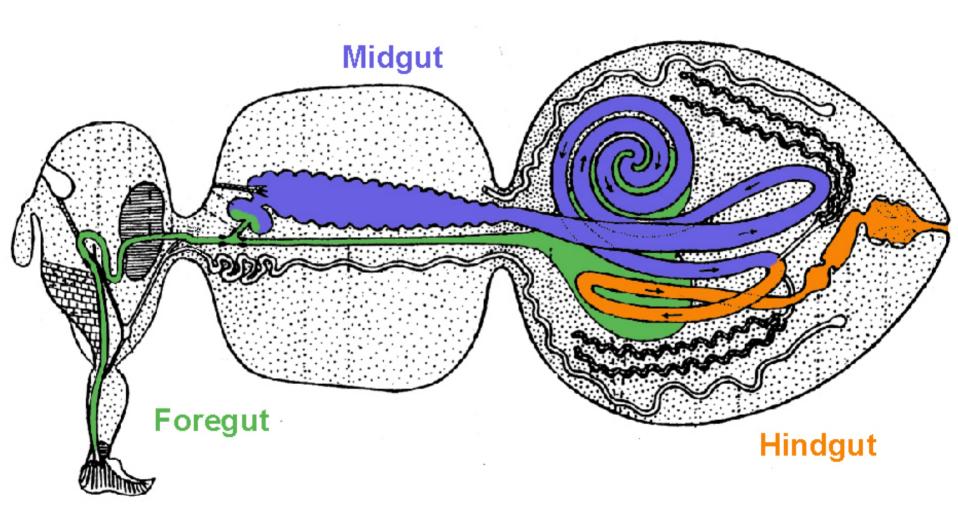
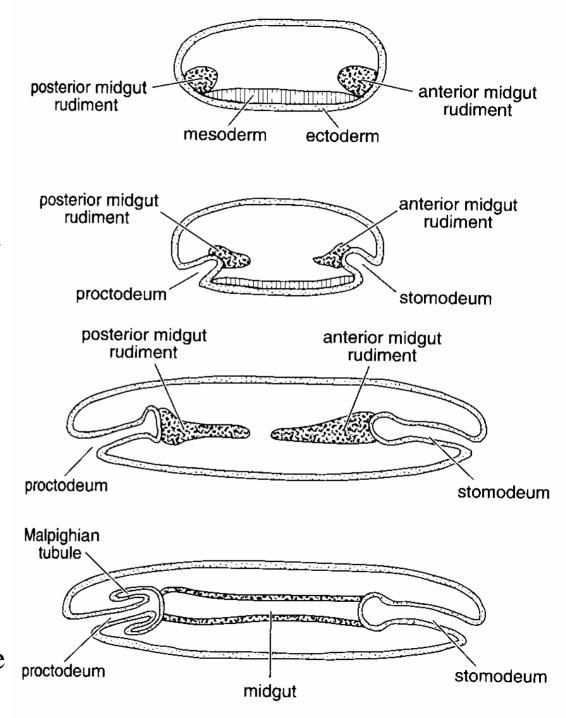
DIGESTIVE SYSTEM



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



DIGESTIVE SYSTEM

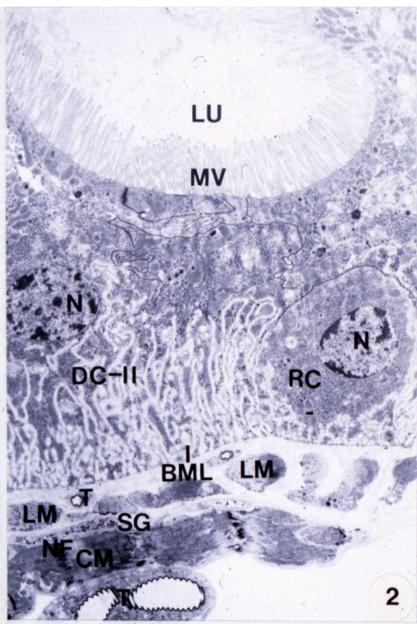
- The digestive tract not only aids in obtaining, processing and digesting food molecules. It is the largest endocrine tissue in both humans and insects.
- The digestive system is involved in:
- 1. Obtaining food
- 2. **Mechanically** breaking it down into smaller particles that facilitate digestive enzymes acting on them
- 3. **Enzymatic** breakdown of larger food molecules into molecules that can pass through the digestive tract and enter the hemolymph
- 4. Producing molecules, such as 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 glandsa. Sexual dimorphism in mosquitoesb. 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

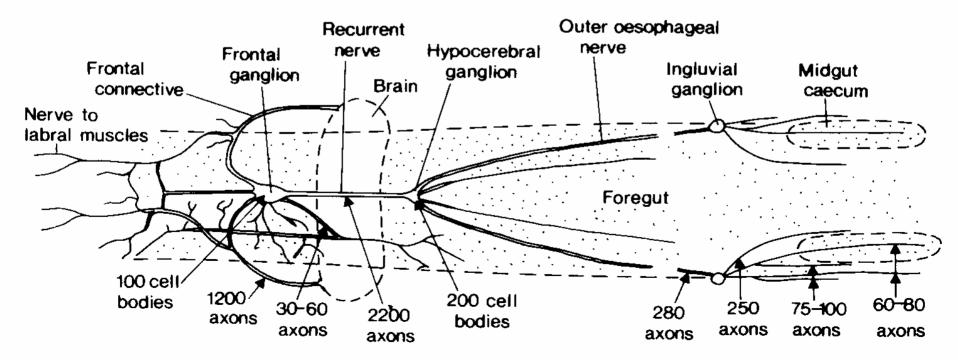
- 1. Tube starting at mouth and ending at anus
- 2. Tube surrounded by circular + longitudinal muscles
- TEM of *Phorma* adult midgut (see on right, notice the LM, which is **longitudinal muscle** and the CM, which is **circular muscle** surrounding the gut. MV=microvilli, LU=lumen, BML= Basal membrane labyrinth, RC=regenerative cell).



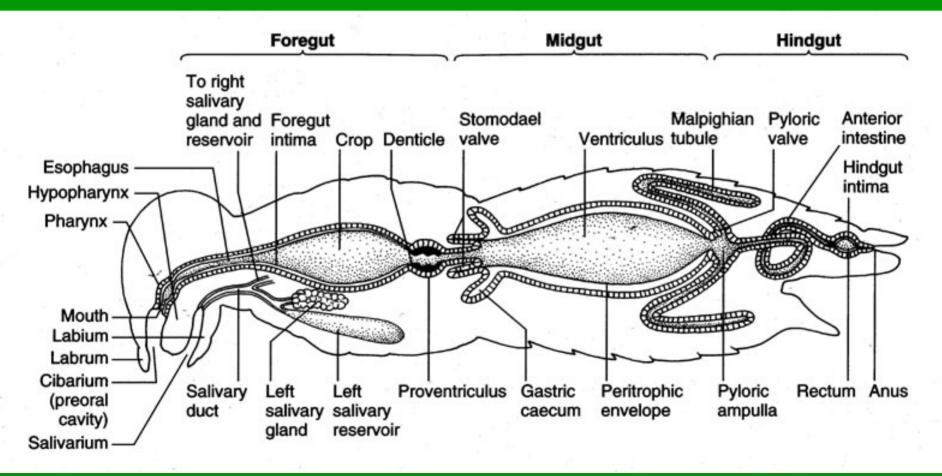
GENERALIZED STRUCTURE

3. Is innervated by the stomadeal or visceral nervous sytem

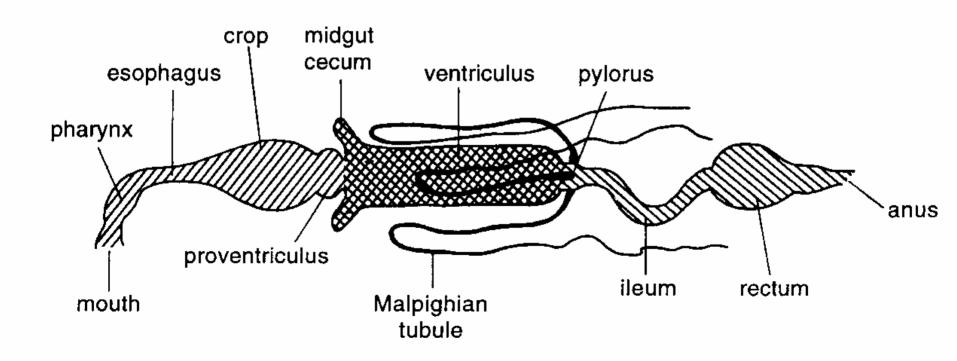
Stomadeal nervous system of Locust (below)

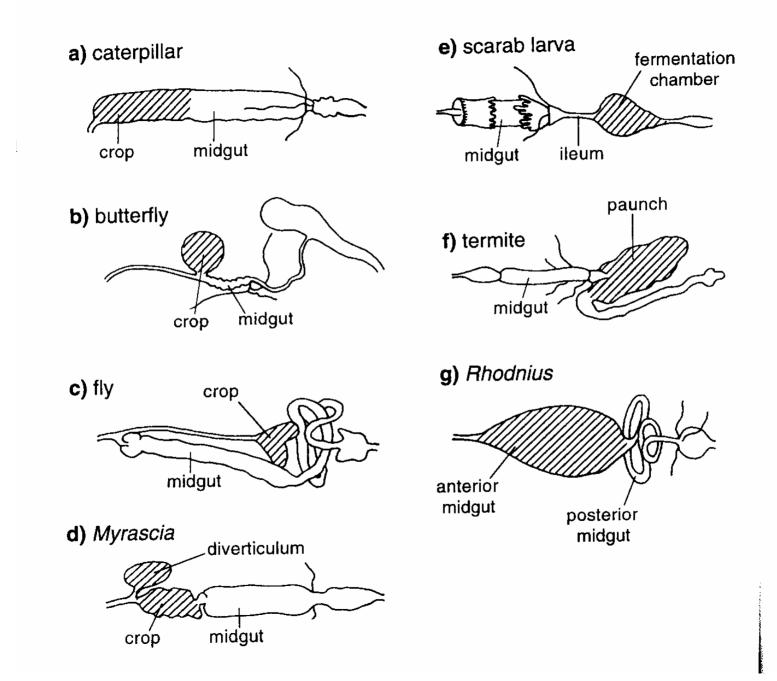


Generalized insect alimentary tract



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.





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MECHANISMS OF FOOD INGESTION

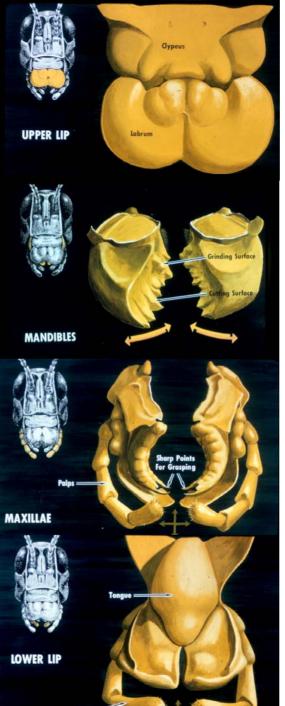
Different types of mouthparts have added greatly to insect diversity and evolution.

