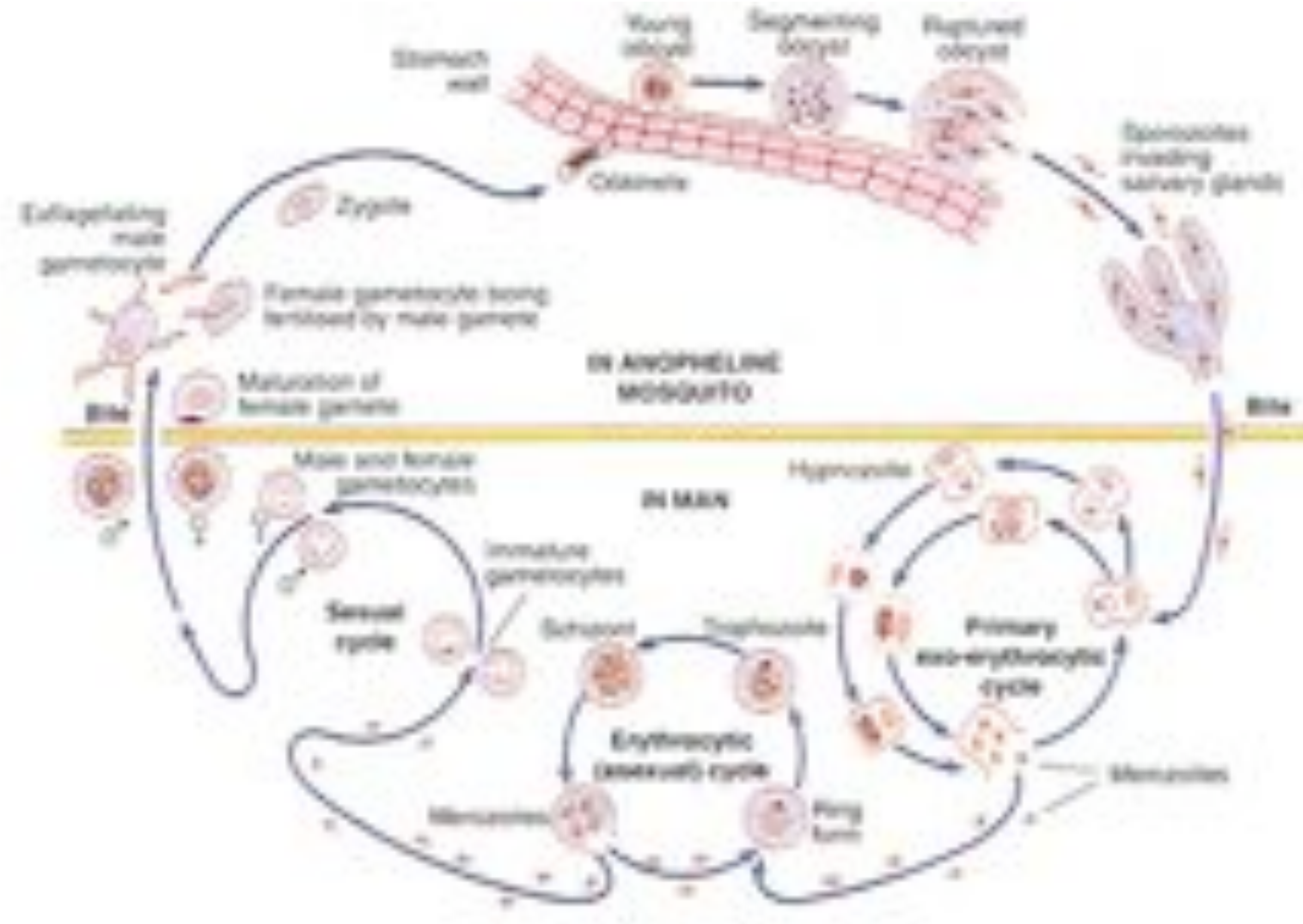
Virus transmission in aphids involves the passage through the midgut, then into the hemolymph and finally into the salivary glands where they are then passed onto another host while feeding.

To the right are the oocysts that form on the mosquito's midgut epithelia cells. Note the digested blood in the gut lumen. Inside these oocysts the parasite matures and changes to a sporozoite.



The sporozoites break out of the oocysts and swim through the mosquito's hemolymph and find their way to the salivary glands. They penetrate and invade a specific region of the female's salivary gland. When the now infected mosquito feeds on another host, it transfers the sporozoites with its saliva into the capillaries of the host.

Blood feeders



Proc Natl Acad Sci U S A. 2001 Nov 6;98(23):13278-81. Epub 2001 Oct 30.

Targeting Plasmodium ligands on mosquito salivary glands and midgut with a phage display peptide library.

Ghosh AK, Ribolla PE, Jacobs-Lorena M.

Department of Genetics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4955, USA.

***Anopheles gambiae* salivary gland proteins as putative targets for blocking transmission of malaria parasites**

Justin D. G. Brennan[*], Melissa Kent[*], Ravi Dhar[*], Hisashi Fujioka[*,], and Nirbhay Kumar[*,]

[*] Johns Hopkins University School of Hygiene and Public Health, The W. Harry Feinstone Department of Molecular Microbiology and Immunology, 615 North Wolfe Street, Baltimore, MD 21205; and Case Western Reserve University, School of Medicine, Institute of Pathology, 2085 Adelbert Road, Cleveland, OH 44106

HOST RESPONSE

Hemostasis has 3 major components:

1. Platelet aggregation
2. Blood coagulation
3. Vasoconstriction

Inflammation has 3 components:

1. Pain-nociception
2. Redness-tissue vasodilation
3. Heat-----tissue vasodilation

Blue jay and tick

Dermacenter varibialis

Saliva of hematophagous insects

1. Anticoagulant or antihemostatic
2. Aids in finding blood vessels
3. Anesthetic quality

To date, all bloodsucking arthropods have 3 things in their saliva

a. Anticlotting mechanism----apyrase
b. Vasodilator mechanism-----Maxidilan from sandfly
c. Antiplatelet mechanism

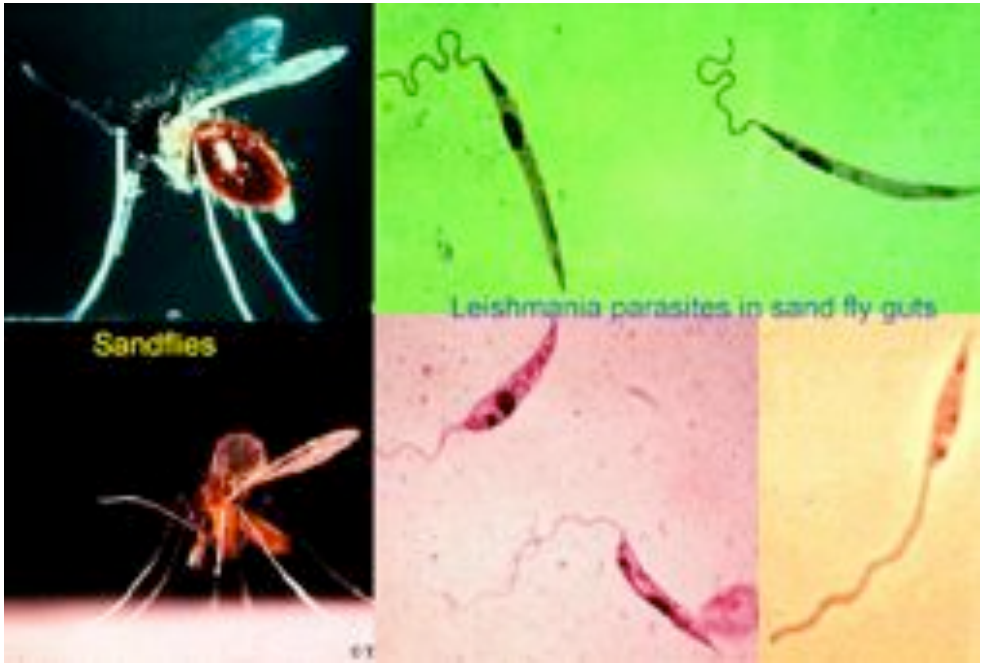
The **blood feeder** wants 3 things — **The host**

a. The blood not to clot-----------------------------hemostasis
b. Blood flow at the site should be intense-------inflammation
c. Host will not bother to kill the blood feeder--anesthetic

Leishmaniasis

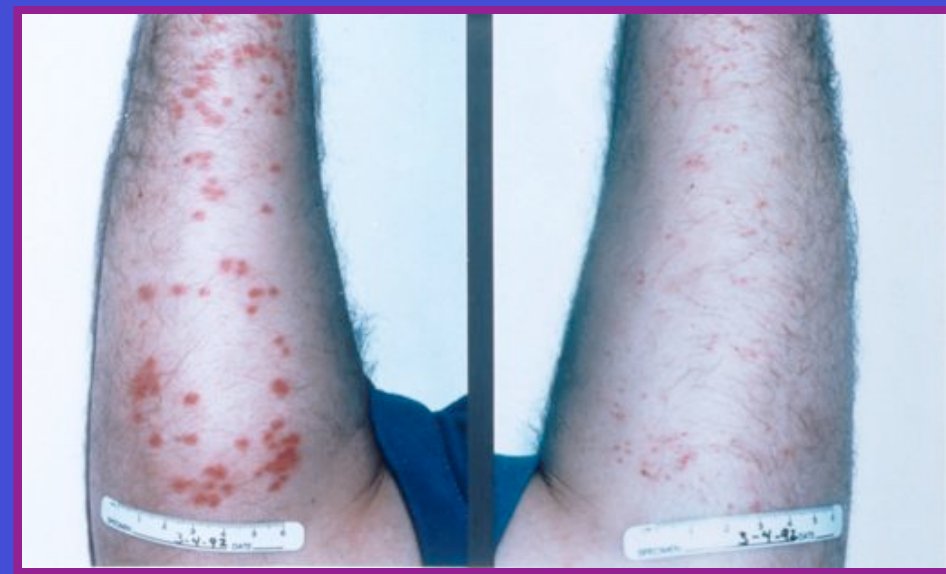
http://www.vetsci.psu.edu/coursedesc/vsc402/slideshows/06 Leishmania.pdf

Maxidilan, a peptide from the saliva of the sandfly is a powerful vasodilator.



Sand flies can vector a flagellated parasite that causes various types of Leismaniasis. While feeding, the fly secretes saliva into the wound. In the saliva are various factors that aid the fly in avoiding a host response, preventing vaso-constriction using a **pepide known as maxidilan** and preventing a clot from forming.

Polymorphism in levels of expression of Maxadilan



Erythemea-redness of skin caused by dilatations and congestion of capillaries

ERYTHEMA CAUSED BY BITE OF UNIFECTED *Lu. longipalpis*

PHOTO TAKEN 12 HRS. POST-FEEDING

From: Warburg et al. Trans. Roy. Phil. Soc. 345:261-267; 1994.

Sexual dimorphism in salivary glands of mosquitoes

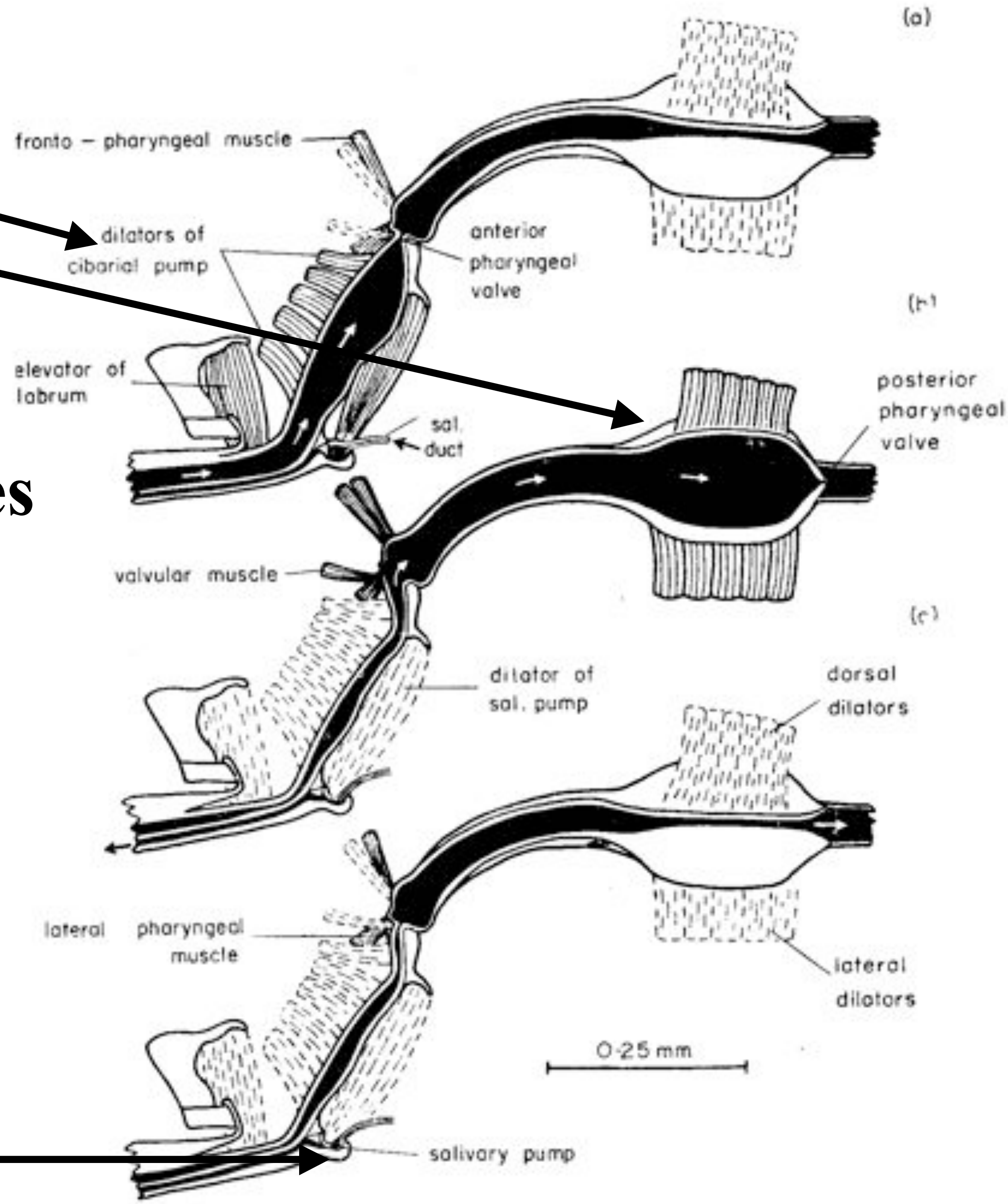
STRUCTURE

COMPLIMENTS

FUNCTION

Cibarial pump
Pharyngeal pump

Muscles and nerves are involved



Salivary pump

MIDGUT

1. Midgut starts at the cardiac sphincter and ends at the pyloric sphincter
2. Midgut contains different cell types:
   a. Digestive cells
   b. Regenerative cells
   c. Endocrine cells
   d. Goblet cells
3. Midgut has a sleeve of tissue, called the peritrophic envelope or matrix that covers the microvilli and separates the food being digested from these digestive cells
4. Midgut is where the food is mainly digested

Digestive cells or Columnar Digestive cells

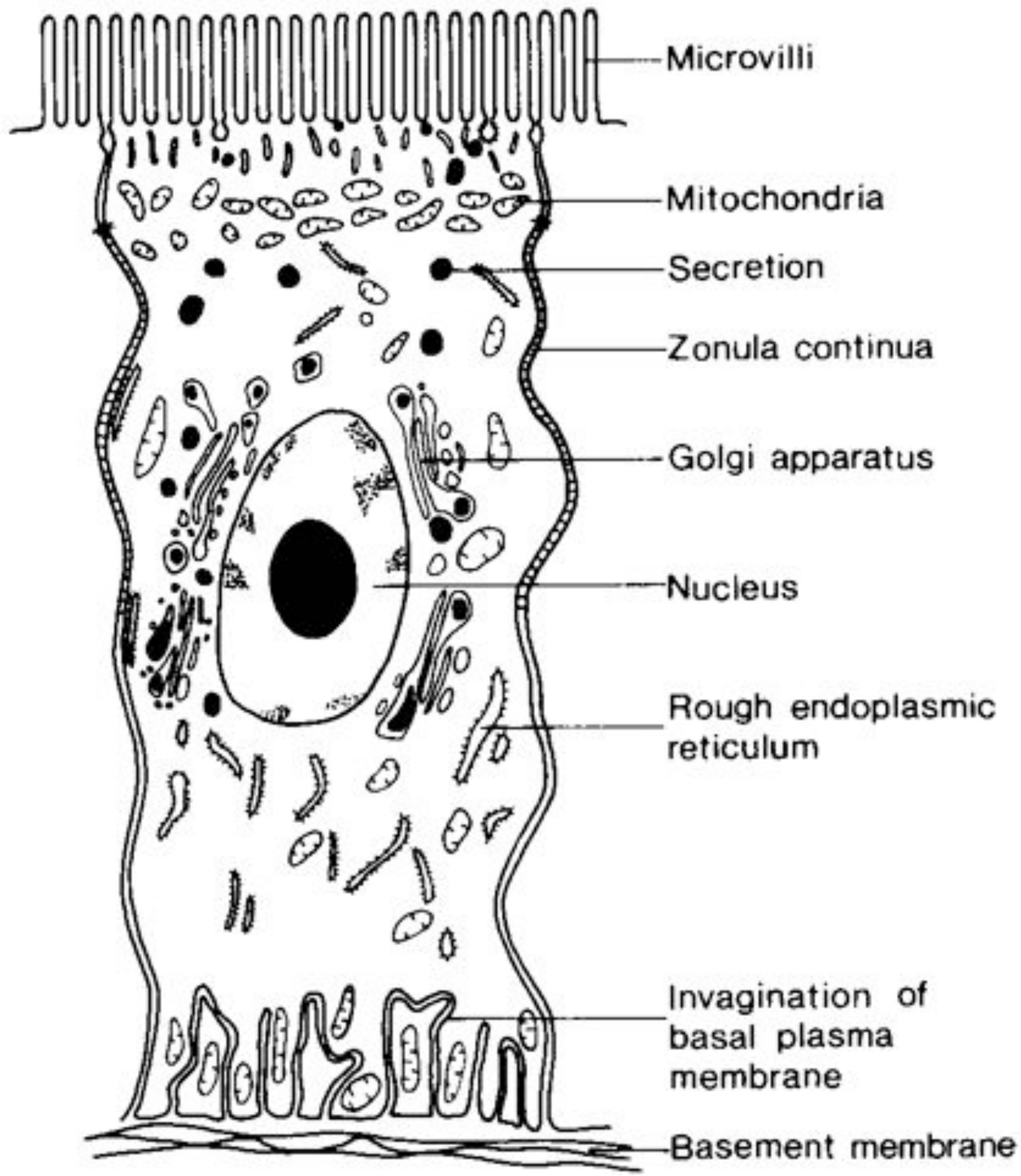
The digestive cells secrete enzymes into the lumen of the midgut that aid in digestion of proteins, carbohydrates and fats. They also are short lived and need to be replaced.

Enzyme breakdown to:

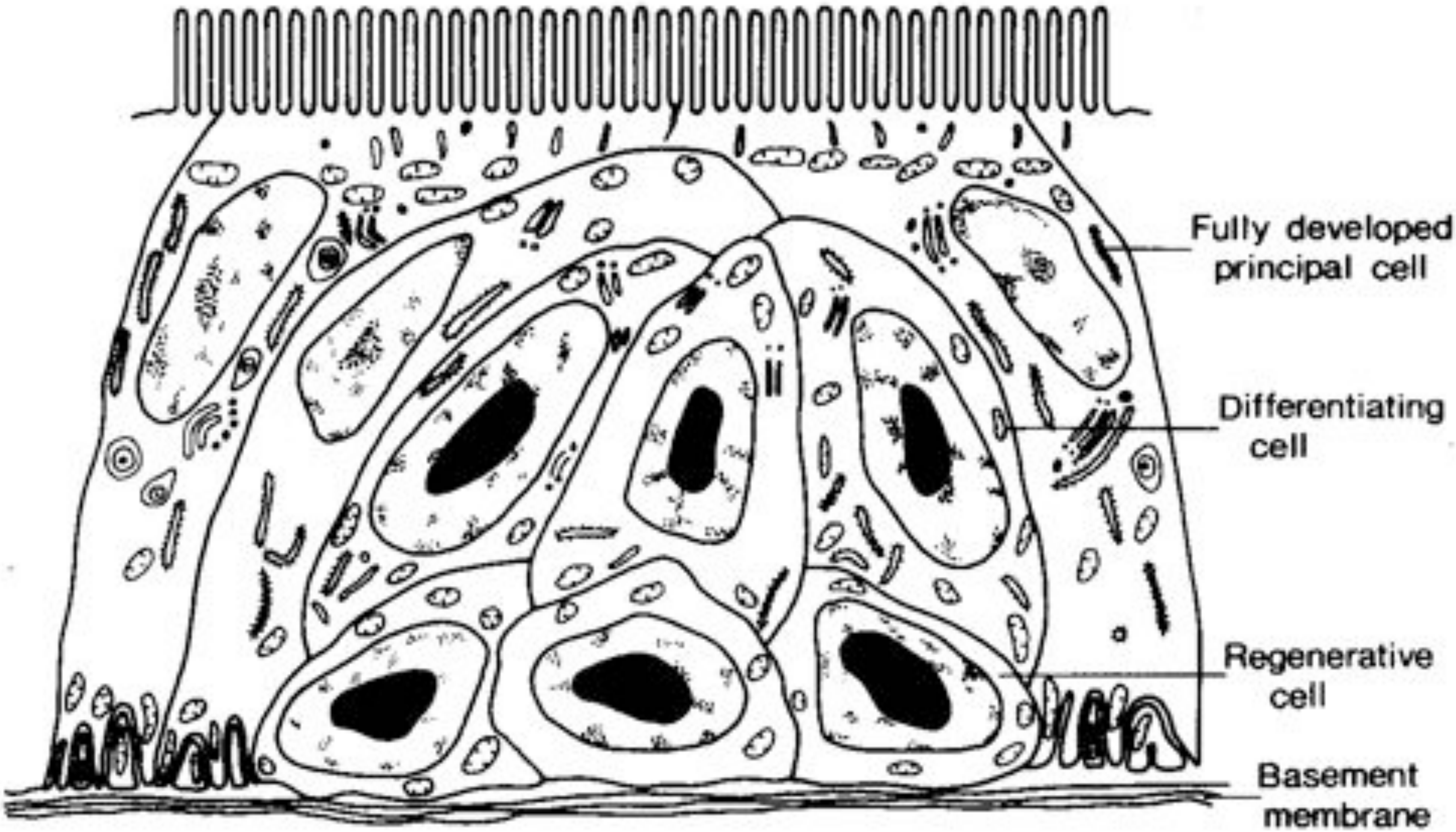
Proteins=amino acids

Carbs.=glucose

Fats=fatty acids.

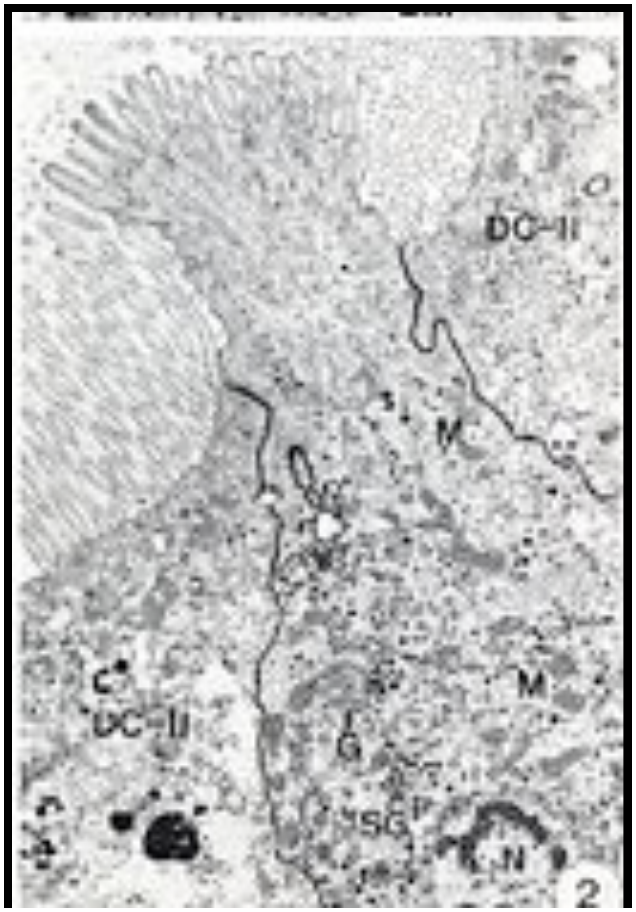


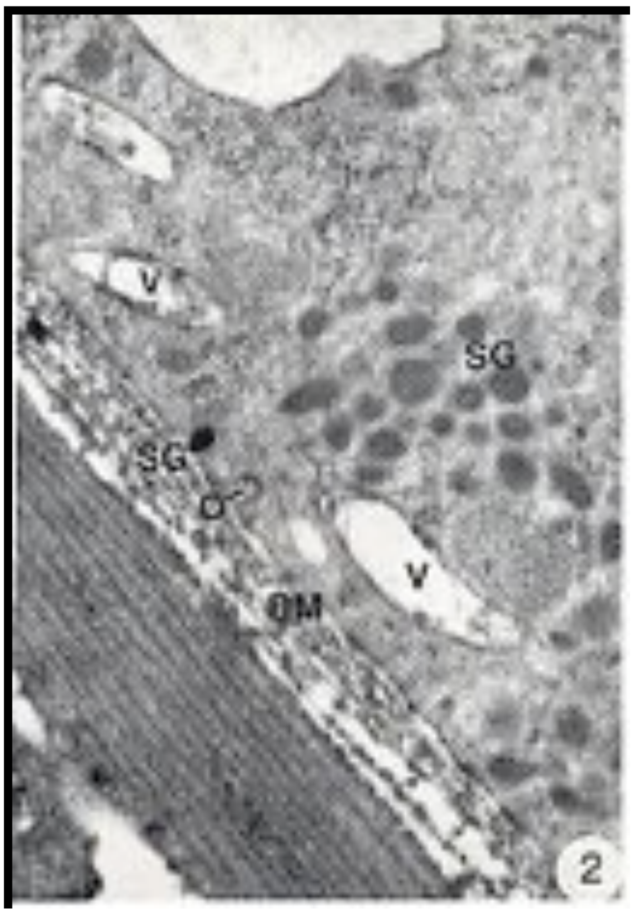
Regenerative cells- Also called nidi. They produce new digestive cells to replace worn out digestive cells.

