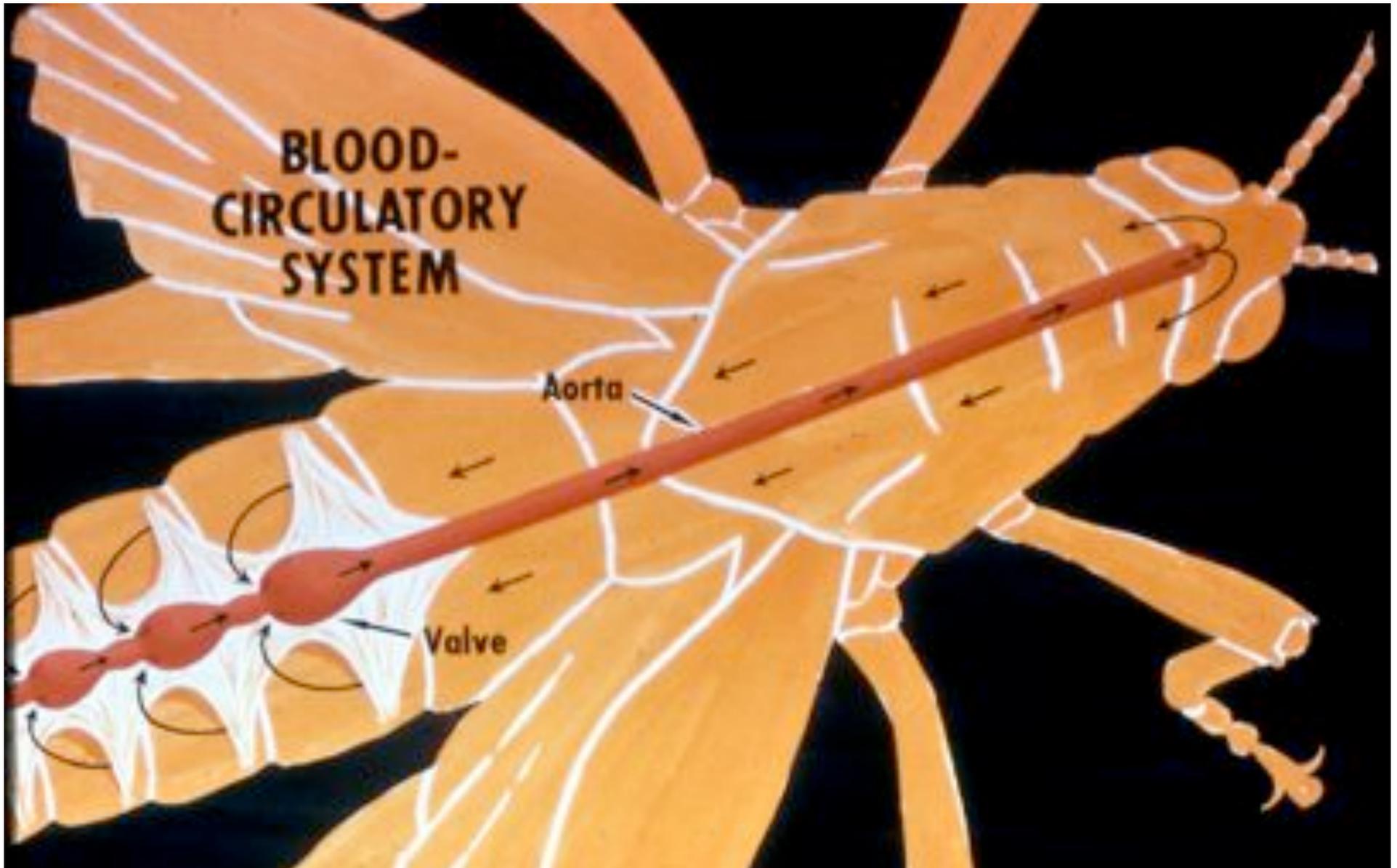
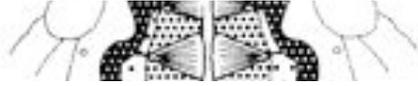
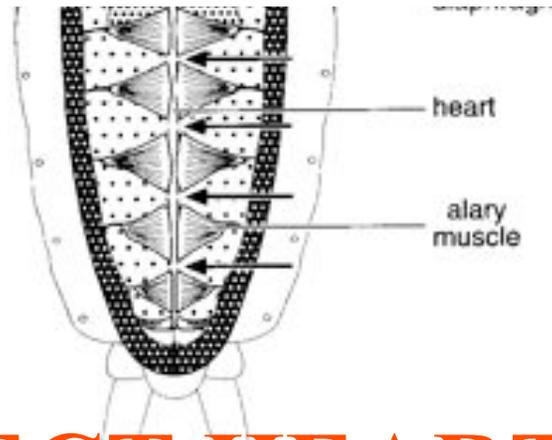


# CIRCULATORY SYSTEM





**HAPPY LATE VALENTINE'S DAY**



## **INSECT HEART**

Locke, M. 1997. Caterpillars have evolved lungs for hemocyte  
Gas exchange. J. Insect Physiol. 44: 1-20.

# **FUNCTIONS OF THE INSECT CIRCULATORY SYSTEM**

## **HEMOLYMPH OR PLASMA**

- Lubricant for tissues
  - Hydraulic medium (caterpillars, molting, extrusion of osmeteria, ptilinum and wing expansion)
4. Transport medium for many molecules, including wastes
  5. Storage (amino acids and glycerol)
  6. Protection (reflex bleeding)
  7. Non-cellular (non-encapsulating) immune response molecules and pathways

## **HEMOCYTES**

1. Phagocytosis
2. Coagulation
3. Encapsulation of foreign objects (eg., including parasite)

# CIRCULATORY SYSTEMS OF HUMANS + INSECTS

## HUMANS

1. Closed system with blood vessels (capillaries, veins, arteries) and heart
2. Heart is located ventrally
3. Red blood cells carry oxygen and carbon dioxide. Use a hemoglobin carrier molecule
4. Have a true immune response, long-term recognition system  
Acquired immunity

## INSECTS

1. Open system-hemocoel  
Dorsal blood vessel
2. Heart and aorta are dorsal
3. No red blood cells.  
Some insects have hemoglobin.  
Oxygen delivered by tracheal system
4. Do not have a long-term immune recognition system  
No acquired immunity

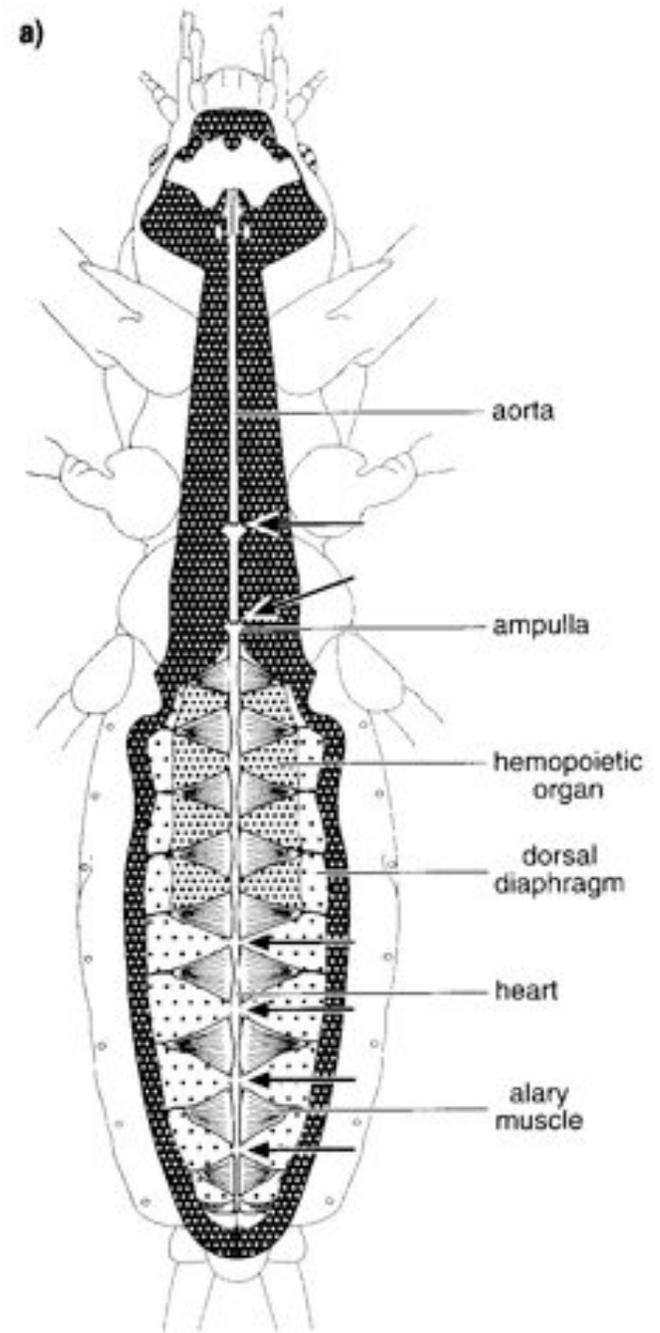
- A. Dorsal blood vessel
  - 1. Heart – pacemaker cells
    - a. Ostia
  - 2. Aorta
- B. Hemolymph
- C. Hemocytes
- D. Hemopoietic organs
- E. Wound healing
- F. Cellular immune responses
- G. Non-cellular immune responses
- H. Accessory pulsatile organs
- I. Dorsal and ventral diaphragms
- J. Neurohormonal control of heartbeat
- K. Alary muscles
- L. Reflex bleeding
- M. Thermoregulation and circulatory system
- N. Parasitic adaptation (giant cells and virus)
- O. Cytochrome P450 enzymes present in hemolymph

## A. DORSAL BLOOD VESSEL

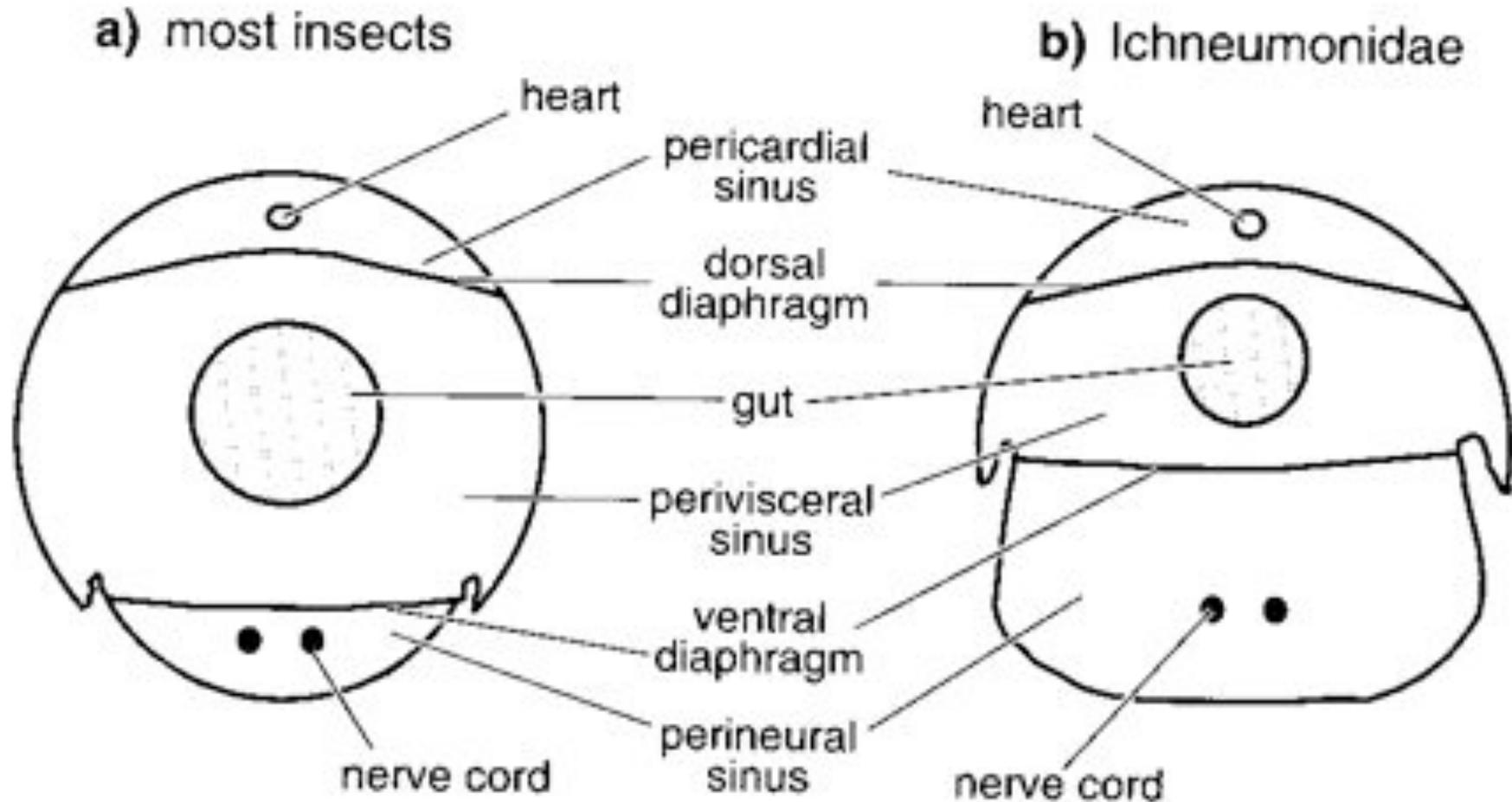
The dorsal blood vessel is open in the heart area by small openings called **ostia**. In the relaxed stage, the ostia open and hemolymph enters. When the heart contracts, the valves of the ostia are closed by pressure and the hemolymph is moved forward and dumped into the head area.

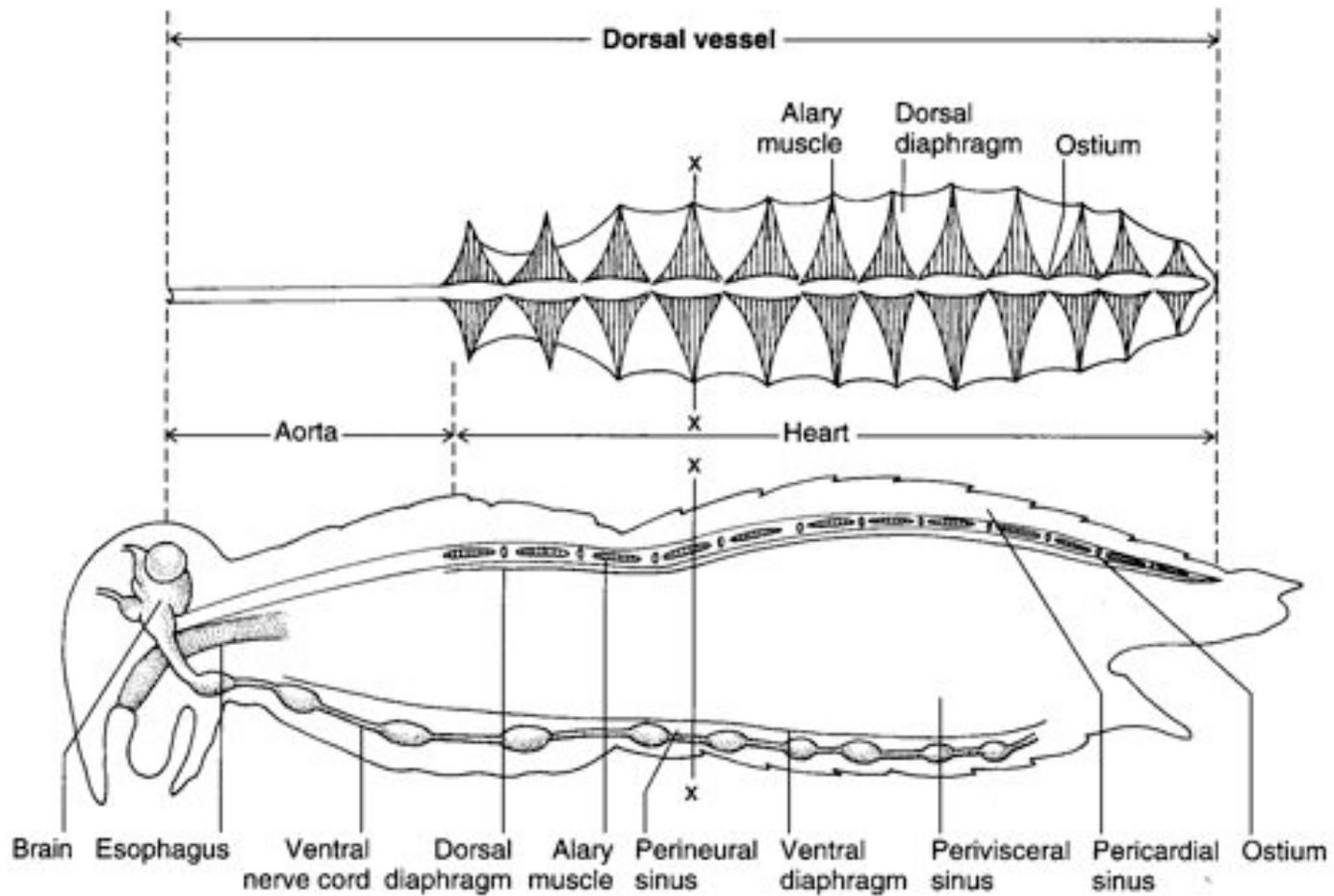
The heart is also aided in contractions by a special set of muscles known as the **alary muscles**.