- **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

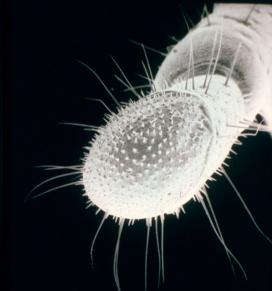
MANDIBULATE

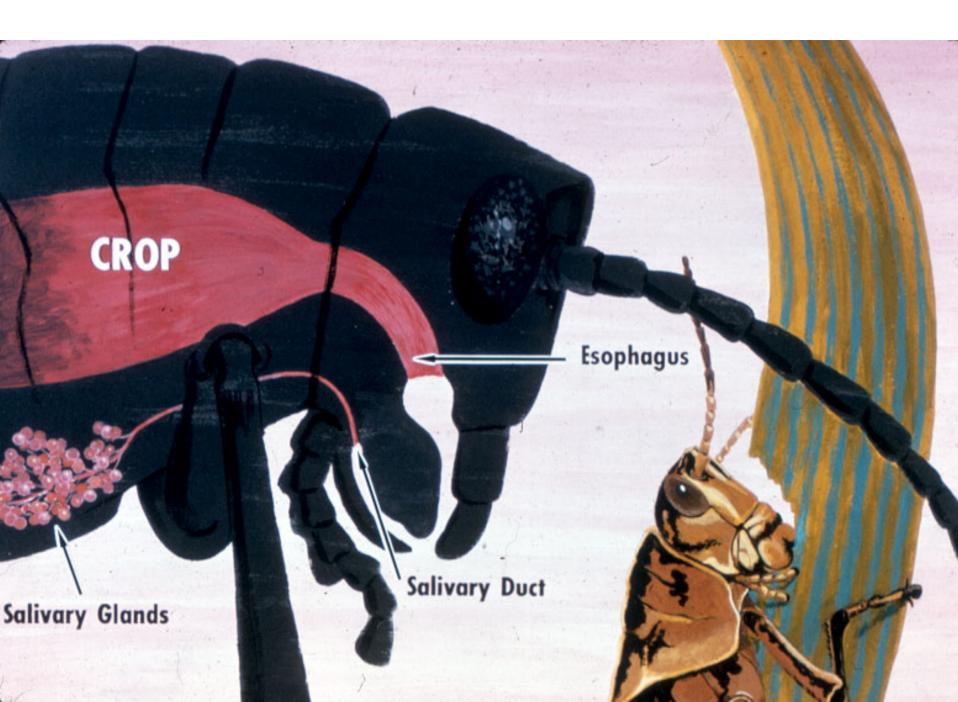


Mouthparts of lubber grasshopper and some associated sensilla

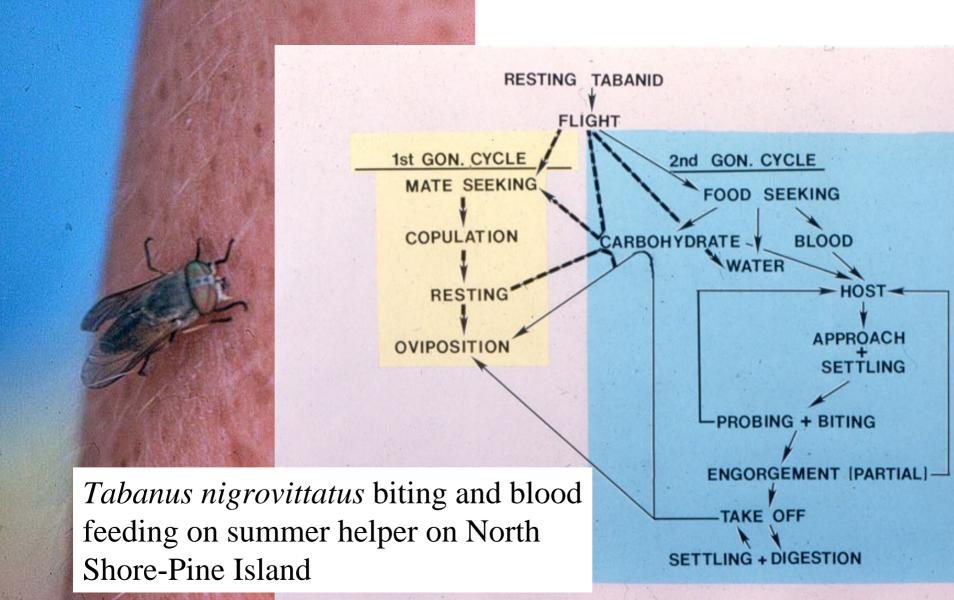


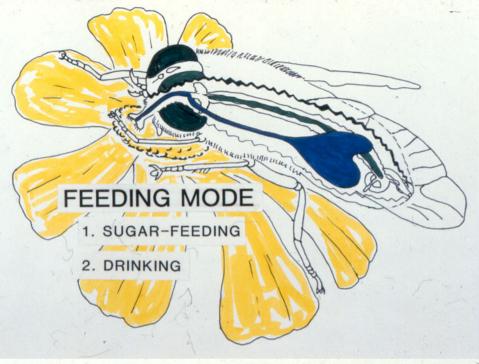
Chemosensilla on inner surface of labrum (top and arrow) and on tip of maxillary palp (below)

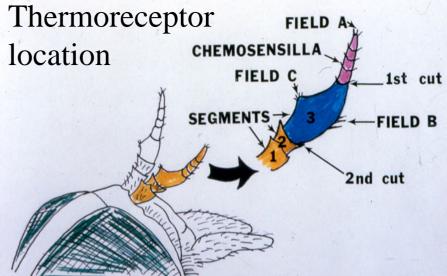


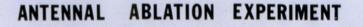


Haustellate or sucking mouthparts of *Tabanus nigrovittatus*





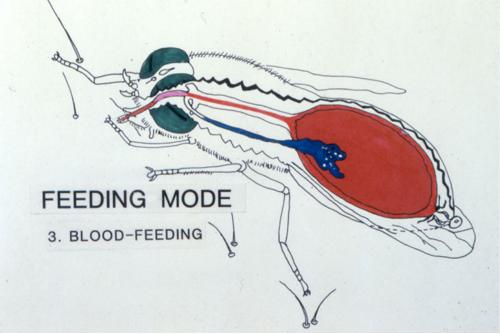


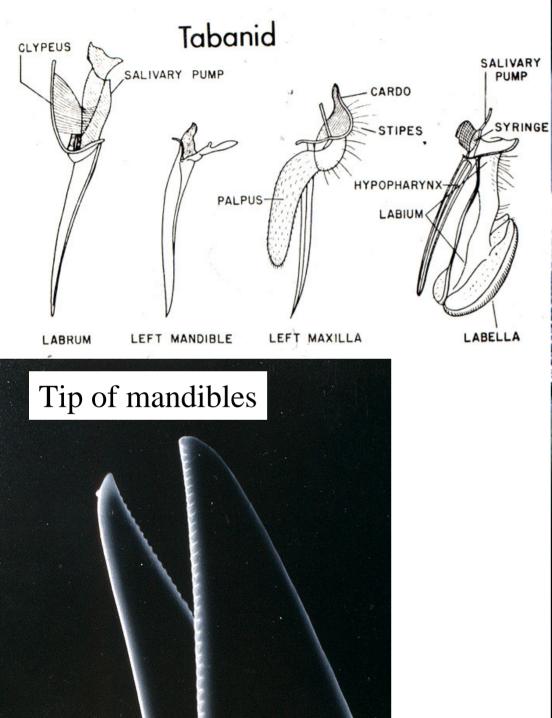


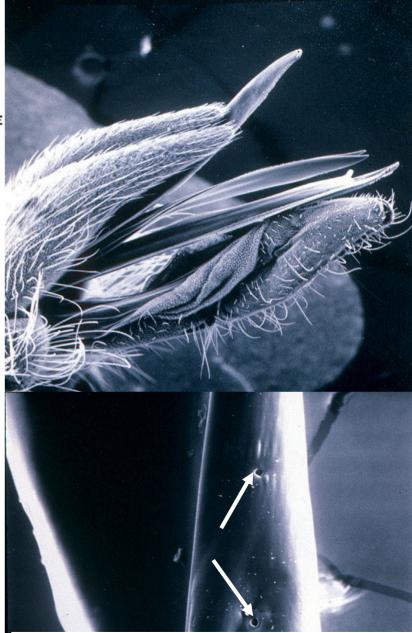
EFFECT OF REMOVING VARIOUS APPENDAGES ON BITING THE HUMAN HAND

| 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

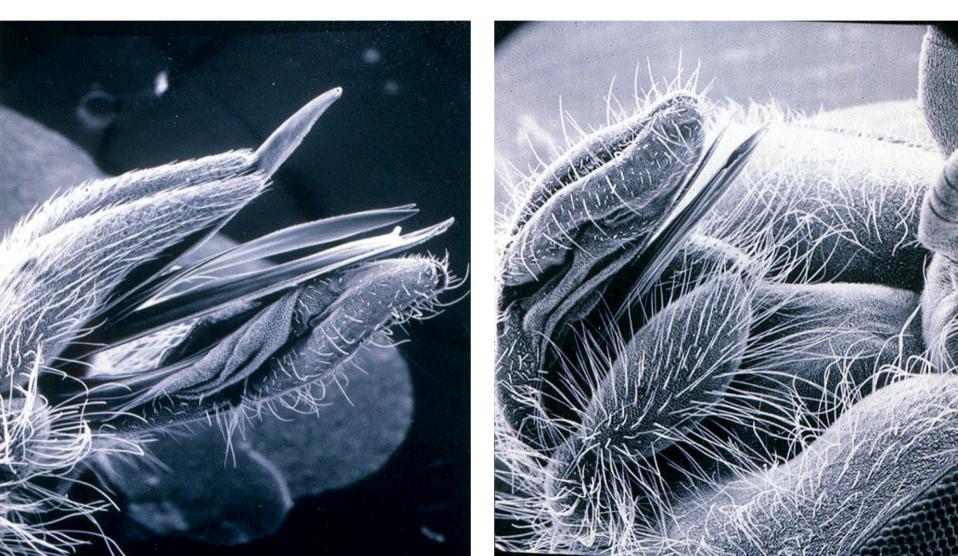






Mechanosensilla on mandible of *Tabanus nigrovittatus*

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*



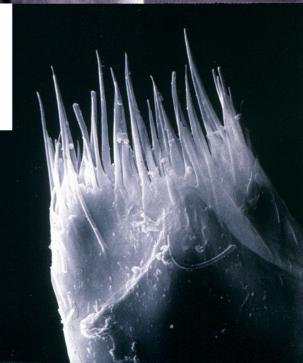
Female on left Male on right

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



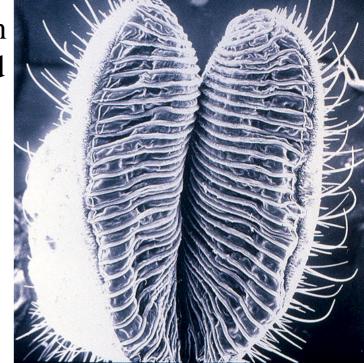
Tip of labrum-Note rasping teeth and chemoreceptors in female

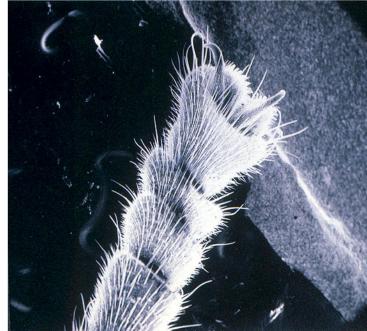
> Females on right and males on the left showing sexual dimorphism in mouthpart stylets.



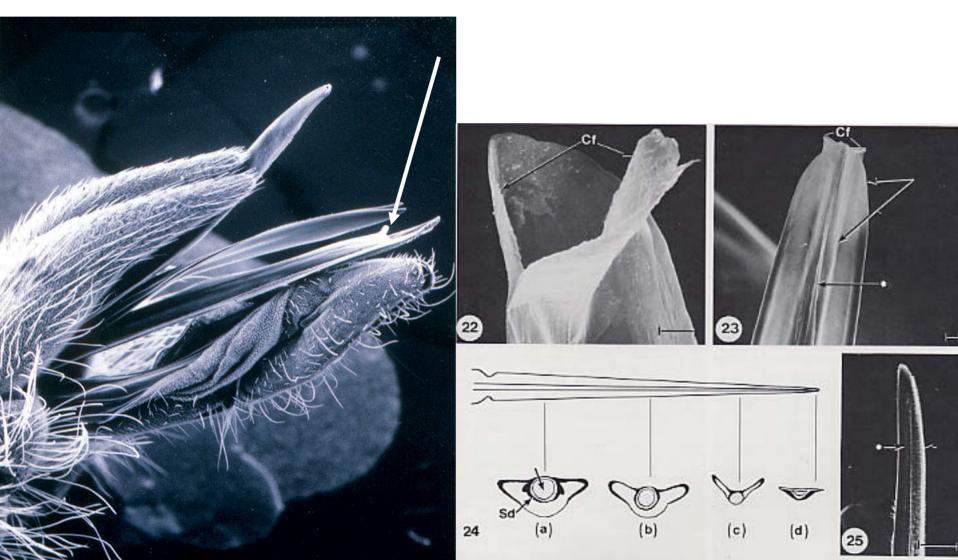
Information must be obtained by the insect in order to feed. Chemoreceptors on labium and also on the tarsi. *Tabanus nigrovittatus*