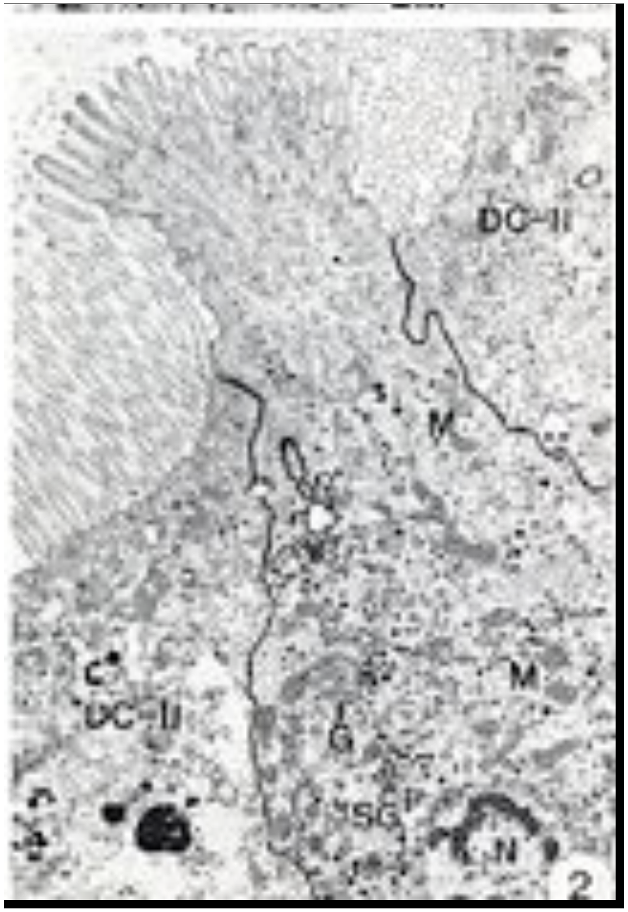


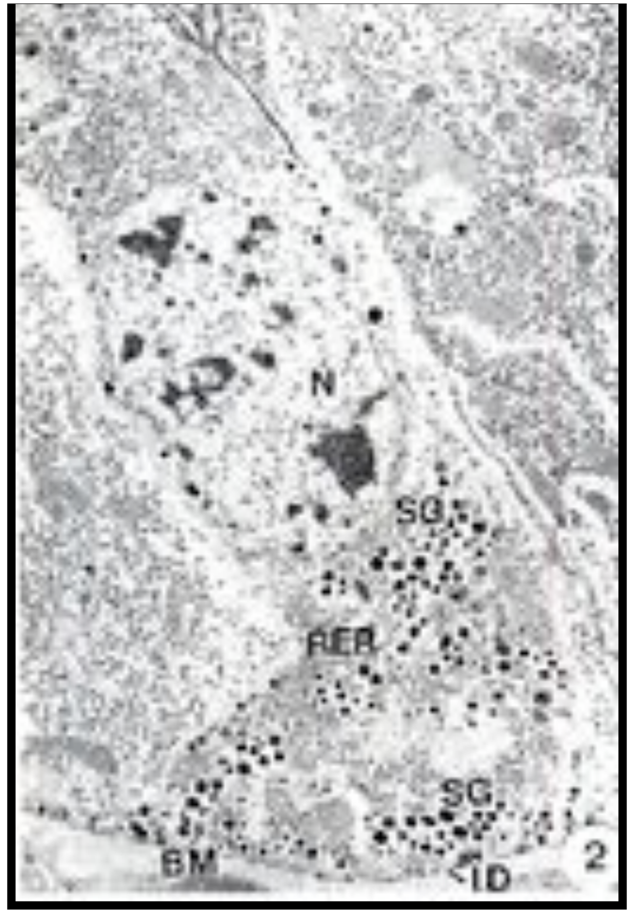
GUT ENDOCRINE CELLS

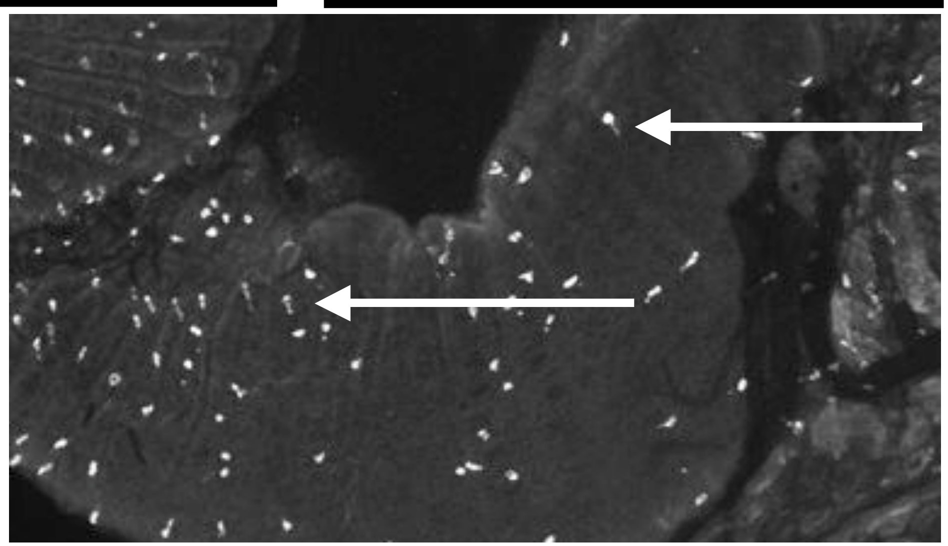
Two types of endocrine cells occur in the midgut of insects: closed and open. The closed types contact the basement membrane but do not reach to the gut lumen. Open cells extend from the lumen to the hemolymph (Shown in two TEM of *Phormia* gut below. On the right it shows a dark secretory granule near the membrane of the cell and the O=omega body that shows how the material is released here into the hemolymph and into the basement envelope.







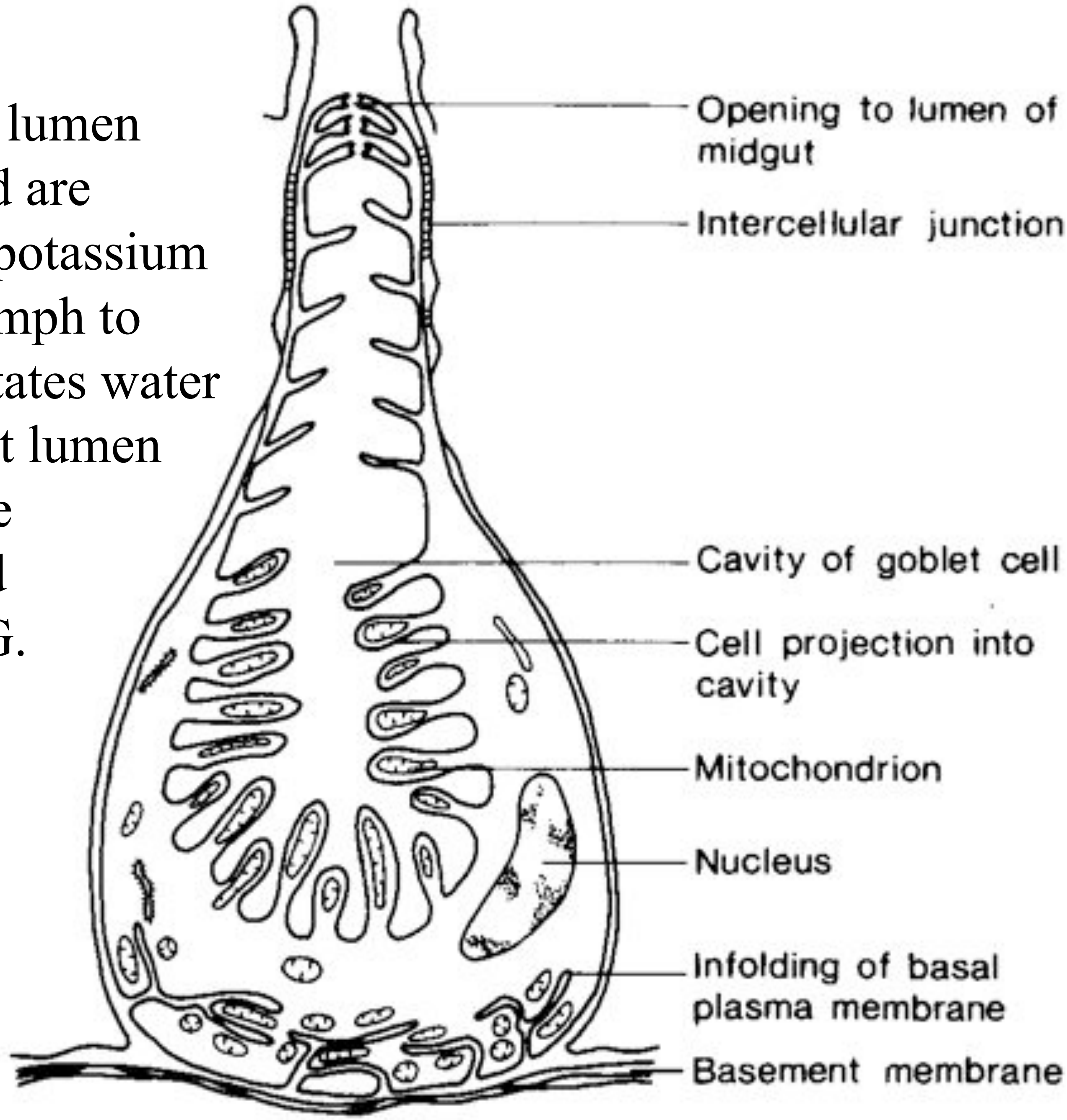




Photos of *Phormia regina* midgut. Fluorescent antibody against one of the MG peptides. Note the white arrow pointing to cells that show a larger circle-like spot and a connecting long duct-like arrangement. These are open gut endocrine cells that connect the cell to the lumen of the gut and to the basement matrix

Goblet cells

Cells that connect the lumen to the hemolymph and are involved in pumping potassium ions from the hemolymph to the lumen. This facilitates water movement into the gut lumen that is essential for the absorption of digested nutrients from the MG.



FUNCTIONS OF PERITROPHIC MATRIX

1. Ultrafilter-proposed by Wigglesworth, 1929
2. Protection against infection by ingested pathogens
3. Division of midgut into endo- and ectoperitrophic spaces, which could improve digestion efficiency
4. Protection against toxic plant allelochemicals, such as tannins

IS PRODUCED BY SPECIALIZED CELLS JUST BEHIND THE CARDIAC SPHINCTER AND IS PRODUCED AS A CONTINUAL SLEEVE THAT IS CONSTANTLY BEING FORMED

COMPOSITION OF THE PERITROPHIC MATRIX/ENVELOPE

Mucin is any of a group of glycoproteins found especially in the secretions of mucous membranes.

Enhacin is a metalloprotease that aids different types of pathogens in penetrating the peritrophic matrix. Enhacins of various types and produced by the pathogen can elicit a host immune response without the involvement of blood cell aggregation (i.e., not the encapsulation response).

Thus, the baculovirus enhancin, which is encoded and carried by specific baculoviruses, has mucin-degrading activity both *in vitro* and *in vivo*.

Microbial metalloproteinases mediate sensing of invading pathogens and activate innate immune responses in the lepidopteran model host Galleria mellonella. Altincicek B, Linder M, Linder D,
•Preissner KT, Vilcinskas A.
Institute of Phytopathology and Applied Zoology, Justus-Liebig-University of Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.
Thermolysin-like metalloproteinases such as aureolysin, pseudolysin, and bacillolysin represent virulence factors of diverse bacterial pathogens. Recently, we discovered that injection of thermolysin into larvae of the greater wax moth, Galleria mellonella, mediated strong immune responses. Thermolysin-mediated proteolysis of hemolymph proteins yielded a variety of small-sized (<3 kDa) protein fragments (protfrags) that are potent elicitors of innate immune responses. In this study, we report the activation of a serine proteinase cascade by thermolysin, as described for bacterial lipopolysaccharides (LPS), that results in subsequent prophenoloxidase activation leading to melanization, an elementary immune defense reaction of insects. Quantitative real-time reverse transcription-PCR analyses of the expression of immune-related genes encoding the inducible metalloproteinase inhibitor, gallerimycin, and lysozyme demonstrated increased transcriptional rates after challenge with purified protfrags similar to rates after challenge with LPS. Additionally, we determined the induction of a similar spectrum of immune-responsive proteins that were secreted into the hemolymph by using comparative proteomic analyses of hemolymph proteins from untreated larvae and from larvae that were challenged with either protfrags or LPS. Since G. mellonella was recently established as a valuable pathogenicity model for Cryptococcus neoformans infection, the present results add to our understanding of the mechanisms of immune responses in G. mellonella. The obtained results support the proposed danger model, which suggests that the immune system senses endogenous alarm signals during infection besides recognition of microbial pattern molecules.
PMID: 17074843 [PubMed - indexed for MEDLINE]

PERITROPHIC MATRIX

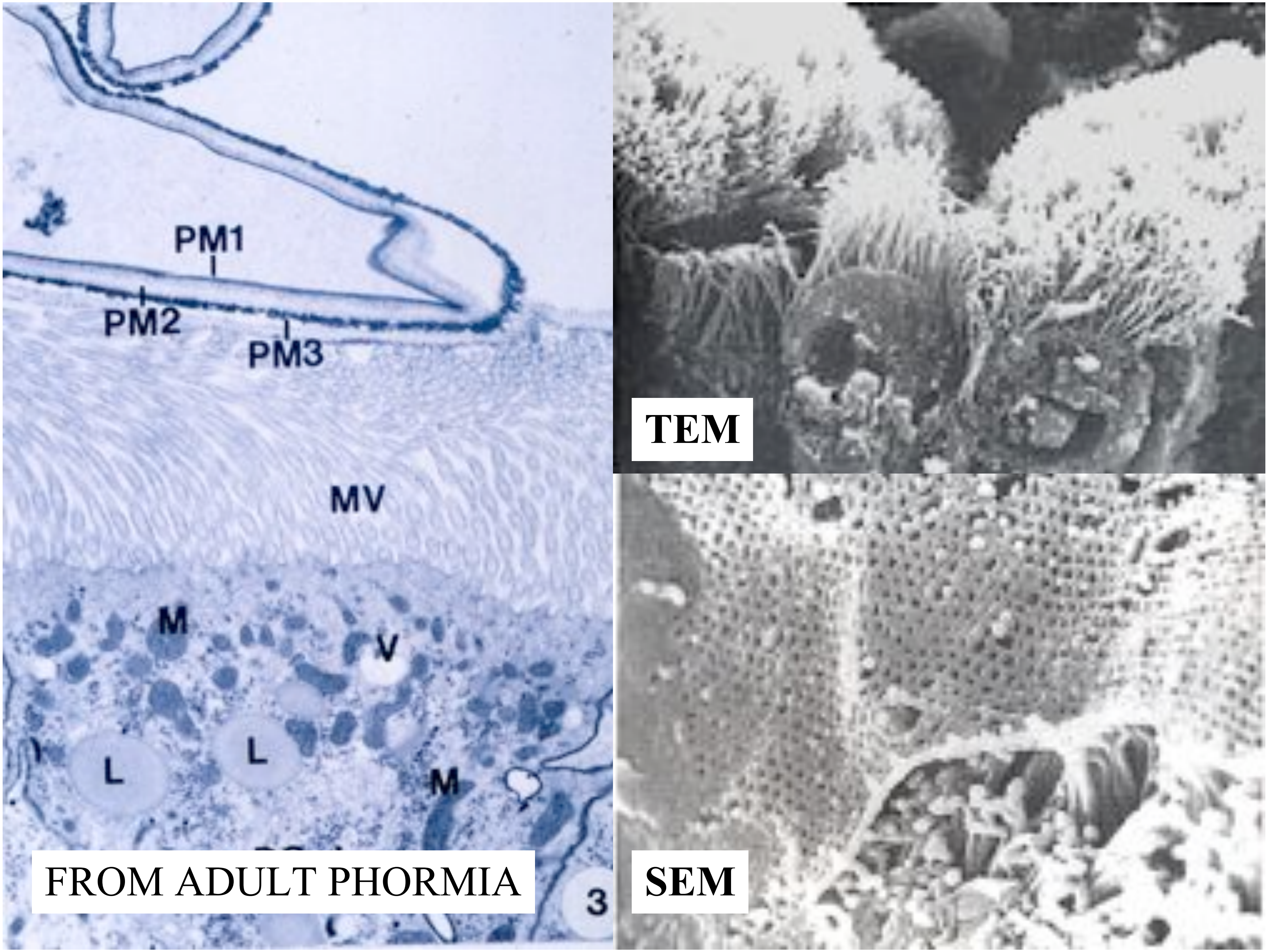
1. Importance for pathogen and parasite passage
2. Permeability to various food molelcules

Barbehenn, R. V. and M. M. Martin. 1995. Peritrophic envelope permeability in herbivorous insects. J. Insect Physiol. 41: 303-311.

Based on their work they concluded:

a. Pore diameters are small enough to exclude bacteria and virons of baculoviruses

b. Pores are too large to function as a filter against digestive enzymes and free plant allelochemicals

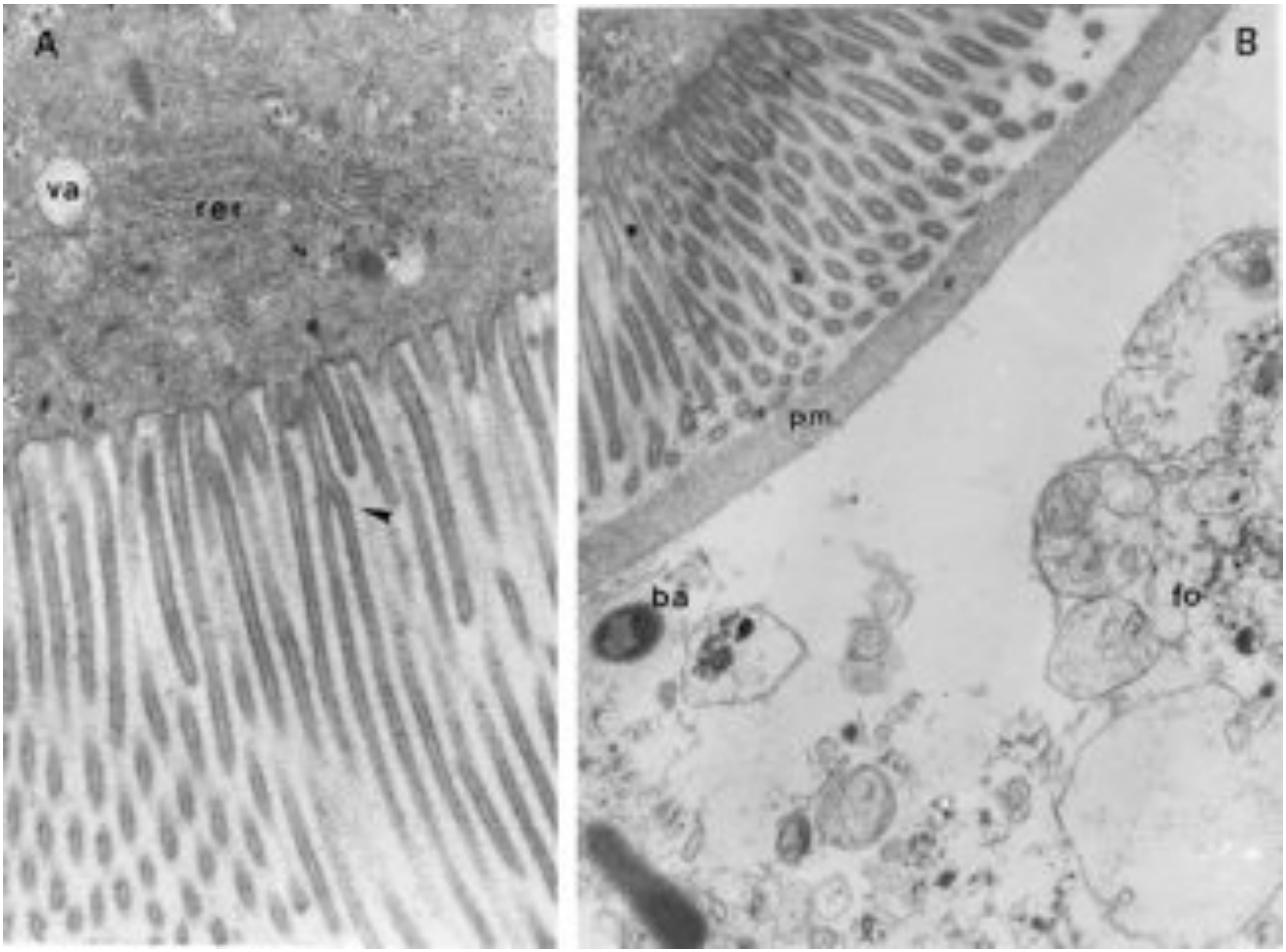

PM1
PM2
PM3
MV
M
V
L
L
M
3
FROM ADULT PHORMIA
TEM
SEM

Midgut Ultrastructure of the Third Instar of *Dermatobia hominis* (Diptera: Cuterebridae) Based on Transmission Electron Microscopy. J. Med. Entomol., vol. 40