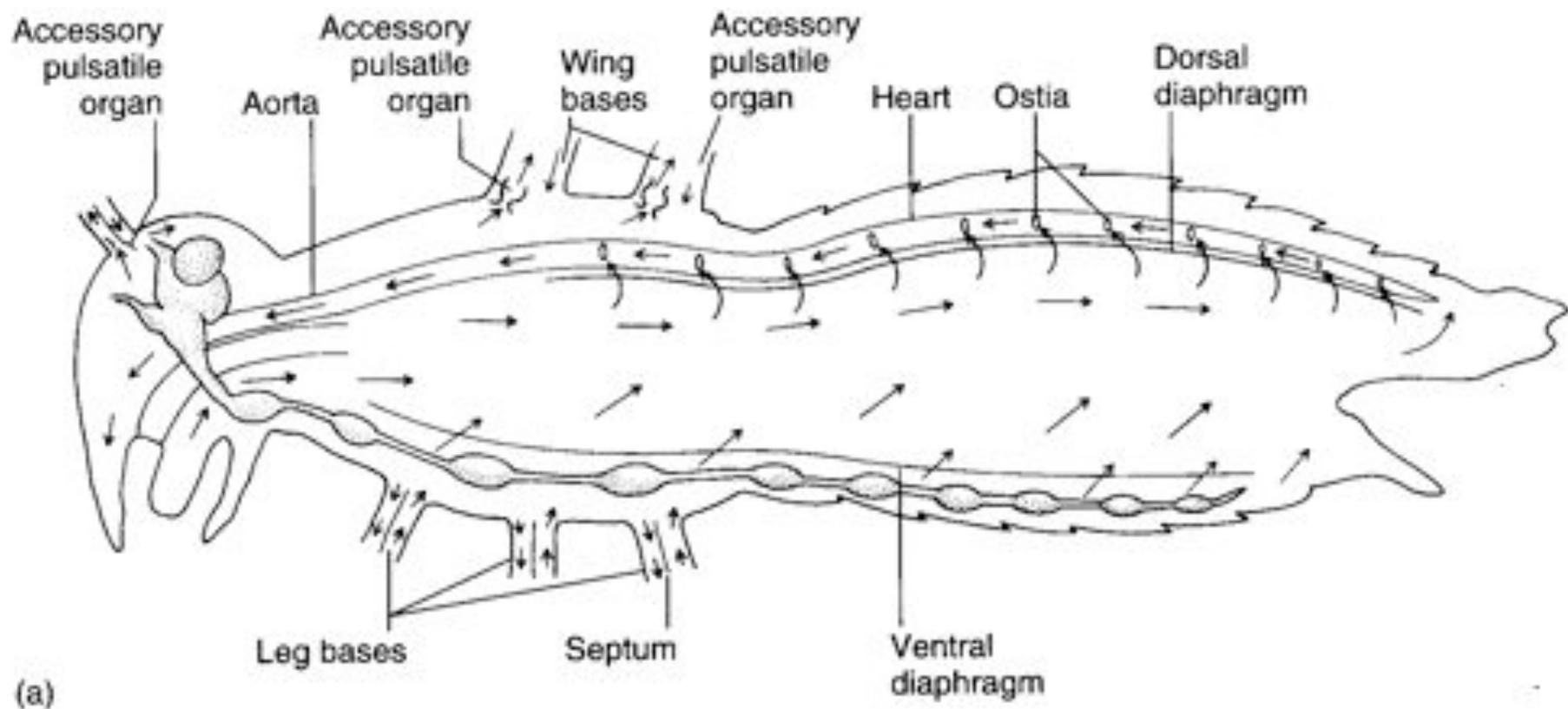
Blood circulation is also aided by the presence of a **dorsal and ventral diaphragms**.



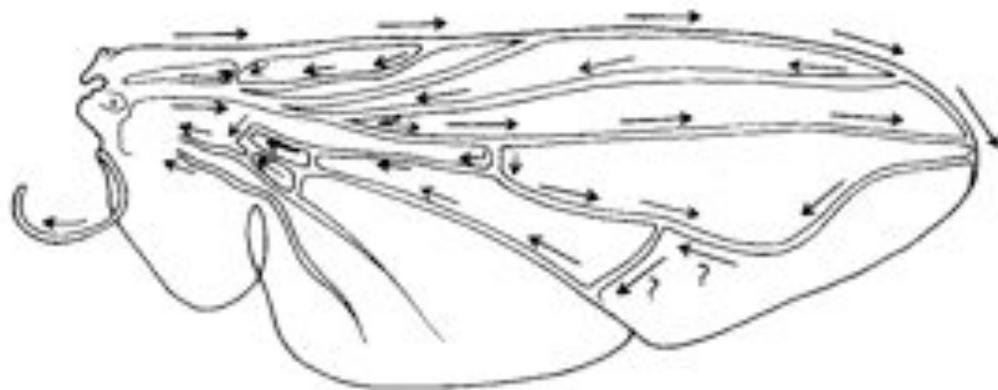
An open system, like the insects, has problems with circulation. To aid in hemolymph movement, insects have evolved a dorsal and ventral diaphragm, which consists of sets of muscles that divide the hemocoel into various sinuses. Their contraction aids in movement within these sinuses.







(a)



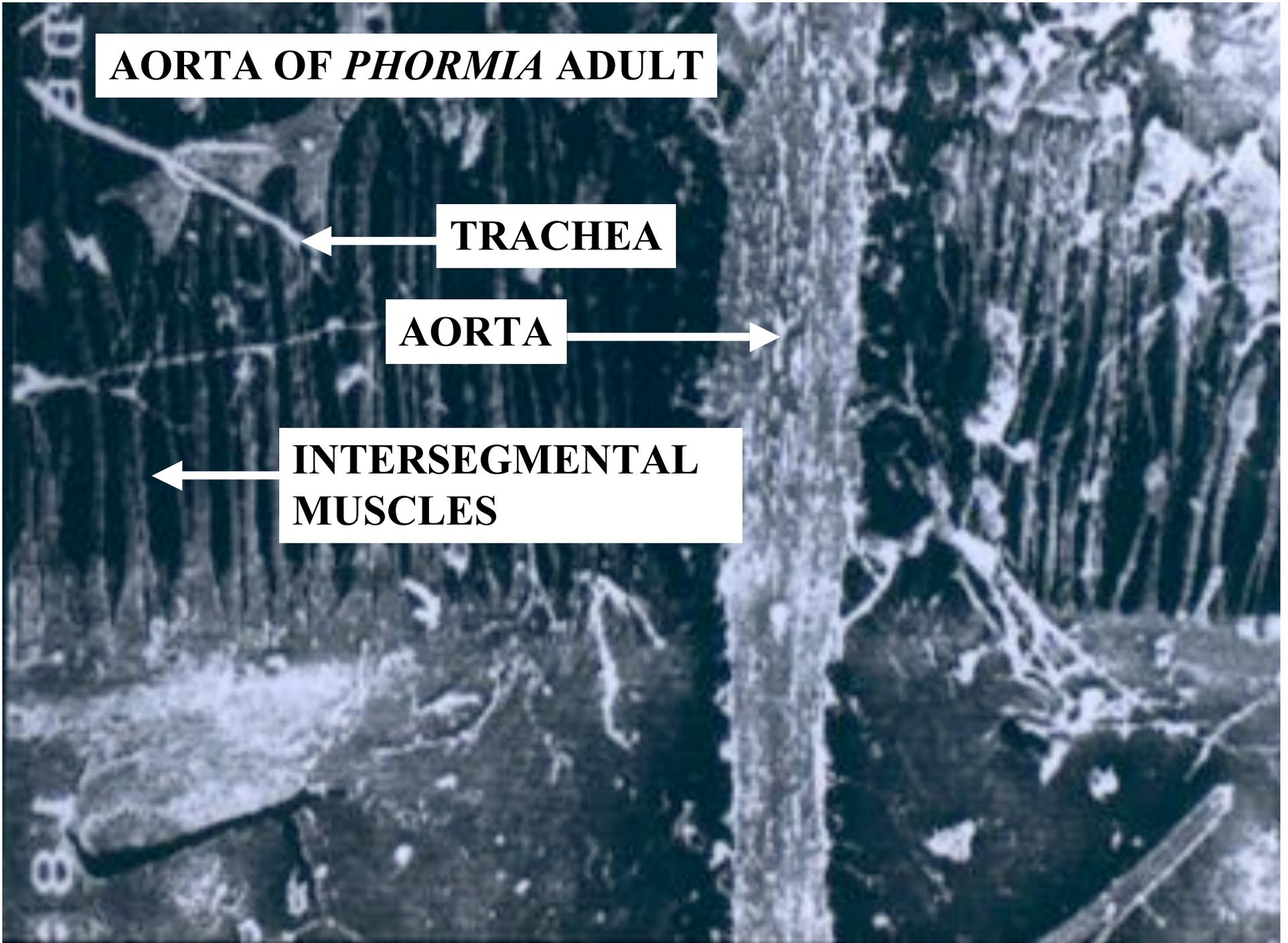
(b)