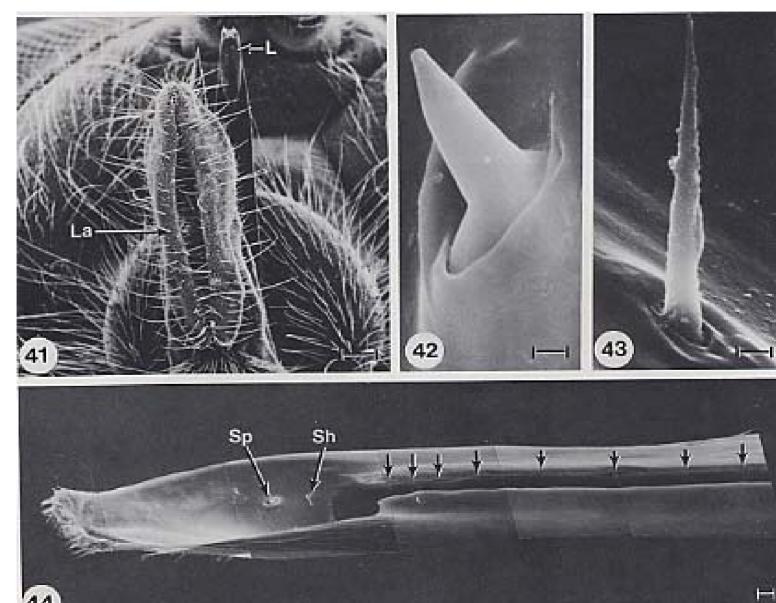


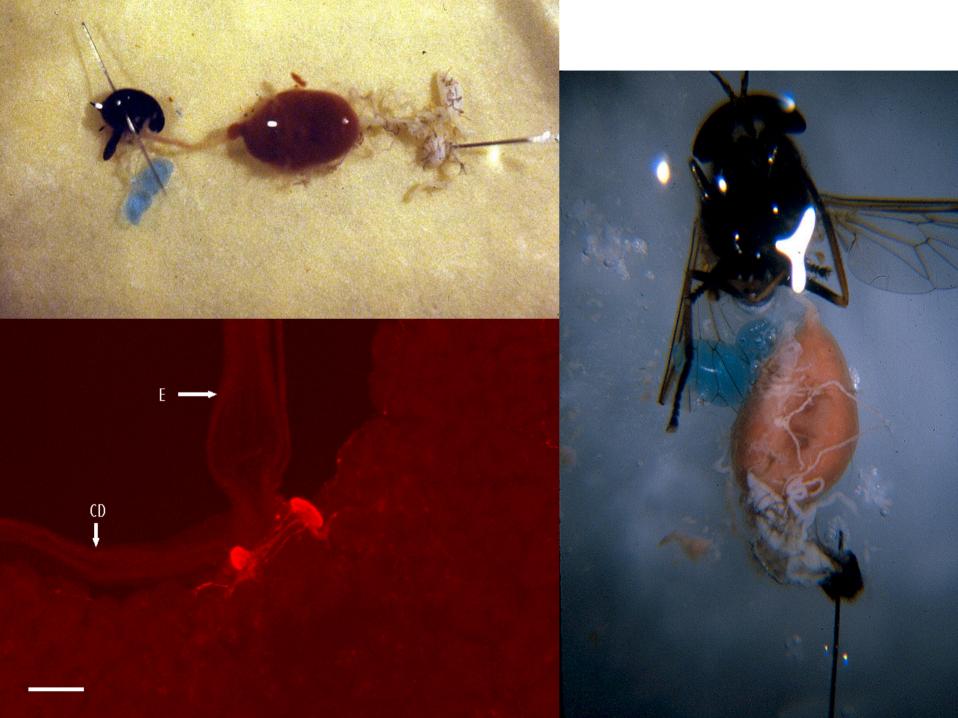


Once the stylets are inside the wound, the tabanid secretes salivary secretions that come out of the hypopharynx (see white arrow on the left and the openings of it in figs. 22 and 23). Note the midrib and flanging that occurs to give the hypopharynx rigidity.



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 mechanoreceptors to monitor blood flow and composition (ATP).

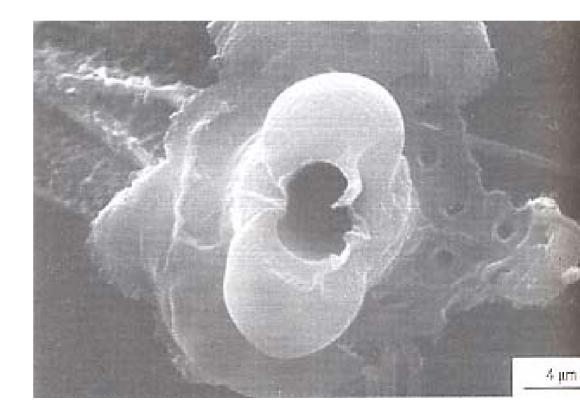


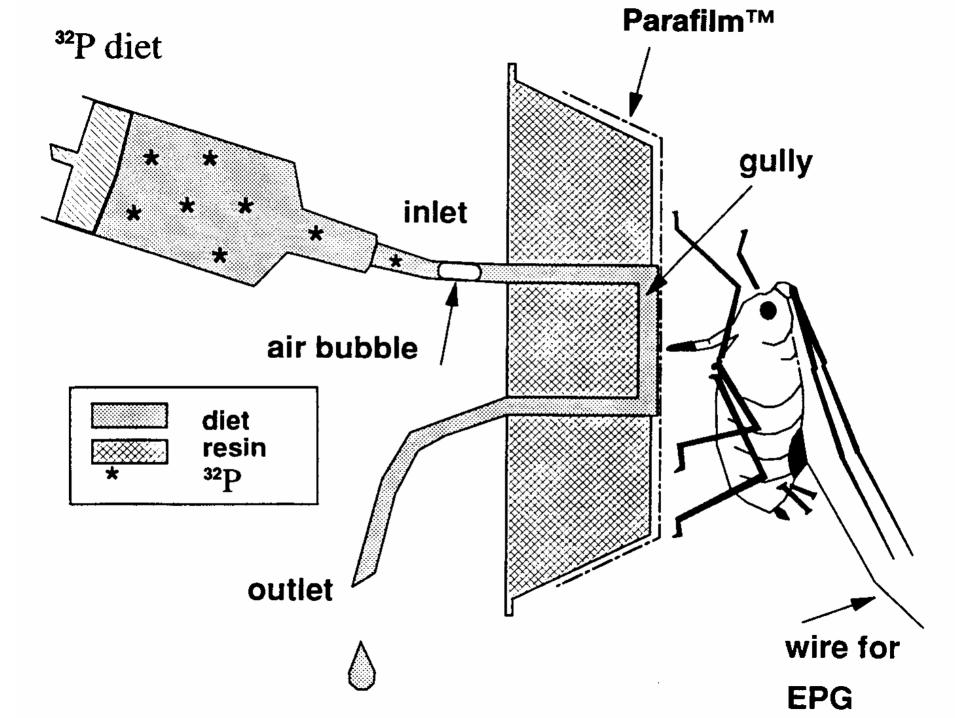


Feeding on pressure systems for long periods of time could lead to the fluids leaking from between the mouthparts and the system the insect is feeding on. Several groups produce a **sieve tube** from saliva that forms a secure tube and also seals the feeding apparatus. Aphids, plant-hoppers, and ticks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

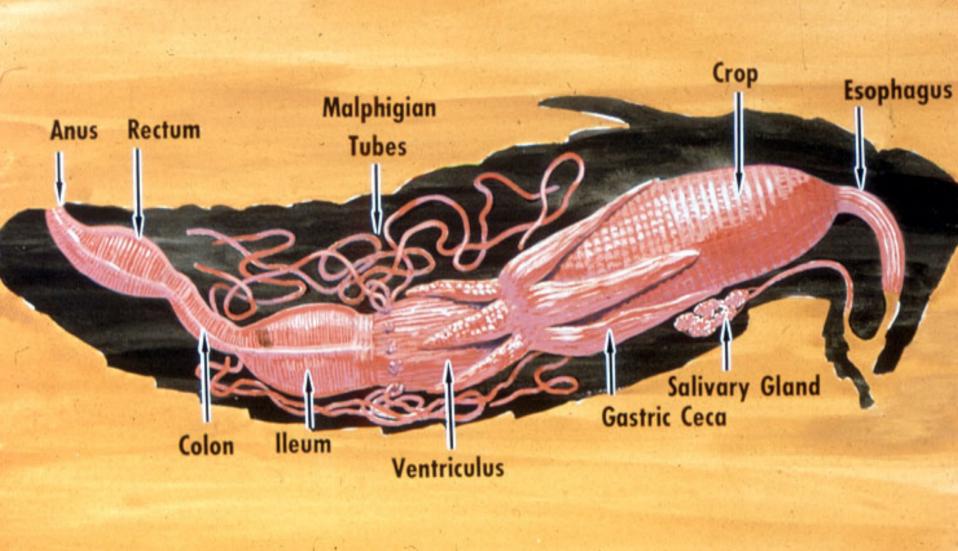


Salivary flange produced by plant-hopper feeding on sorghum. The hole is where the stylets were removed from the plant while the small holes to the right of the larger hole were made by chemoreceptors on the tip of the labium.



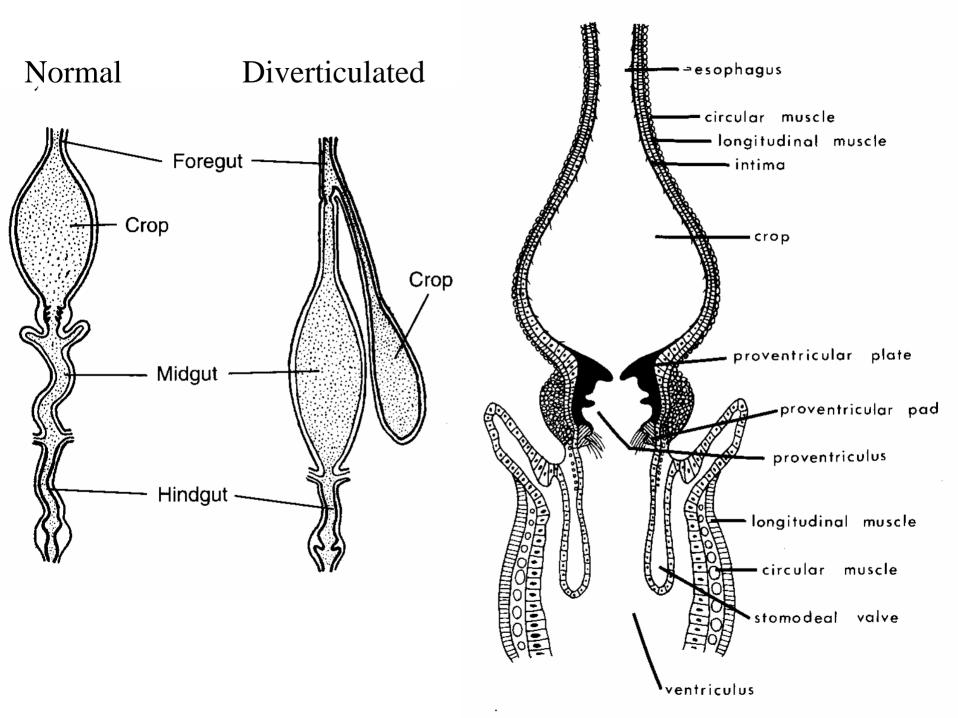


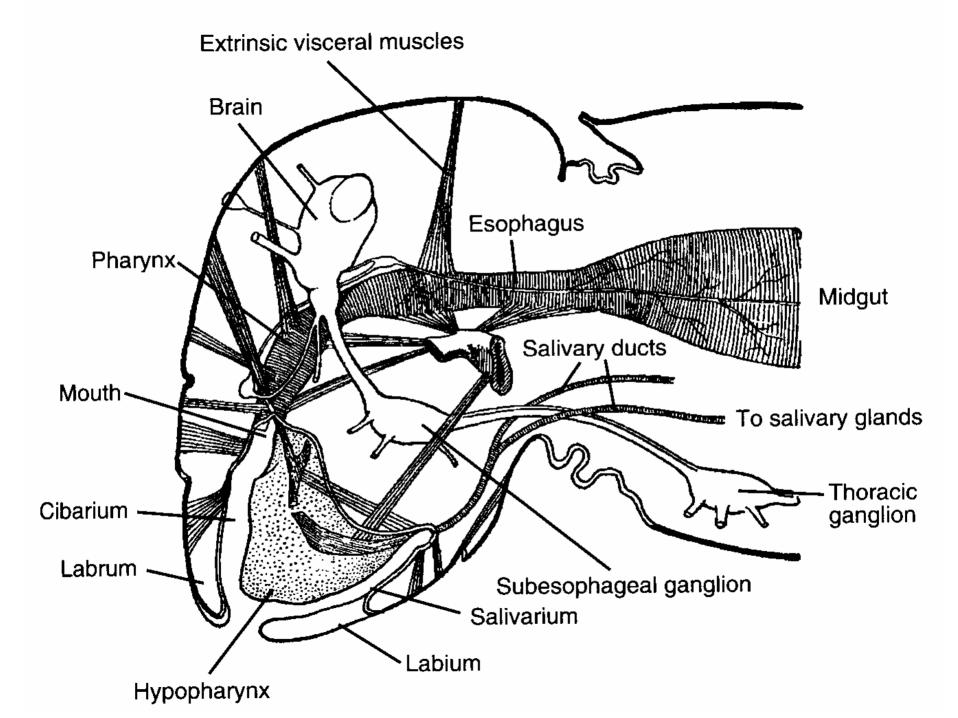
DIGESTIVE SYSTEM



FOREGUT

- Foregut starts at mouth and ends just after proventriculus but before the cardiac sphincter Little or no digestion occurs in the foregut
- 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
- 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
- The salivary glands empty into the foregut or mouth area depending on the insect





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 sugarsb. 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
 - c. Anticoagulants
 - d. Formation of silk-Lepidopterans
 - e. Contain bacteria in myiasis producing flies