L. G. Evangelista,[b] and A.C.R. Leite[a, b]

pm=peritorphic matrix; ba=bacteria; fo=food

PM=peritrophic matrix



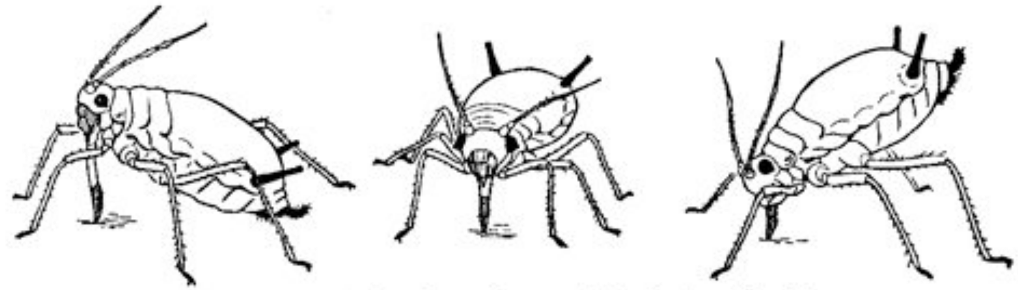
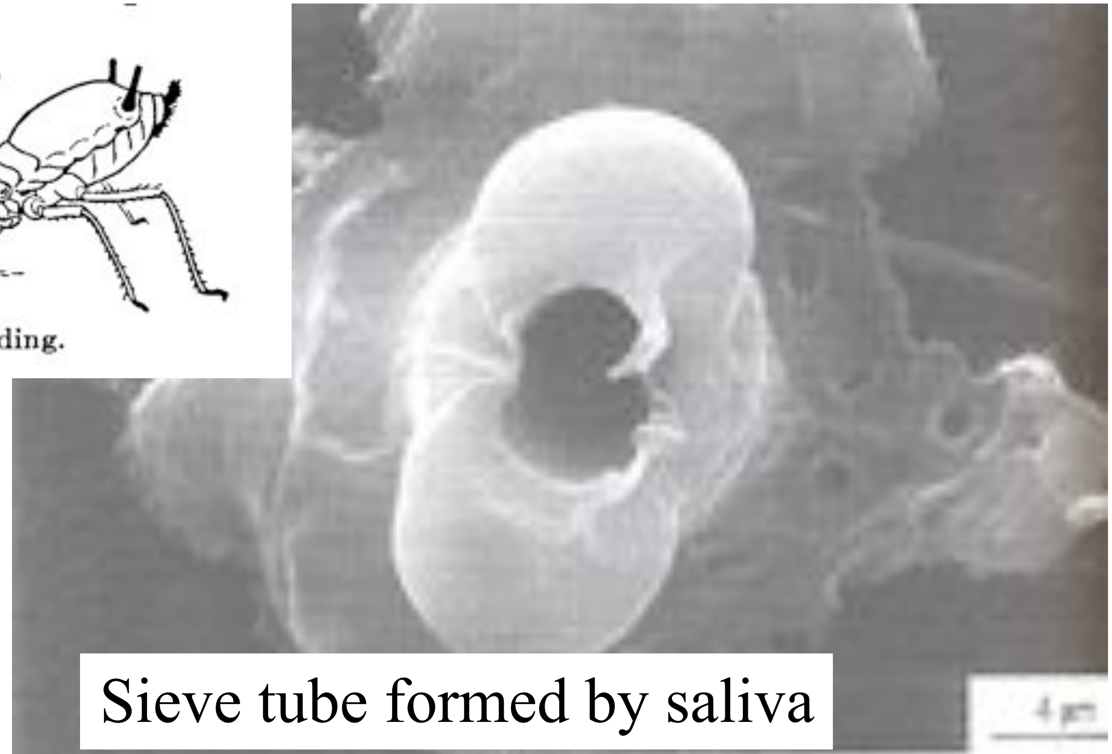
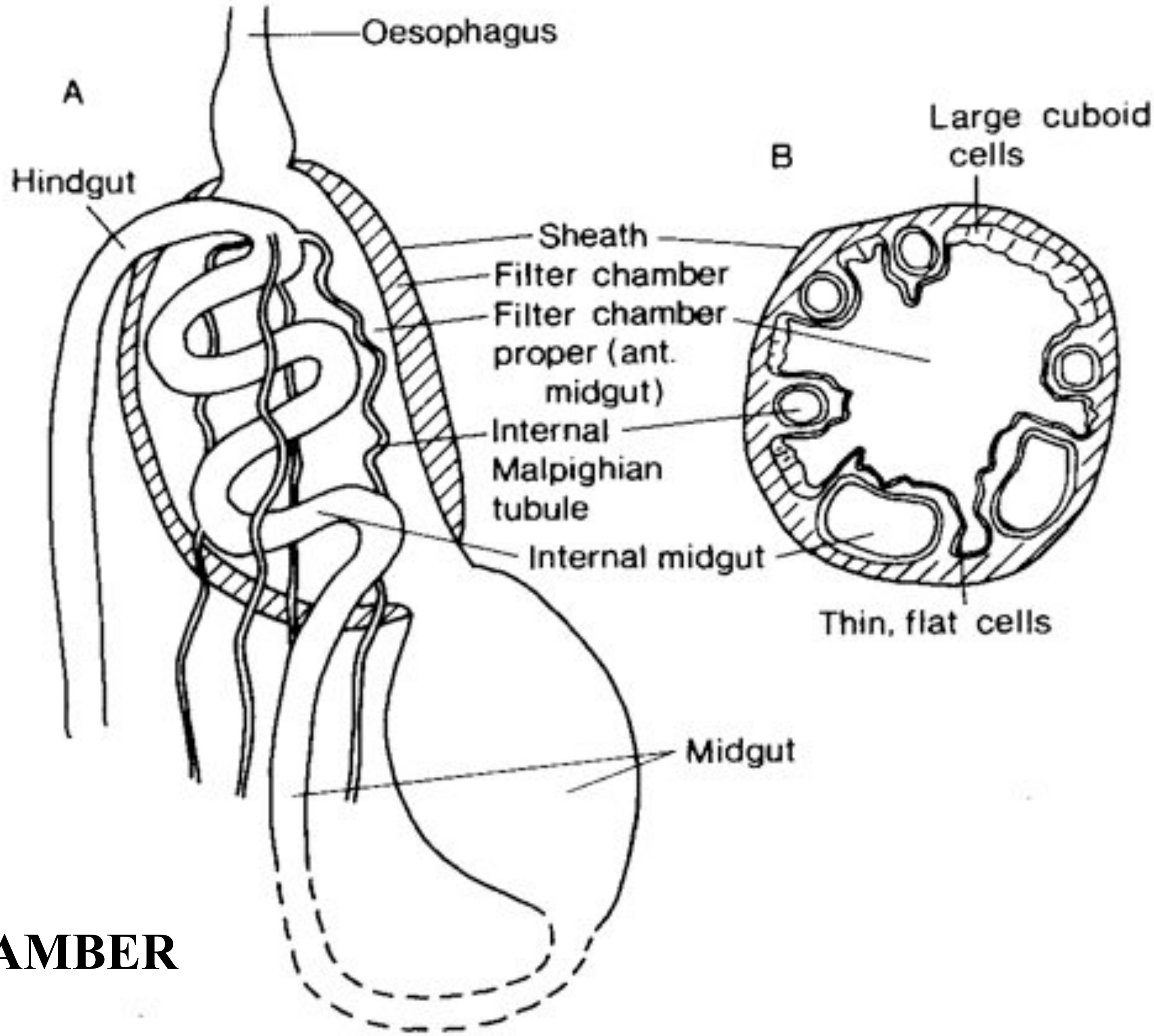


FIG. 185.—Attitudes of an aphis during feeding.

Aphid feeding using the sieve tube and also the specialized filter chamber of the foregut



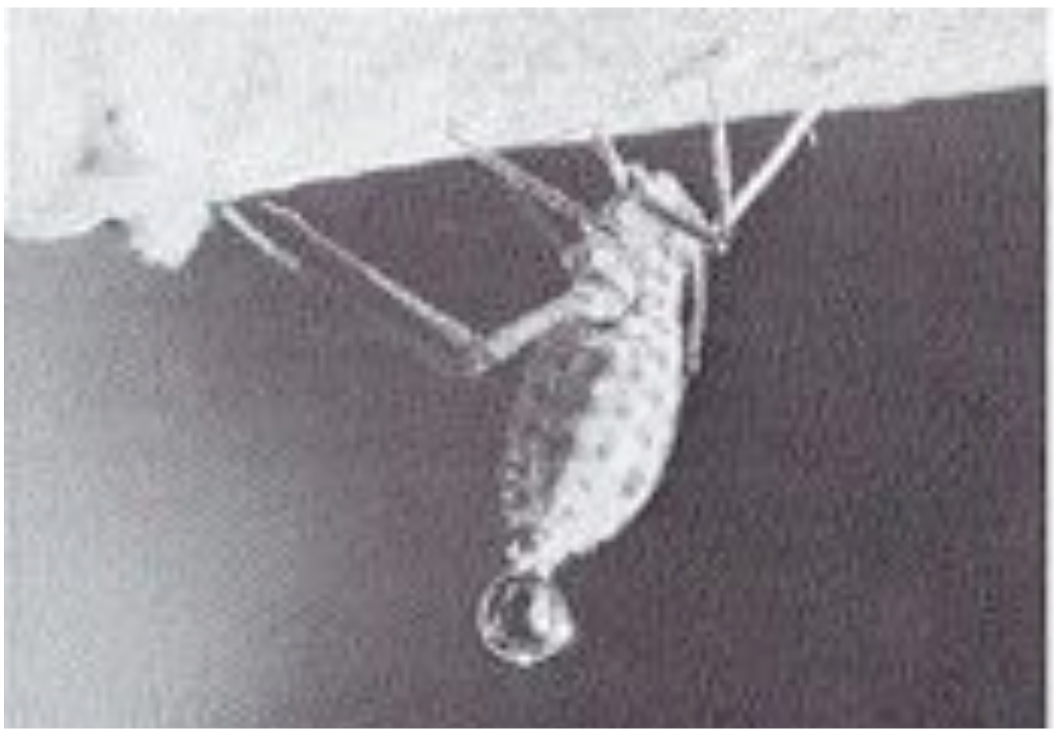
Sieve tube formed by saliva

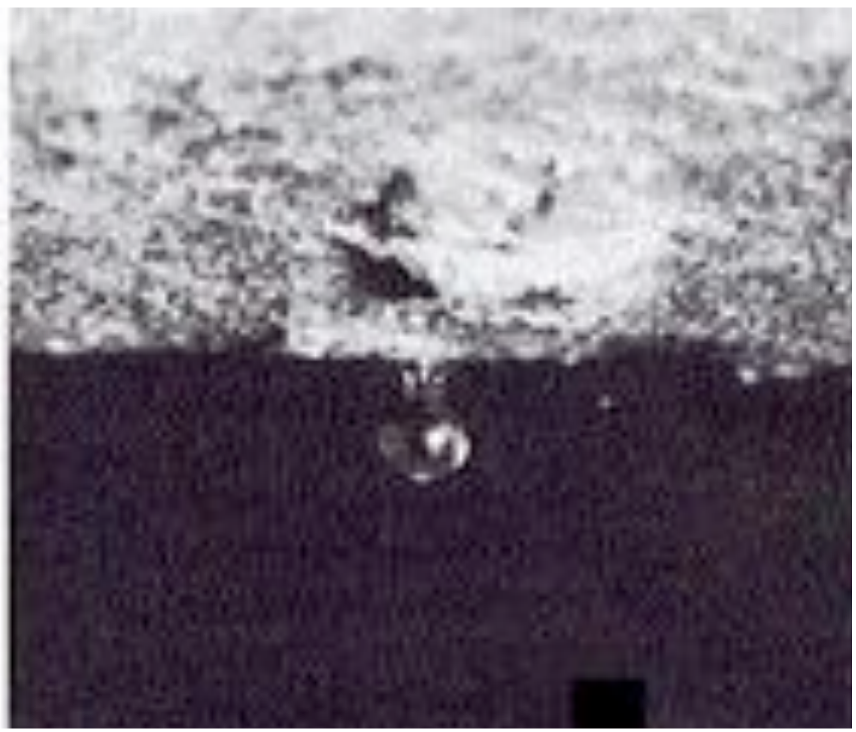


FILTER CHAMBER

FILTER CHAMBER OF APHIDS-A SPECIALIZED CASE FOR FEEDING ON NITROGEN DILUTE PLANT SAPS

Aphids feed on plant saps that are low in amino acids (nitrogen based). In order to obtain these, they have a specialized digestive tract system known as a filter chamber. The liquid they feed upon is under pressure and when they remove their mouthparts the sap continues to drip (see photo on right). After recycling the sap and removing the amino acids a sticky droplet of carbohydrate emerges from the aphid's anus (see photo on left). This is called honeydew; ants love it; food for parasites





Blood feeding insects undergo rapid diuresis

1. Diuretic hormone is released
2. Rapid uptake of water from the midgut, thus from the bloodmeal
3. Water enters hemolymph and then is rapidly taken up by the Malpighian tubules and excreted very rapidly as water droplets from the anus

Parasite and pathogen penetration through the midgut

Currently, the hot area of research on the midgut and arthropod borne disease agents is the **molecular biology of the midgut and the peritrophic matrix**. The MG area is believed to be selected out by the pathogens or parasites from other parts of the digestive tract because of **receptors** that the pathogen or parasite tune into on these tissues. By better understanding these receptors, they believe they can devise better control strategies against these agents and prevent them from either recognizing these receptors (blocking them) or interfering somehow with their recognition of these areas on the agent itself. Thus, lack of recognition of the midgut should prevent infection and entrance into the insect's hemocoel.

Dalbulus maidis, leafhopper vector of corn stunt spiroplasma. (Courtesy A. Wayadande)

TEM of spiroplasm

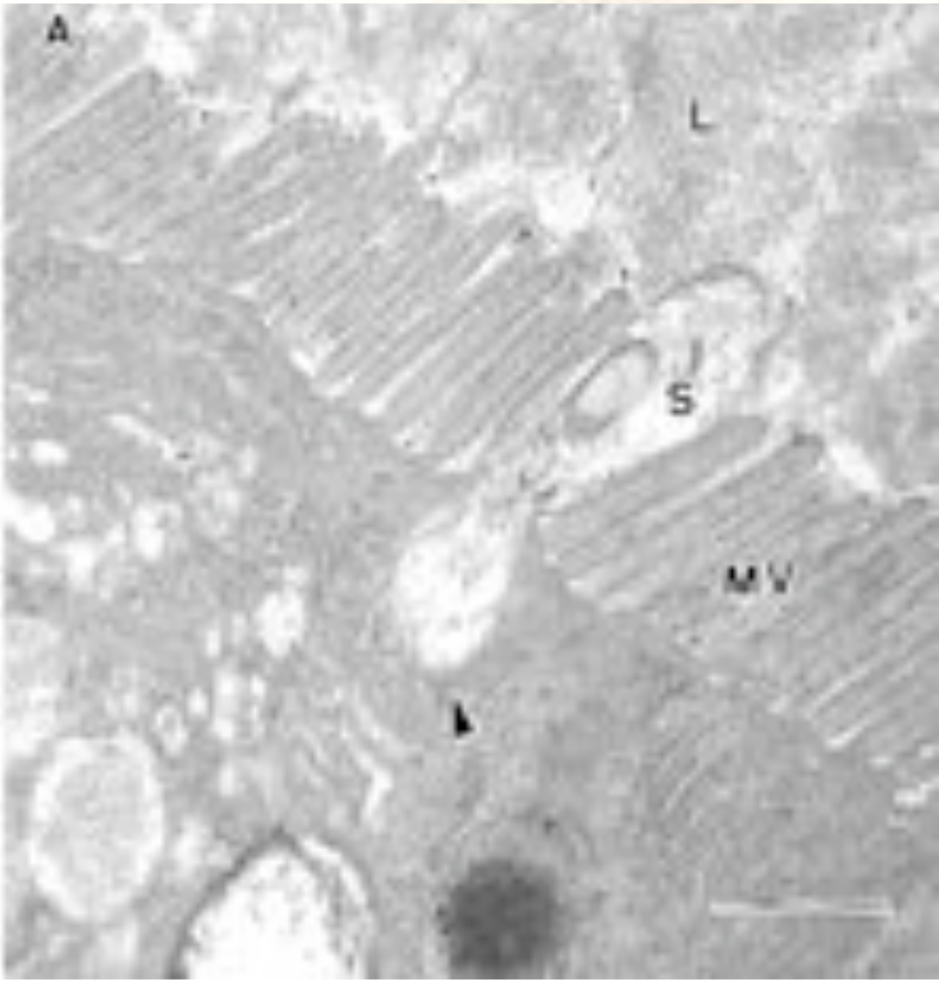


Photo to the right shows the spiroplasm penetrating the midgut wall through the microvilli.

http://www.apsnet.org/education/IntroPlantPath/PathogenGroups/fastidious/

EATING FROM THE POISONOUS PLATTER

Many plants contain ‘nasty’ chemicals that have adverse effects on herbivores

Organisms that eat plants are potentially eating from a poisonous platter

What is the first major structure involved in meeting or contacting toxins that are eaten?

MIDGUT

Just think of all of the poisonous plants and fungi that one could eat and get real sick or even die. No different for many insects. How do they protect themselves from these naturally occurring toxins?

MFO’s (mixed function oxidases) or P450 system-Oxidative enzymes that detoxify toxins in the food. Found in the midgut cells. What organ is involved in detoxification of ‘nasty’ chemicals in humans?

HINDGUT

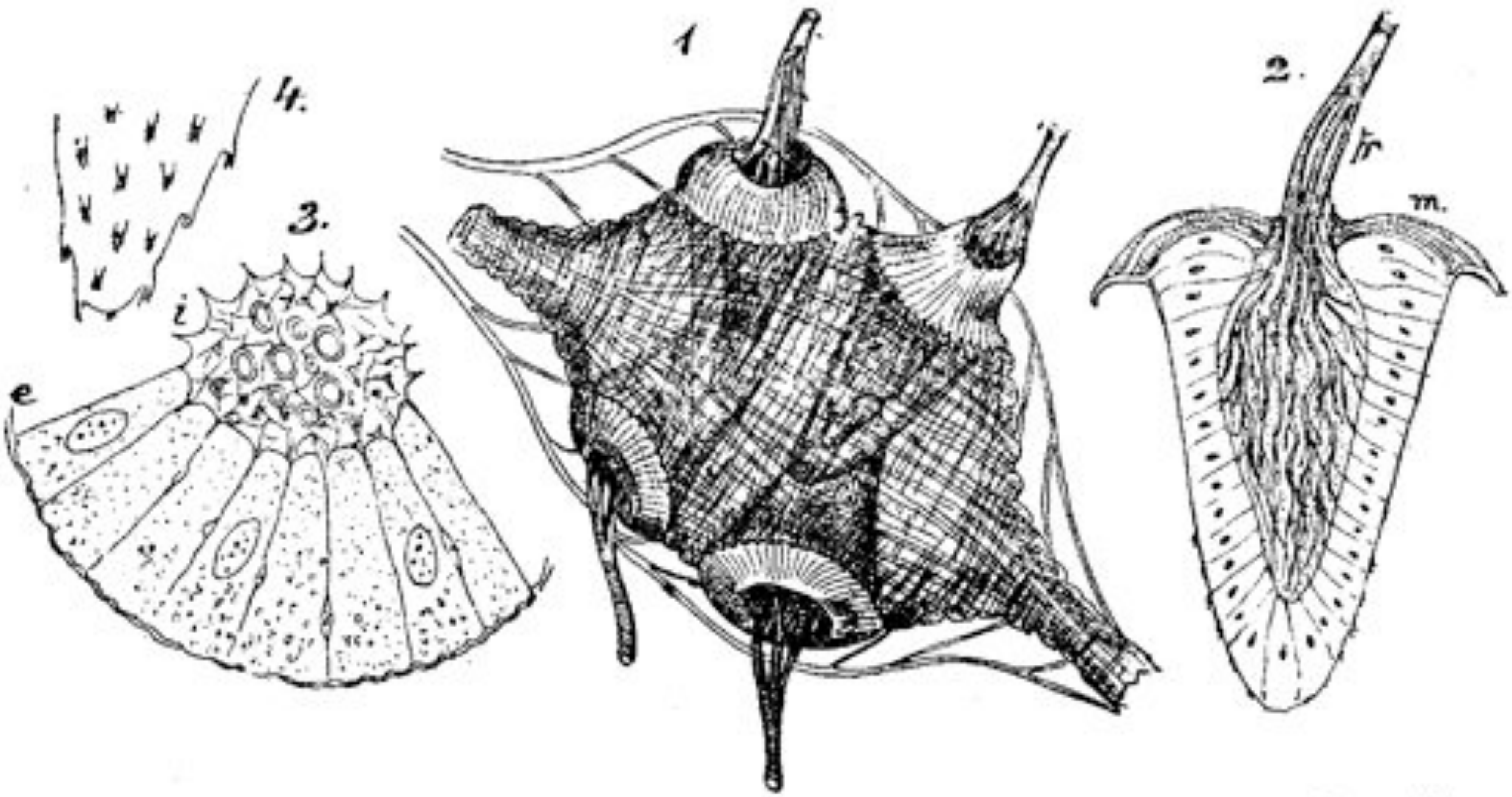
1. Major refuse dumping area for waste products from midgut and Malpighian tubules
2. In insects that feed on dilute foods (i.e., low in amino acids), such as plant saps or blood, the hindgut is involved in getting rid of excessive water and also in housing symbionts that use these waste products to produce substances the insect needs.
3. Many insects have special adaptations of the hindgut region that aid in reabsorption of certain salts and amino acids. Helps in maintaining osmotic pressure of the hemolymph.

HINDGUT

4. Water absorption from feces into the hemolymph
5. Pheromone production-Male scolytid beetles produce an aggregation pheromone. Also in *Dacus tryoni* in males
6. Respiration in larvae of Anisoptera (Dragonflies)

Rectal papillae of flies and rectum

The various types of papillae in the rectum of insects are involved in reabsorption of water and the movement of ions for osmoregulation



HINDGUT AND ITS SYMBIONTS

- Because of the following foods, insects have relied upon and have taken up symbionts to either aid in digestion of molecules they can't digest (e.g., Cellulose) or provide the insect with essential nutrients, especially various vitamins, etc., that they would otherwise be unable to get from diets poor in these substances.

1. Termites and digestion of cellulose
2. Insects feeding on blood or on plant saps.

Trophallaxis or feeding on the feces of another insect provides that organism with the symbionts that it lost, usually from the molt since the hindgut and its cuticular lining are shed at the molt. Take a newly emerged termite and isolate it with filter paper to eat and it will starve to death because it can't digest the cellulose without the symbionts.

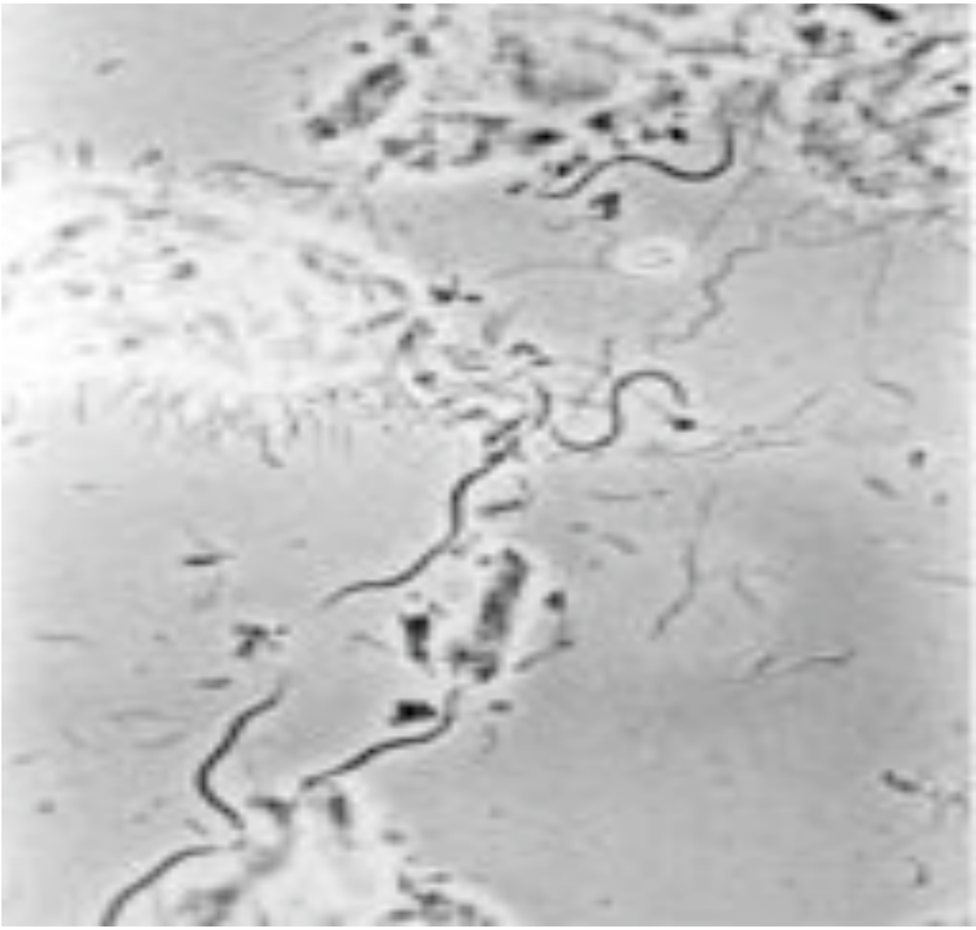
Insects depending on this feces feeding:

- Fleas
- Termites-the dependance of termites on one another for reinfection with symbionts certainly had a role to play in the evolution of social behavior in this group.
- Beetle grubs

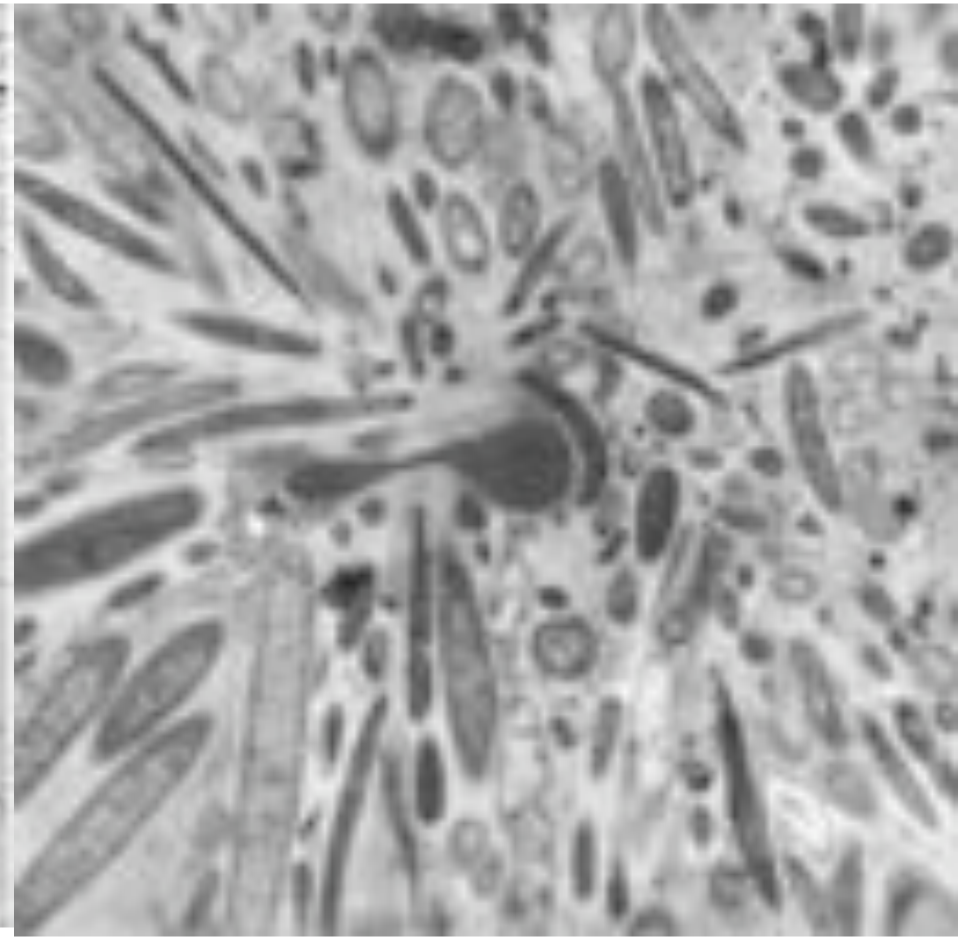
Note the dark abdomens of the workers and soldiers, which are mainly taken-up by the fermentation chamber, thus it appears dark.