**AORTA OF *PHORMIA* ADULT**

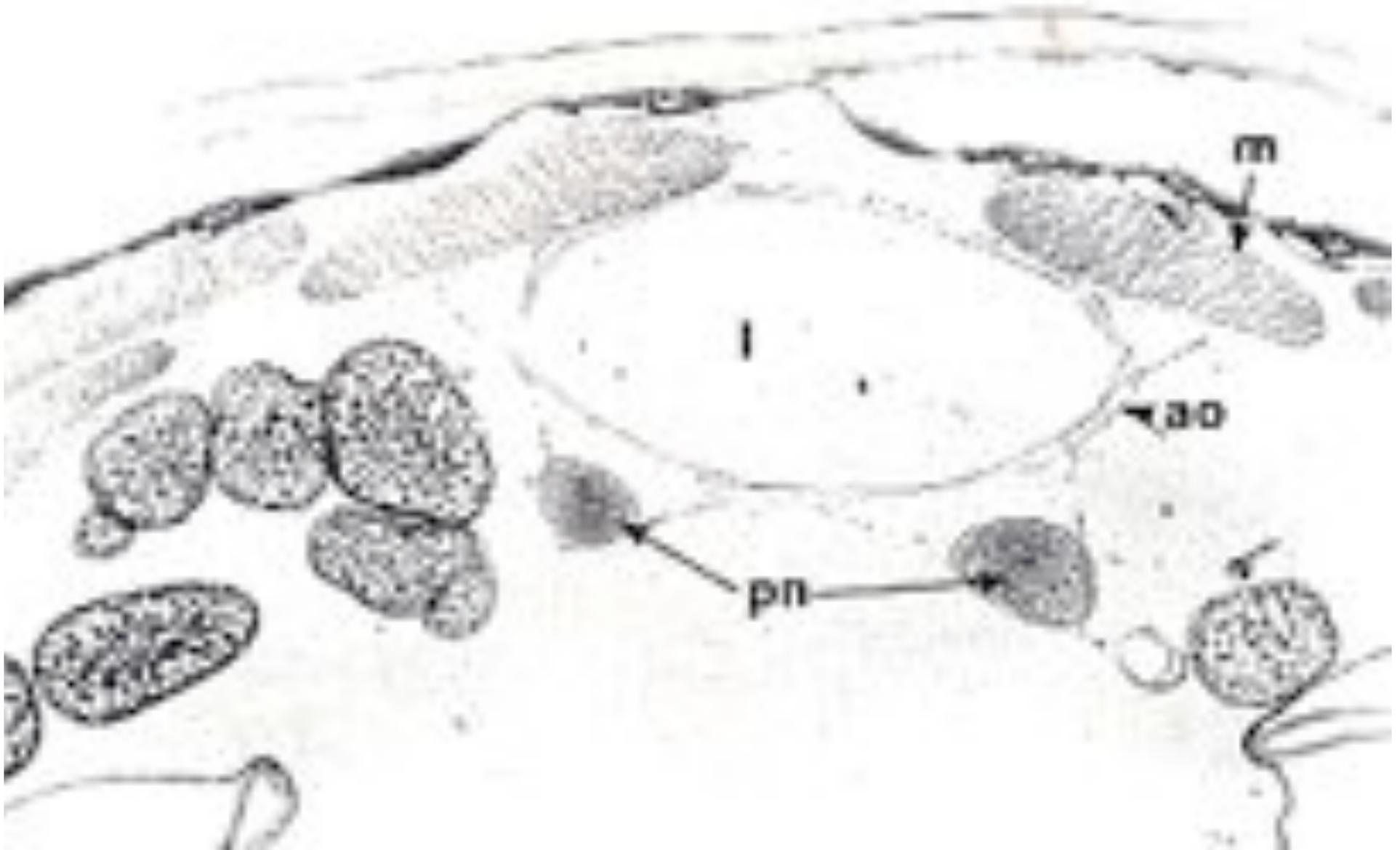
**TRACHEA**

**AORTA**

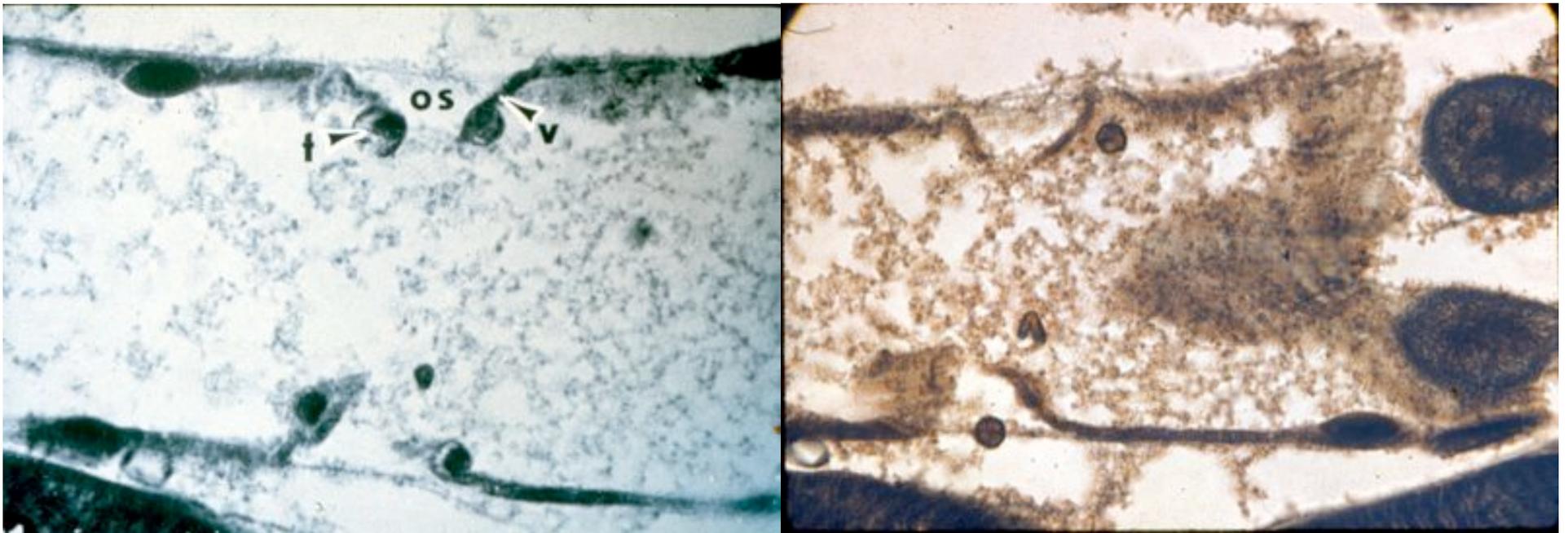
**INTERSEGMENTAL  
MUSCLES**

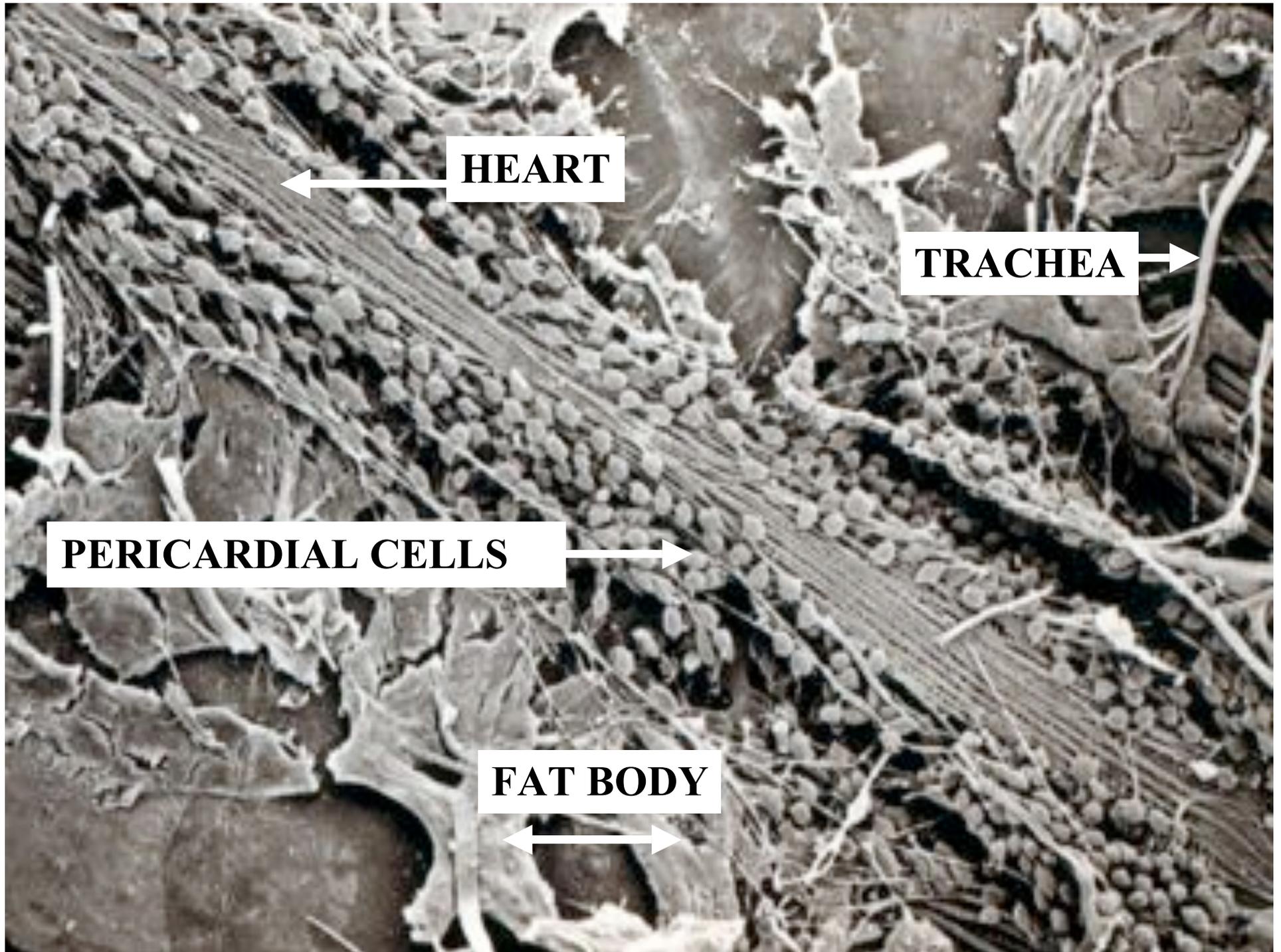


Cross-section of *Phormia* larva showing the aorta (ao), its lumen (l) and the surrounding pericardial cells (pn). Note the net-like fat body.



Two different longitudinal sections of the heart of a fly larva showing the interior lumen of the heart and coagulated hemolymph, the ostia (os) and the valves (v) that close the opening when the heart contracts. Note that no hemocytes are present inside the heart. Insect hemocytes do not normally enter the heart but remain in areas of hemopoietic tissue unless a foreign object gets into the system or the 'self' of the basement matrix is destroyed.





**HEART**

**TRACHEA**

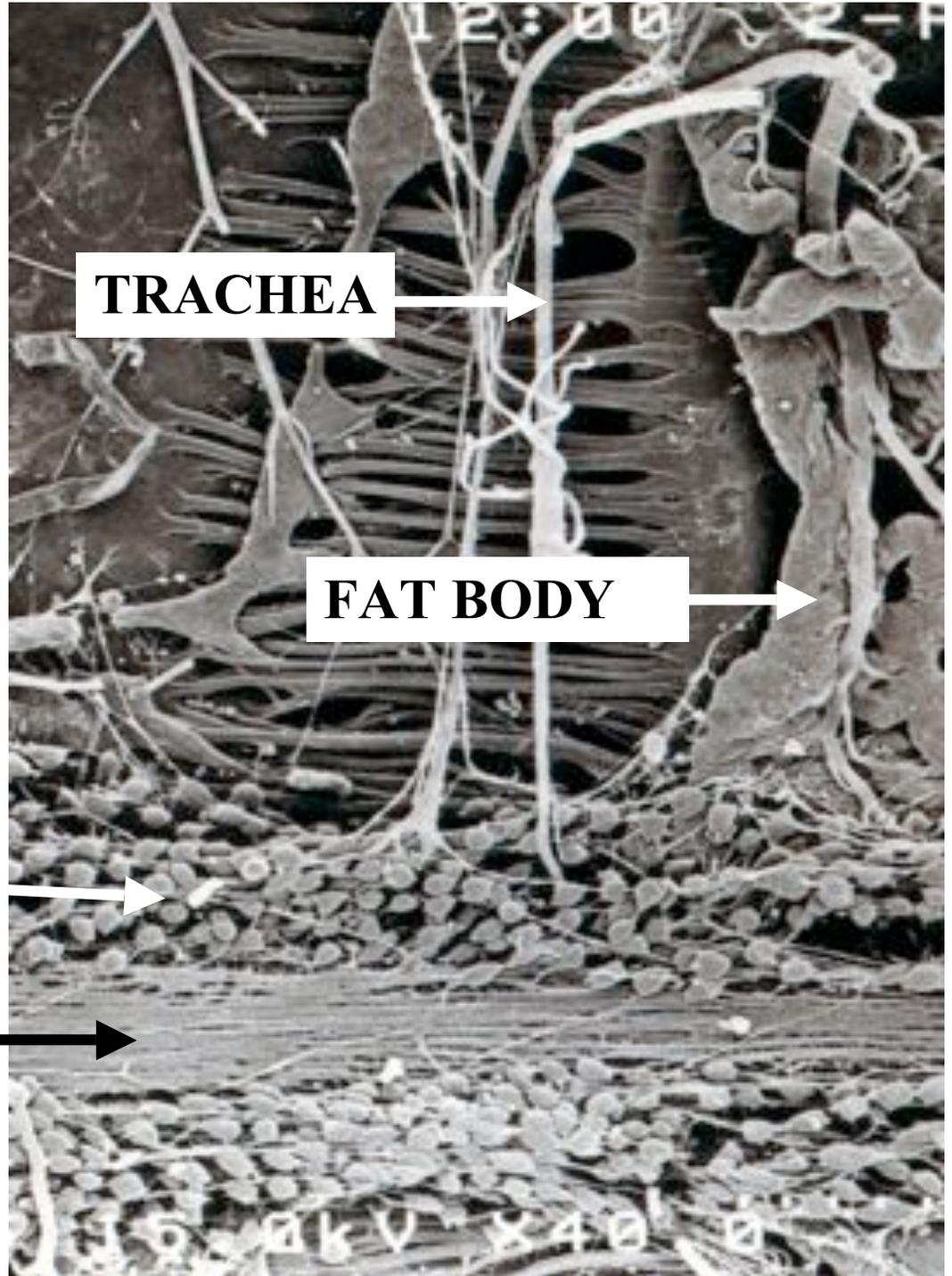
**PERICARDIAL CELLS**

**FAT BODY**

SEM of the heart and the surrounding pericardial cells. Notice the massive network of trachea, the globular fat body, and the intersegmental muscles between two abdominal sclerites. Notice the heart appears as a series of longitudinal muscles.

**PERICARDIAL CELLS**

**HEART**



# Heart Pacemakers

## Humans

1. More than one pacemakers
2. Hierarchy of pacemaker activity:
  - a. sinoatrial node
  - b. atrioventricular node
  - c. His bundle
3. Fastest pacemaker drives heart

## Insects

1. May be a few pacemakers but not in each individual segment
2. Each segment can maintain or assume pacemaker role with last posterior one setting the pace.
3. Not driven by one single pacemaker.

**A. Top trace shows intracellular recording from the last (6<sup>th</sup>) cardiac segment (EII). Lower trace is extracellular recording of aortic muscle fibers (EI).**

**B. As in A, but immediately after the heart was sectioned (see 's' in top drawing).**

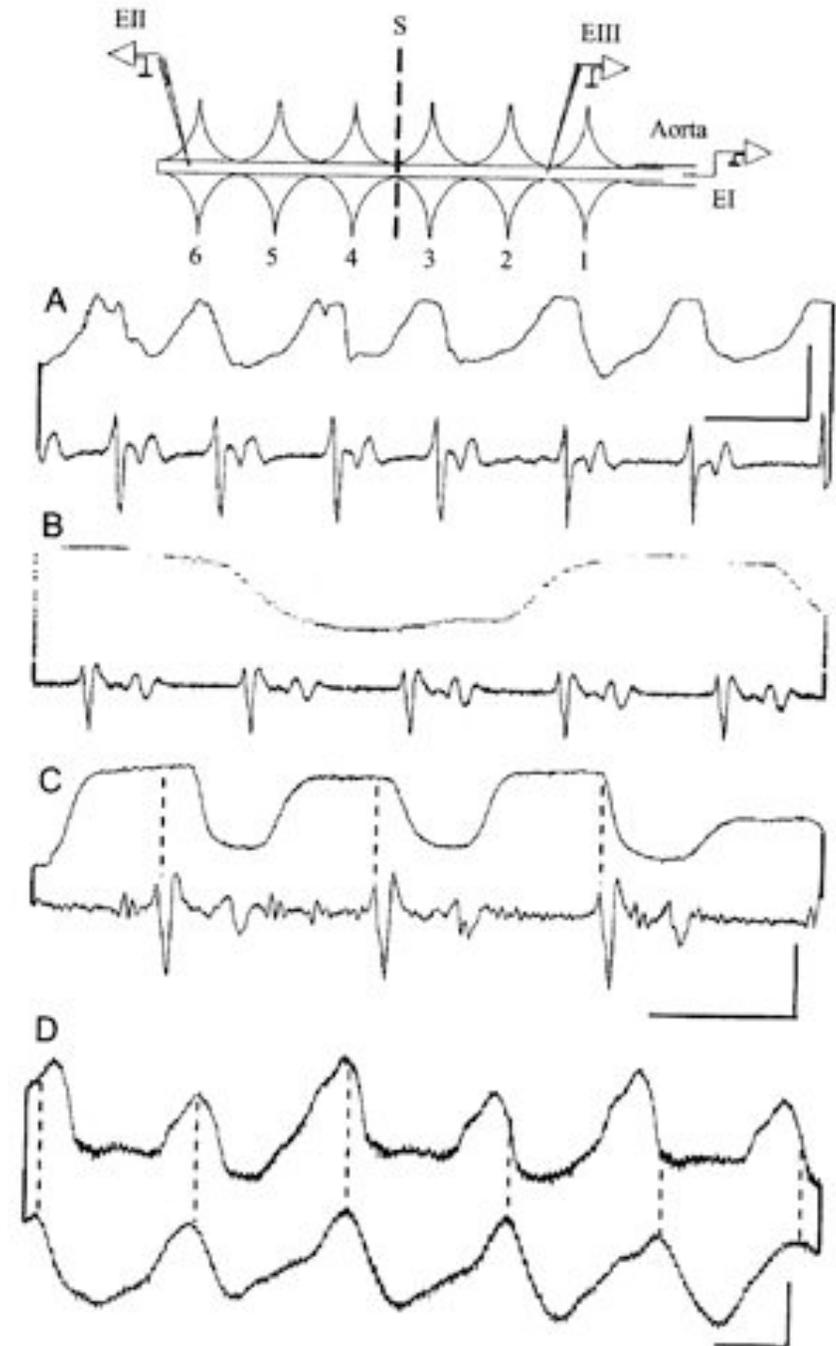
**C. As in B, but 10-15 mins. After sections.**

**D. Intracellular recordings of the two isolated halves 50 min. after sectioned. Top trace is intracellular recording from last 6<sup>th</sup> segment (EII) and lower trace intracellular recording from 2<sup>nd</sup> cardiac segment EIII.**

**Broken lines shows pacemaker action potentials drifting out of phase.**

#### **Conclusions:**

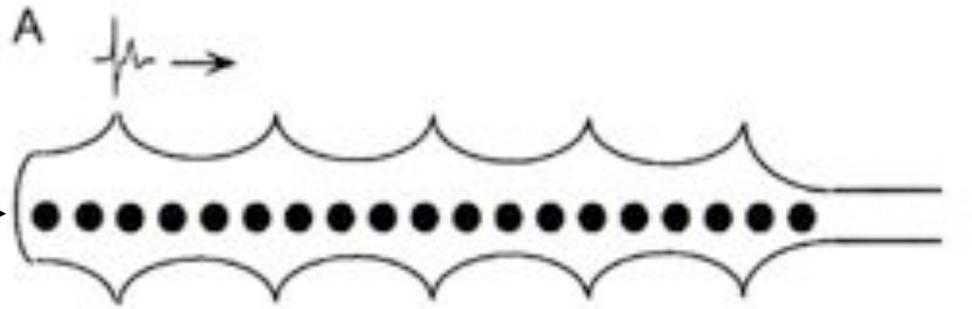
- 1 Pacemaker cells are only found in the heart region and in individual segments, not the aorta.**
- 2. Pacemaker cells are believed to not reside in each segment but there maybe single muscle fibers that extend the length of the heart.**
- 3. No pacemaker in the aorta.**



# Pacemaker cells

Markou, T. and G. Theophilidis. 2000. The pacemaker activity generating the intrinsic myogenic contraction of the dorsal vessel of *Tenebrio molitor* (Coleoptera). J. Exp. Biol. 203: 3471-3483.