SALIVARY TOXINS

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

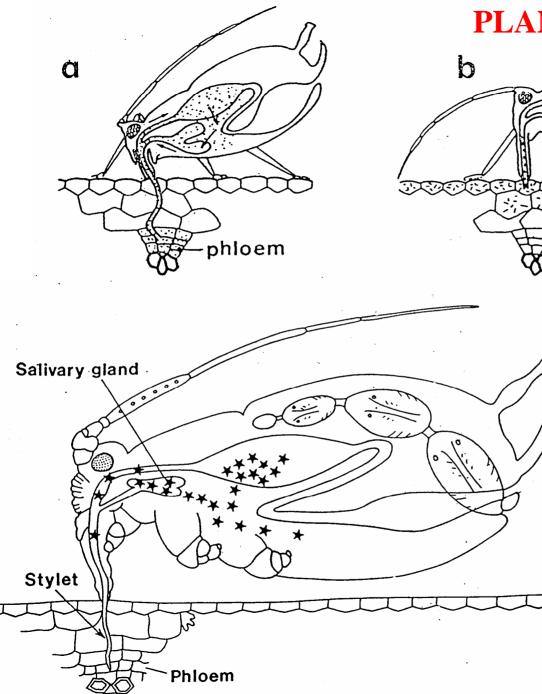




The asilids have large salivary glands that produce a toxin that can kill an 85 gm. mouse. Above you can see the fly has taken down a bigger and better flyer, a dragonfly adult. **Involvement of salivary glands in pathogen or parasite tranmission**

Plant feeders a. Aphids and viruses

Blood feeders a. Mosquito and plasmodium



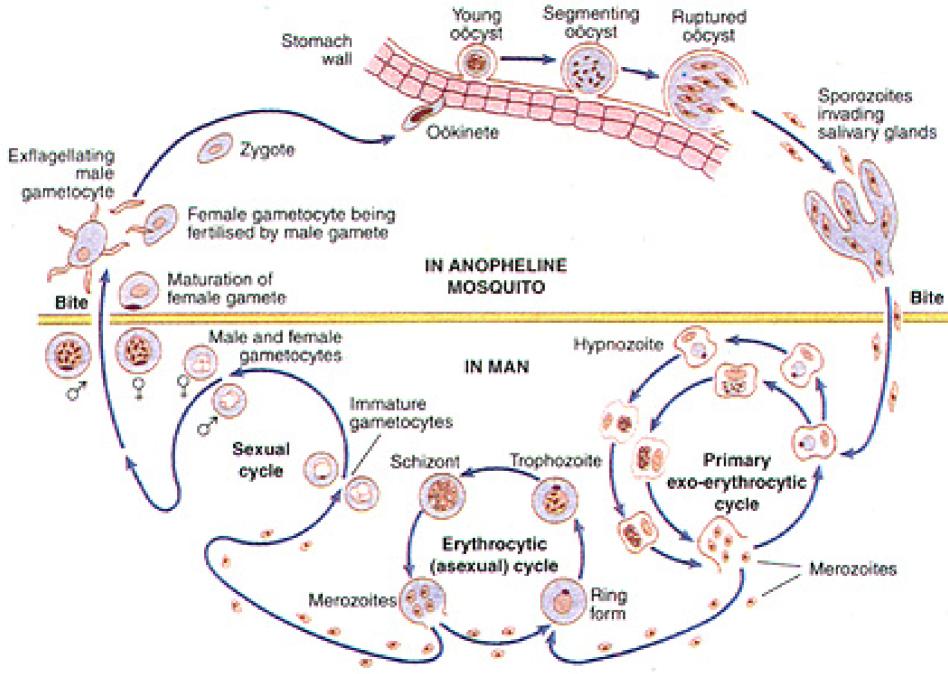
PLANT FEEDERS

N. X. J. T. Y.

epidermis

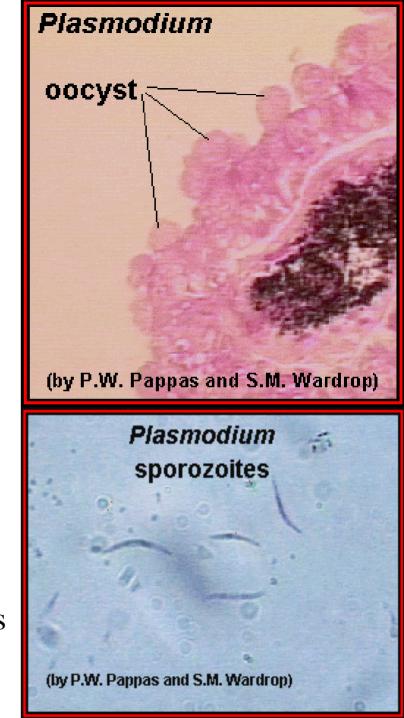
Virus transmission in aphids involves the passage through the midgut, then into the hemolymph and finally into the salivary glands where they are then passed onto another host while feeding.

Blood feeders



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.



- 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

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

- 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--immunity

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.





Sandflies





Phlebotomus dubosci

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 vasoconstriction using a pepide known as maxidilan and preventing a clot from forming.

Polymorphism in levels of expression of Maxadilan

Atypical Cutaneous Leishmaniasis

American Visceral Leishmaniasi





ERYTHEMA CAUSED BY BITE OF UNIFECTED Lu. longipalpis

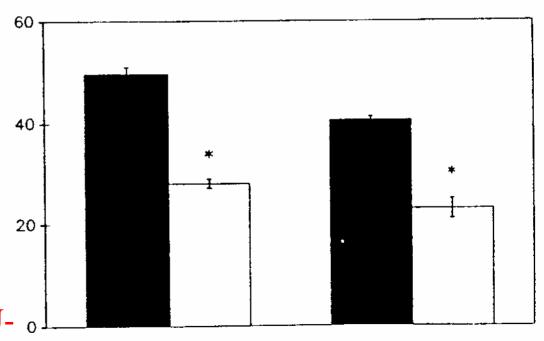
PHOTO TAKEN 12 HRS. POST-FEEDING

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

- Sexual dimorphism in salivary glands of mosquitoes Would one expect their to be a difference in salivary gland structure between the sexes in mosquitoes?
- Female mosquitoes have their glands clearly divided into an anterior and posterior region. This is not true for the males.
- Apyrase is produced in the section found only in females. Apyrase in the mosquito saliva prevents human blood platelets from aggregating by breaking down ADP, which is a signal for platelet aggregation, thus preventing clotting. Enzym

Jnits∖mg protein

- Effect of blood feeding on salivary apyrase activity in the bedbug, *Cimex*. Solid bars=8-10 days after bloodmeal
- Open bars=1 hr after the Bloodmeal
- ATP MAJOR PHAGOSTIMU- 0 LANT FOR BLOOD FEEDERS

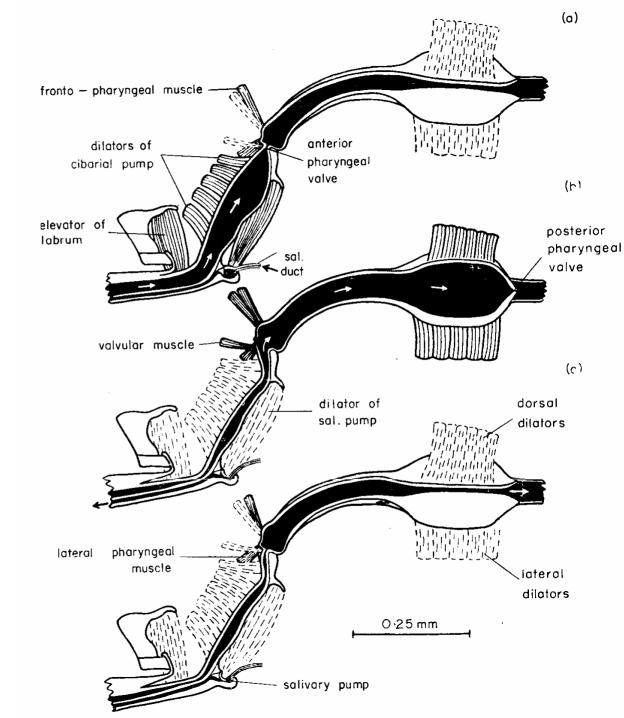


ATPase activity

ADPase activity

Salivary pump Cibarial pump Pharyngeal pump

Muscles and nerves are involved.



Hemostasis has 3 major components:

- 1. Platelet aggregation
- 2. Blood coaguation
- 3. Vasoconstriction
- Inflammation has 3 components:
- 1. Pain-nociception
- 2. Redness-tissue vasodilation
- 3. Heat----tissue vasodilation



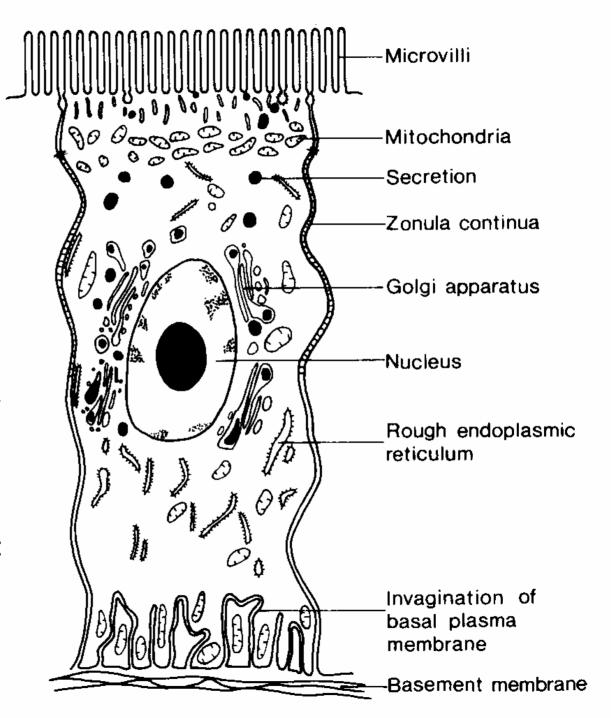
MIDGUT

- Midgut starts at the cardiac sphincter and ends at the pyloric sphincter
- Midgut contains different cell types:
 - a. Digestive cells
 - b. Regenerative cells
 - c. Endocrine cells
 - d. Goblet cells
- 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
- Midgut is where the food is mainly digested

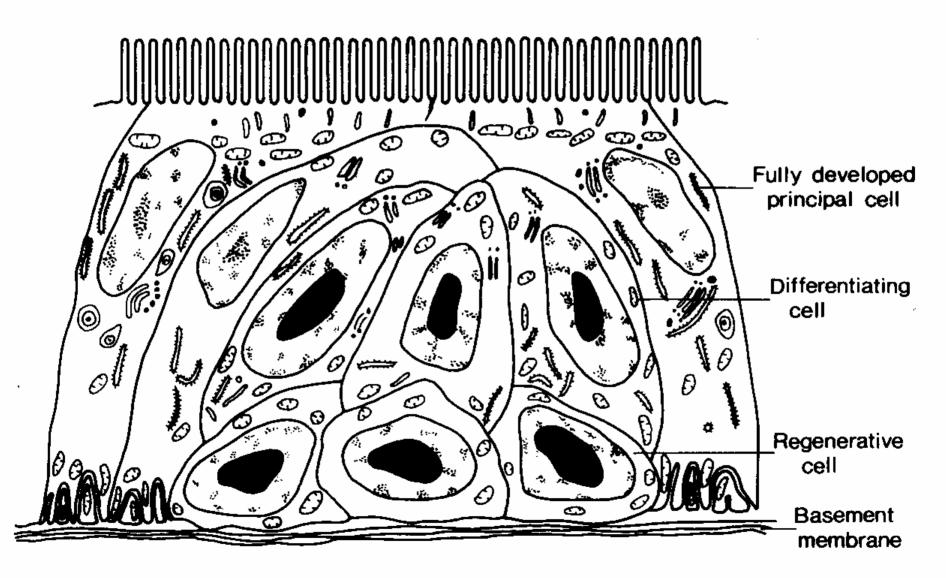
Digestive cells or Columnar Digestive cells

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.

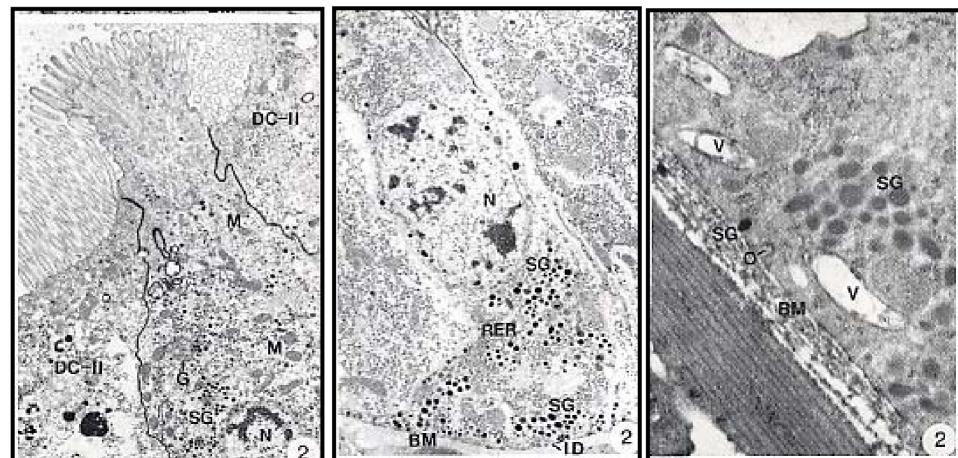


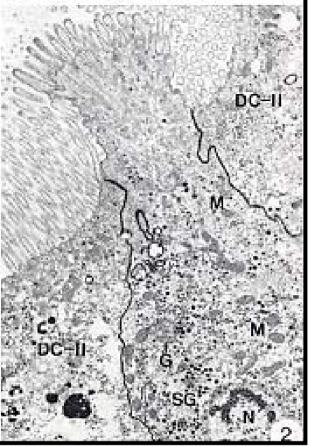
Regenerative cells- Also called nidi. They produce new digestive cells to replace worn out digestive cells.