The dark area of the midgut is often called the fermentation chamber. This is where the symbionts aid in digestion of cellulose and other molecules that are essential to the life of the host.

Kostas Bourtzis and Thomas Miller (editor). 2003. Insect Symbiosis. CRC Press, Boca Raton, FL

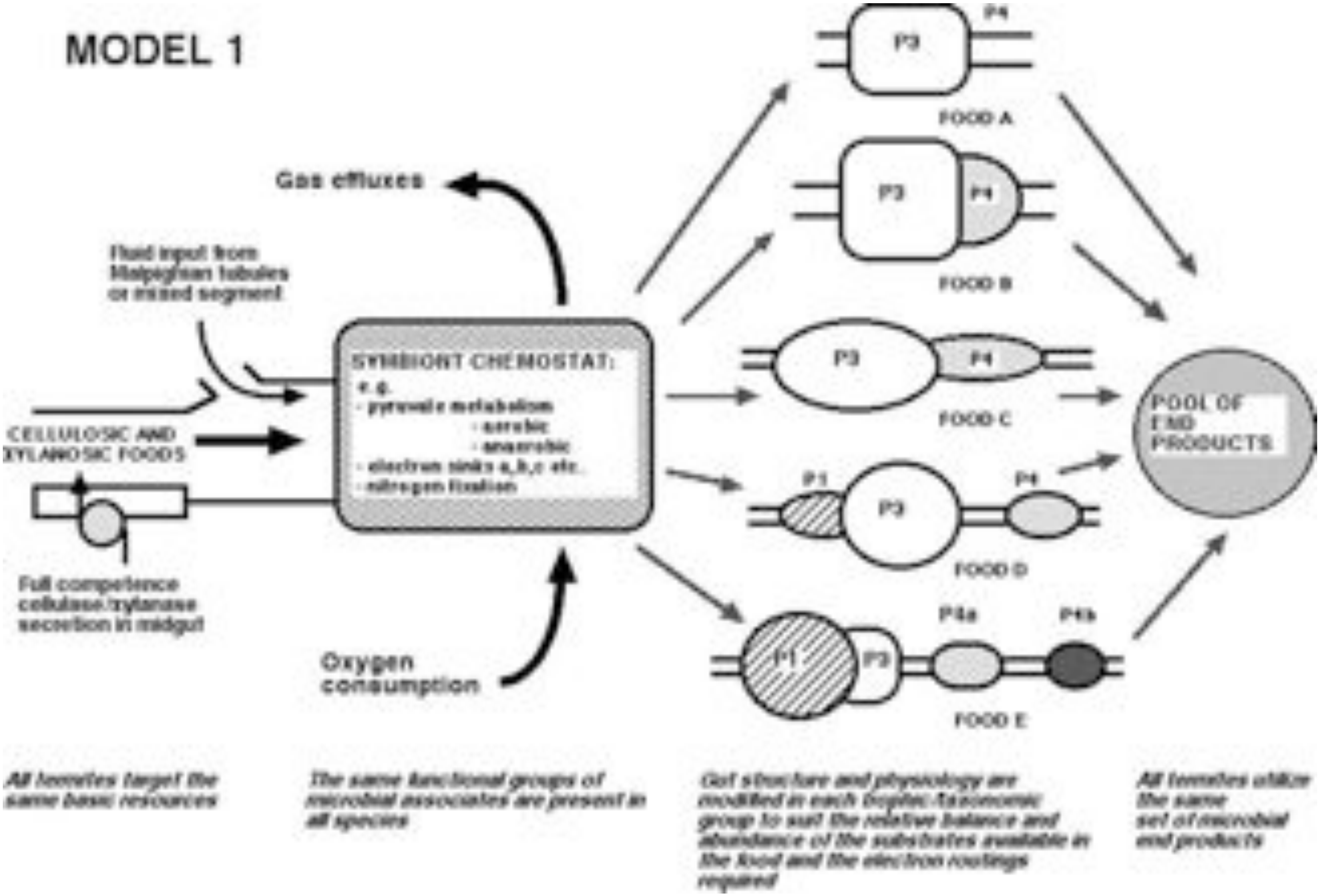


Spirochaetes from the intestine of *Reticulitermes flavipes*, magnified 2000 times. Large cells in the upper part of the micrograph (with attached bacteria) are *the protists. Photo, J. Breznak.*

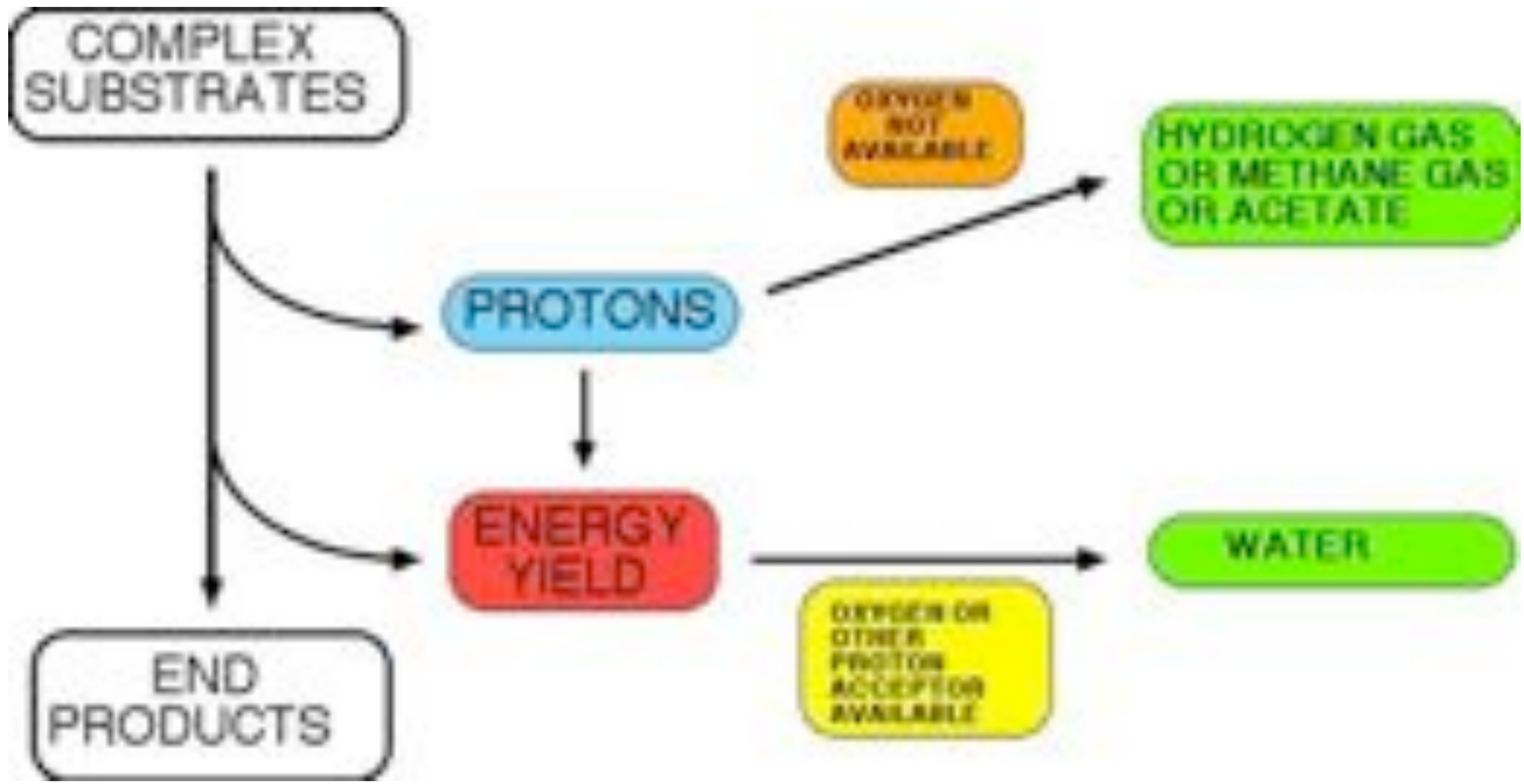


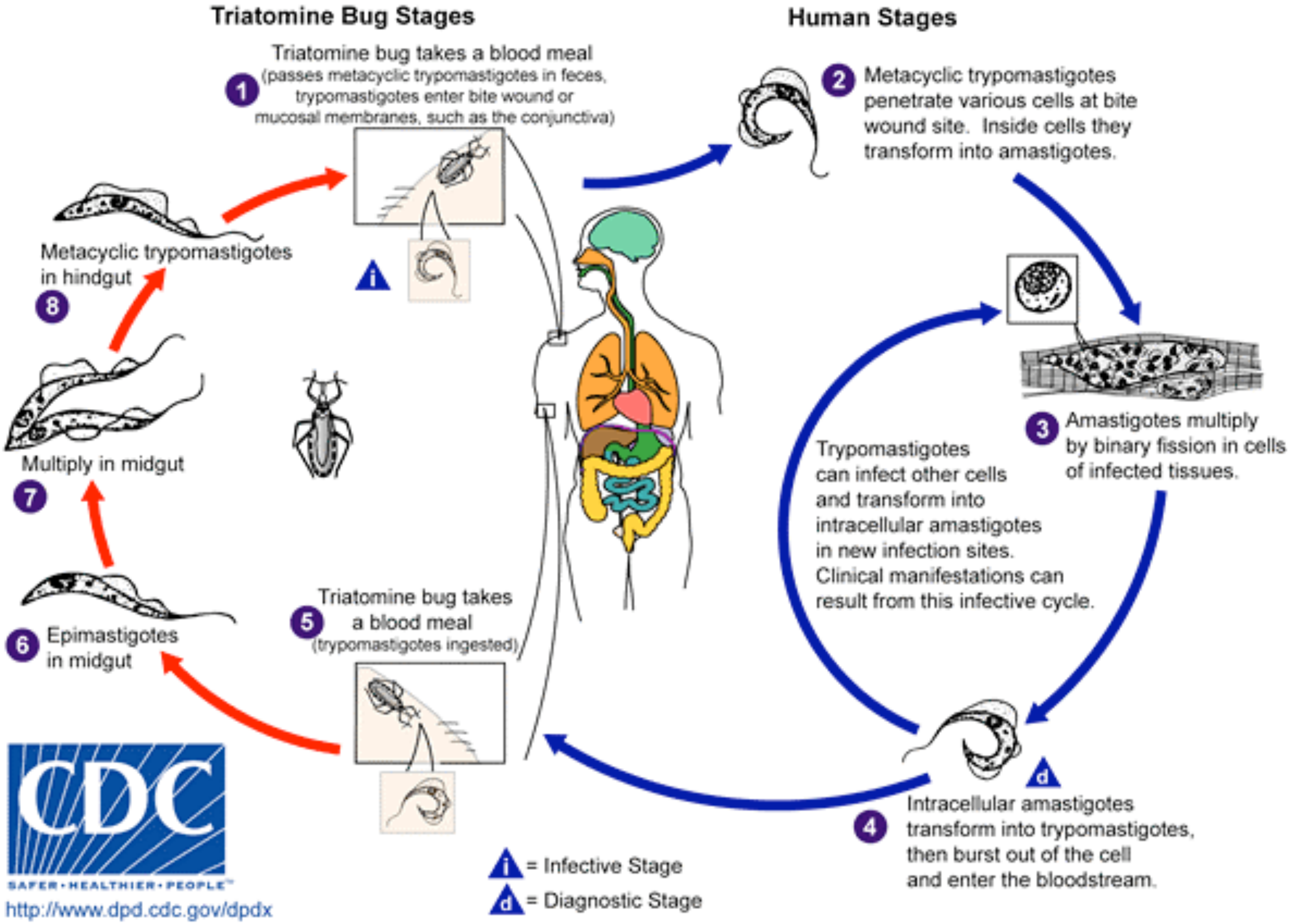
Transmission electron micrograph of the contents of the posterior hindgut of *Procubitermes aburiensis* , clustered around a protruding cuticular spine (central dumbell). More than a dozen prokaryotic morphotypes can be distinguished in this section. From Journal of Zoology (London) 201, 445-480 (1983).

MODEL 1


Gas effluxes
Fluid input from
Malpighian tubules
or mixed segment
CELLULOSIC AND
XYLANOSIC FOODS
Full competence
cellulase/xylanase
secretion in midgut
SYMBIONT CHEMOSTAT:
e.g.
- pyruvate metabolism
- aerobic
- anaerobic
- electron sinks a,b,c etc.
- nitrogen fixation
Oxygen
consumption
P3
P4
FOOD A
FOOD B
FOOD C
P1
FOOD D
P4a
P4b
FOOD E
POOL OF
END
PRODUCTS
All termites target the
same basic resources
The same functional groups of
microbial associates are present in
all species
Gut structure and physiology are
modified in each trophic/taxonomic
group to suit the relative balance and
abundance of the substrates available in
the food and the electron routings
required
All termites utilize
the same
set of microbial
end products

An environmental concern by some is that from all of these insects that house symbionts, especially termites, hydrogen gas or methane gas is being given off in large quantities when you consider termites world-wide, thus air pollution.




Triatomine Bug Stages
Human Stages
1 Triatomine bug takes a blood meal (passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
2 Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
3 Amastigotes multiply by binary fission in cells of infected tissues.
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
4 Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
5 Triatomine bug takes a blood meal (trypomastigotes ingested)
6 Epimastigotes in midgut
7 Multiply in midgut
8 Metacyclic trypomastigotes in hindgut
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx

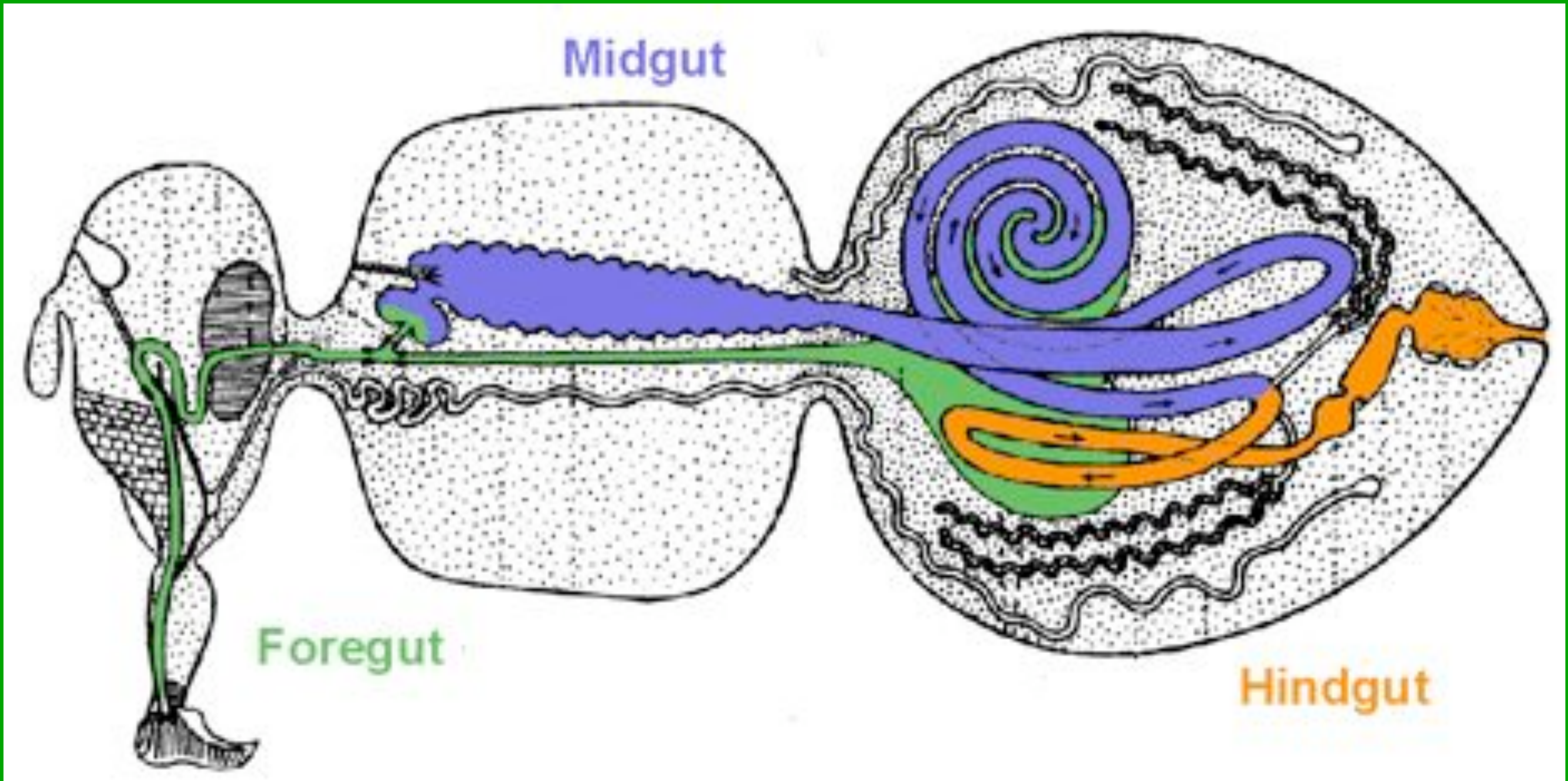
http://www.who.int/tdr/media/multimedia/lifecycle.htm

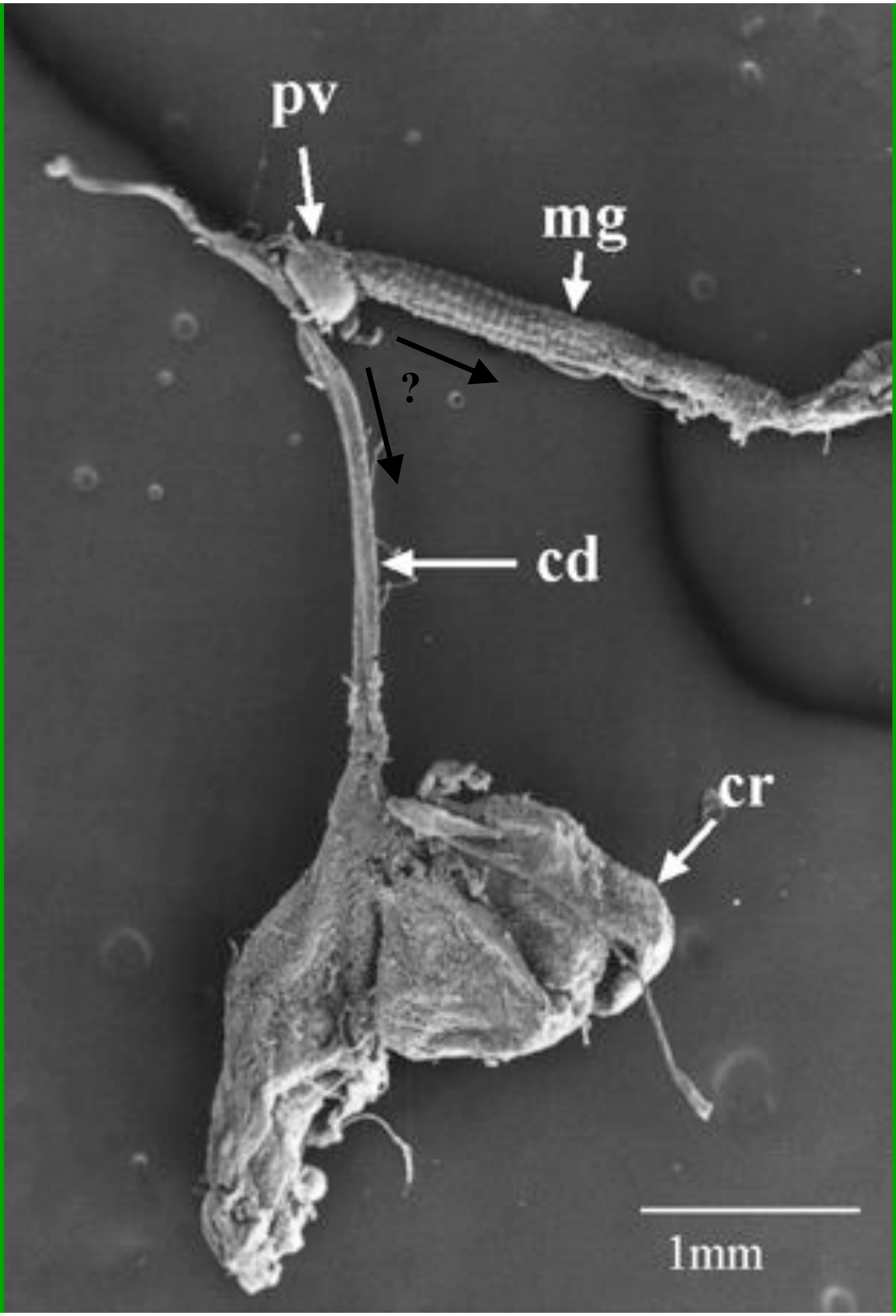
tsetse and African Sleeping Sickness

Neurohormones in the digestive tract of Adult Diptera

The occurrence and putative functions of gut associated neuropeptides and biogenic amines in adult flies

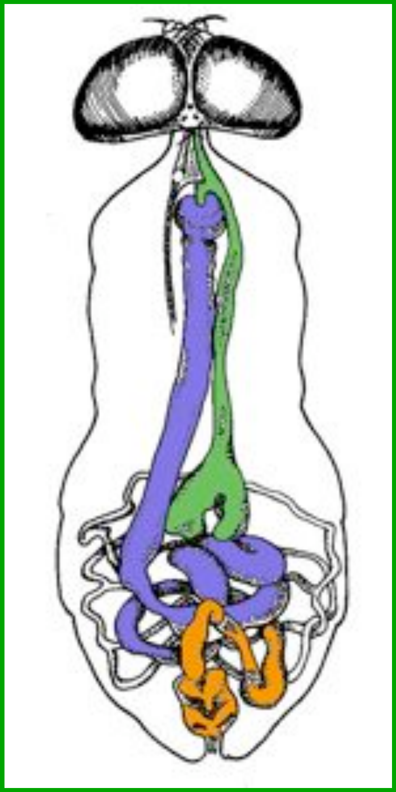
Dipteran alimentary tract

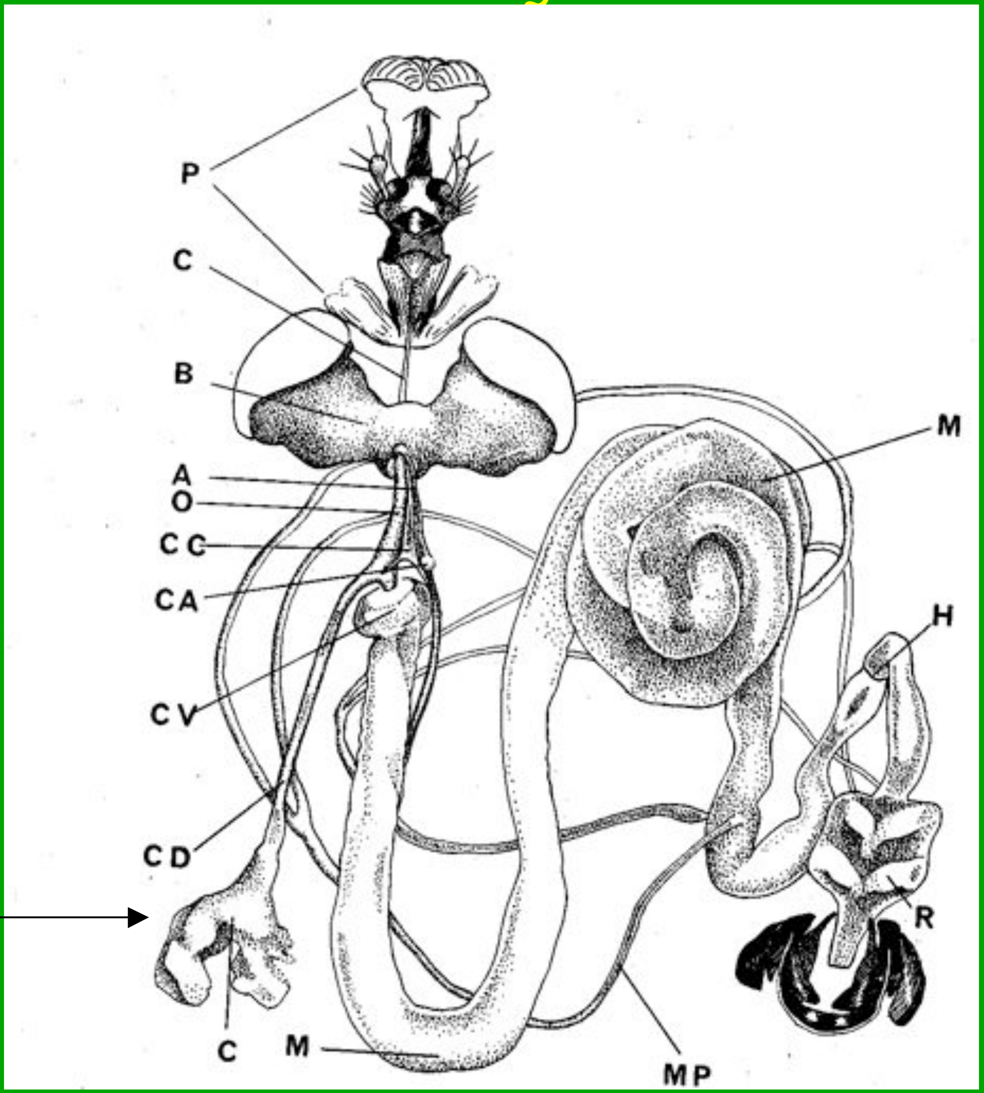




- bilobed blind sac in abdomen
- storage reservoir for patchily distributed resources