Old model with individual or many pacemaker cells in each segment



New model with a few pacemaker cells running the length of the heart

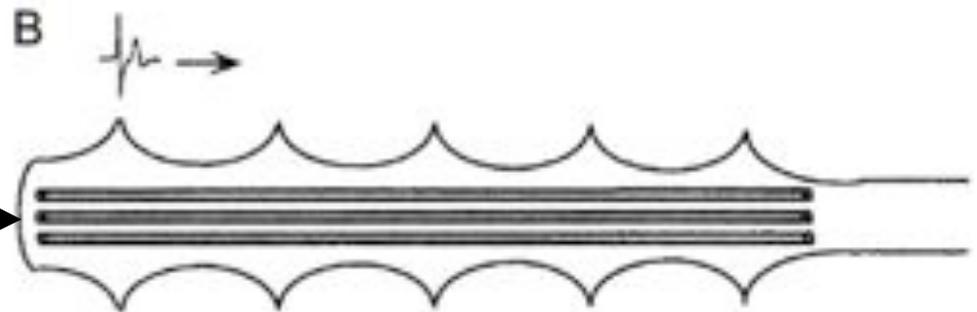


Fig. 8. Two possible mechanisms involved in the control of the spontaneous rhythmic contractions of the dorsal vessel of *Tenebrio molitor*. (A) A model with a series of individual myocardial cells with pacemaker properties. (B) A model of single longitudinal differentiated muscle fibres with pacemaker properties running along the length of the heart.

## B.

### HEMOLYMPH OR PLASMA

- Lubricant for tissues
- Hydraulic medium (caterpillars, molting, extrusion of osmeteria, ptilinum and wing expansion)
- 4. Transport medium for many molecules, including wastes
- 5. Storage (amino acids and glycerol)
- 6. Protection (reflex bleeding)
- 7. Non-cellular immune response molecules and pathways

### COMPOSITION OF HEMOLYMPH

1. **90% Water**
2. **Inorganic ions, ex. Na, Ca, K,**
3. **Nitrogenous wastes, uric acid**
4. **Carbohydrates-Alpha trehalose (most imp. insect blood sugar)  
glucose, glycerol (antifreeze)**
5. **Lipids**
6. **Proteins and enzymes (MFO's)**
7. **Antifreeze proteins**
8. **Pigments**
9. **Hormones**

# **Proteins found in insect hemolymph**

- 1. Storage proteins**
- 2. Lipophorins or lipid transport proteins**
- 3. Proteins taken up by the ovary (e.g., vitellogenin)**
- 4. Antibacterial and antifungal proteins**
- 5. Lectins**
- 6. Protease inhibitors**
- 7. Enzymes**
- 8. Peptides**
- 9. Chromoproteins**
- 10. Transport proteins**
- 11. Ice nucleators**
- 12. Heat shock and cold shock proteins**

## Physiological Insect Defense Mechanisms

### External

- a. Deterrents and antimicrobial agents
- b. Cuticular barrier

### Internal-external

- a. Reflex bleeding
- b. Cuticular encystment

### Internal

- a. Coagulation
- b. Humoral
  1. Inducible factors
    - Antibacterial/antifungal proteins
    - Lysozymes
  2. Constitutive factors
    - Lectins (agglutinins)
    - Phenoloxidasases
  3. Cellular
    - Phagocytosis
    - Nodule formation
    - Encapsulation

The father of insect Hemocytic and Immune responses was

**George Salt. 1970.**

**The cellular defence reactions of insects.**

**Cambridge Univ. Press., England**

KING'S COLLEGE,  
CAMBRIDGE.

26 Sept. 1973

Dear Professor Stoffolano,

Thank you for sending me copies of your two recent papers. I am especially glad to have your useful review of the host specificity of entomophilic nematodes because I have now returned, after several years, to the subject of host specificity among insect parasitoids. It is interesting to find that there is much in common between your problems and mine.

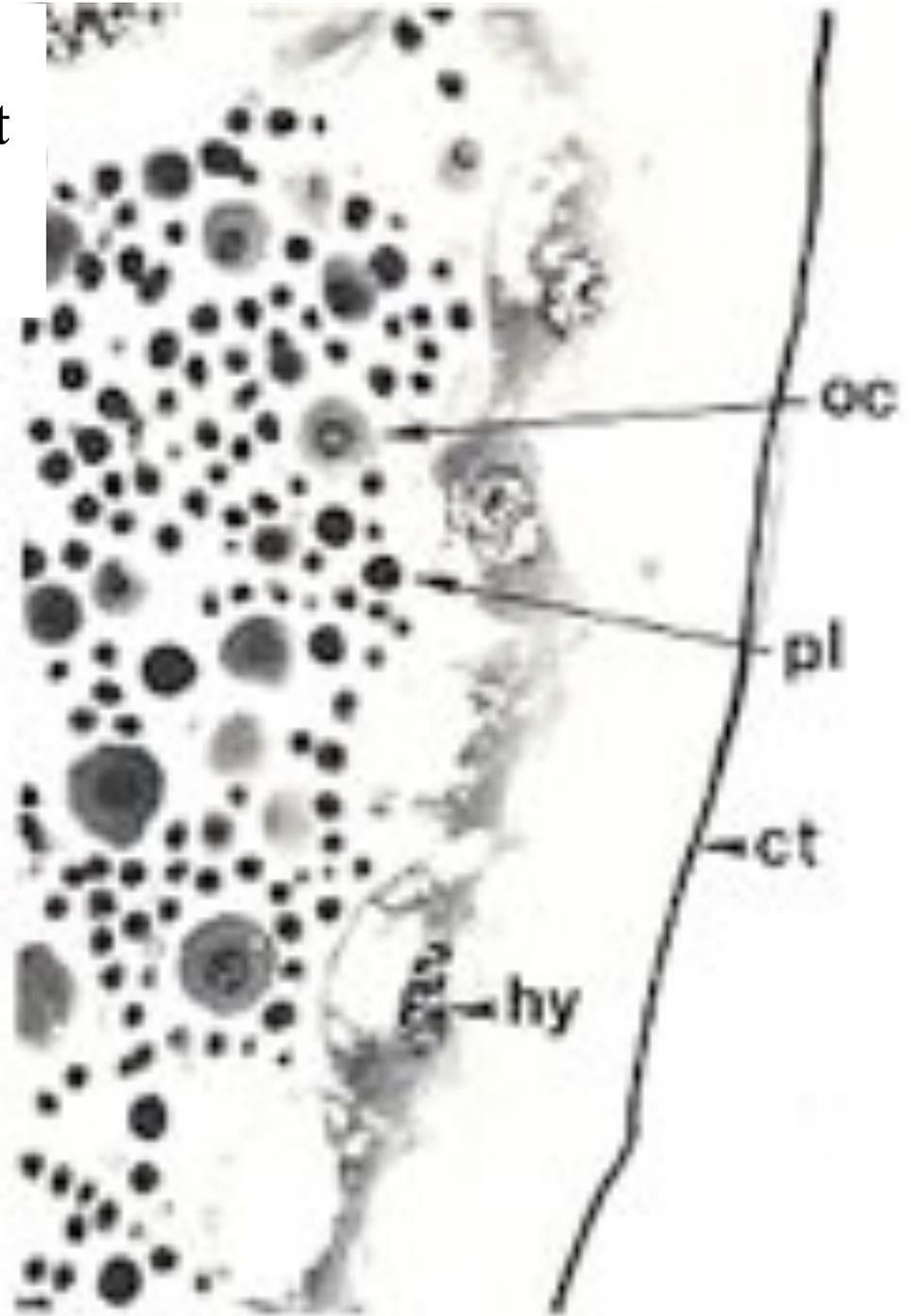
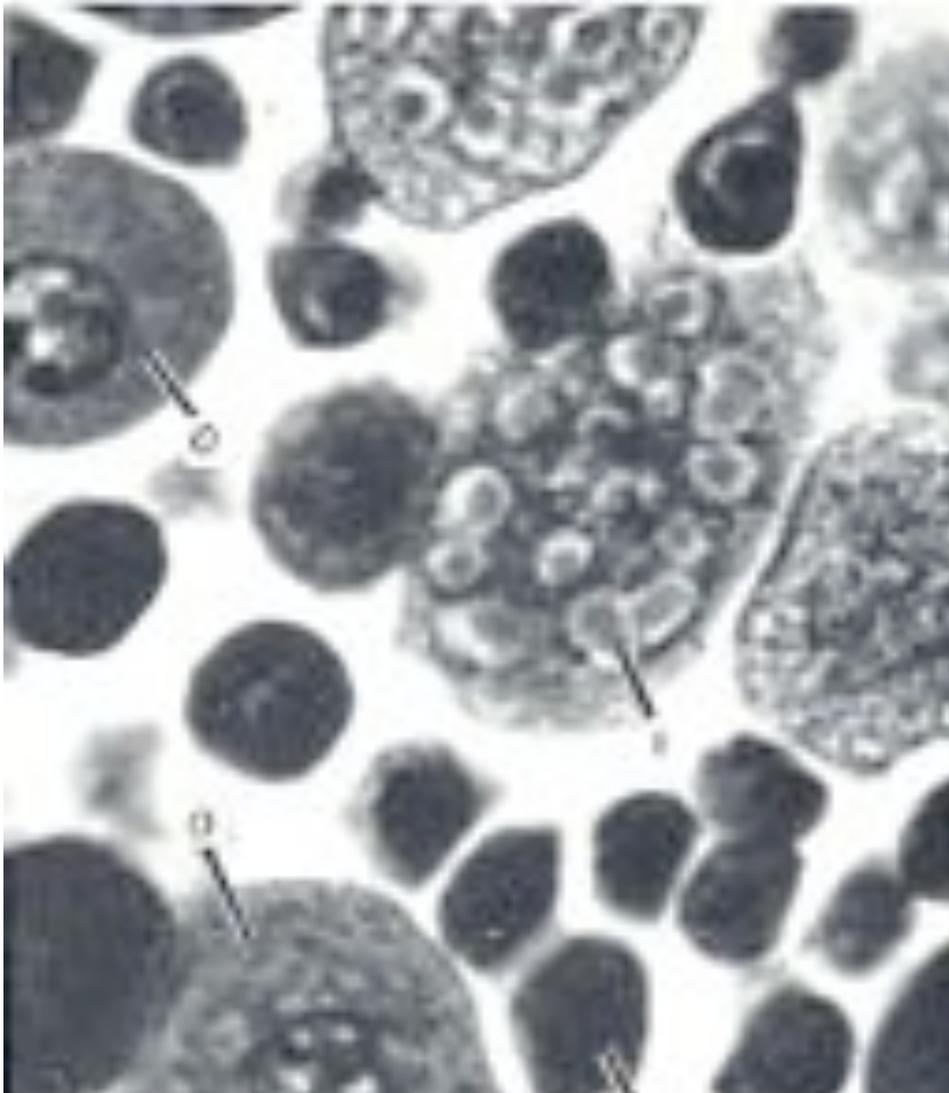
With good wishes for the progress of your research,

Yours sincerely,  
George Salt

Professor J. G. Stoffolano  
Dept. of Entomology  
University of Massachusetts

## C. HEMOCYTES-

Are not surrounded by a basement membrane-like matrix as all other cells and tissues are in insects.



# TYPES OF HEMOCYTES

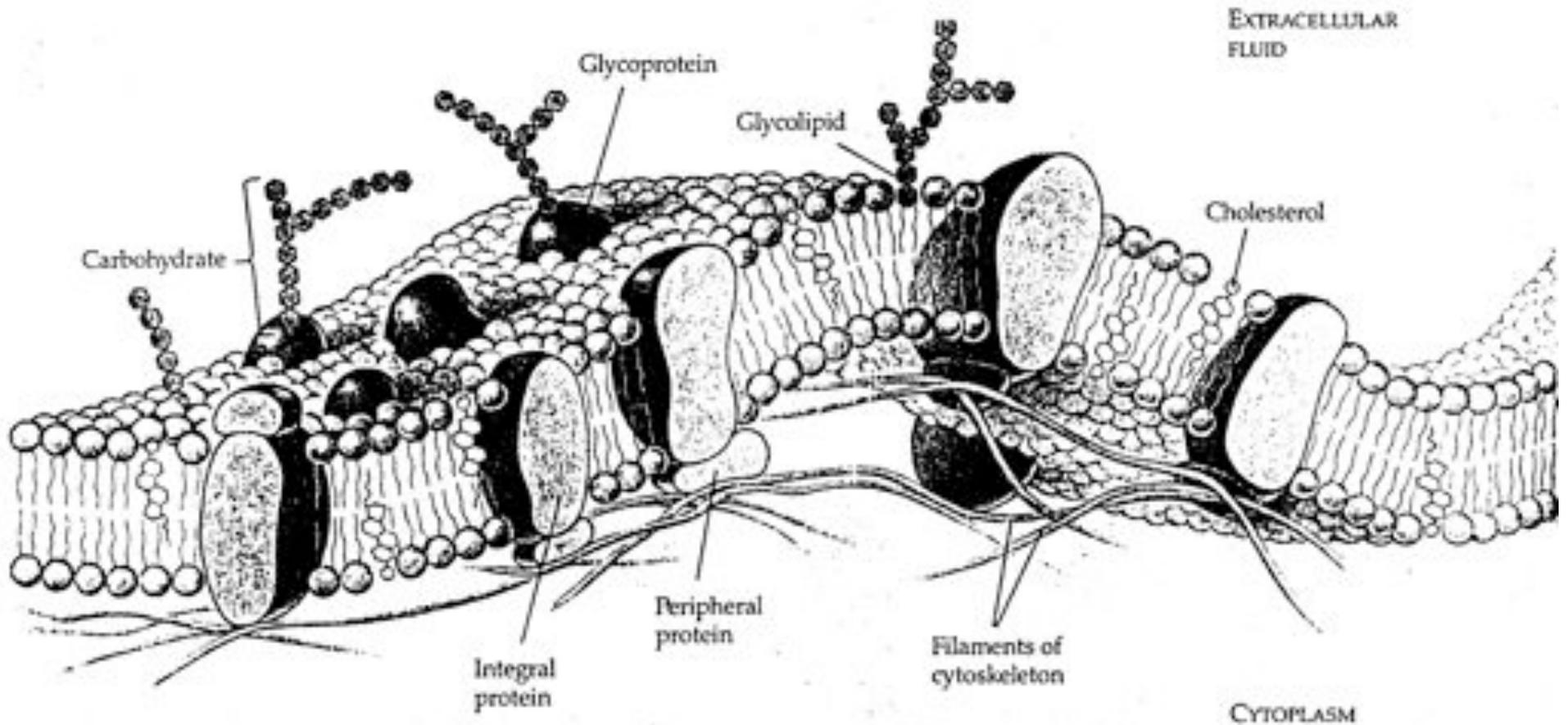
1. Prohemocytes-the ‘stem-cells’ that produce other blood cells.
2. Plasmacytes-large cells with lots of cytoplasm and involved in encapsulation response
3. Granulocytes-contain granules of materials that aid the plasmacytes in recognition
4. Coagulocytes-involved in rupturing and forming coagulant, sticky material