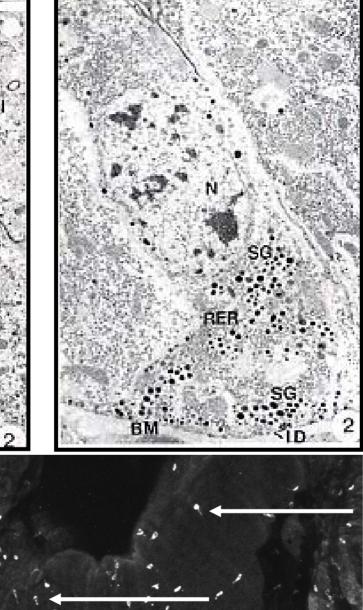


GUT ENDOCRINE CELLS

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.



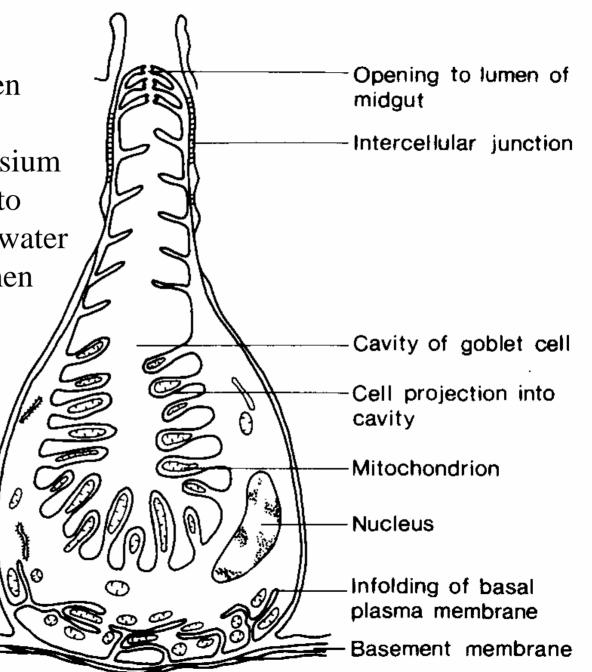




Photos of *Phormia* regina midgut. Fluorescent antibody against one of the MG peptides. Note the white arrow pointing to cells that show a larger circle-like spot and a connecting long duct-like arrangment. These are open gut endocrine cells that connect the cell to the lumen of the gut and to the basement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

Is not really a membrane, thus term peritrophic membrane is incorrect

- 1. Chitin
- 2. Glycoprotein of mucin-type

An intestinal mucin is the target substrate for a baculovirus enhancin. Wang P, Granados RR.

Department of Entomology, Cornell University, Ithaca, NY 14853, USA.

An invertebrate intestinal mucin (IIM) was identified from a lepidopterous insect, Trichoplusia ni. The IIM is a major protein constituent of the peritrophic membrane that facilitates the digestive process, as well as protecting invertebrate digestive tracts from microbial infections. The IIM demonstrated biochemical characteristics similar to vertebrate mucins, but exhibited strong association with the chitincontaining peritrophic membrane matrix. We have demonstrated that a baculovirus enhancin, which is encoded and carried by specific baculoviruses, has mucindegrading activity both in vitro and in vivo. The in vivo degradation of IIM by enhancin was correlated with the enhancement of baculovirus infections in insects. These findings have shown that viruses have evolved a novel strategy to overcome intestinal mucinous barriers against microorganisms by utilizing a mucin-degrading enzyme.

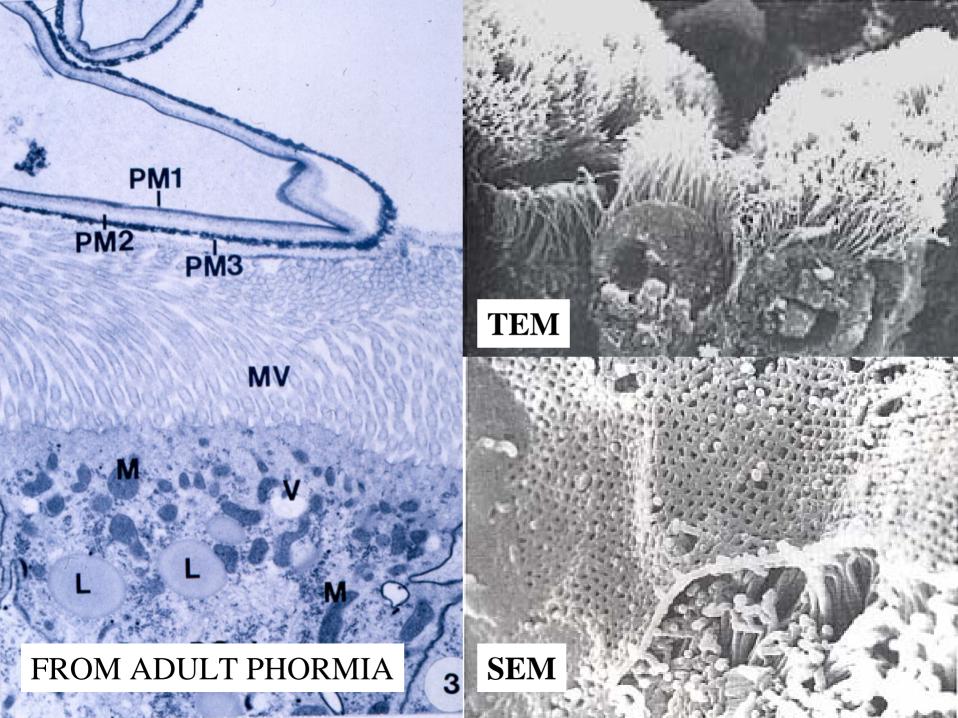
PERITROPHIC MATRIX

- 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

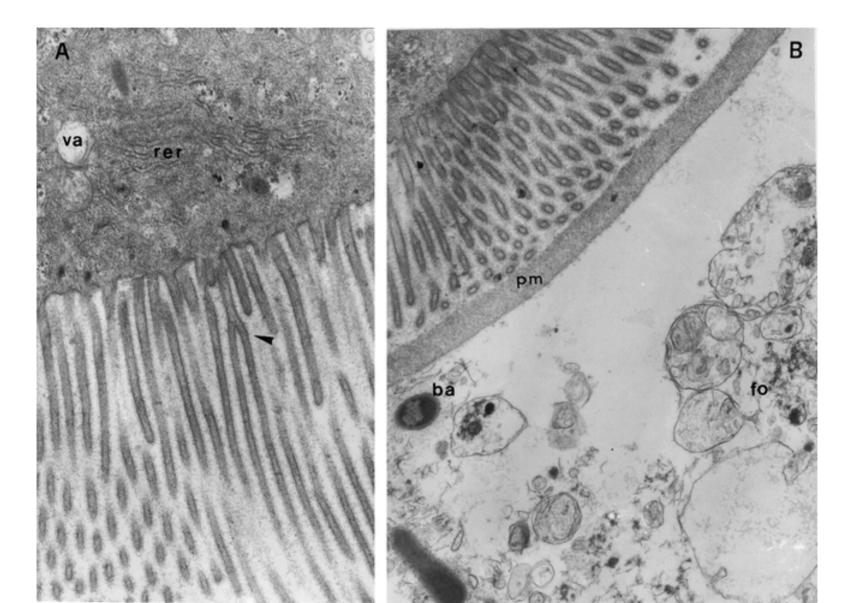


Midgut Ultrastructure of the Third Instar of *Dermatobia hominis* (Diptera: Cuterebridae) Based on Transmission Electron Microscopy. J. Med. Entomol., vol. 40

L. G. Evangelista,^b and A.C.R. Leite^{a, b}

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

PM=peritrophic matrix



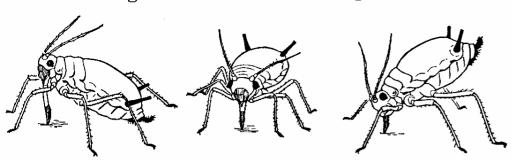
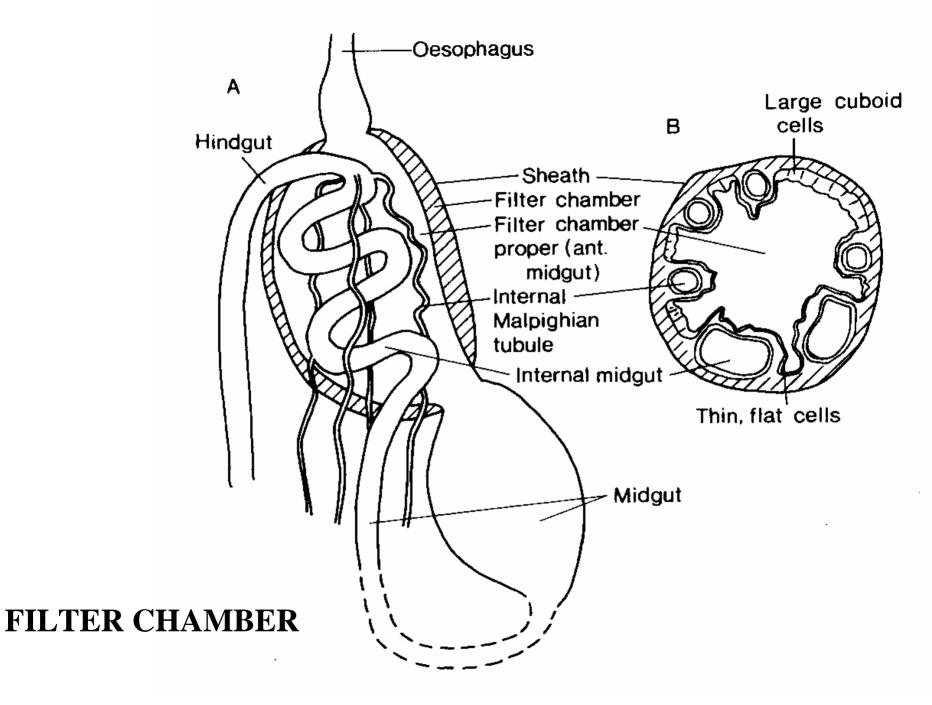


FIG. 185.—Attitudes of an aphis during feeding.

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

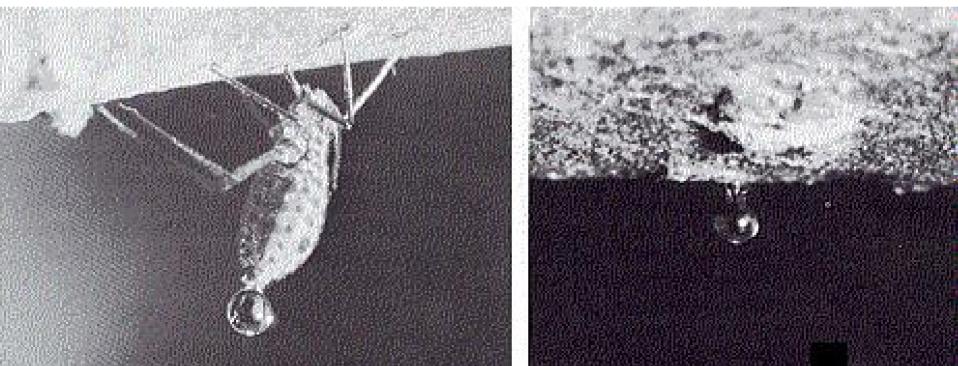
Sieve tube formed by saliva

 $4 \,\mu m$



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



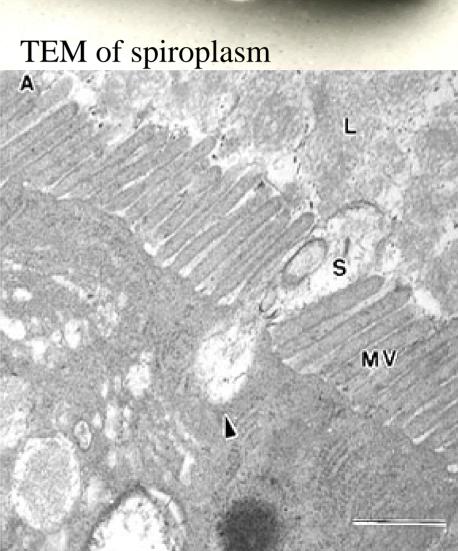
Parasite and pathogen penetration through the midgut

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.