Dipteran alimentary tract



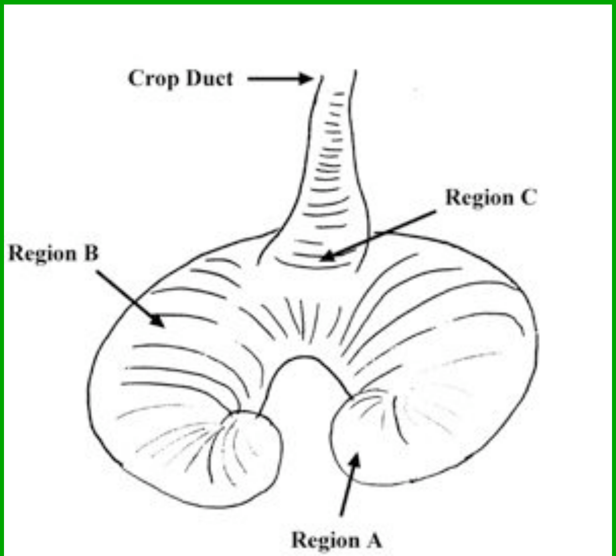


Crop:
Bilobed, blind sac in abdomen

The Dipteran crop

- as crop fills, it is constantly contracting (myogenic)

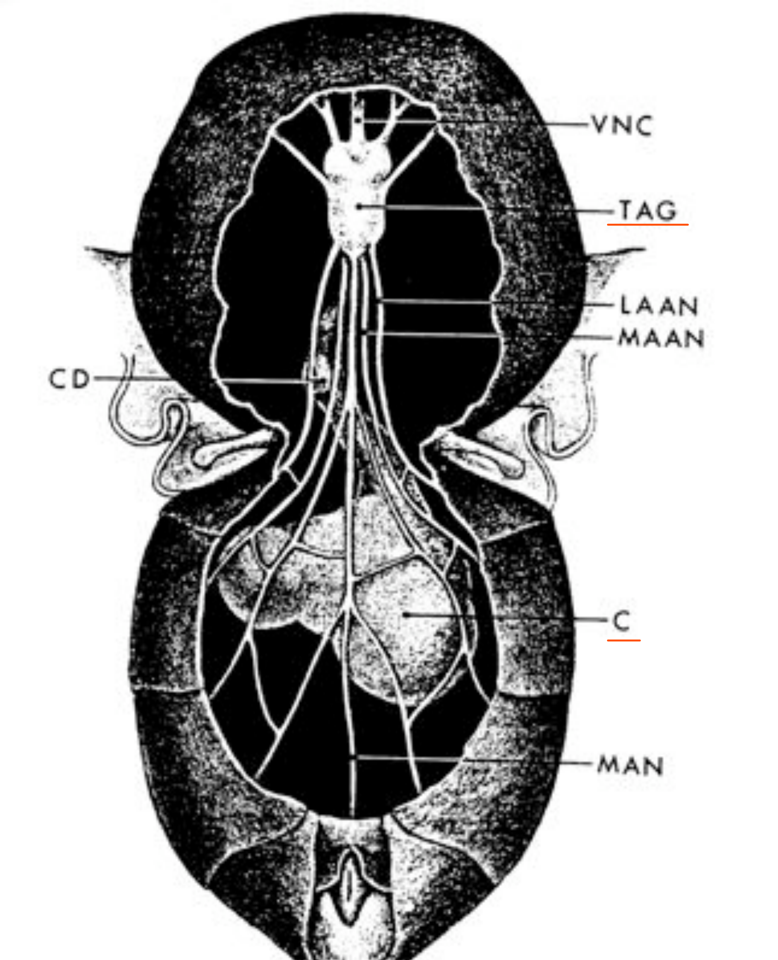
- contraction frequency increases with volume ingested



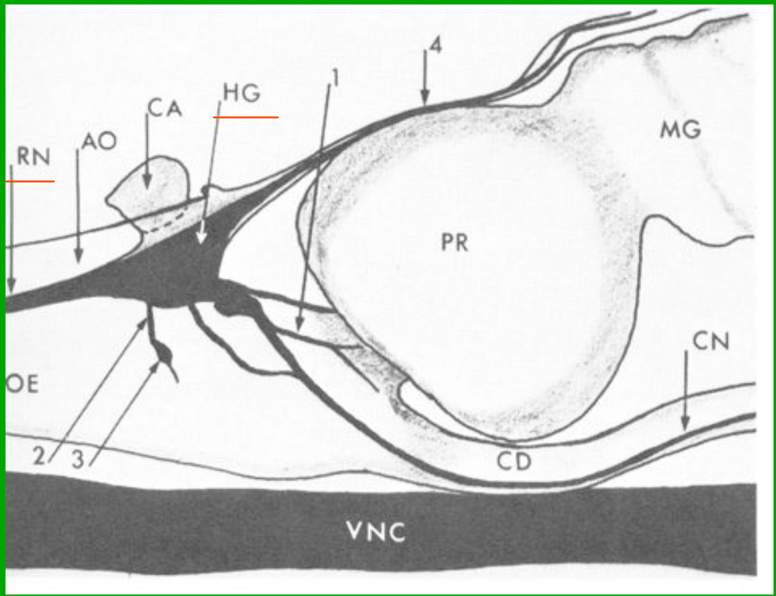
- filled crop is detected by stretch receptors in the abdominal nerve net

- stretch receptors send inhibitory feedback to brain and feeding ceases – food is later forced into midgut

Abdominal nerve net



(Dethier and Gelperin 1967)



Bubbling

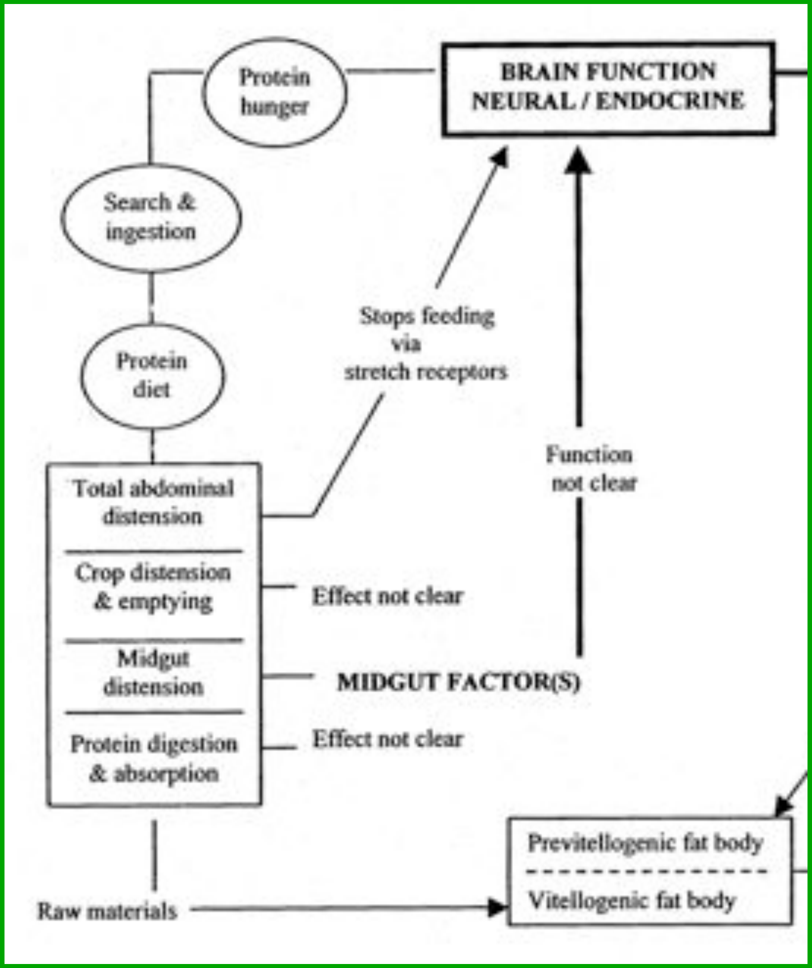
- crop contents are regurgitated and ingested repeatedly
- concentrates crop contents via evaporation
- bacterial and enzyme mixing

In the midgut

- digestive enzyme secretion
- muscle contraction
- diuresis
- brain must be notified of nutritional state
- signal to start reproductive cascade is given (in anautogenous female flies)

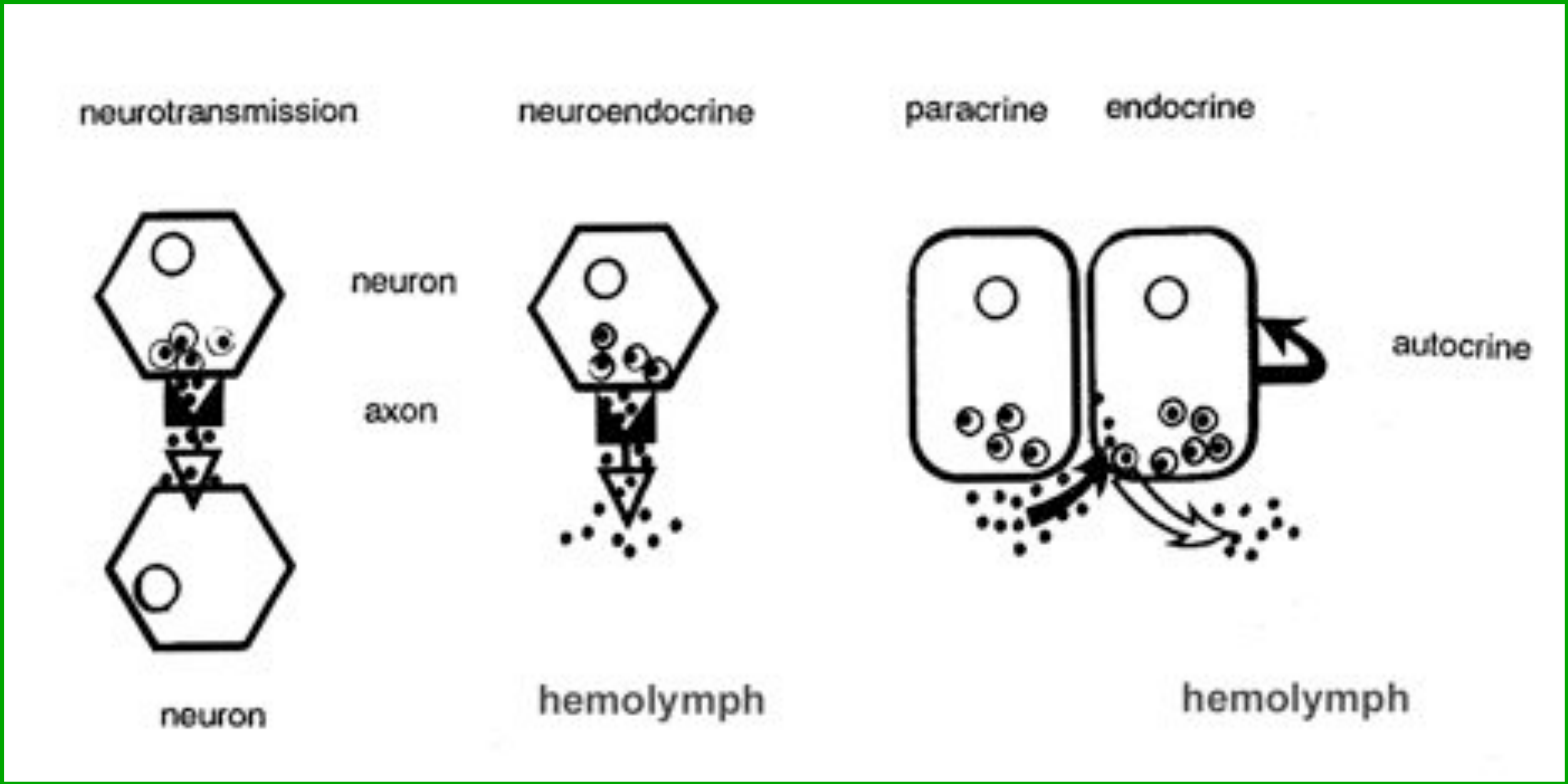
How is this all controlled?

- direct innervation (meal control)
- through endocrine and/or neuroendocrine systems
  - peptide hormone (neuropeptides)
  - biogenic amine (octopamine, serotonin)



Messenger molecules

Transmitters, hormones, and/or modulators

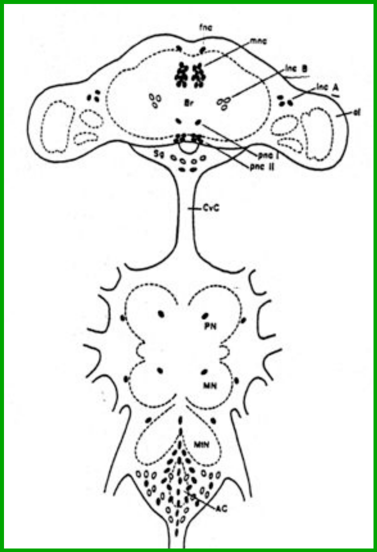


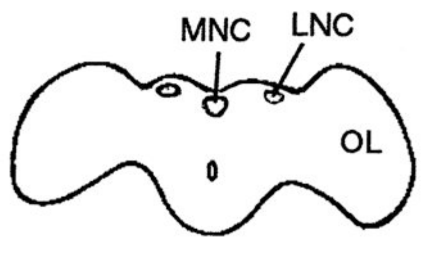
Neuropeptides

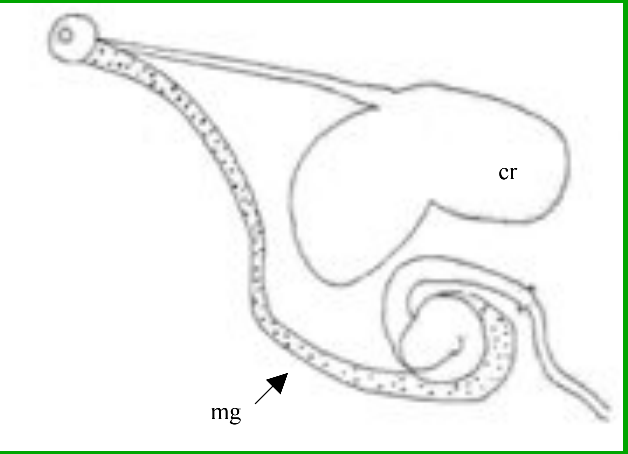
- peptides produced in brain, gut, or other tissues that affect neurons and non-nervous tissues
- act as neurohormones, neuromodulators, and neurotransmitters
- neuropeptides integrate brain function and systems of the body

Sources of messenger molecules

- CNS neurosecretory cells
- CA/CC complex
- various endocrine cells
- the gut



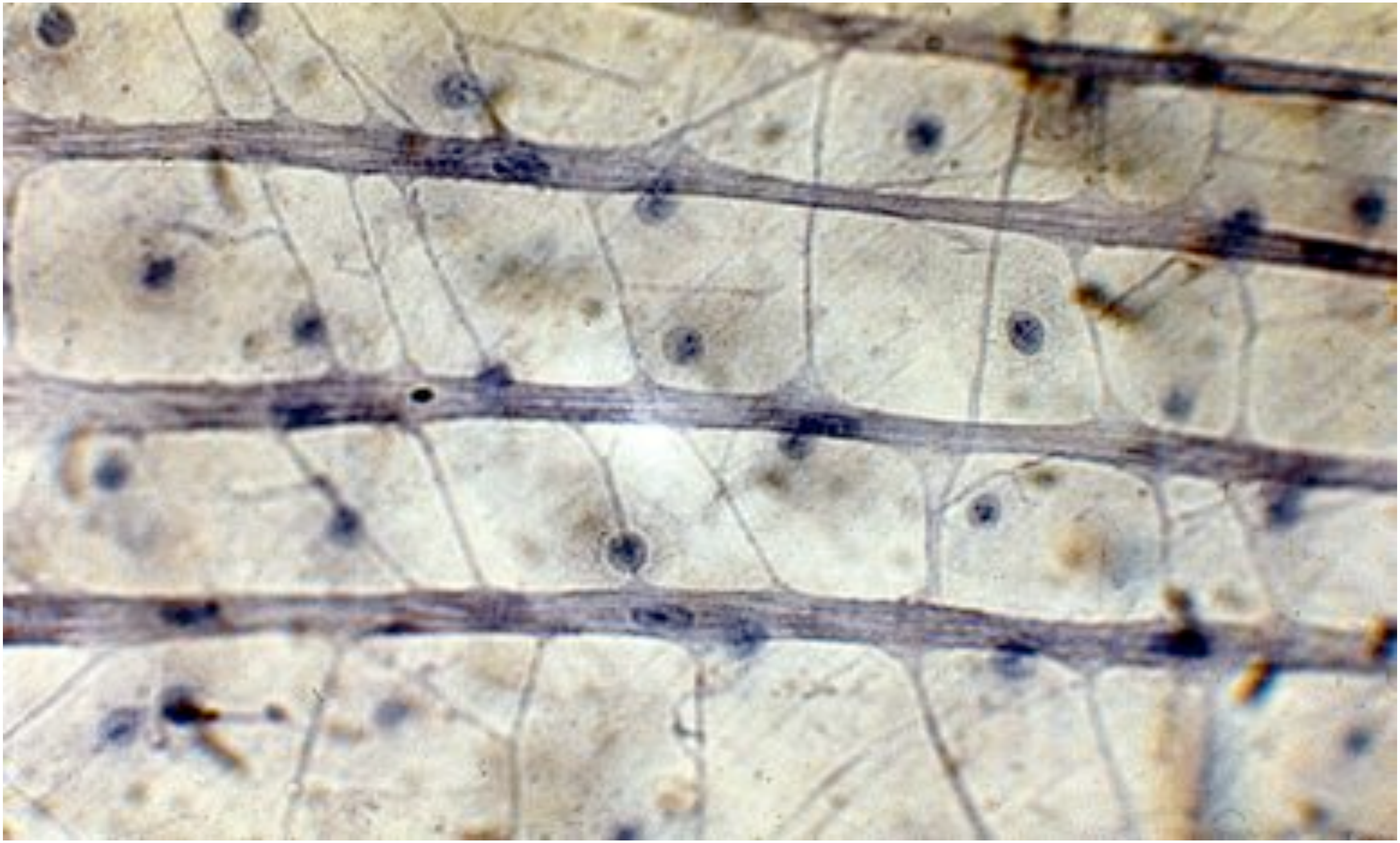




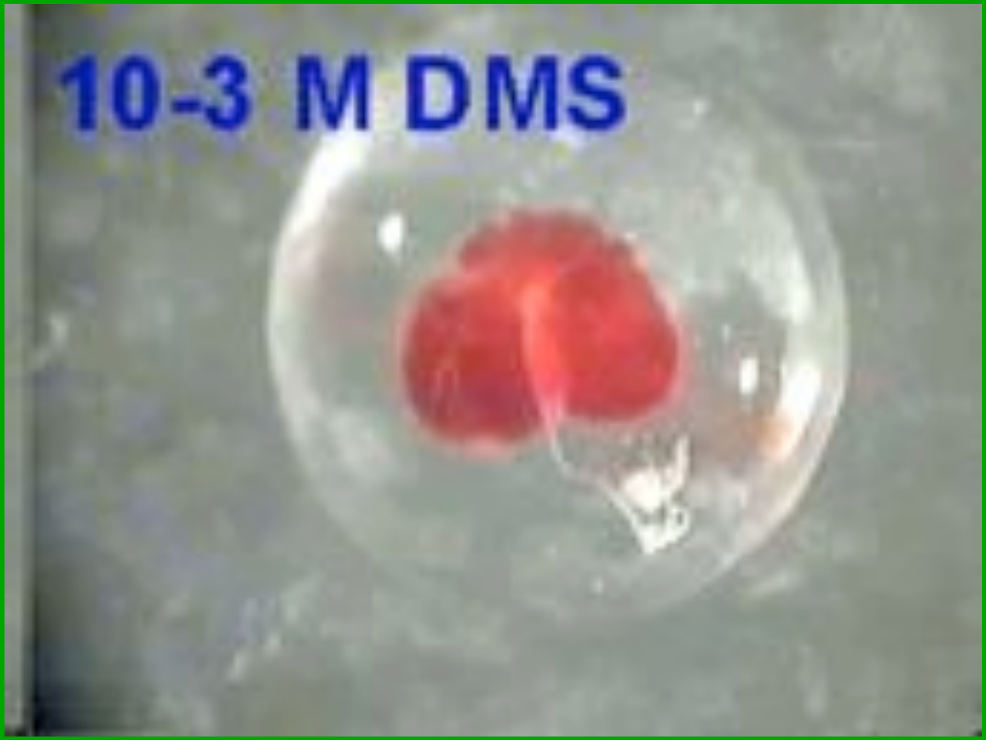
Dromyosuppressin (DMS) and crop contractions

- myosuppressins isolated from several insects, including *Drosophila*
- myotropic myoinhibitors
- dromyosuppressin (DMS)
- CNS, alimentary tract with DMS IR cells and processes
- DMS applied to *in vitro* crop preparation

MUSCLES OF THE CROP OF ADULT *PHORMIA REGINA*



DMS and crop contractions



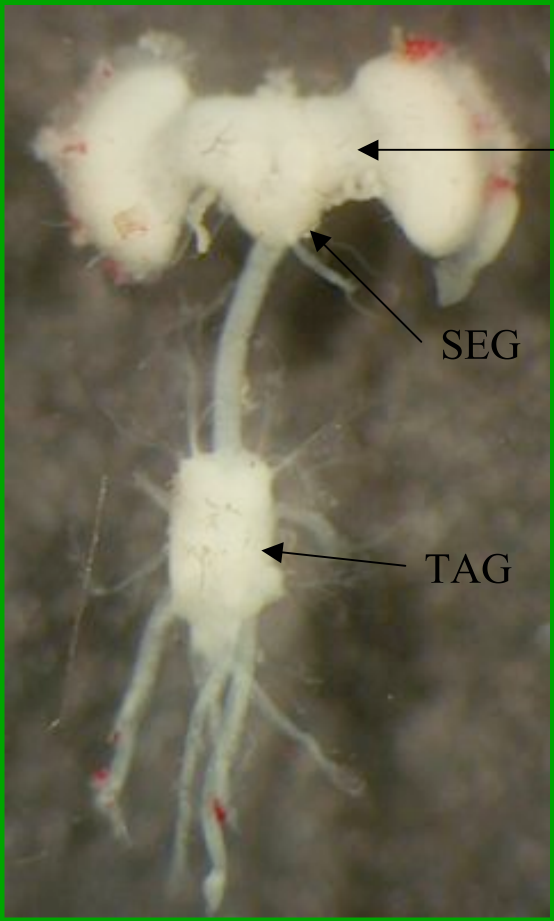
Application of 10-6 M DMS reduced crop contractions by 95% (from 46 to 2 contr./min)

Messengers investigated

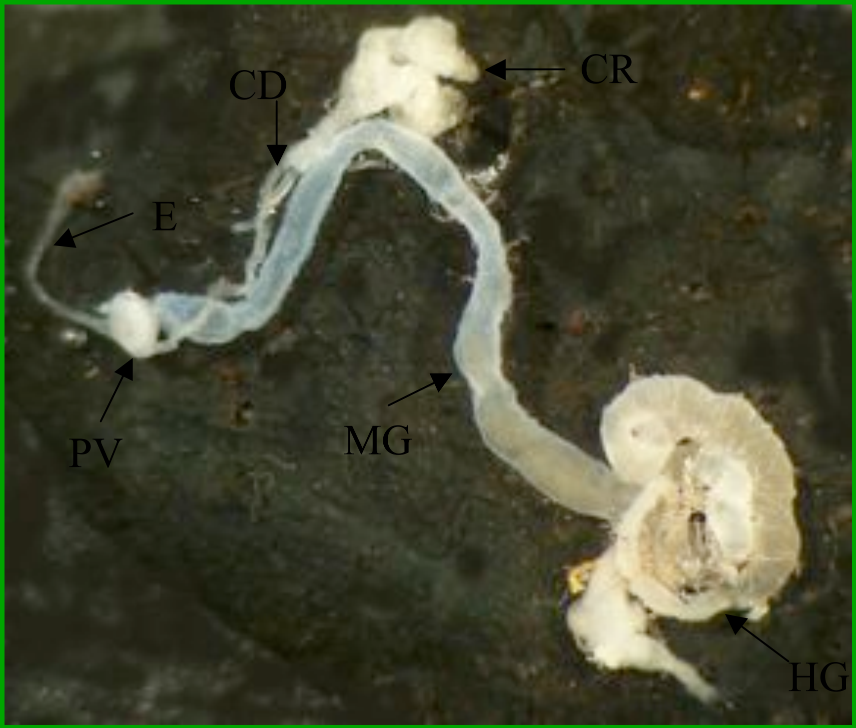
- Serotonin (5-hydroxytryptamine or 5-HT)

- FMRFamide related peptides
  - FMRFamide
  - Perisulfakinin (PSK)