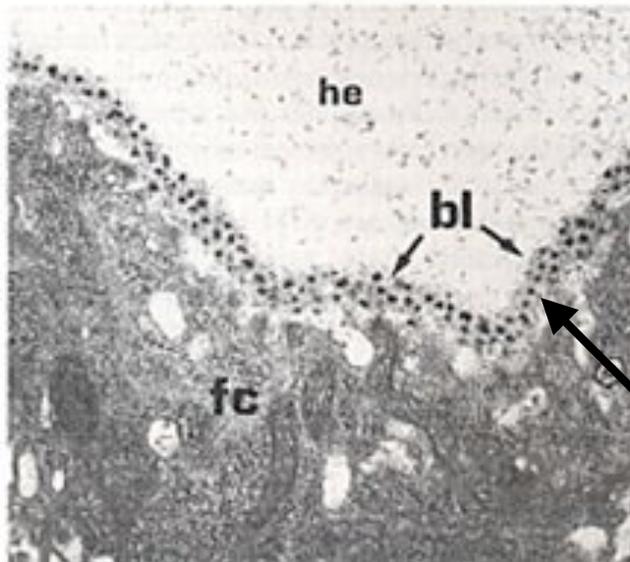
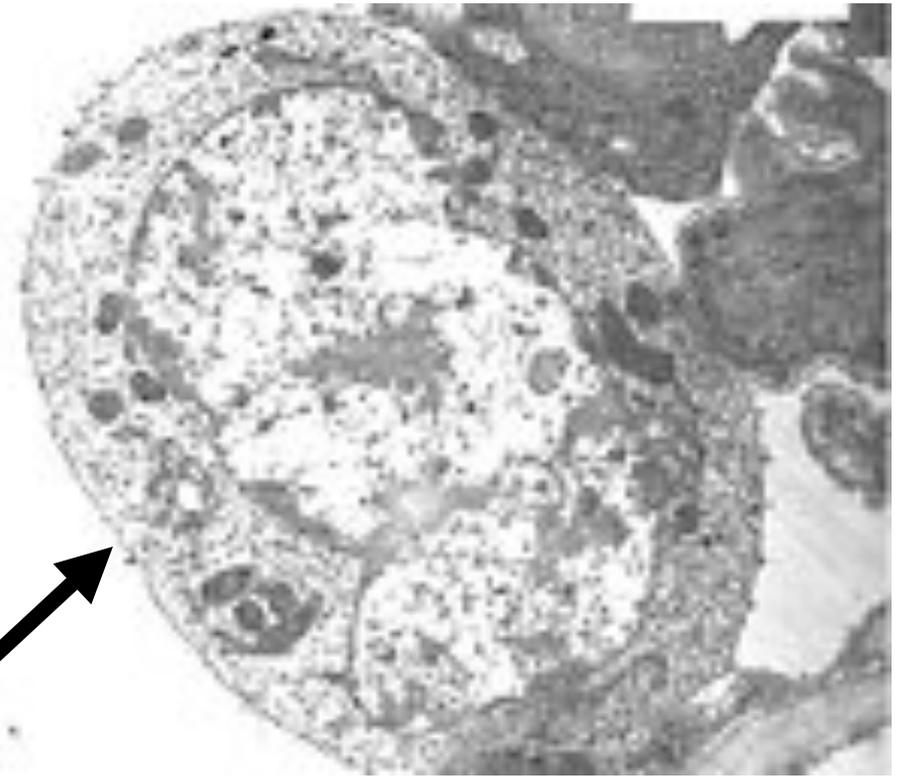
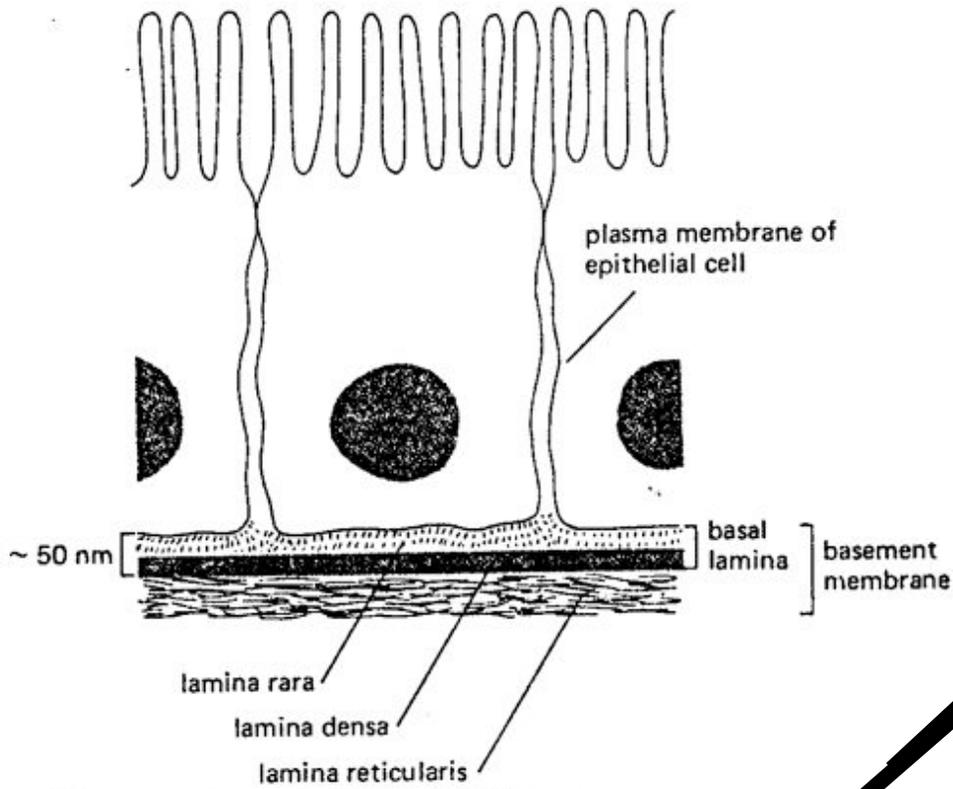
**Hemocyte classification is still far from perfect and one will see different names used for the same blood cell type.**

Hemocytes are involved in:

- a. Wound healing
- b. Repair of the basement matrix
- c. Encapsulation response

Plasma membranes have on their surface molecules involved in **recognition** (ligands) and molecules involved in **reception** (receptors). Protein molecules operate on exposed surface residues that aid in binding. When a protein molecule binds to another molecule, the second molecule is called a ligand. The region where the binding is taking place is called the **binding site**.





Note the basement matrix or basal lamina in the TEM of the follicle cells on the left and its absence in the above TEM of a prohemocyte. Hemocytes lack basement matrices

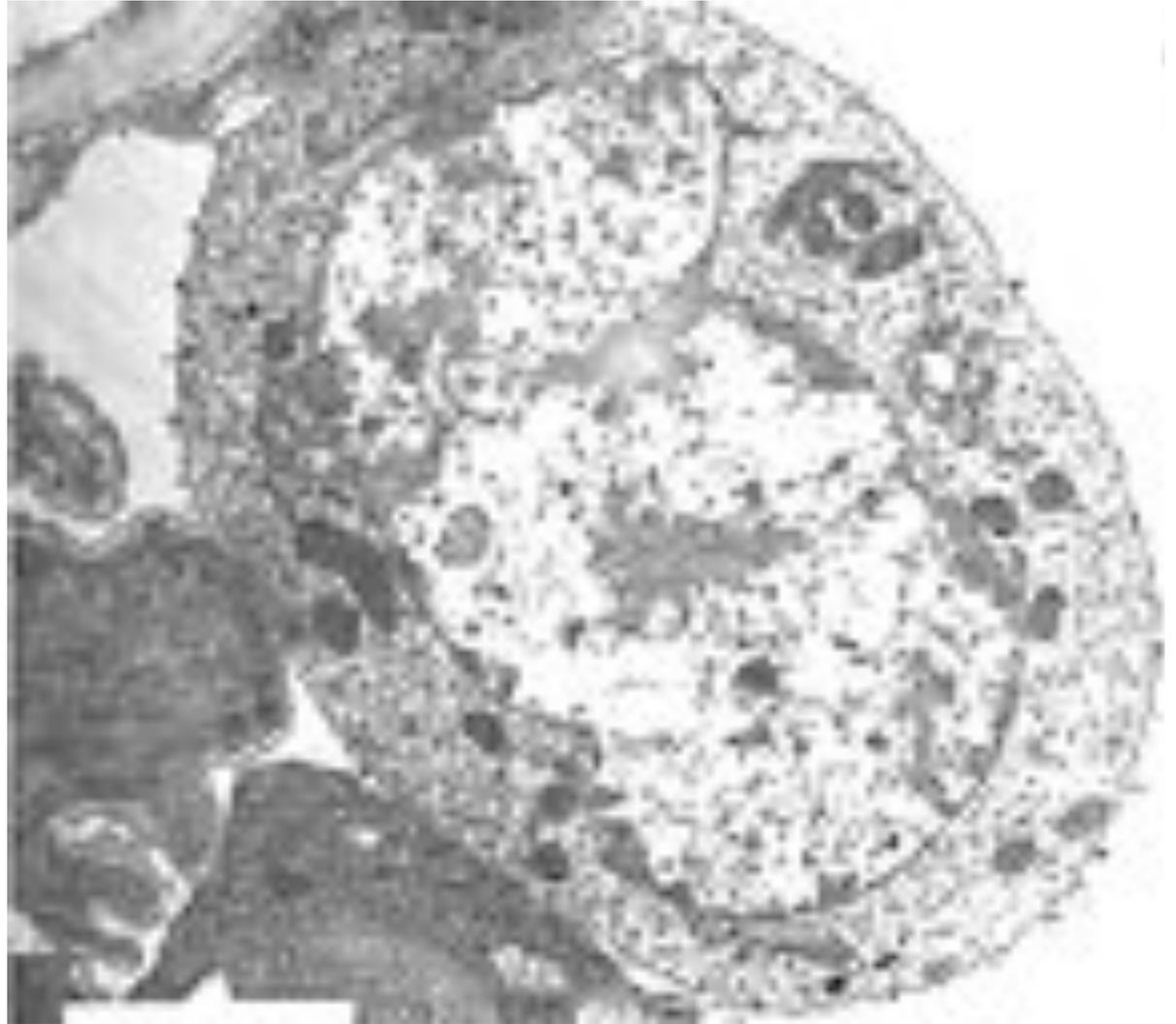
In this micrograph cationized ferritin molecules was added and one can see that they are attracted to the anionized sites in the basal lamina. This was in *Phormia regina* follicle cells (fc). He=hemolymph

## **TEM of prohemocyte**

These hemocytes are considered 'stem cells' and give rise to other types of blood cells.

### **IMPORTANT**-Note

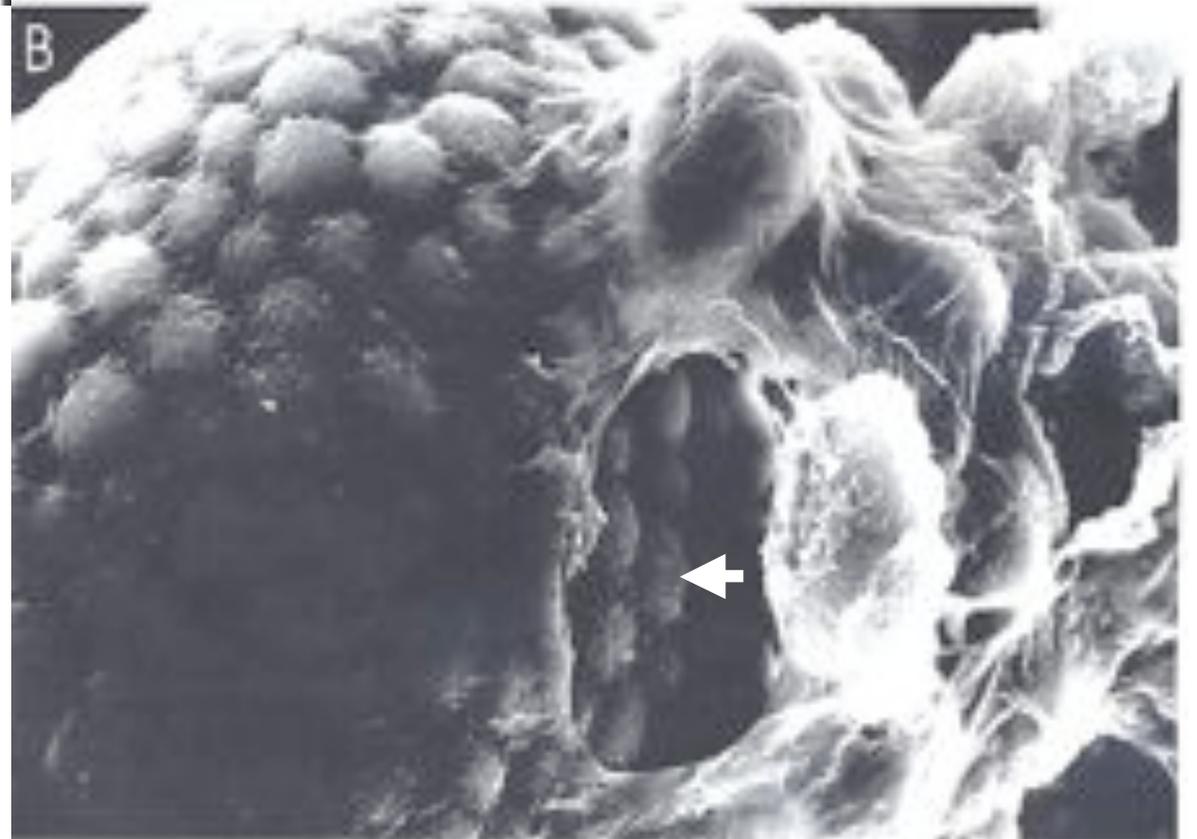
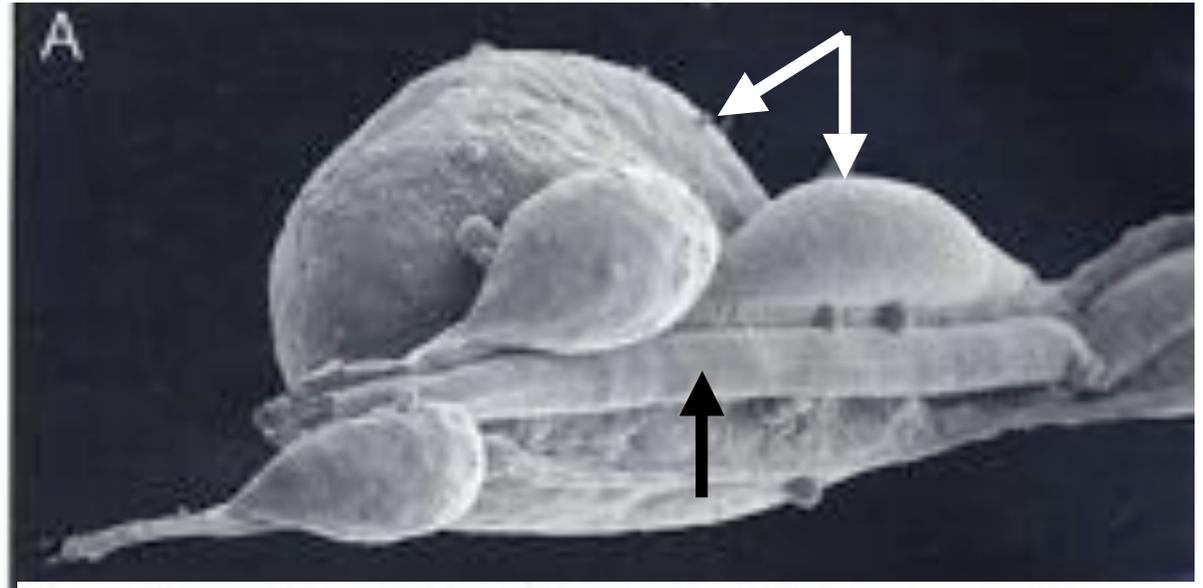
That there is no basement matrix or 'self' around hemocytes but, that this layer surrounds every other cell or tissue in insects. Without this matrix, the hemocytes recognize it as foreign and either encapsulate or phagocytize it..