- *Dalbulus maidis*, leafhopper vector of corn stunt spiroplasma. (Courtesy A. Wayadande)
- Photo to the right shows the spiroplasm penetrating the midgut wall through the microvilli.

http://www.apsnet.org/education/In troPlantPath/PathogenGroups/fastid ious/



EATING FROM THE POISONOUS PLATTER



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 this in humans?

HINDGUT

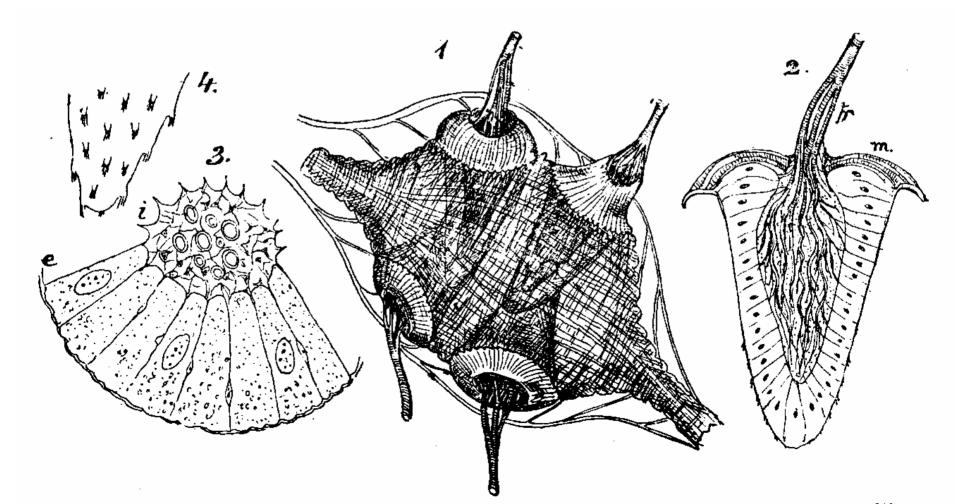
- 1. Major refuse dumping area for waste products from midgut and Malpighian tubules
- 2. In insects that feed on dilute foods (i.e., low in amino acids), such as plant saps or blood, the midgut is involved in getting rid of excessive water and also in housing symbionts that use these waste products 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 s 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 digestions 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

Rhodnius

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

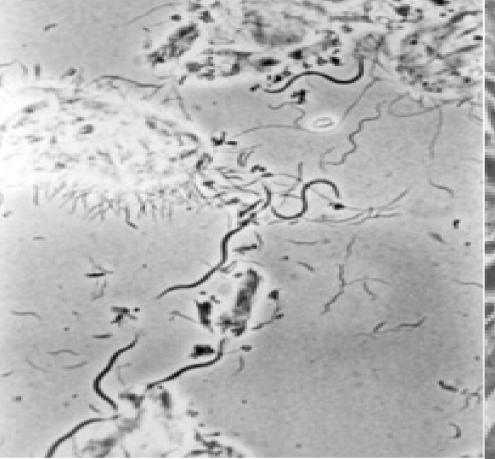


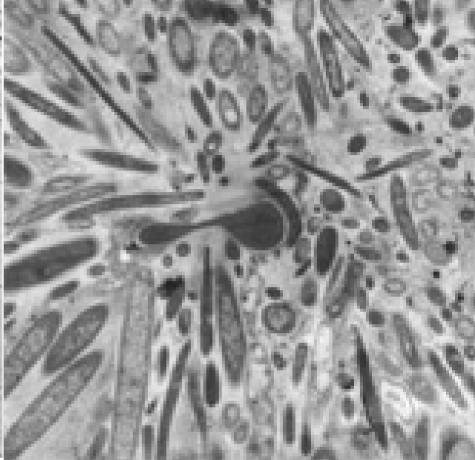
Note the dark abdomens of the workers and soldiers, which are mainly taken-up by the fermentation chamber, thus it appears dark.



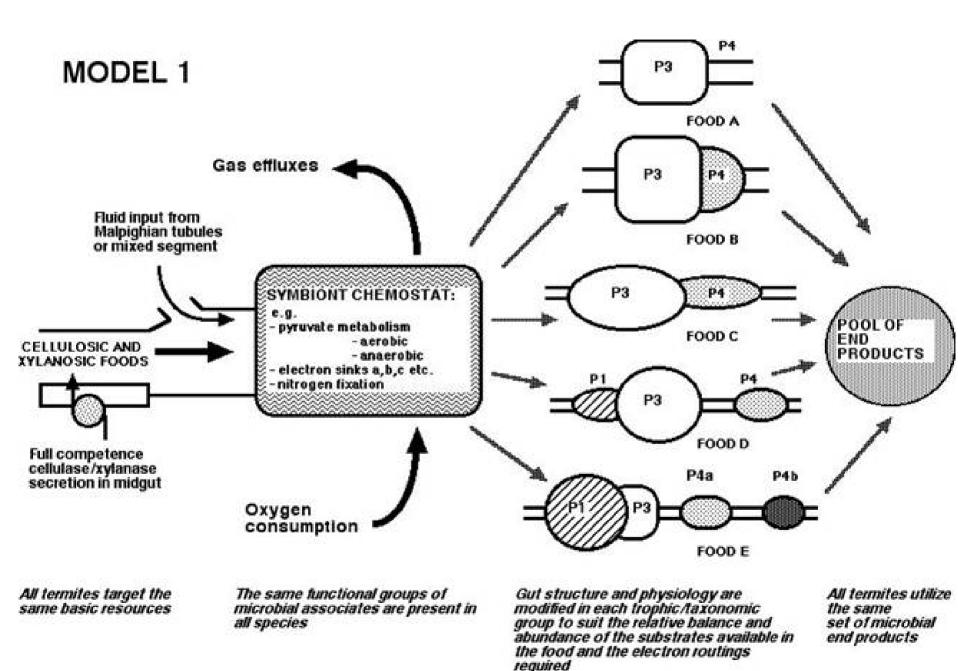


The dark area of the midgut is often called the fermentation chamber. This is where the symbionts aid in digestion of cellulose and other molecules that are essential to the life of the host. Kostas Bourtzis and Thomas Miller (editor). 2003. Insect Symbiosis. CRC Press, Boca Raton, FL

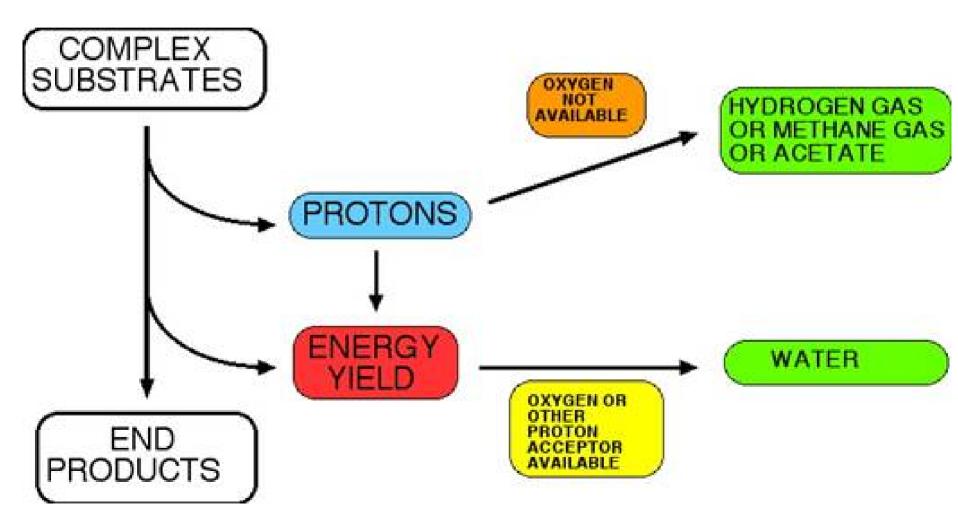




Spirochaetes from the intestine of Reticulitermes flavipes, magnified 2000 times. Large cells in the upper part of the micrograph (with attached bacteria) are the protists. Photo, J. Breznak. Copyright, Kluwer Academic Publishers. Transmission electron micrograph of the contents of the posterior hindgut 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).



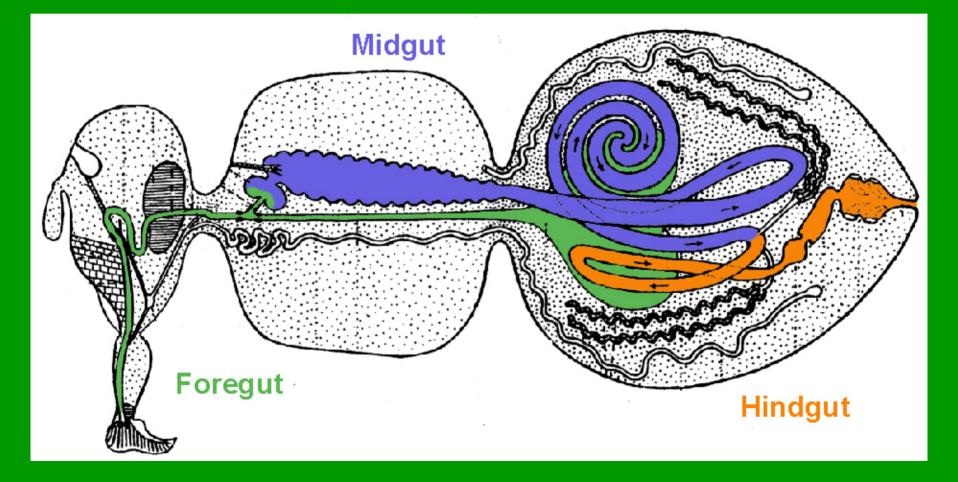
An environmental concern by some is that from all of these insects that house symbionts, especially termites, hydrogen gas or methane gas is being given off in large quantities when you consider termites worldwide, thus air pollution.

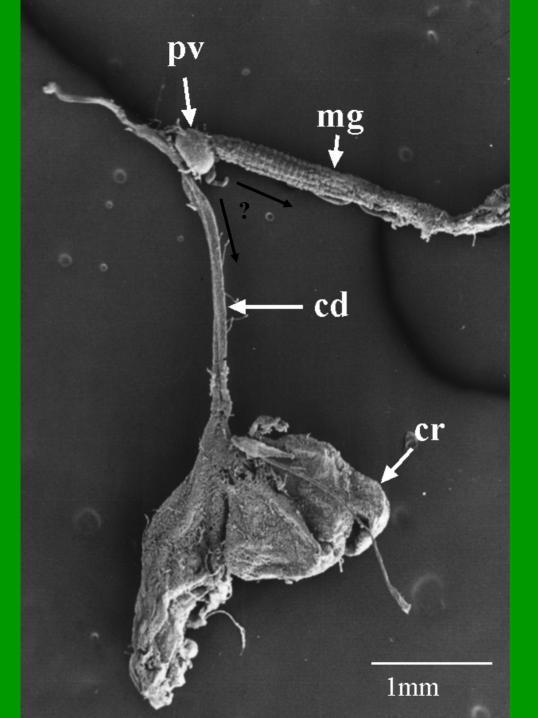


Neurohormones and Feeding in Adult Diptera

The occurrence and putative functions of feeding-related neuropeptides and biogenic amines in adult flies

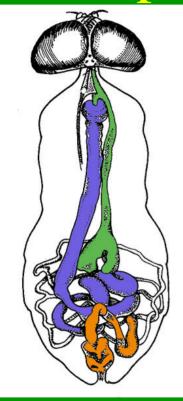
Dipteran alimentary tract



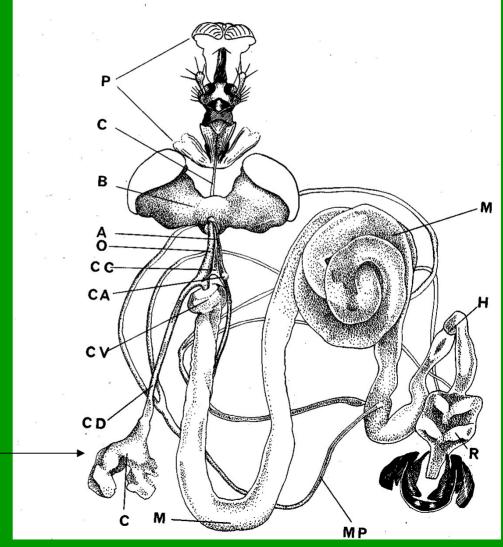


- bilobed blind sac in abdomen
- storage reservoir for patchily distributed resources

Dipteran alimentary tract



Crop: Bilobed, blind sac in abdomen

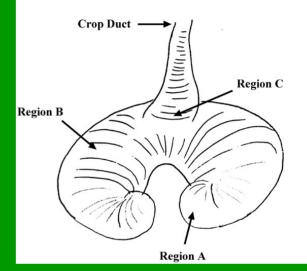


Meal destination and control

- in hematophagous flies, protein goes to distensible midgut, carbohydrates go to crop
- in *Phormia*, protein and carbohydrates go to crop first
- meal is terminated when crop is fully distended