Phormia CNS and alimentary tract



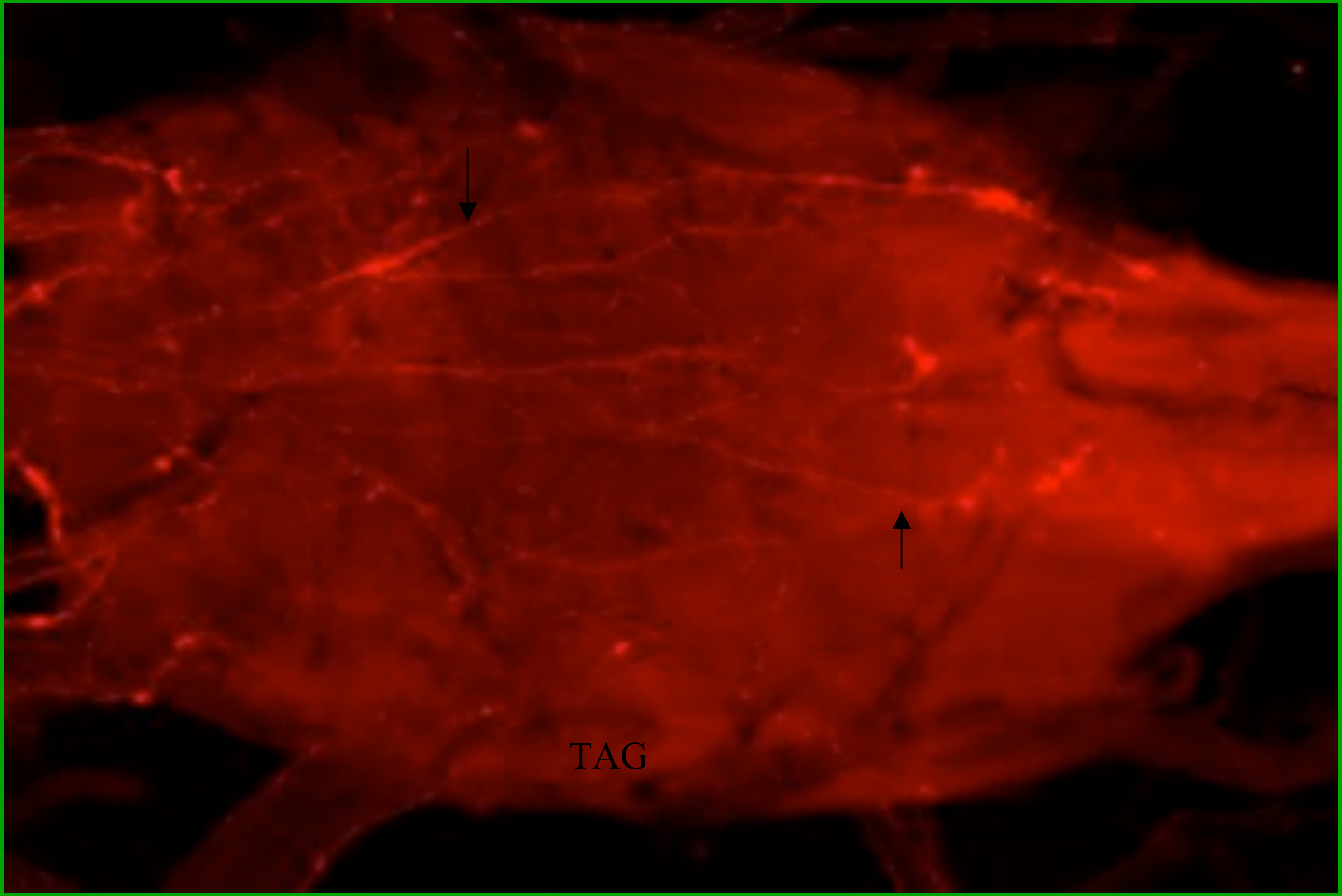
CNS



Alimentary tract

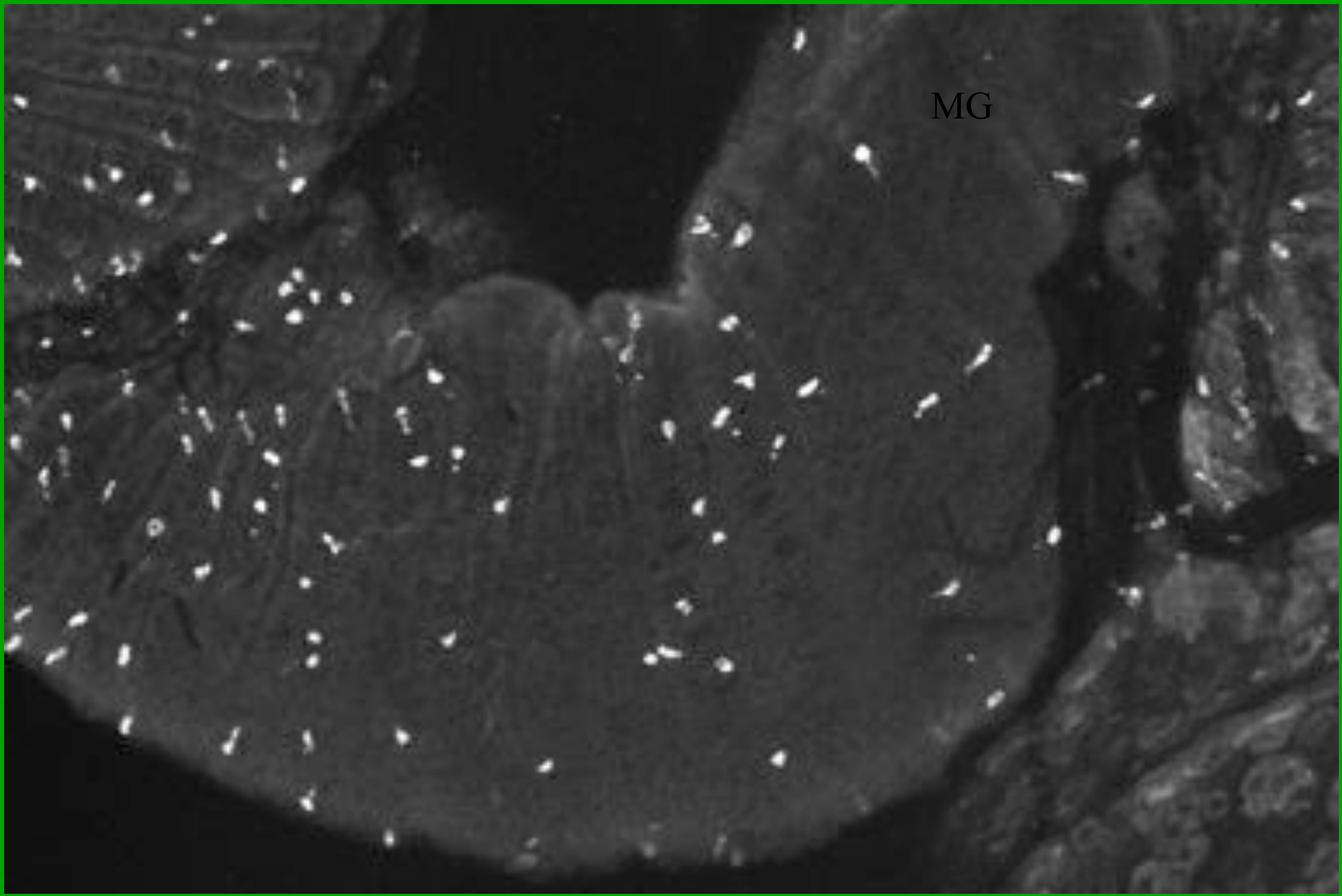
Phormia – Serotonin

possibly a neurohemal organ like in Calliphora



5-HT immunoreactivity in the TAG of the blow fly

Midgut endocrine cells
Phormia FMRFamide-like IR



FMRFamide-like immunoreactivity in the midgut of the blow fly

Effect of PSK on crop contractions

FMRFamides are myotropic - both stimulatory and inhibitory

Phormia neuropeptide summary

- FMRFamide IR cells and processes were immunolocalized throughout CNS and midgut
- many characterized neurosecretory regions were IR to FMRFamide antisera
- region of immunoreactive cells in midgut region anterior to posterior coil suggest specialized function, i.e. digestion
- 10^{-6} M PSK increases "intensity" of crop contractions *in vitro* - a native PSK-like peptide may do similar *in vivo*

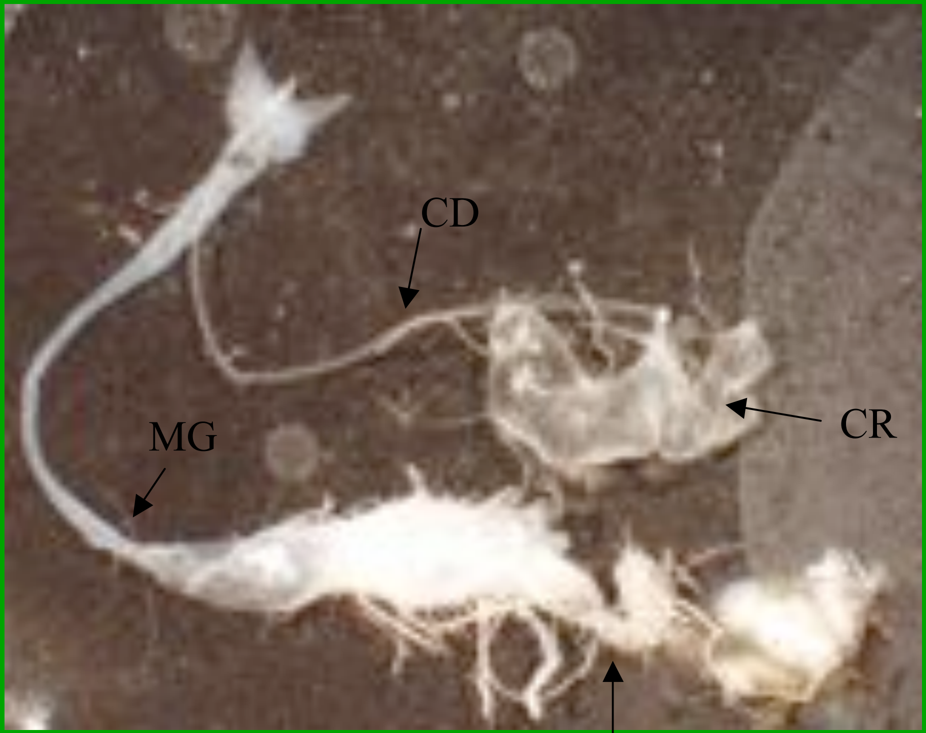
Tabanus nigrovittatus (Macquart)

- eastern coast of North America
- hematophagous
- nuisance pest
- vector of animal disease

Salt marsh Newbury, MA

Tabanus CNS and alimentary tract

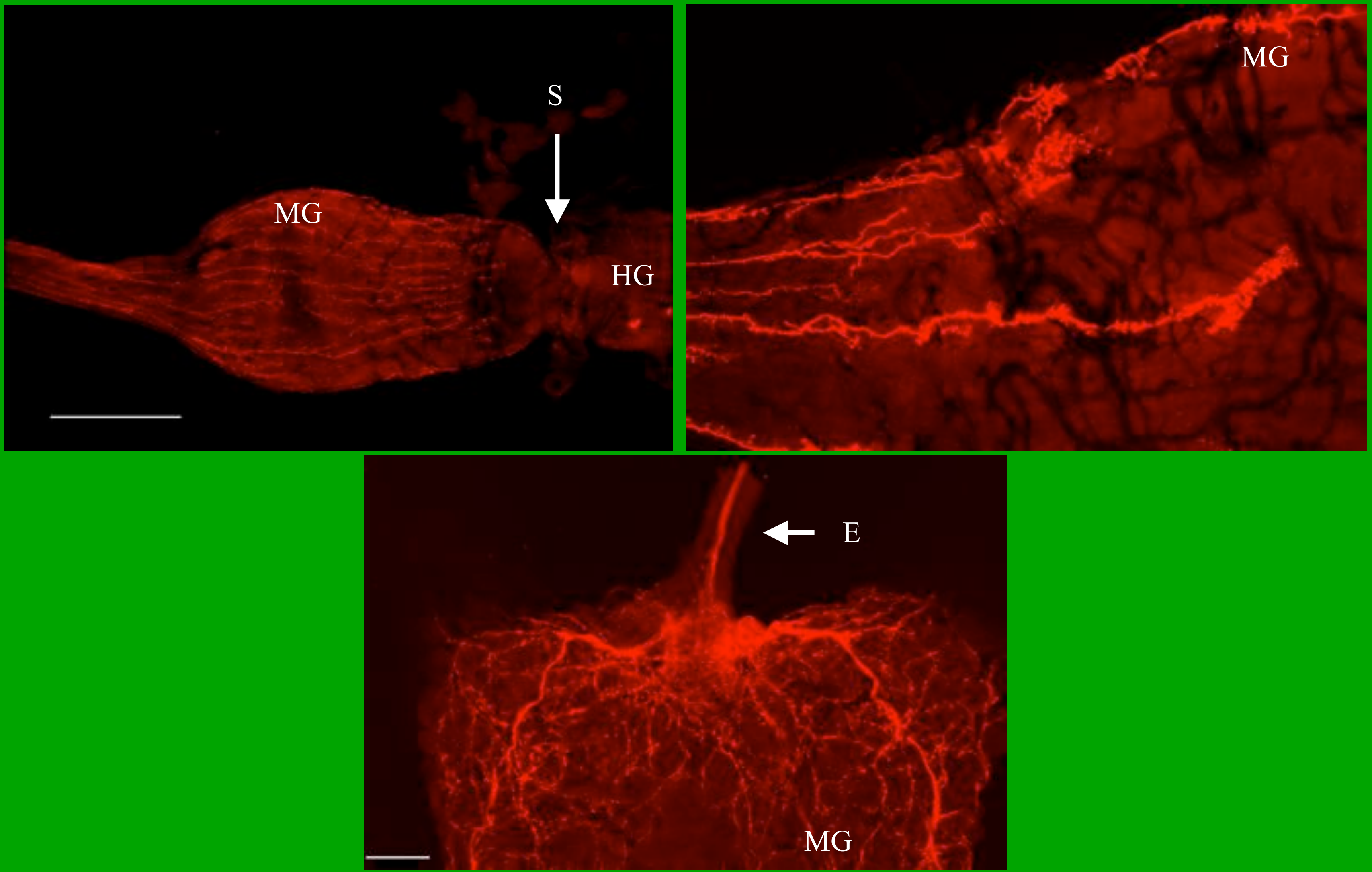
CNS



Alimentary tract

Tabanus - Serotonin IR

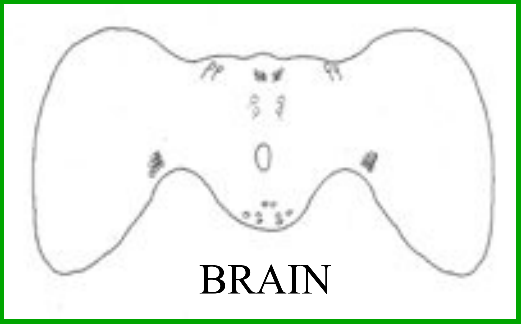
possible involvement in diuresis of bloodmeal

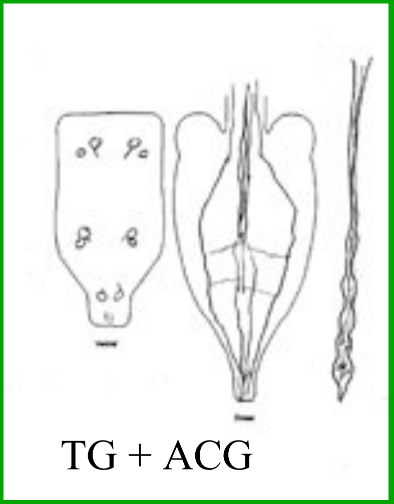


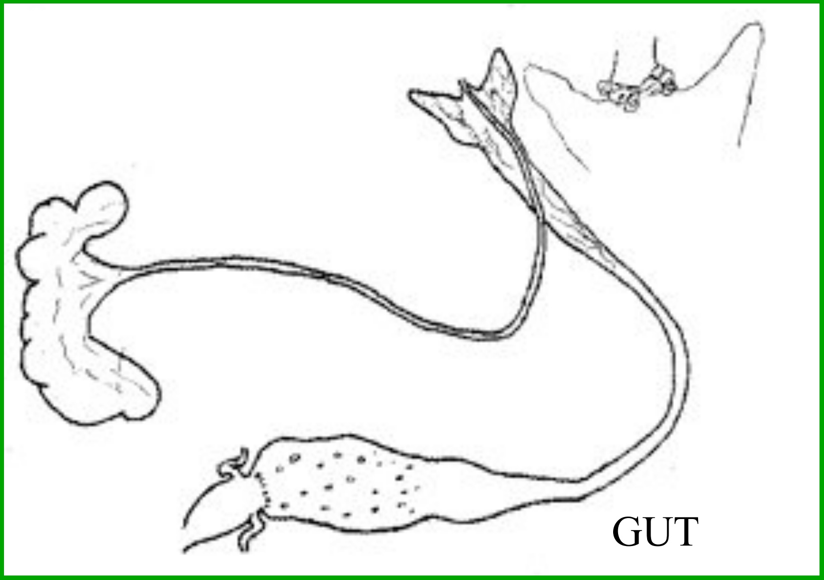
5-HT immunoreactivity in the midgut of the horse fly

Tabanus FMRFamide-like IR

- brain
- thoracic ganglion
- abdominal chain ganglion
- midgut
- crop

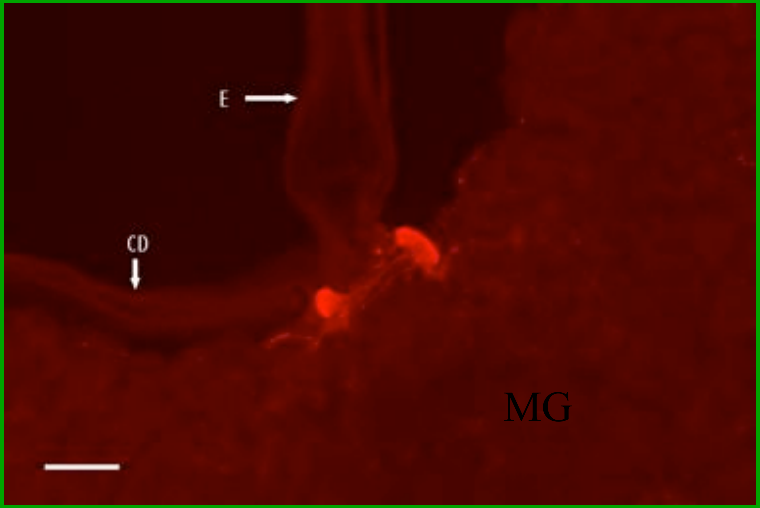




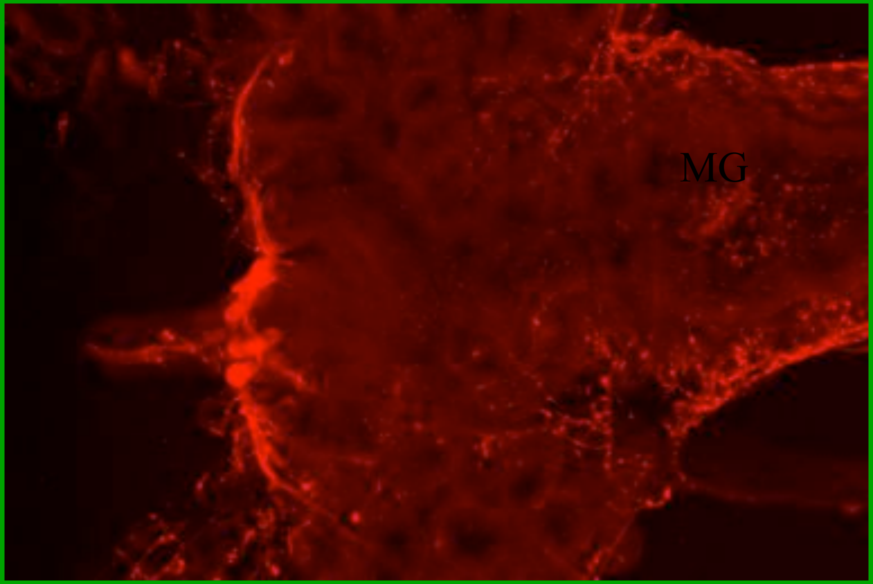


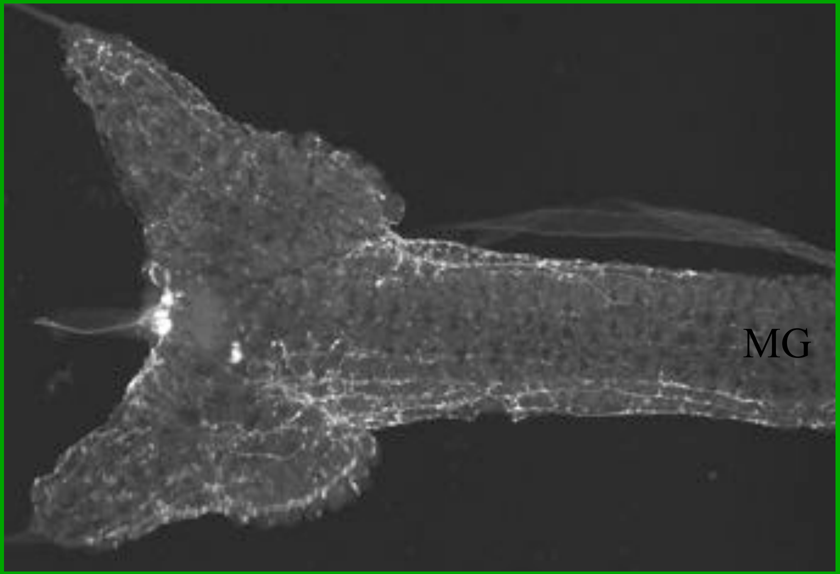
Tabanus FMRFamide-like IR

POSSIBLE INVOLVEMENT IN SPHINCTER CONTROL



MG

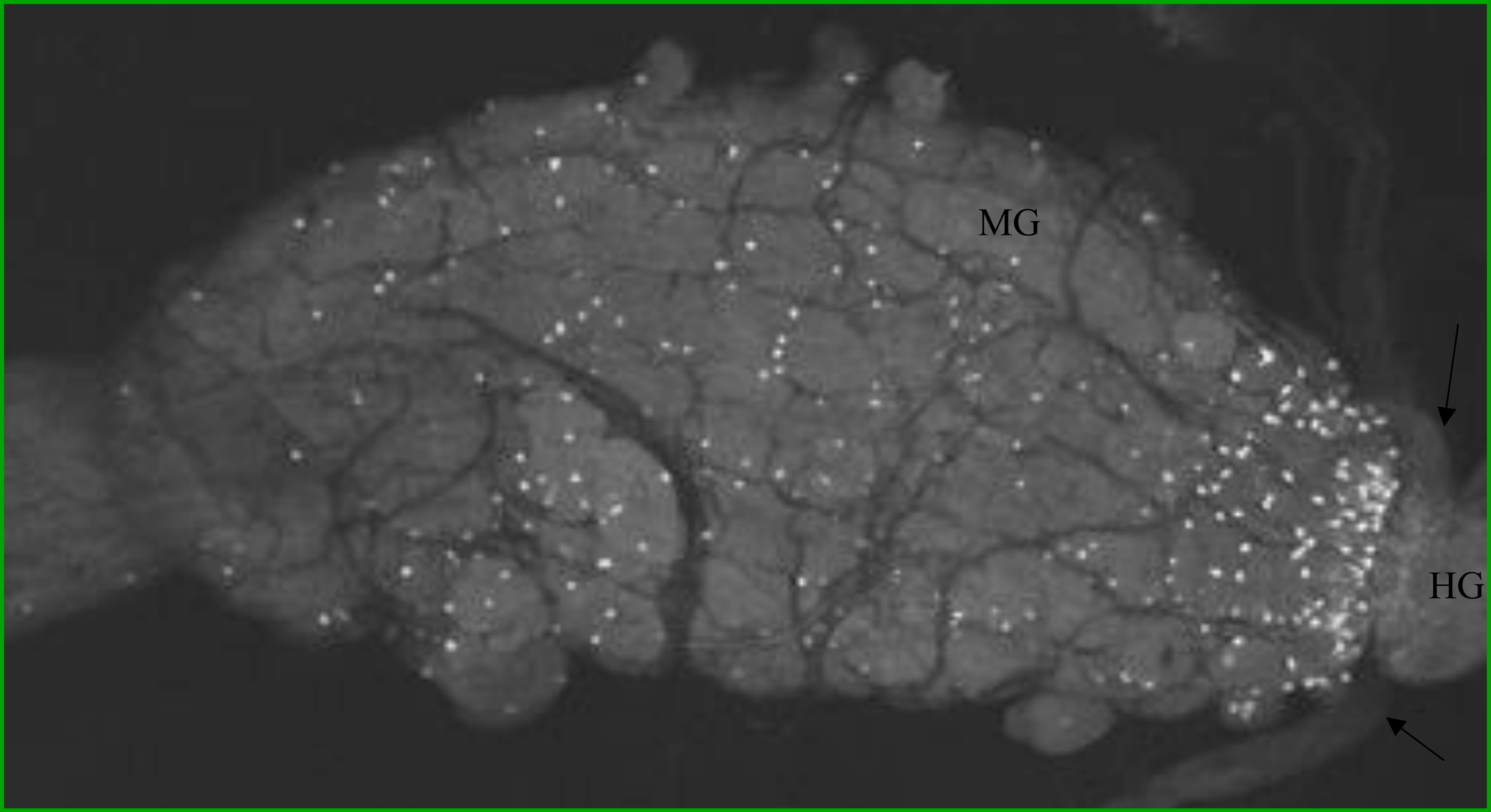




FMRFamide-like immunoreactivity in the midgut of the horse fly

Tabanus FMRFamide-like IR

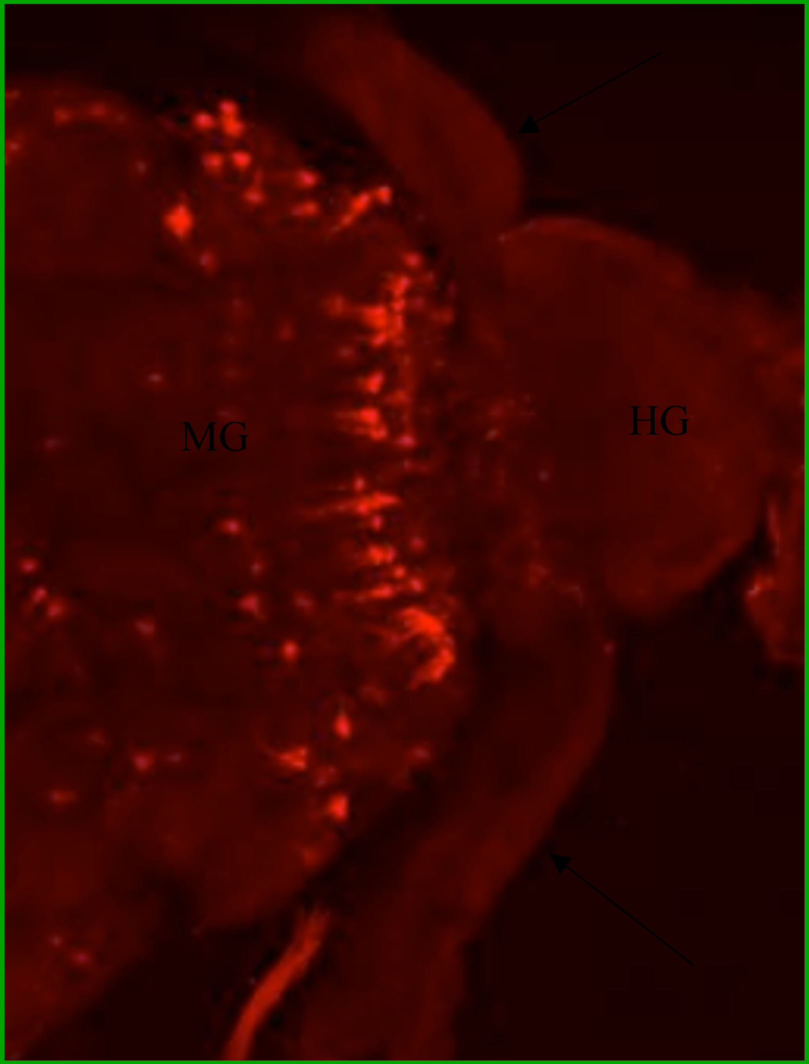
FUNCTION(S) UNKNOWN



FMRFamide-like immunoreactivity in the midgut of the horse fly

Tabanus FMRFamide-like IR

FUNCTION(S) UNKNOWN



FMRFamide-like immunoreactivity in the midgut of the horse fly

Tabanus neuropeptide immunohistochemistry summary

- FMRFamide IR cells and processes were immunolocalized throughout CNS and midgut
- many characterized neurosecretory regions with IR to FMRFamide antisera
- midgut immunoreactivity suggests functions related to digestion, diuresis and muscle control

BASIC ASPECTS OF DIGESTIVE SYSTEM

- Basic-How pathogens and parasites

1. recognize
2. adhere to and
3. gain entrance

through the various membranes of the midgut.