**D. HEMOPOIETIC ORGANS-**Where hemocytes are produced  
Lymph glands or hemopoietic organs of third-instar *Drosophila* larva.  
You can see hemocytes within the covering sacs of the organ.



SEM showing the dorsal blood vessel (black arrow) and lymph glands (white arrows) surrounding the blood vessel. Below is an enlargement of one of the lymph glands showing hemocytes within (white arrow). These are of a *Drosophila* larva.



In house fly and face fly large aggregations of hemocytes are found in the posterior, dorsal area near the spiracles

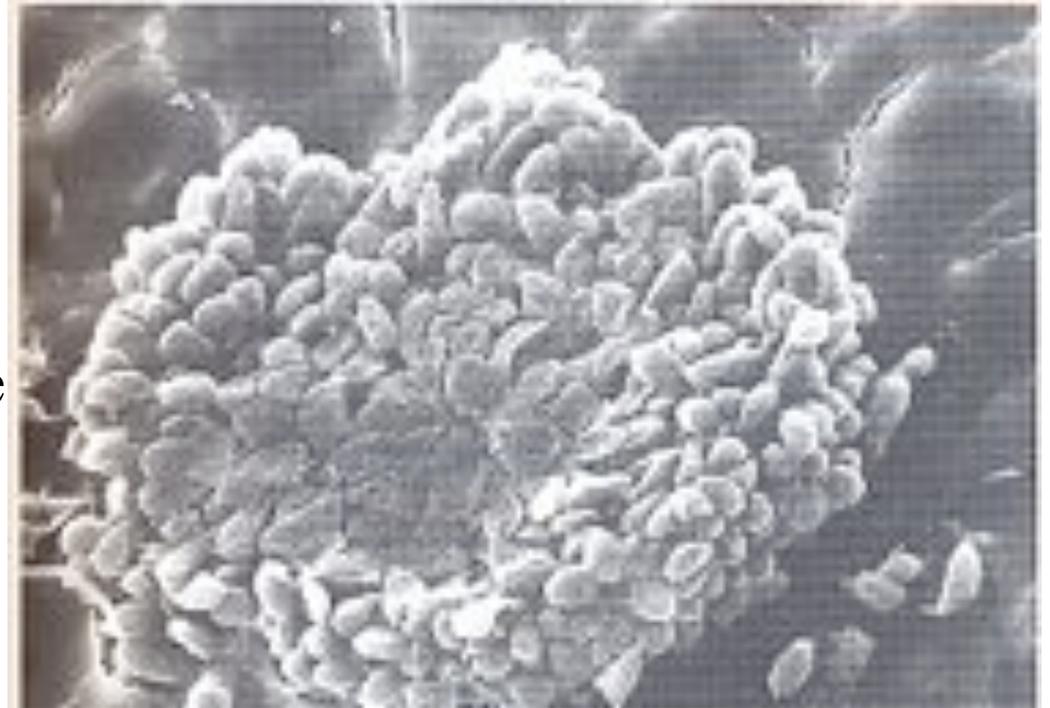
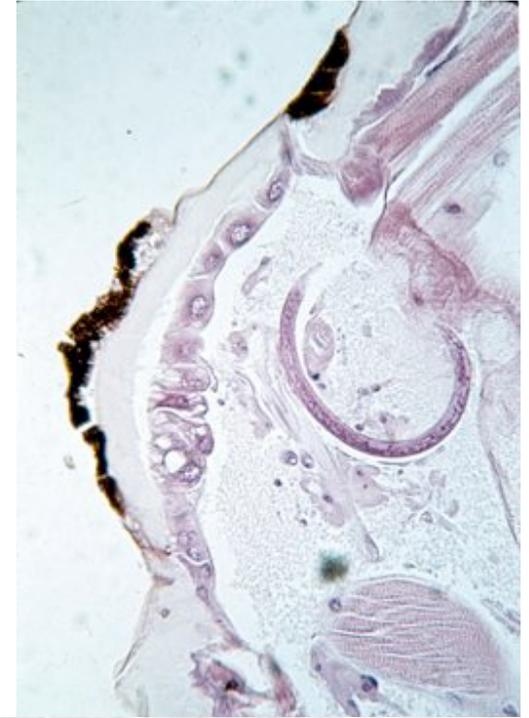




## E. WOUND HEALING

The photo to the right shows cuticular or wound healing of the larva of *Musca domestica* to penetration by the nematode, which is seen curled up in the hemolymph.

The bottom photo shows a mass of hemocytes that have gone to the area where a spore fungus, *Megarhizium*, in *Schistocerca* has penetrated and destroyed the basal lamina, thus ‘self’. To counter this, hemocytes have enmassed at the site to repair the wound and re-establish the basement matrix.



## F. CELLULAR IMMUNE RESPONSES

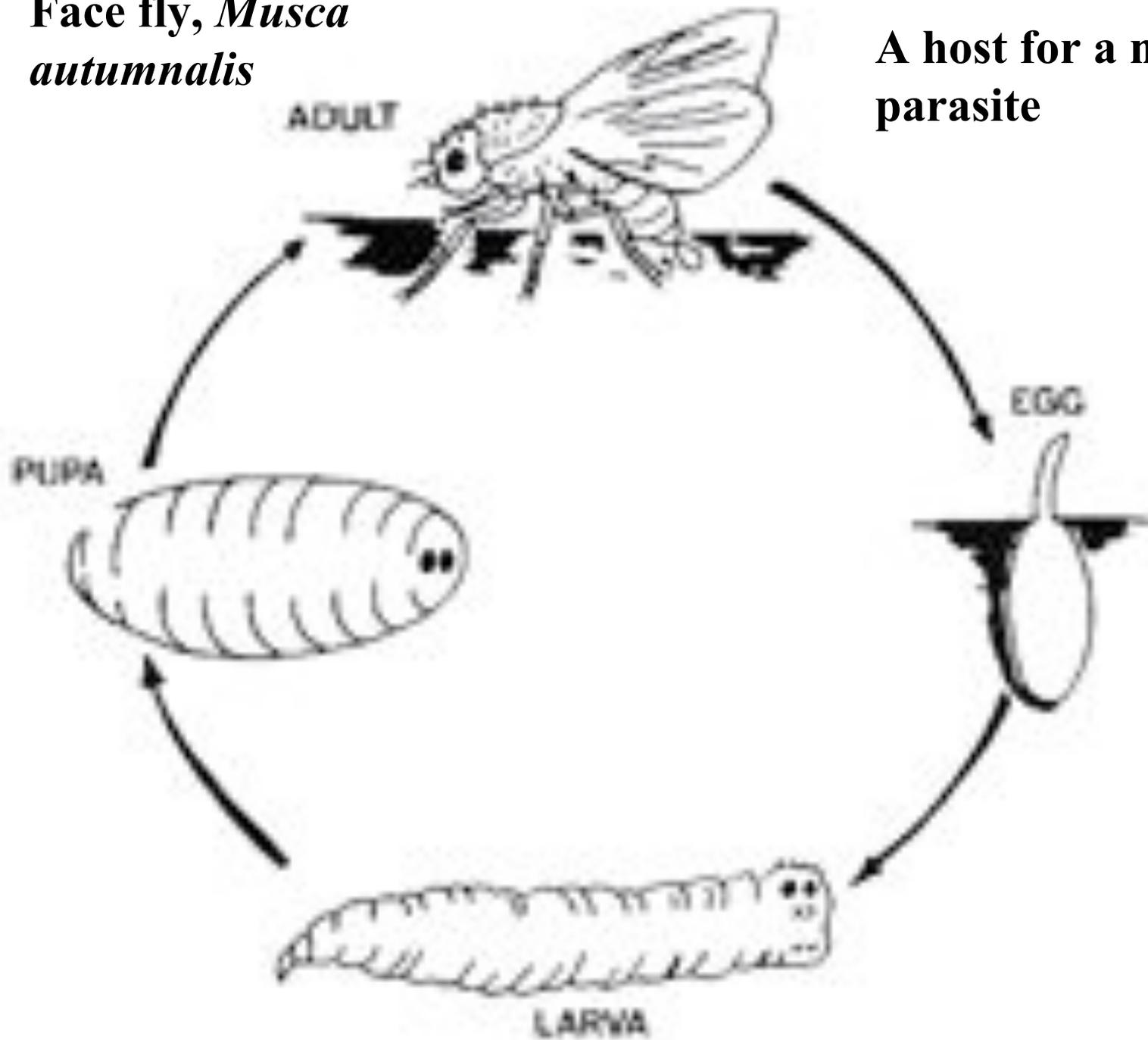
A host defense response against the invasions of parasites, pathogens, and any foreign object that involves **hemocytes**. If successful, the response results in:

1. Encapsulation of the foreign object
2. Melanization of the capsule

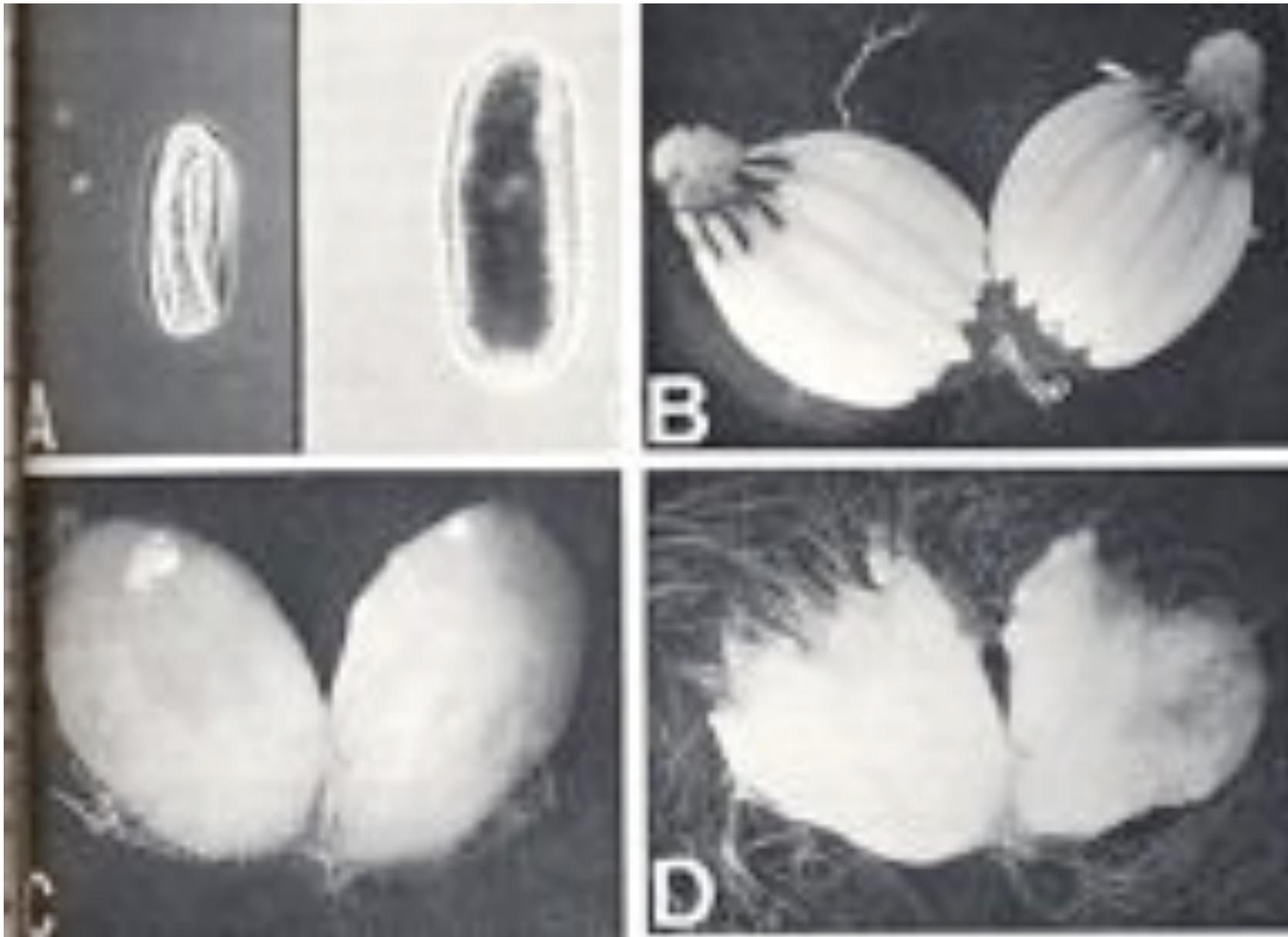
The fact that tracheoles usually go to encapsulated and dying parasites led Wigglesworth to suggest that tracheoles go to areas of low oxygen.