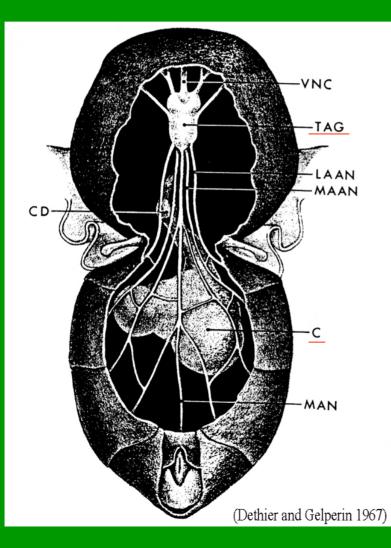
The Dipteran crop

- as crop fills, it is constantly contracting (myogenic)
- contraction frequency increases with volume ingested

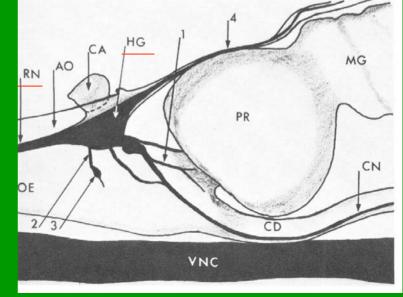


- filled crop is detected by stretch receptors in the abdominal nerve net
- stretch receptors send inhibitory feedback to brain and feeding ceases food is later forced into midgut

Abdominal nerve net







Bubbling

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



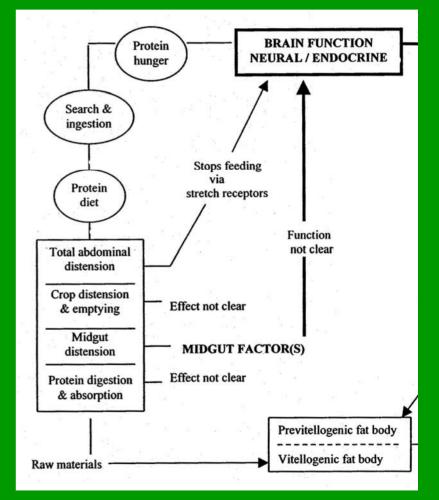


In the midgut

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

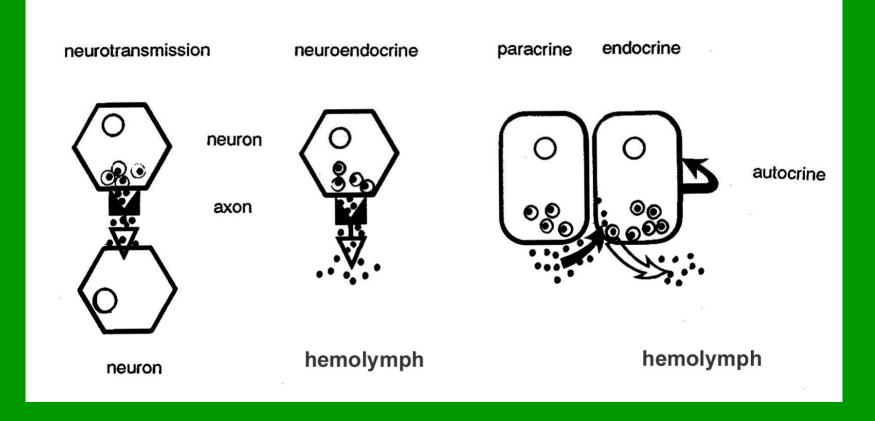
How is this all controlled?

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



Messenger molecules

Transmitters, hormones, and/or modulators

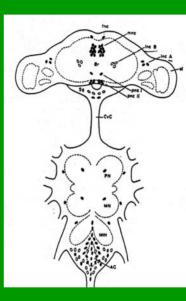


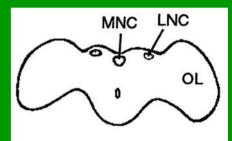
Neuropeptides

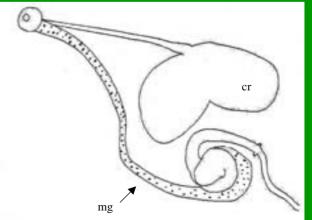
- peptides produced in brain, gut, or other tissues that affect neurons and non-nervous tissues
- act as neurohormones, neuromodulators, and neurotransmitters
- neuropeptides integrate brain function and systems of the body
- grouped into families based on amino acid sequence
 conserved carboxy terminus (usually amidated)

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) structure: TDVDHVFLRFamide
- CNS, alimentary tract with DMS IR cells and processes
- DMS applied to *in vitro* crop preparation

DMS and crop contractions

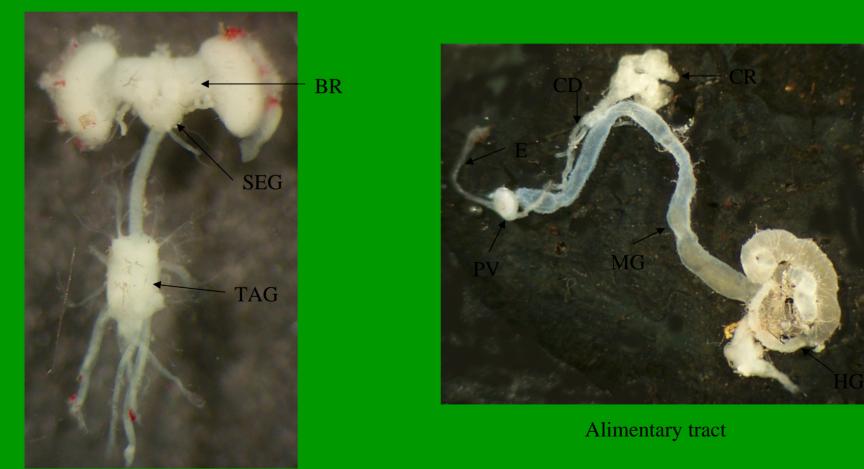


Application of 10-6 M DMS reduced crop contractions by 95% (from 46 to 2 contr./min)

Messengers investigated

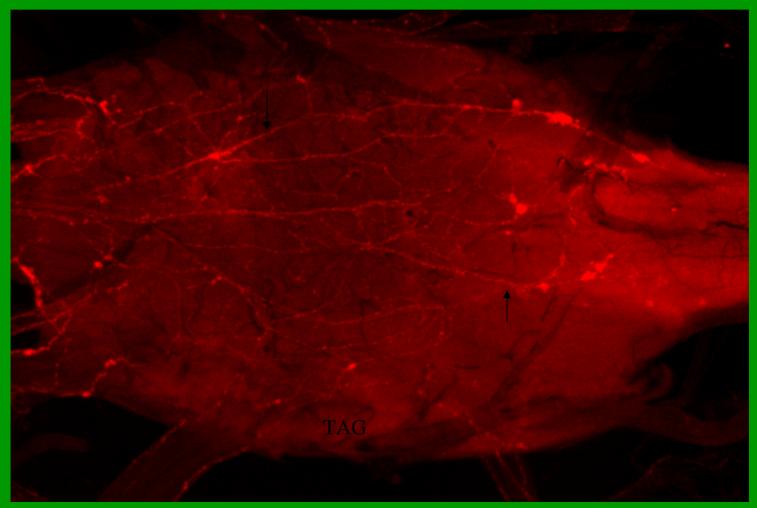
- Serotonin (5-hydroxytryptamine or 5-HT)
- FMRFamide related peptides
 - FMRFamide
 - Perisulfakinin (PSK): EQFDDY(SO₃)GHMRFamide

Phormia CNS and alimentary tract



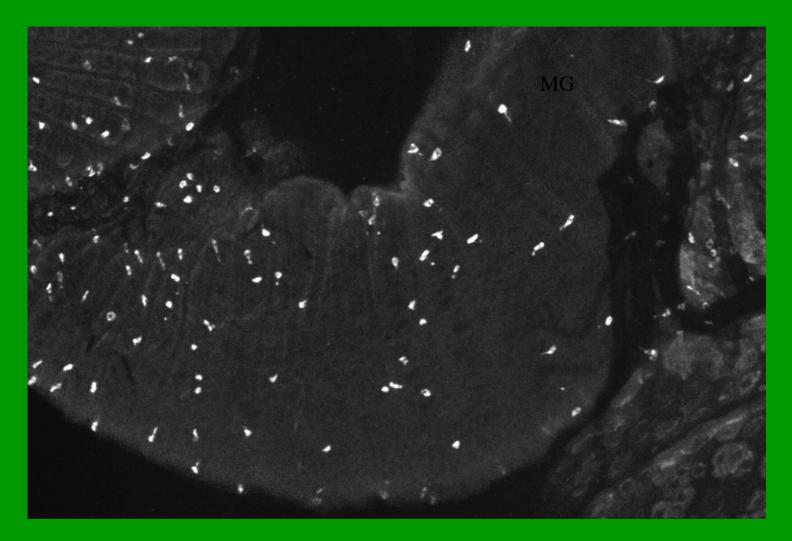
CNS

Phormia - Serotonin



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 (Macauart)

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

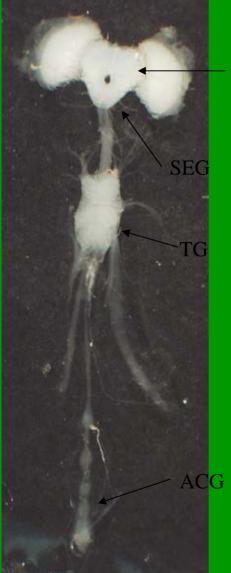




Salt marsh Newbury, MA

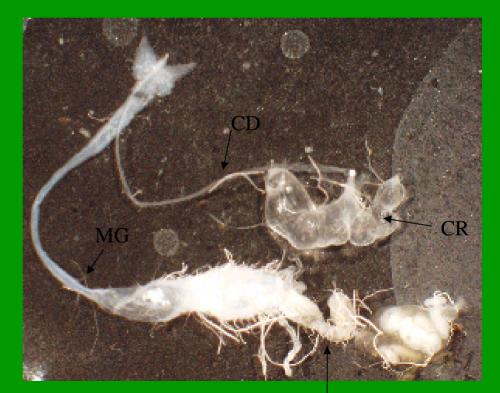


Tabanus CNS and alimentary



tract

BR

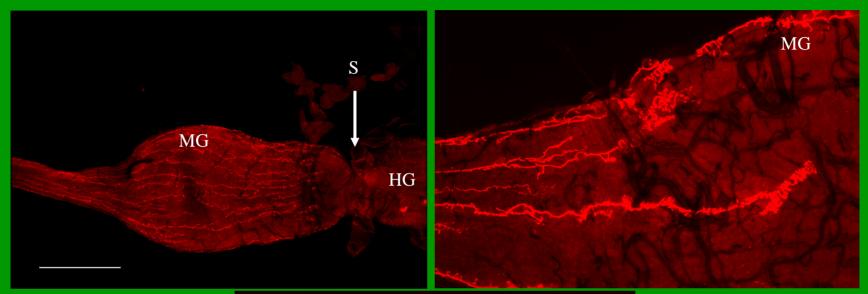


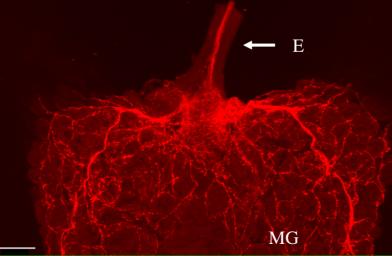
Alimentary tract

CNS

HG

Tabanus - Serotonin IR





5-HT immunoreactivity in the midgut of the horse fly

α-methyl-tryptophan (AMTP) injections

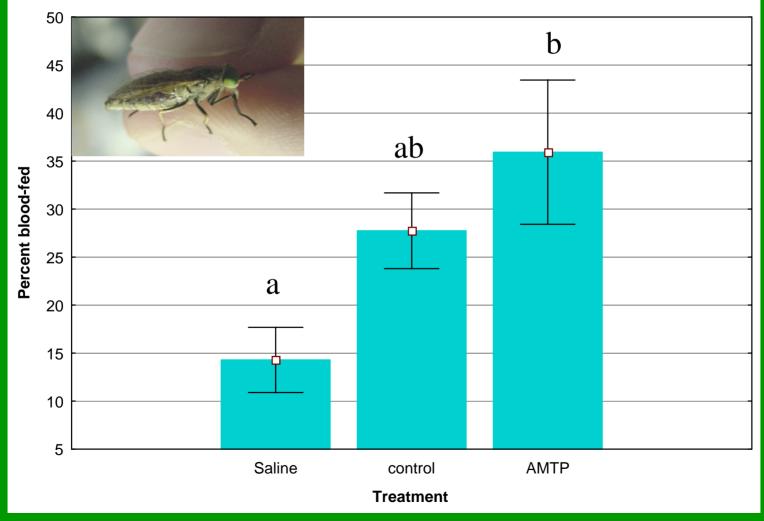
- serotonin has been implicated in feeding control in many insects (*Rhodnius, Calliphora*)
- AMTP selectively depletes serotonin
- horse flies were injected with 50 µg AMTP
- flies were provided with warmed cow's blood at 24 h post-injection and allowed to feed for 30 min