APPLIED ASPECTS OF DIGESTIVE SYSTEM

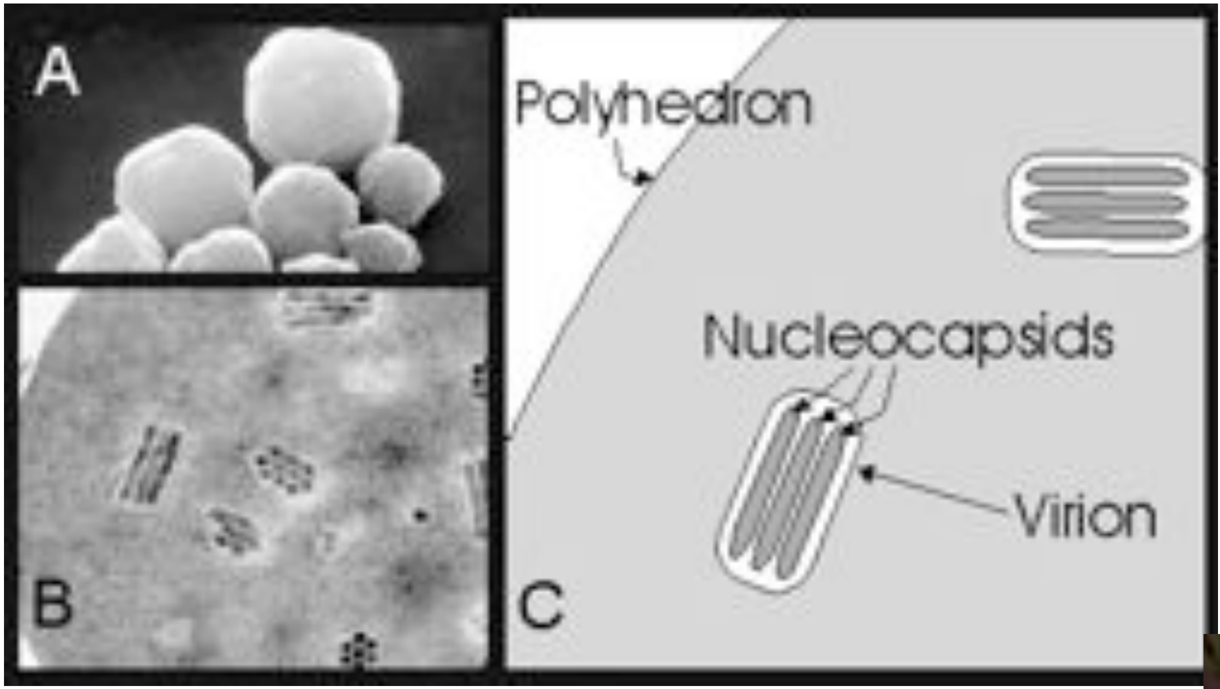
a. Development of transgenic plants (Bt endotoxin and trypsin inhibitor genes PLUS roundup resistant)

b. Development of vertebrate vaccines that disrupt the normal feeding process of hematophagous arthropods. Focus on midgut area and salivary glands. Antibodies to maxidilan for Leishmaniasis

c. Knowing more about specific membrane receptors used by pathogens that could lead to resistant strains of the insect against the pathogen

d. Use of anticoagulant, vasodilators and antiplatelet agents from the saliva of hematophagous arthropods to prevent blood clotting in humans.

Nuclear polyhedrosis virus (NPV), like a baculovirus, alter the peritrophic membrane via a virus enhancement factor (VEF) that enables the virus to gain access to the underlying epithelial cells.

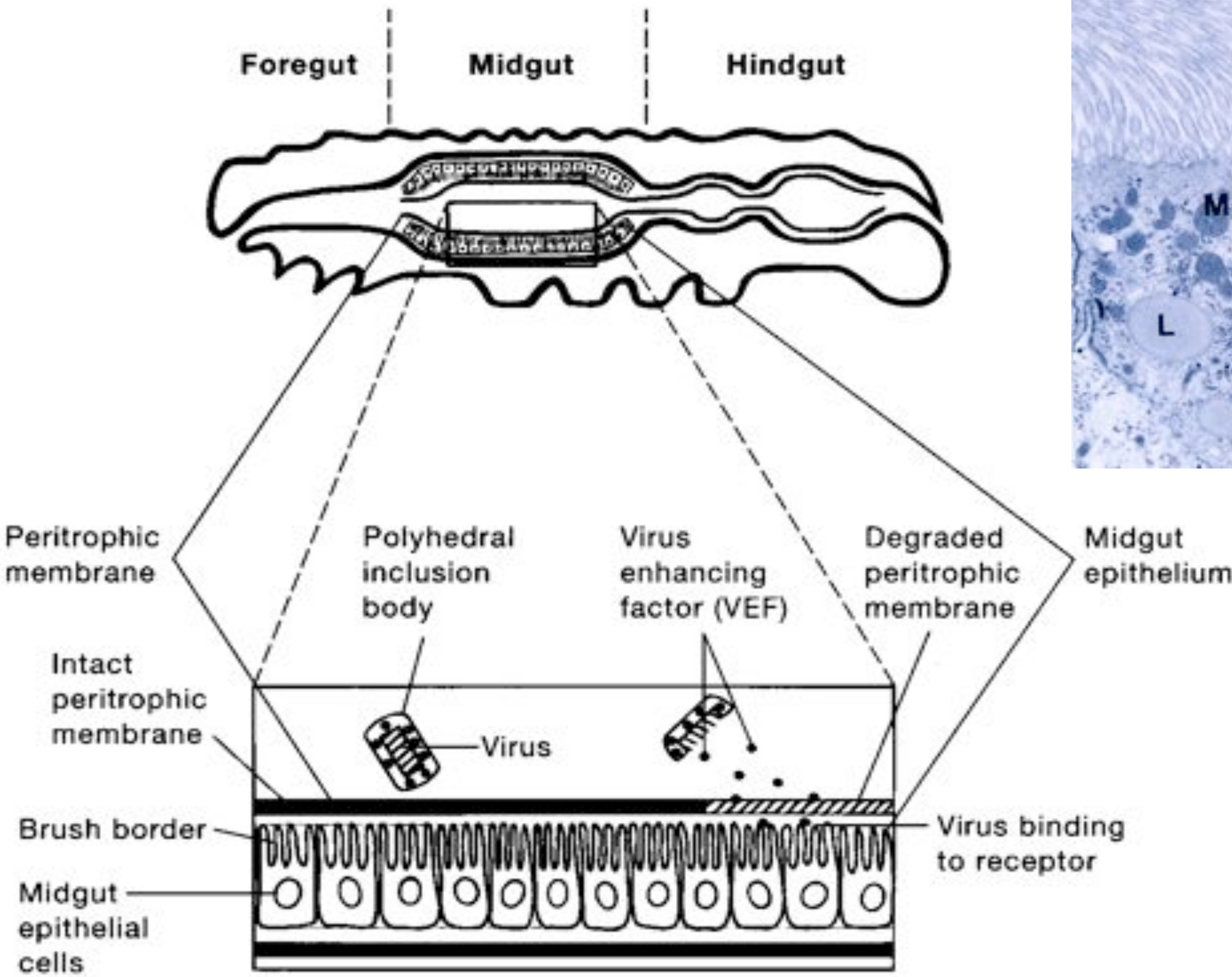


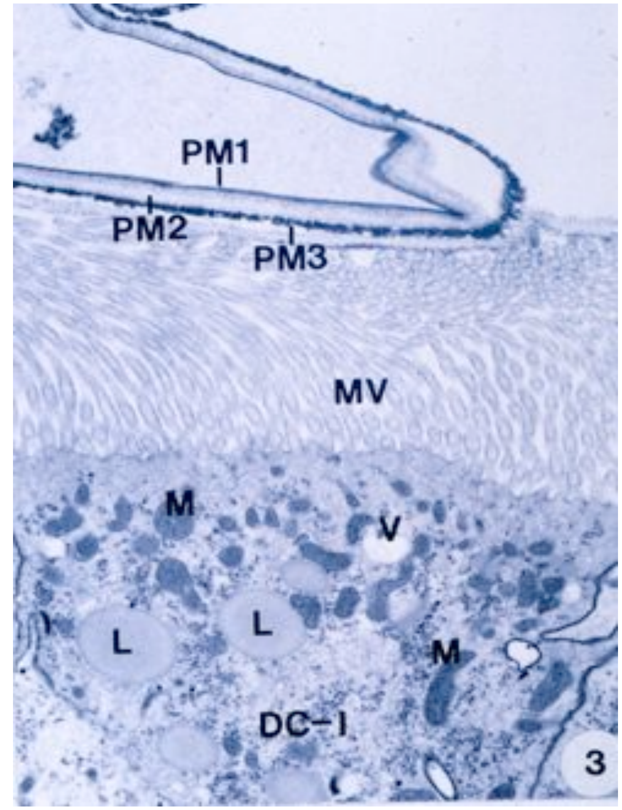
VEF is probably an Enhacin or metalo-protease

Beet armyworm killed By NPV

Characterization of *Mamestra configurata* nucleopolyhedrovirus enhancin and its functional analysis via expression in an *Autographa californica* nucleopolyhedrovirus recombinant

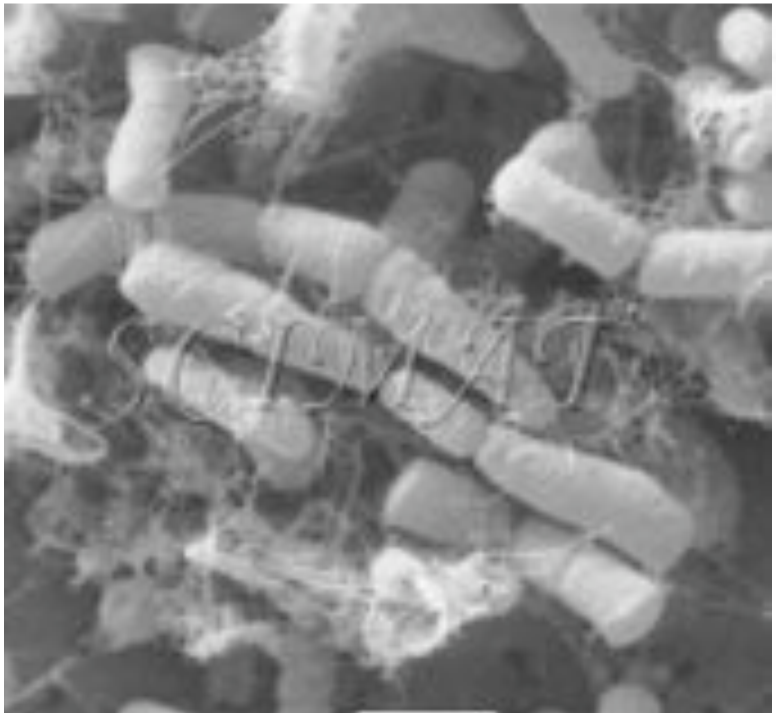
Nucleopolyhedrosis virus and the insect midgut

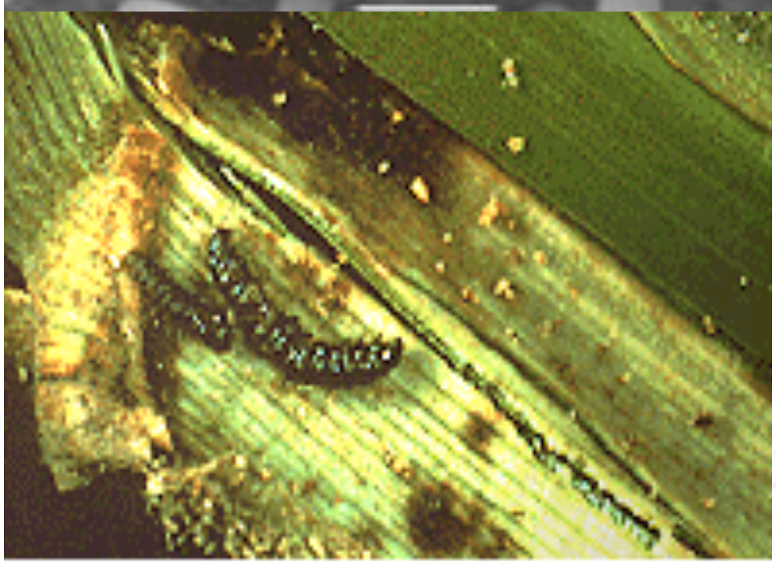




BT OR *BACILLUS THURINGIENSIS*

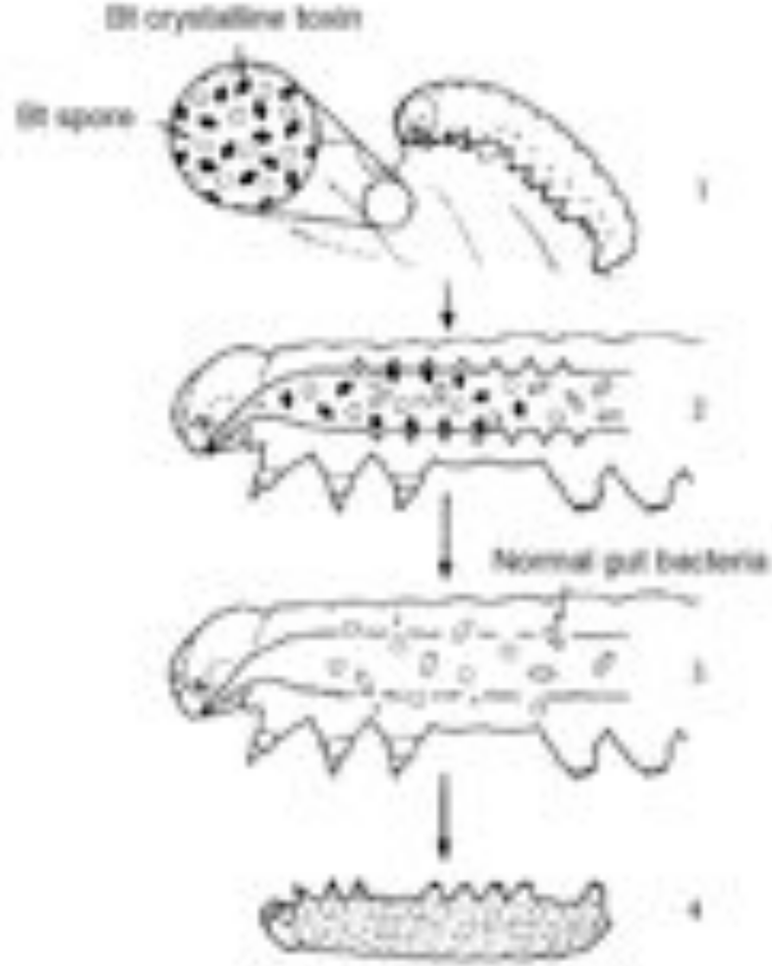
- ***Bacillus thuringiensis*** (known as 'Bt') is an insecticidal bacterium, marketed worldwide for control of many important plant pests - mainly caterpillars of the Lepidoptera (butterflies and moths) but also for control of mosquito larvae, and simuliid blackflies that vector river blindness in Africa. ***Bacillus thuringiensis*** is considered safe to humans and non-target species, such as wildlife. Some formulations can be used on essentially all food crops. Unlike most garden insecticides, *Bacillus thuringiensis* is a highly selective poison. It is effective only against the caterpillars listed and should be used only on the crops on which they feed. Spraying or dusting plants with spores of this bacterium appear to be environmentally safe ways to attack such pests as the gypsy moth, the tent caterpillar, and the tobacco hornworm (which also attacks tomatoes).
- http://www.magma.ca/~scimat/B_thurin.htm





European corn borer larvae infected with *Bacillus thuringiensis*.
Courtesy Nova Nordisk Entotech, Inc.

Action of *Bacillus thuringiensis* var. *kurstaki* on caterpillars



1) Caterpillar consumes foliage treated with Bt (spores and crystalline toxin).
2) Within minutes, the toxin binds to specific receptors in the gut wall, and the caterpillar stops feeding.
3) Within hours, the gut wall breaks down, allowing spores and normal gut bacteria to enter the body cavity; the toxin dissolves.
4) In 1-2 days, the caterpillar dies from septicemia as spores and gut bacteria proliferate in its blood.

MODE OF ACTION OF ALPHA-ENDOTOXIN

1. Bt bacterium enters the insect gut where pH is 6-10. Just right for the action of the bacterium.
2. The crystalline toxin aids in breaks through the peritrophic matrix and binds to a receptor on the brush forming a brush border membrane vesicle.
4. The alpha endotoxin inserts into the membrane of the brush border cells and makes holes. The bacteria now can enter the hemolymph and the cells of the gut are destroyed. The insect is 'sick' and stops eating. It stops feeding and serves as an incubation medium for the bacteria and production of new toxin crystals.

Now 5 biotypes

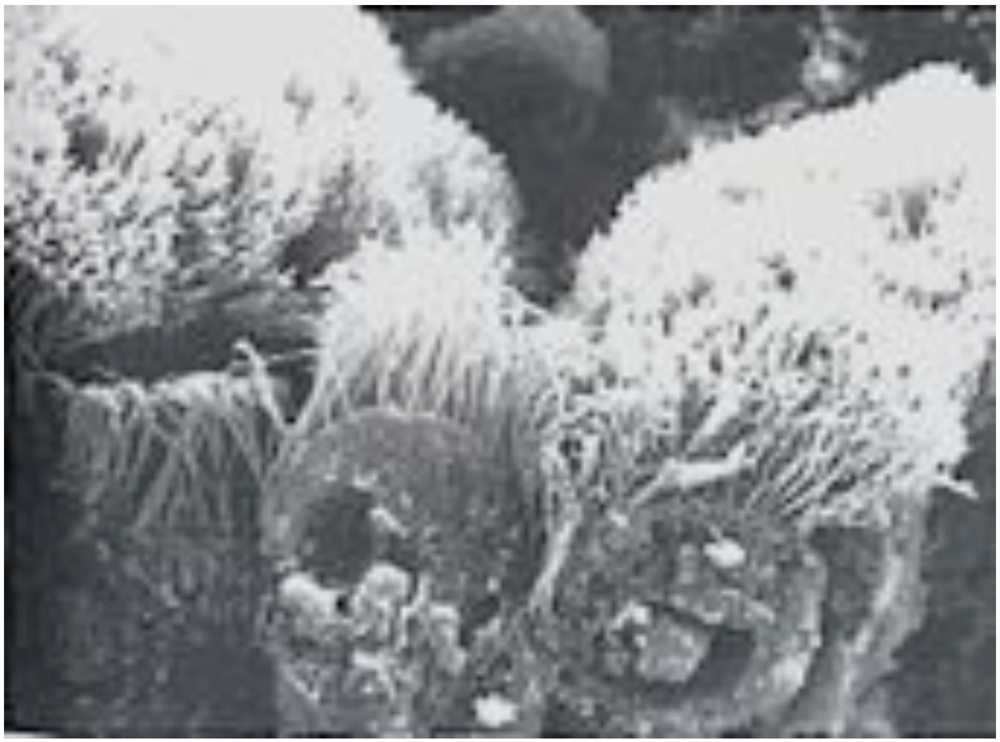
1. ***B.t. thuringiensis*-caterpillars**
2. ***B.t. israelensis*-nematocera dipterans**
3. ***B.t. sandiego*-Coleoptera**

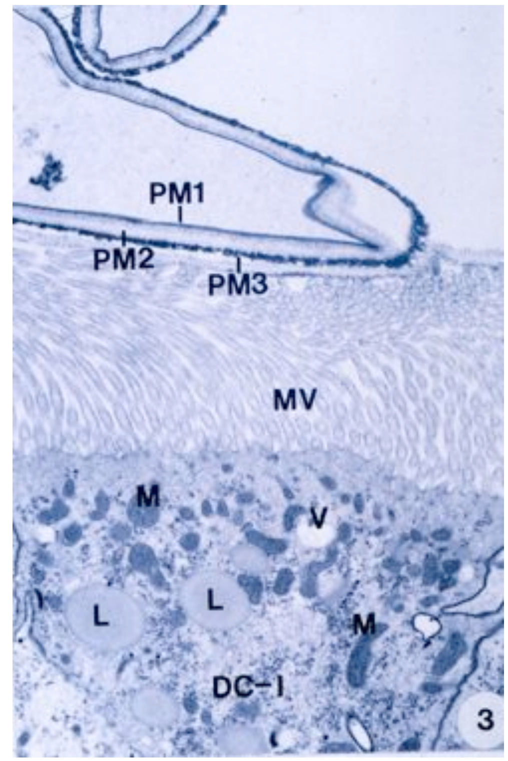
Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity.
Broderick, N.A., Raffa, K.F., and J. Handelsman. Oct. 10, 2006
Proc. Nat. Acad. Sci, USA 103(41): 15196-9.

***Bacillus thuringiensis* is the most widely applied biological insecticide and is used to manage insects that affect forestry and agriculture and transmit human and animal pathogens. This ubiquitous spore-forming bacterium kills insect larvae largely through the action of insecticidal crystal proteins and is commonly deployed as a direct bacterial spray. Moreover, plants engineered with the cry genes encoding the *B. thuringiensis* crystal proteins are the most widely cultivated transgenic crops. For decades, the mechanism of insect killing has been assumed to be toxin-mediated lysis of the gut epithelial cells, which leads to starvation, or *B. thuringiensis* septicemia. Here, we report that *B. thuringiensis* does not kill larvae of the gypsy moth in the absence of indigenous midgut bacteria. Elimination of the gut microbial community by oral administration of antibiotics abolished *B. thuringiensis* insecticidal activity, and reestablishment of an *Enterobacter sp*. that normally resides in the midgut microbial community restored *B. thuringiensis*-mediated killing. *Escherichia coli* engineered to produce the B. thuringiensis insecticidal toxin killed gypsy moth larvae irrespective of the presence of other bacteria in the midgut. However, when the engineered E. coli was heat-killed and then fed to the larvae, the larvae did not die in the absence of the indigenous midgut bacteria. *E. coli* and the *Enterobacter sp.* achieved high populations in hemolymph, in contrast to *B. thuringiensis*, which appeared to die in hemolymph. Our results demonstrate that *B. thuringiensis*-induced mortality depends on enteric bacteria**

Resistance to Bt-

The endotoxin binds to the brush border of the midgut epithelium using a receptor binding site. How do you think an insect might become resistant to Bt?