Face fly, *Musca autumnalis*

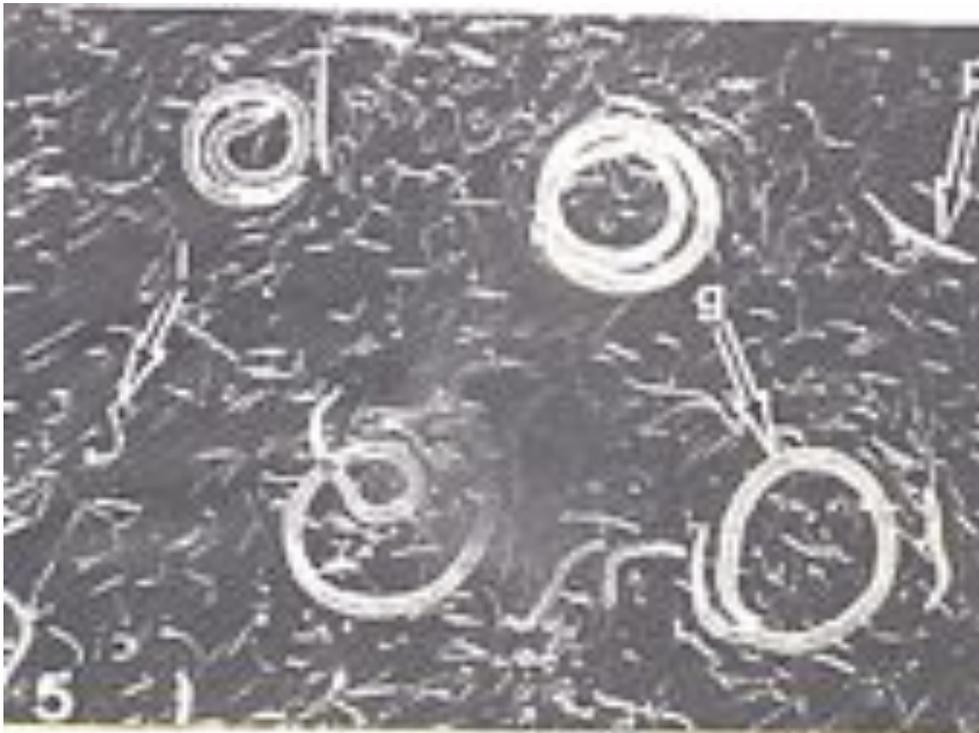
A host for a nematode parasite

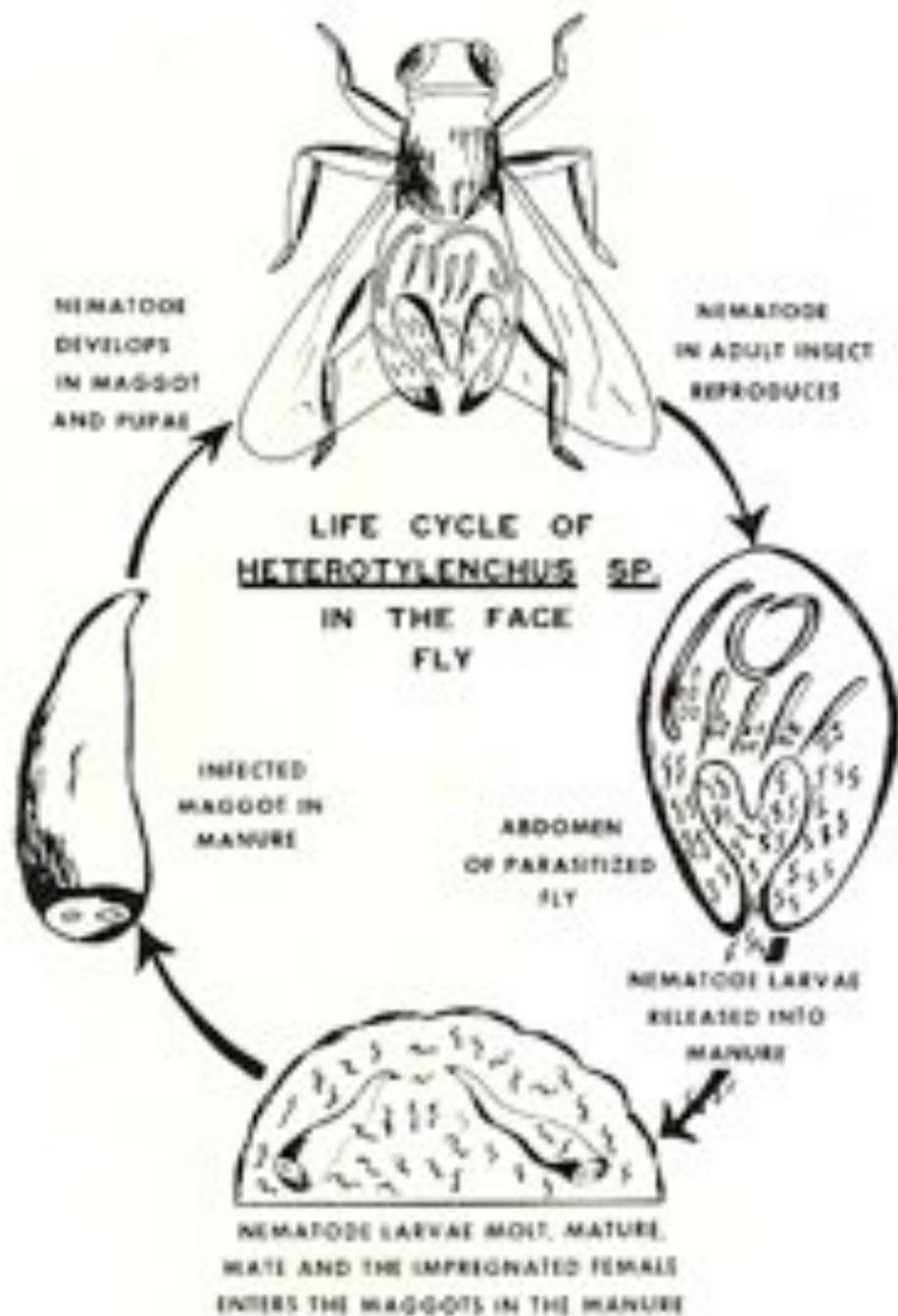


*Heterotylenchus autumnalis* a parasite of *Musca autumnalis* that castrates the female. Now *Paraiotonchium autumnalis*



The nematode, *Paraiotonchium muscadomesticae*, has so far been found only in Serra Talhada, a small town in northeastern Brazil. University of Florida graduate student Reginald R. Coler is credited with the discovery.





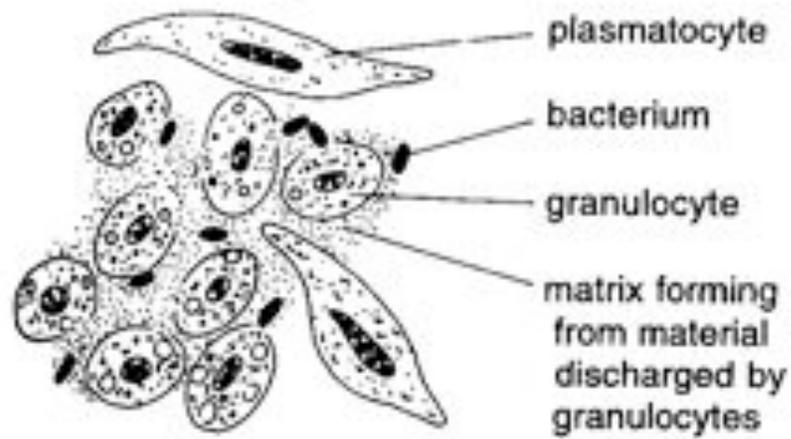
## RECOGNITION AND CHEMOTAXIS OF HEMOCYTES TO WOUNDS OR FOREIGN OBJECTS. Stoffolano and Nappi

Distribution of haemocytes in larvae of *Musca domestica* and *Musca autumnalis* and possible chemotaxis during parasitization. J. Insect Physiol. 18: 169-179.

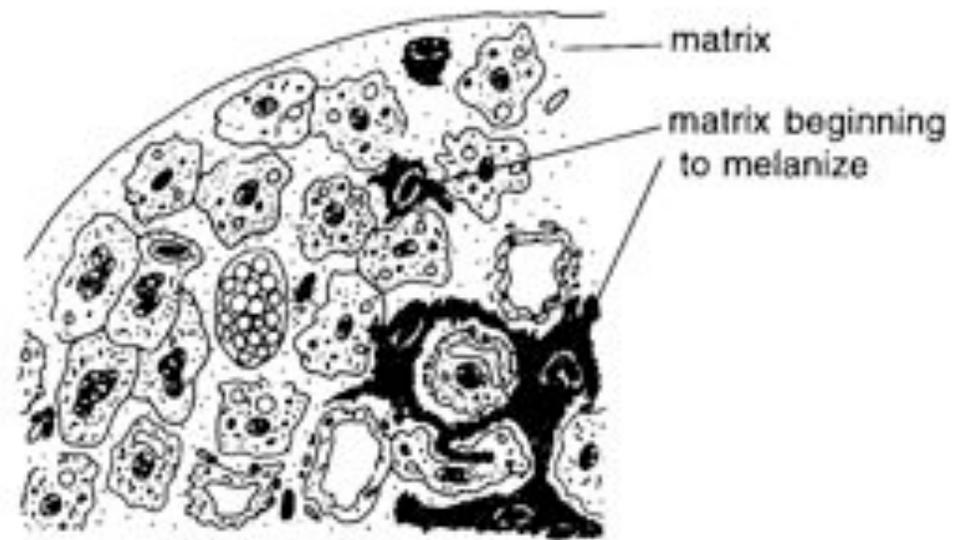
O=oenocytoids; P=plasmatocytes. Ligated larvae and larvae fixed in liquid nitrogen to assure no fixation artifacts of hemocytes moving. Results showed that these masses of hemocytes remained in these areas.



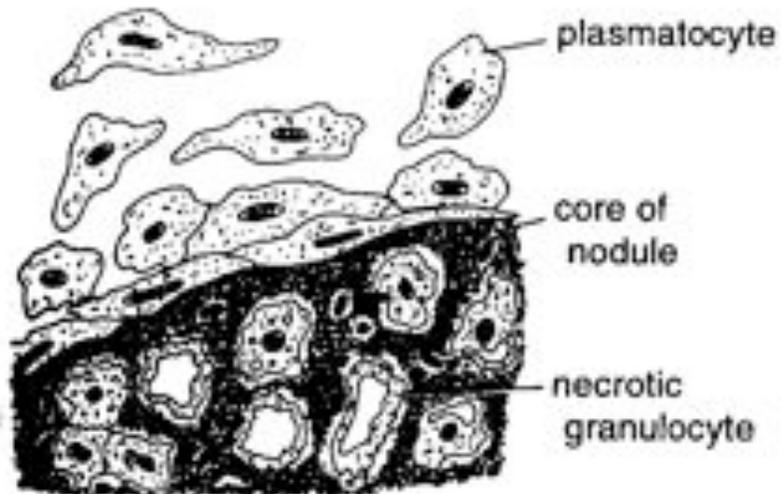
a)



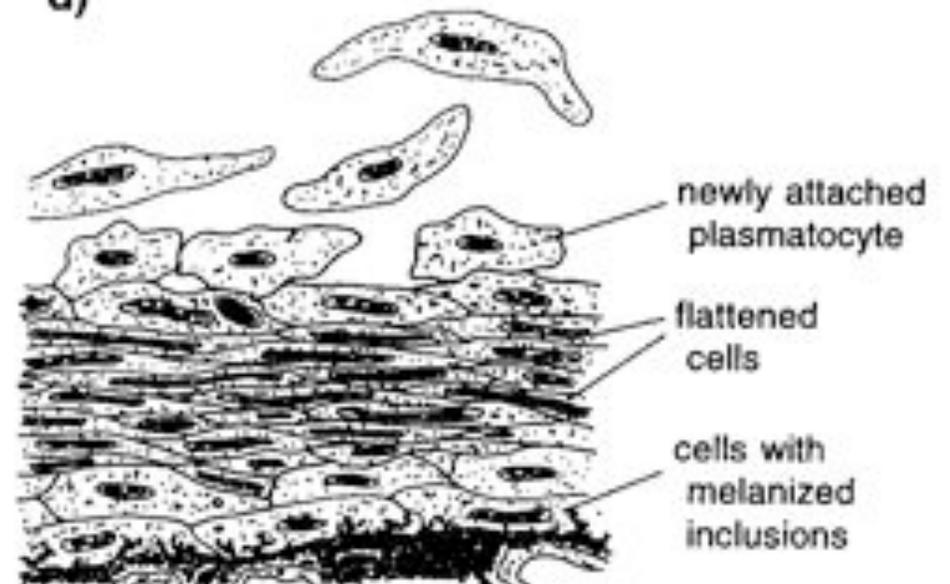
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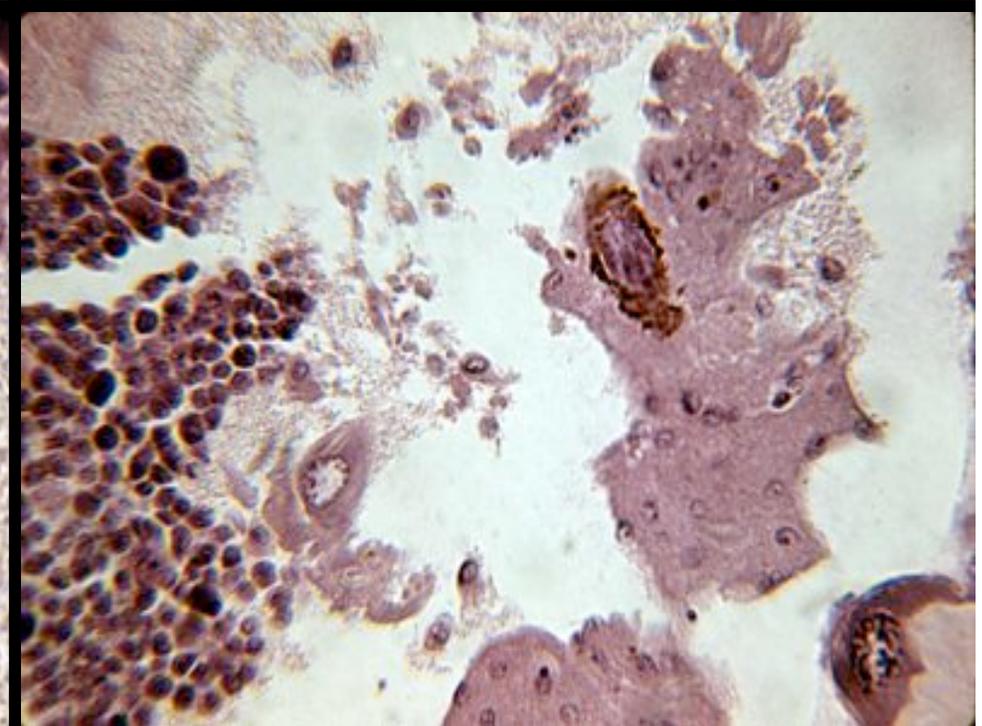
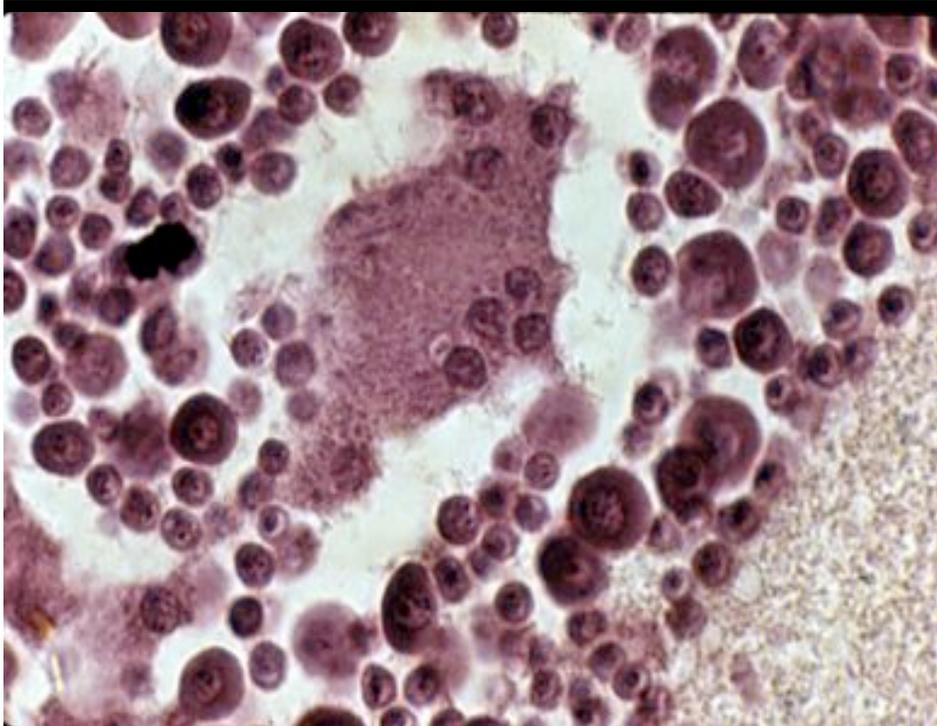
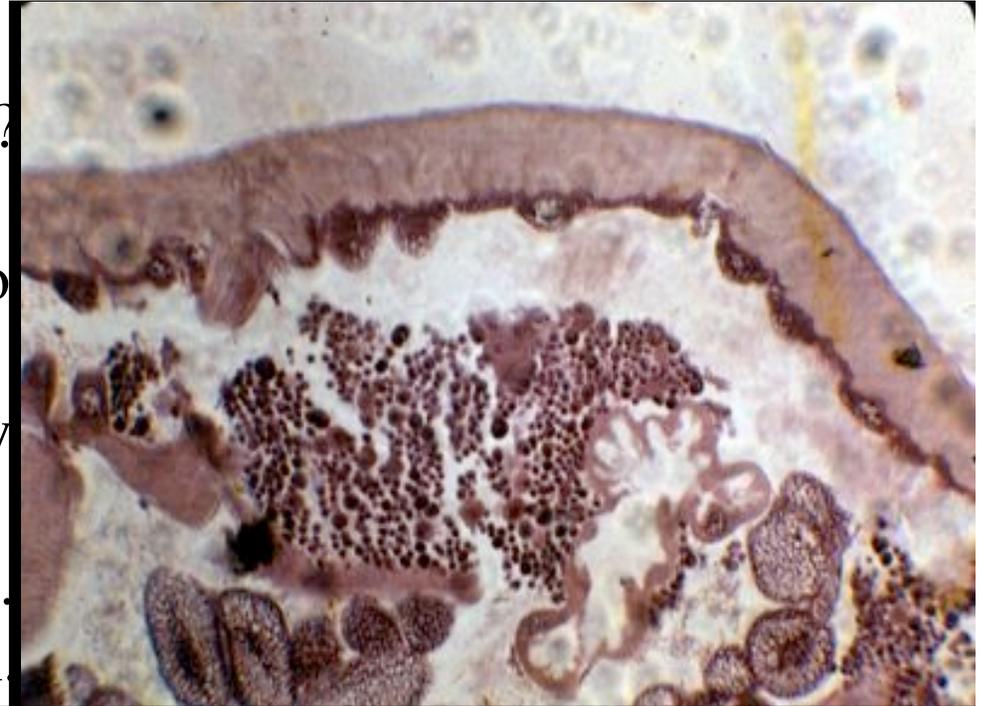
c)