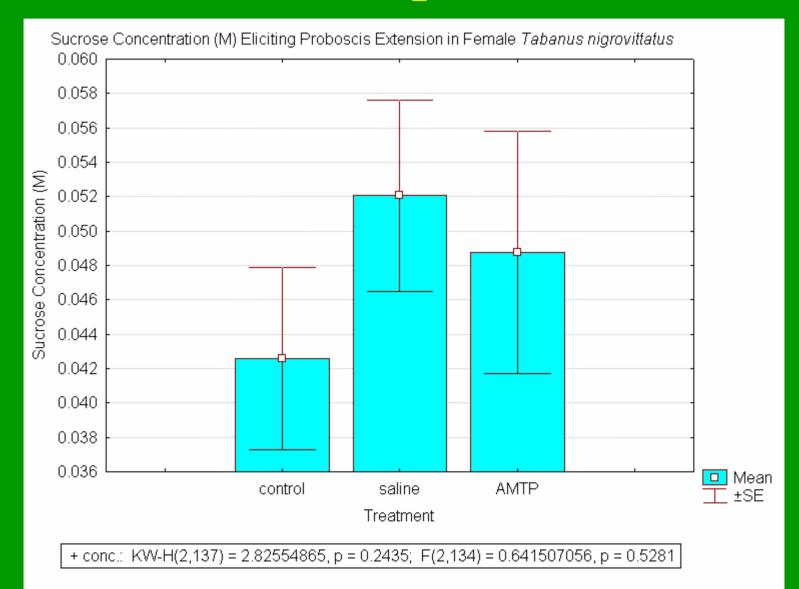
AMTP injections

Percent blood-fed Tabanus nigrovittatus



24 h post-injection of 50 µg AMTP

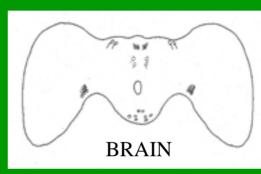
Mean tarsal acceptance threshold

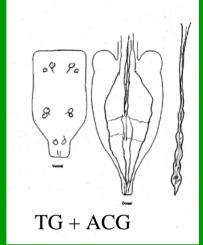


AMTP injection summary

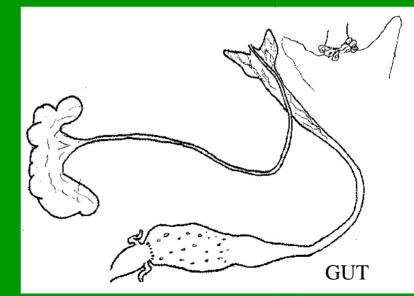
- Injections of 50 µg AMTP result in significant increase of blood fed flies at 24 h post injection relative to saline injected flies, and slight increase over controls
- Injections of 50 µg AMTP have no significant effects on mean tarsal acceptance threshold at 24 h post injection

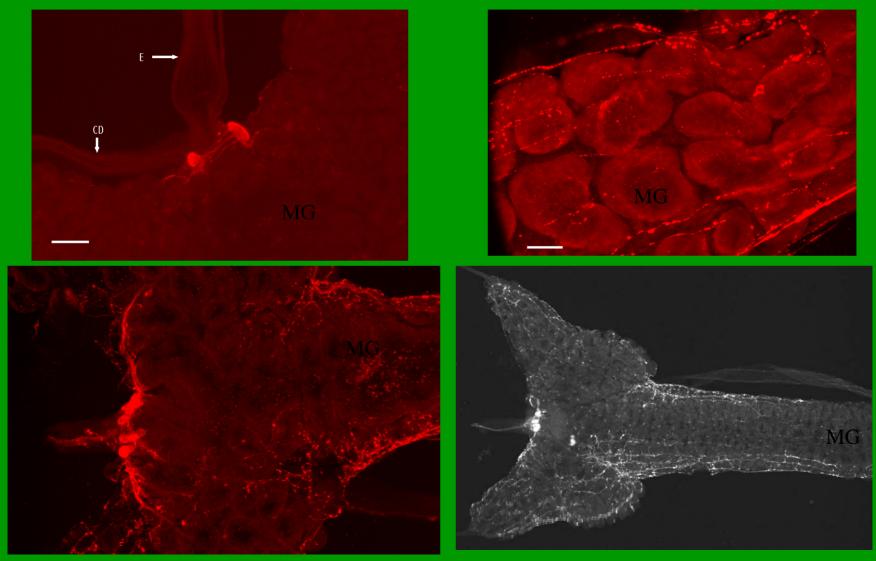
- brain
- thoracic ganglion



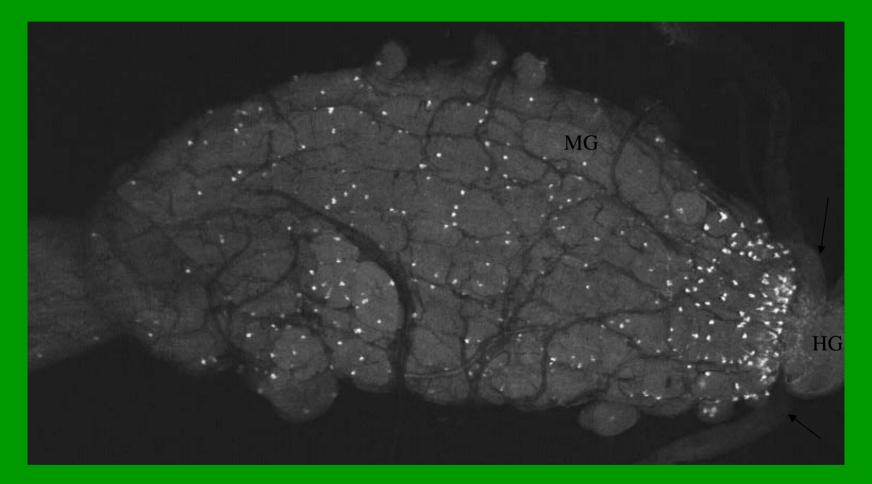


- abdominal chain ganglion
- midgut
- crop

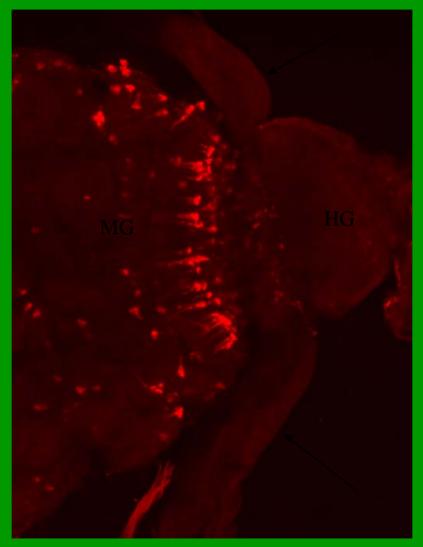




FMRFamide-like immunoreactivity in the midgut of the horse fly



FMRFamide-like immunoreactivity in the midgut of the horse fly



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 and muscle control

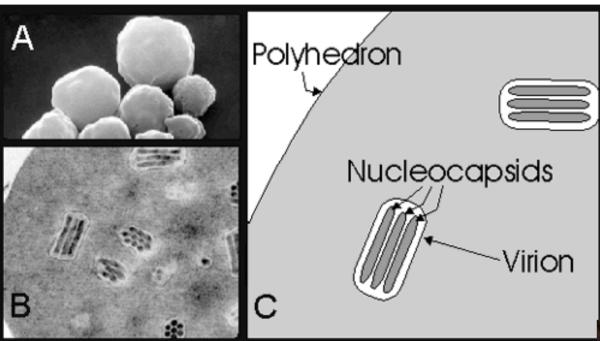
BASIC ASPECTS OF DIGESTIVE SYSTEM

- 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)
- 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.

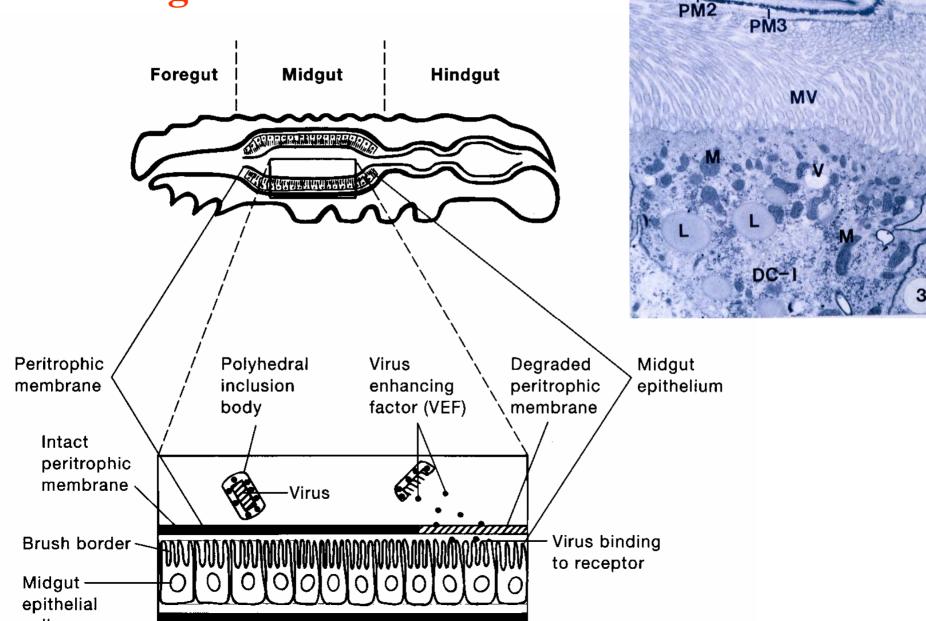


Beet armyworm killed By NPV

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



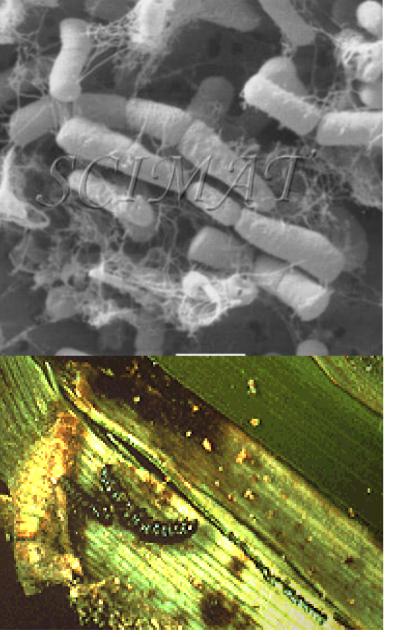
Nucleopolyhedrosis virus and the insect midgut



PM1

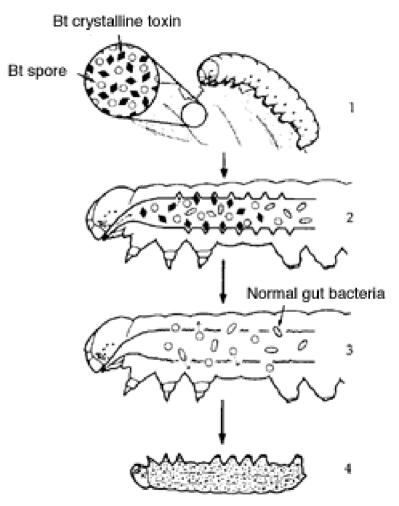
BT OR BACILLUS THURINGIENSIS

- 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* yar. *kurstaki* on caterpillars



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

MODE OF ACTION OF ALPHA-ENDOTOXIN

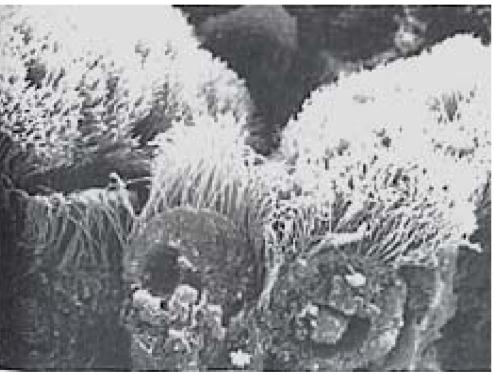
- 1. Bt bacterium enters the insect gut where pH is 6-10. Just right for the action of the bacterium.
- 2. The crystalline toxin aids in breaks through the peritrophic membrane and binds to a receptor on the brush forming a brush border membrane vesicle.
- 4. The alpha endotoxin inserts into the membrane 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.

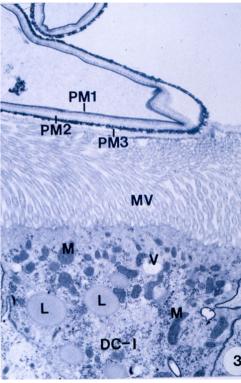
Now 5 biotypes

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

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.**

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.