“Reduced binding of Bt toxin to the brush border membrane of the
midgut epithelium has been identified as a primary mechanism of resistance in *Plodia interpunctella* and *P. xylostella*.”

PROTEINASE INHIBITORS

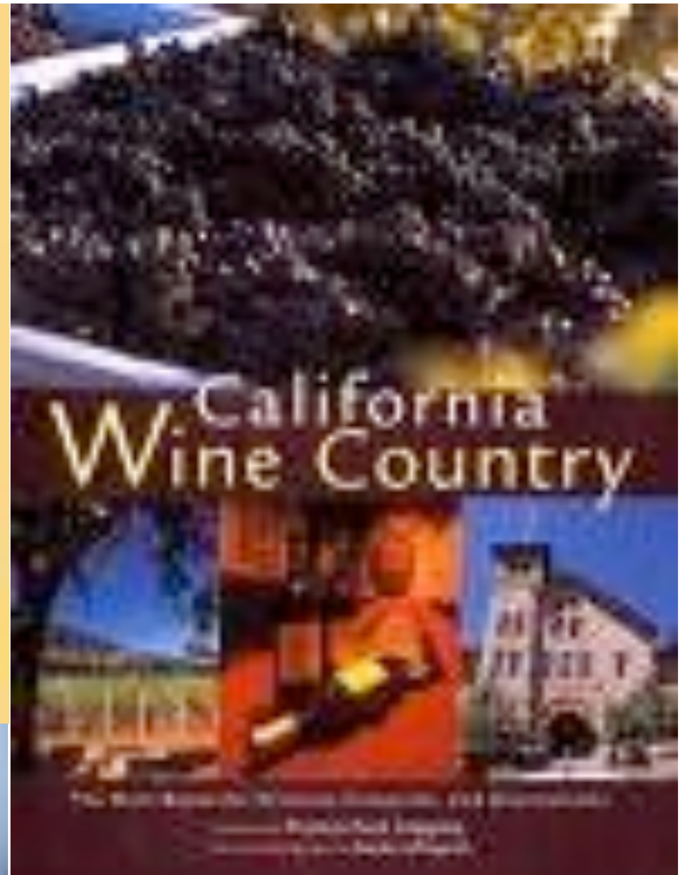
Many plants, especially in their seeds, produce this type of inhibitor to avoid damage by seed or plant predators, which includes insects.

Trypsin inhibitor gene put into tobacco plant (=transgenic plant) against tobacco hornworm.

One novel way to confer insect resistance in plants is to genetically engineer the plant by introducing foreign genes that will express the desired form of resistance when the introduced gene is expressed. Since protease inhibitors are widely present in plants naturally and appear to confer pest resistance by preventing digestion of the plant protein by the herbivore, scientists in England used this information and engineered the trypsin inhibitor gene, which imparts naturally occurring resistance to the cowpea, *Vigna unguiculata*, plant, into the tobacco plant. When completed, these tobacco plants were resistant to the tobacco hornworm, *Heliothis virescen*. Failure of the insect to grow normally is attributed to the interference of digestion by the protease inhibitor preventing normal protein digestion from occurring. **Will resistance occur? For sure, since there is a natural case where hemipterans feeding on seeds rich in trypsin inhibitors evolved cathepson-like proteinases, instead of using trypsinases, to circumvent the problem of proteinase inhibitors from seeds interfering with digestion.**

Glassy-Winged Sharpshooter (LEAFHOPPER)
Scientific name: *Homalodisca coagulata* (Say, 1832)

Vector of Pierce's disease (lethal disease of grapevines)



BIG MONEY AND BIG BUSINESS

Endeavor™
Insecticide

Endeavor penetrates the leaf and stem tissues so that when these insects feed they “suck-up” pymetrozine causing feeding to be inhibited. Pymetrozine does have some initial contact activity, but its primary mode of action and residual control occurs through feeding inhibition.

Back to Top

Suggested the site of action is on the cibarial pump

J Insect Sci. 2004; 4: 34. Published online 2004 October 22.

Impact of pymetrozine on glassy-winged sharpshooter feeding behavior and rate of *Xylella fastidiosa* transmission

B.R. Bextine,[1,2] D. Harshman,[1] M.C. Johnson,[1] and T.A. Miller[1]
[1]University of California, Riverside; Department of Entomology; Riverside, California 92521, USA[2]Email: blake.bextine@ucr.edu

Pymetrozine is a compound that interferes with insect feeding and interrupts transmission of plant pathogens. The glassy-winged sharpshooter, *Homalodisca coagulata* Say (Hemiptera, Cicadellidae), is a vector of *Xylella fastidiosa*, the foregut-borne, propagative bacterium that causes Pierce's disease of grapevine. Pymetrozine, a novel compound sold under the trade names Fulfill™, Endeavor™, and Chess 250 WP™, is a systemic antifeedant, belonging to the class of chemicals known as pyridine-azomethines (MSDS 2001).

Once foods are eaten, mechanically processed, enzymatically processed and moved to the midgut, they are absorbed into the hemolymph. Now the transporation system of the insect, THE **CIRCULATORY SYSTEM** takes over and delivers these nutrients to all of the cells.

proboscisclose.mov

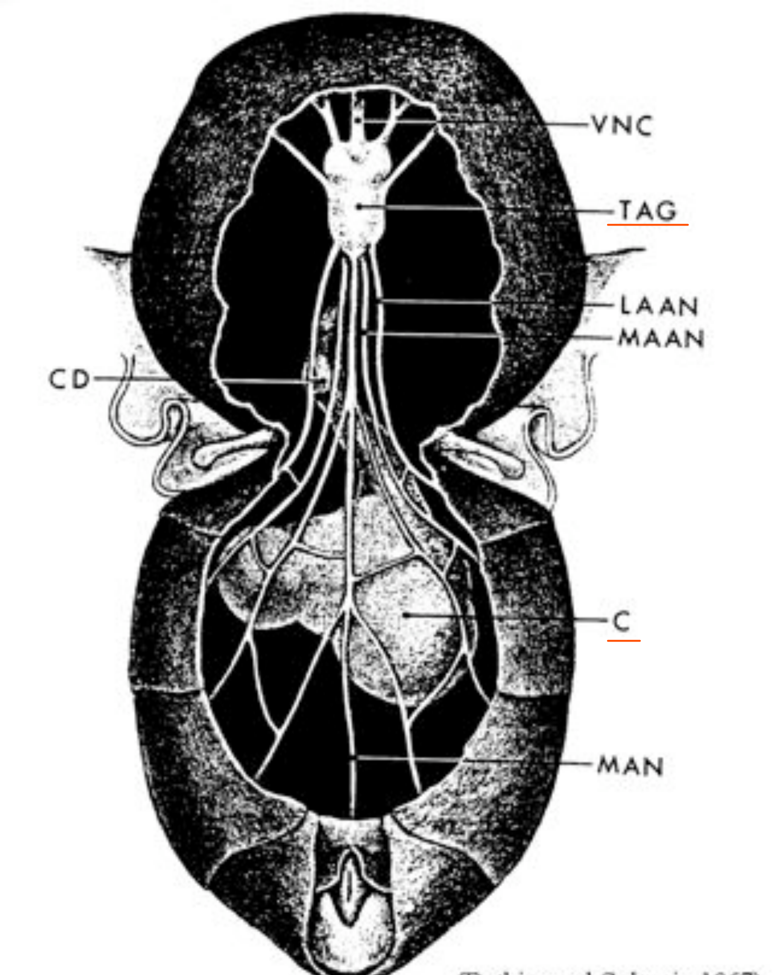
dragonflystrike.mov

mantidfeeding.mov

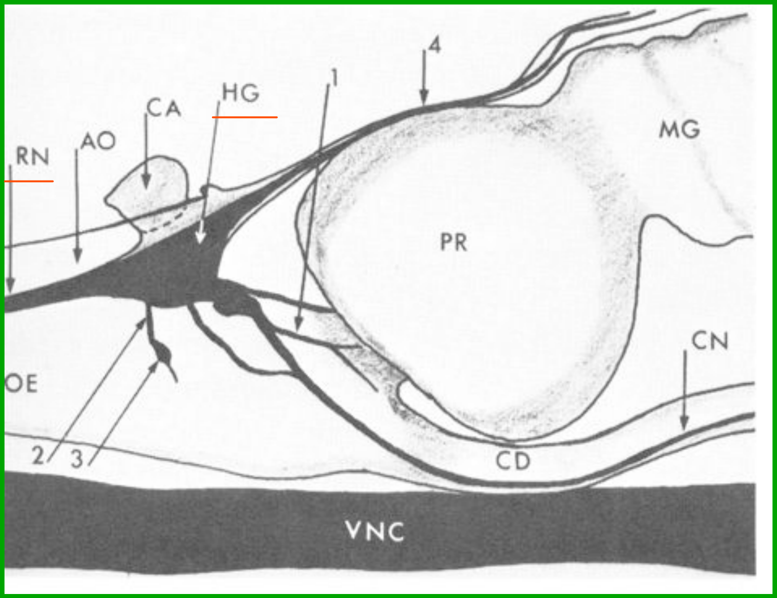
beefeedingside.mov

proboscisclose.mov

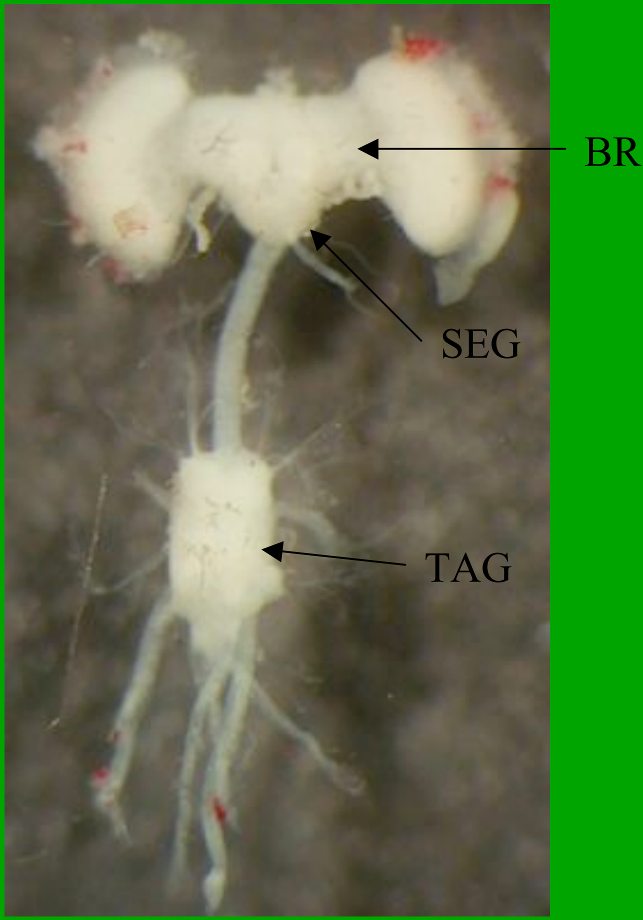
Abdominal nerve net



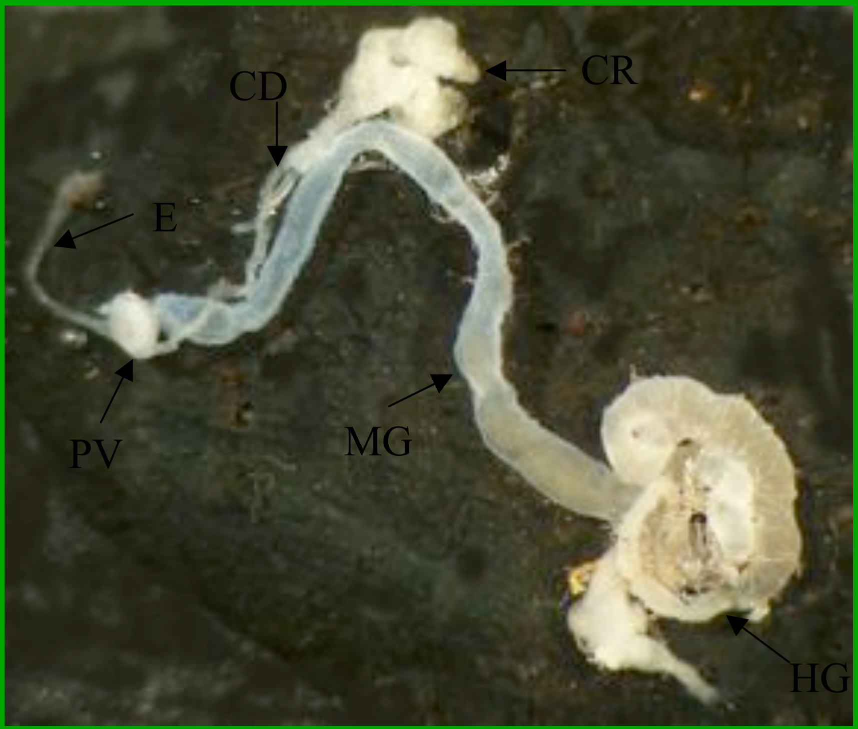
(Dethier and Gelperin 1967)



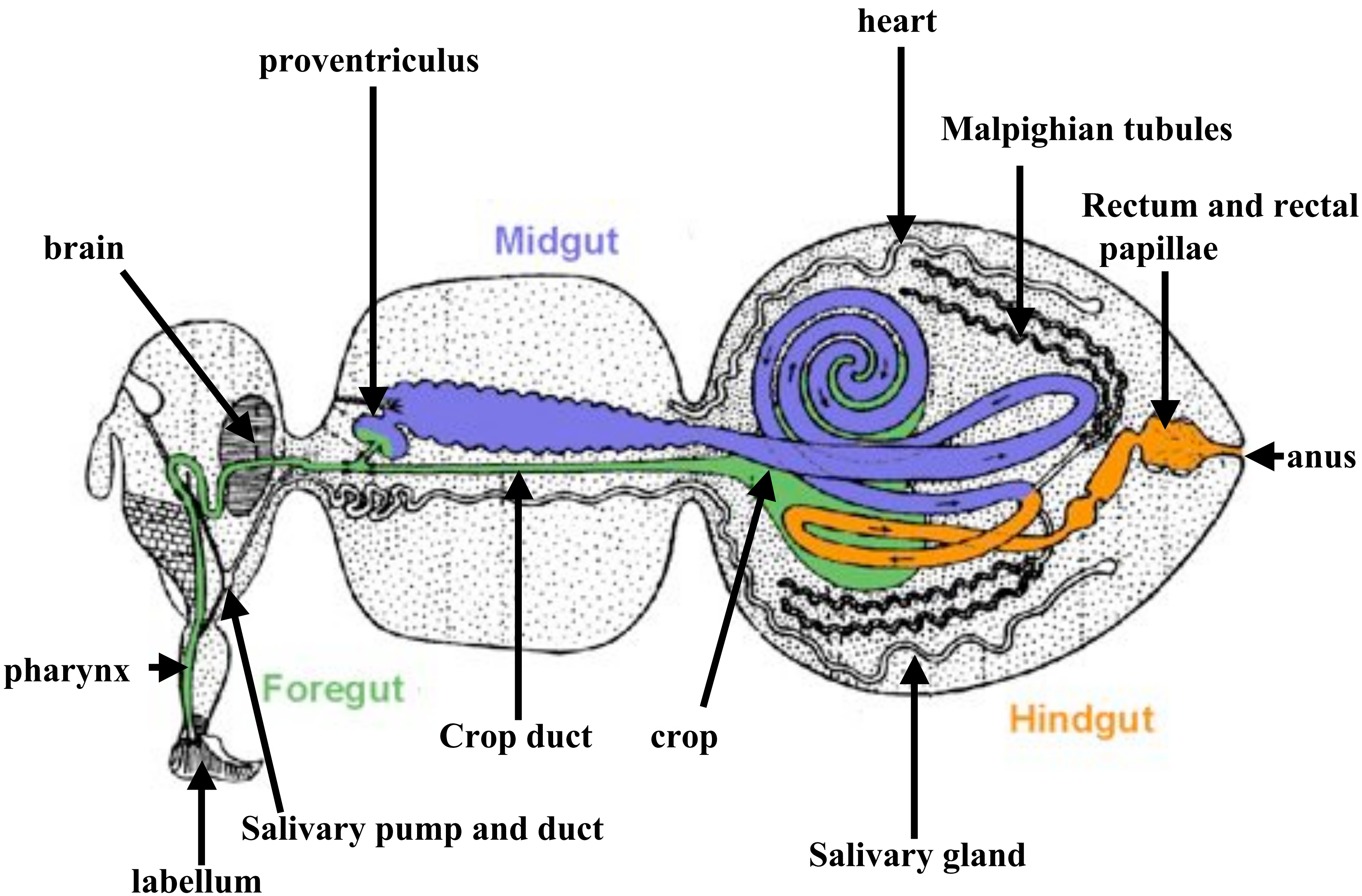
Phormia CNS and alimentary tract



CNS

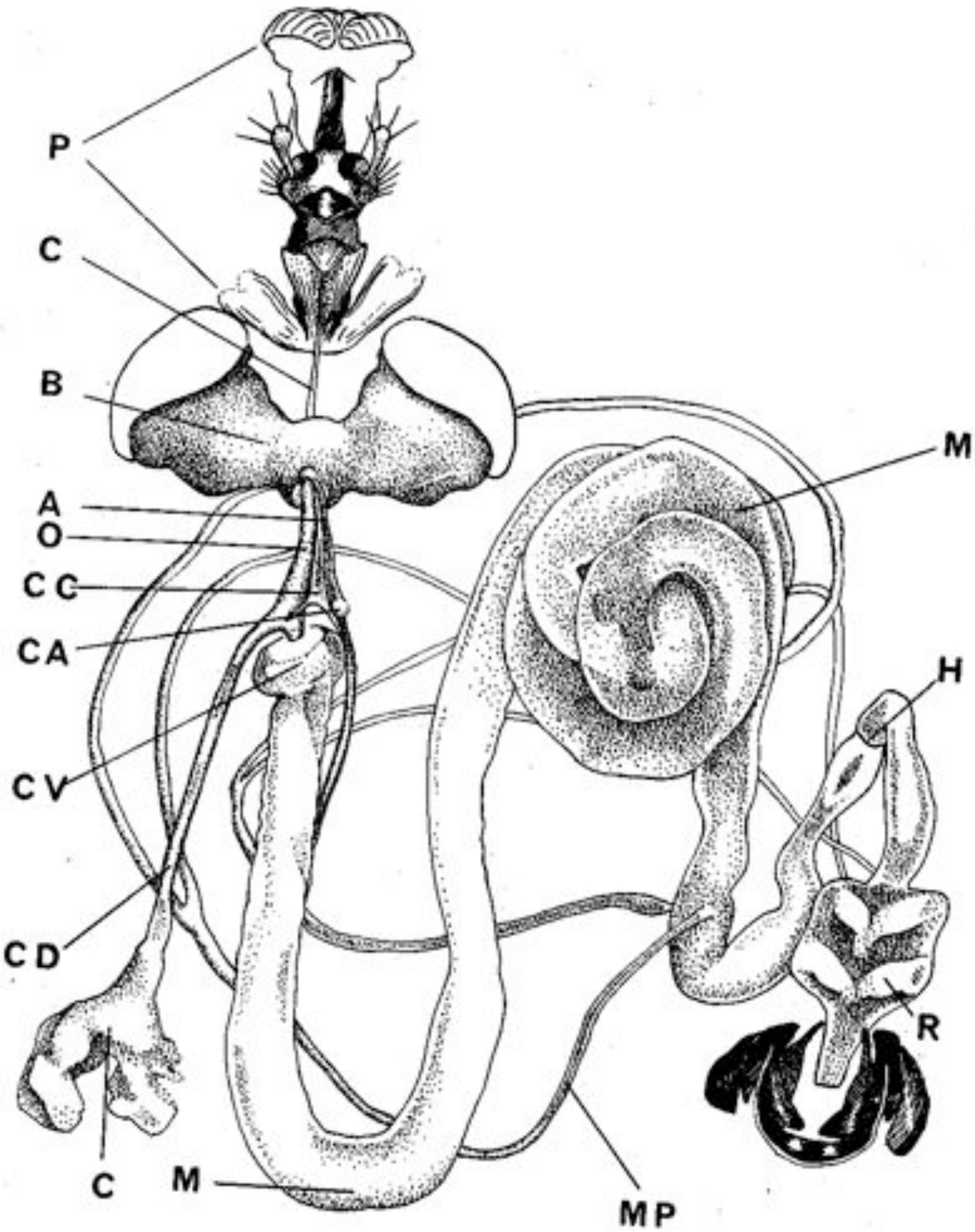


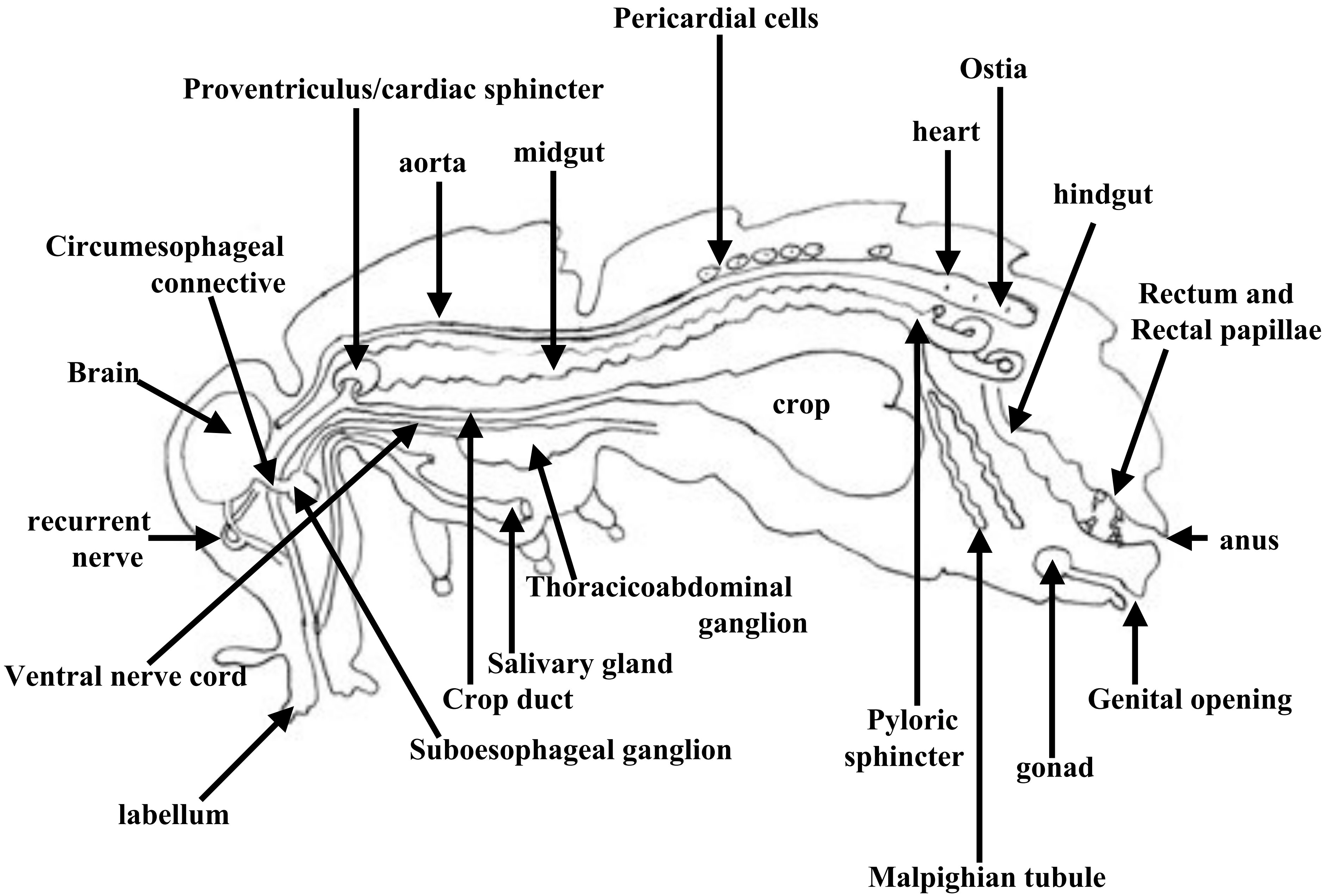
Alimentary tract



heart
proventriculus
Malpighian tubules
Rectum and rectal papillae
Midgut
brain
anus
pharynx
Foregut
Crop duct
crop
Hindgut
Salivary pump and duct
Salivary gland
labellum

P=proboscis
C=cibarium
B=brain
A=aorta
O=oesophagus
CC=corpus cardiacum
CA=corpus allatum
CV=proventriculus
cardiac valve
CD=crop duct
C=crop
M=midgut
H=hindgut
MP=Malpighian tubule
R=recturm





Pericardial cells
Ostia
Proventriculus/cardiac sphincter
heart
aorta
midgut
hindgut
Circumesophageal connective
Rectum and Rectal papillae
Brain
crop
recurrent nerve
anus
Thoracicoabdominal ganglion
Salivary gland
Ventral nerve cord
Crop duct
Genital opening
Pyloric sphincter
Suboesophageal ganglion
gonad
labellum
Malpighian tubule