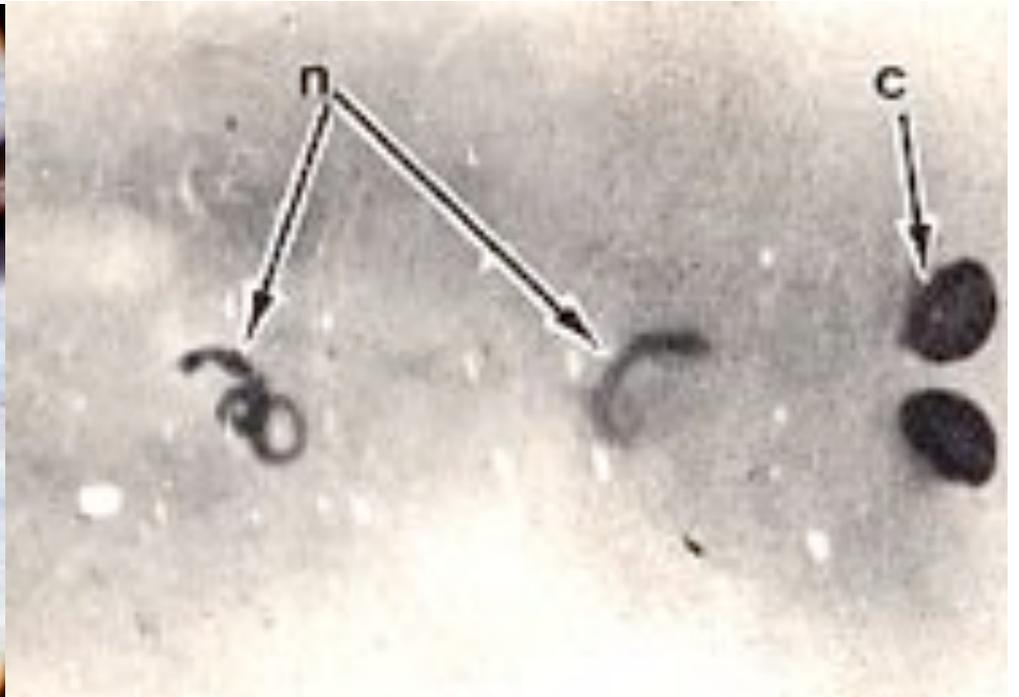
d)



Nematode encapsulated in *Musca domestica*. Note hemopoietic organ? on the right. Once a parasite gets into a non-host, the hemocytes go to that area and start rupturing (see below). This continues until a sticky matrix is formed that entraps the parasite, which is then encapsulated. Coagulocytes produce sticky matrix.



**Parasitic expulsion**-Here the dead and encapsulated nematode is surrounded by trachea, is stuck in a cytoplasmic matrix and is encapsulated or encrusted with melanin. Bottom right shows the nematodes moved to the cuticle surface where they are discarded at the larval/pupal molt.



# WHAT HAPPENS TO THE EGG OR LARVA OF A PARASITE THAT GETS INTO AN ABNORMAL HOST?

IT IS USUALLY **ENCAPSULATED**

The following slides on *Drosophila* are from  
Vass, E. and A.J. Nappi. 2000. Developmental and immunological aspects of  
*Drosophila*-parasitoid relationships. J. Parasitol. 86(6): 1259-1270.

AVIRULENT PARASITOID

VIRULENT PARASITOID

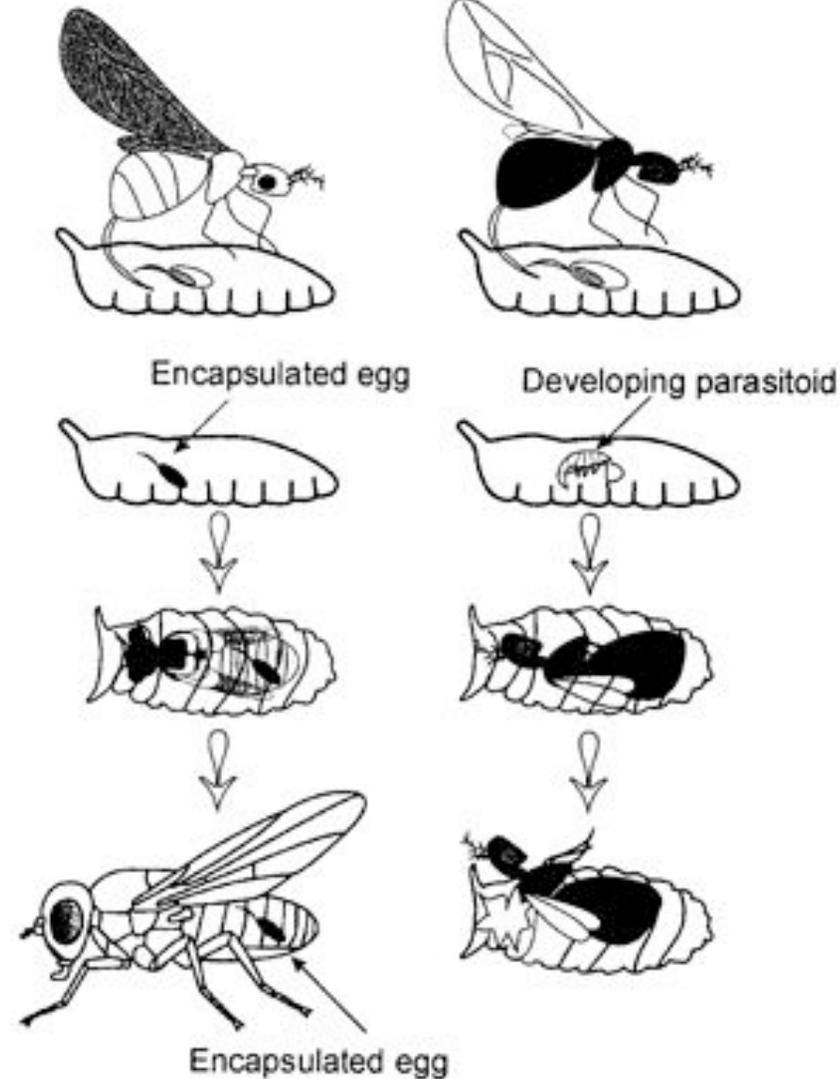


FIGURE 1. The contrasting developmental fates of avirulent and virulent parasitoids in *Drosophila melanogaster*. The avirulent parasitoid succumbs to the immune response of the host and becomes sequestered in a melanotic capsule that is retained within the body of the host throughout its development. The virulent parasitoid suppresses the immune system of its host and emerges as an adult from the puparium of the host.

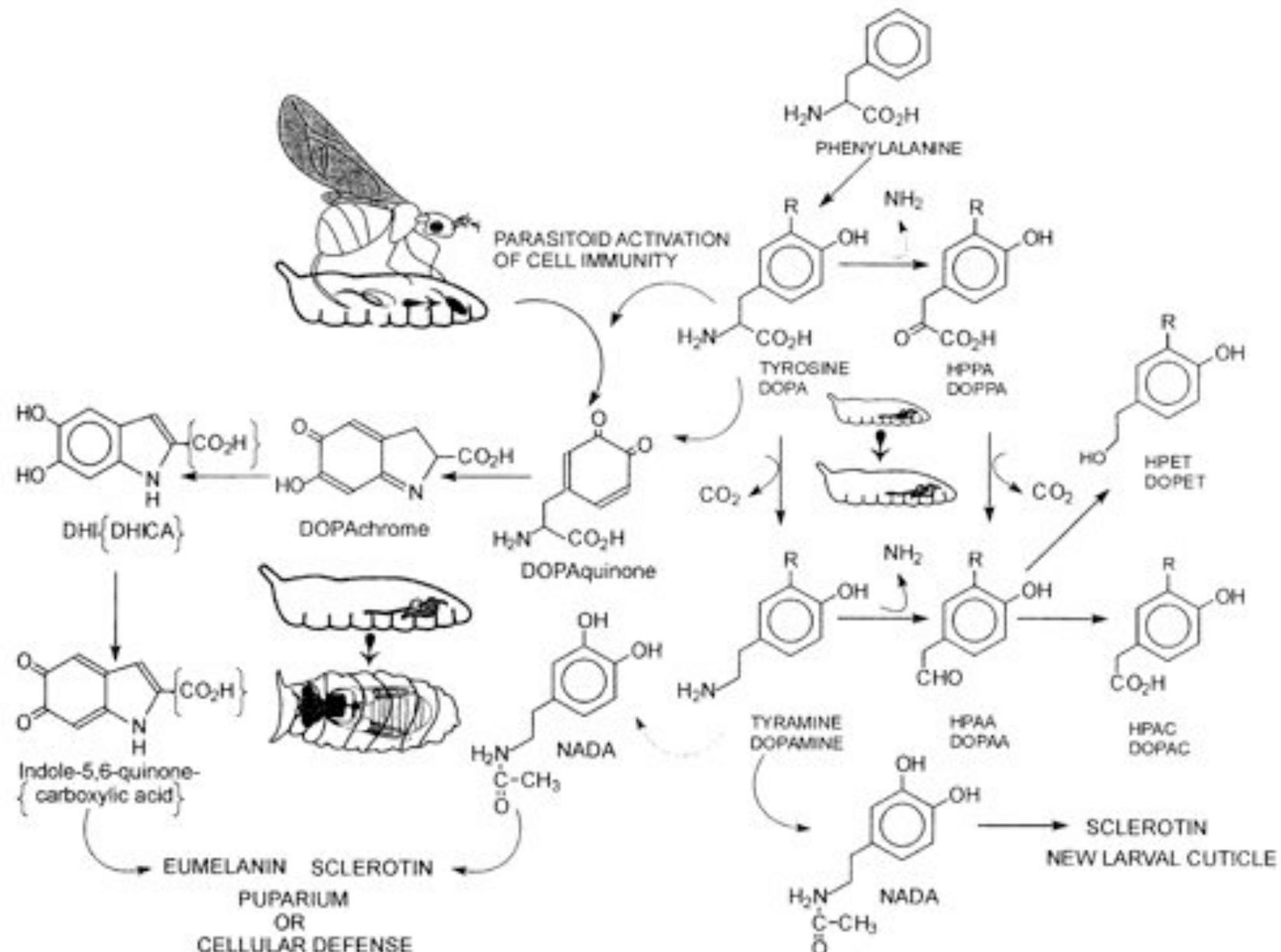
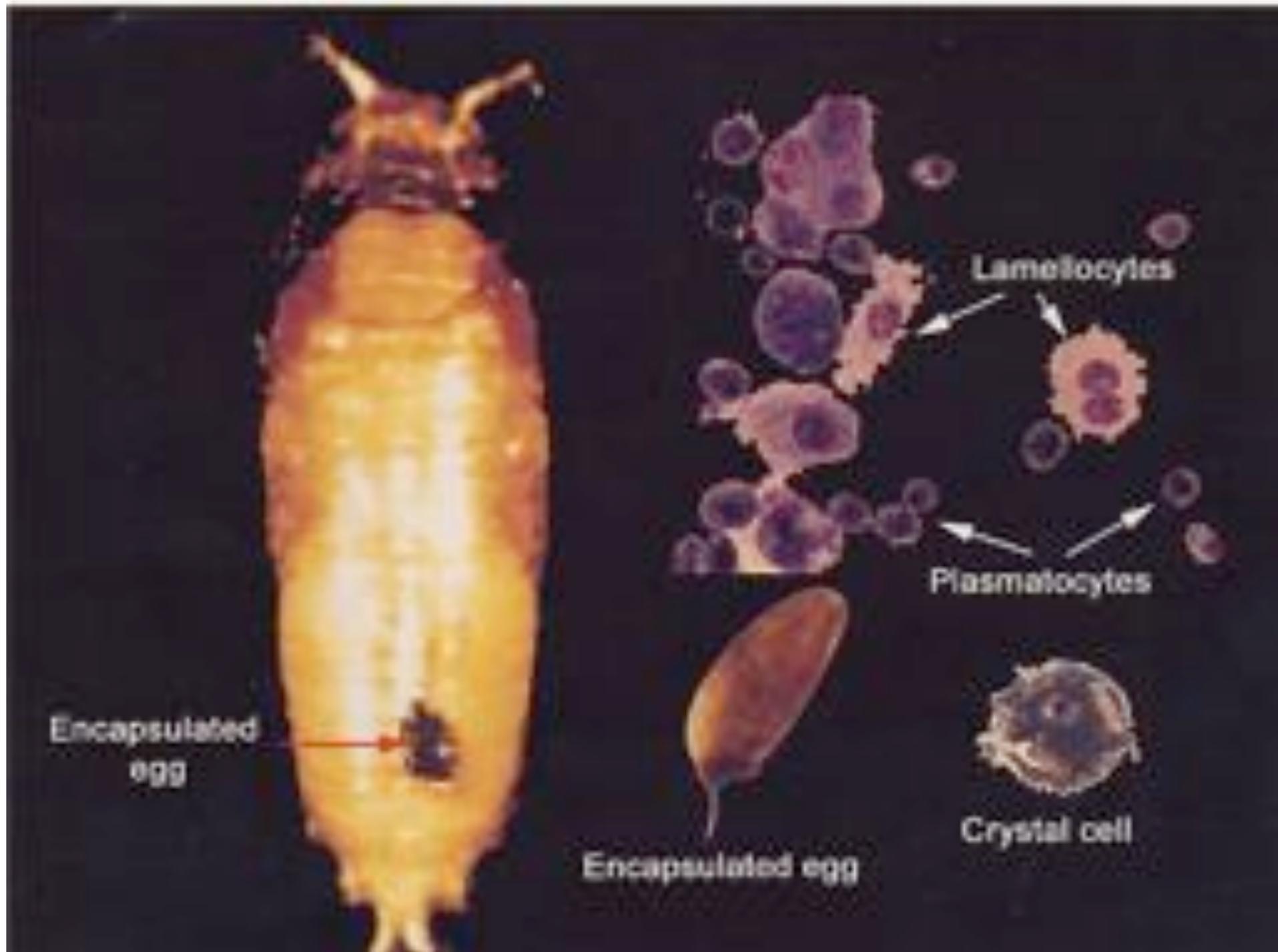


FIGURE 2. Proposed major routes of tyrosine metabolism during normal *Drosophila* development and during immune reactions to parasitoid infection. Although the various pathways for tyrosine metabolism have yet to be identified, deamination and decarboxylation are suspected of being major routes during much of larval development. Normally, catecholamines and related *o*-diphenols are used to produce sclerotin during new cuticle formation in larvae. During the later stages of larval development and early pupariation, melanogenic pathways become activated. This switch in tyrosine metabolism also is triggered by avirulent parasitoids, and the premature production of quinones and indole precursors of eumelanin and sclerotin are utilized to form melanotic capsules.



Parasitized Larvae	Hours post infection when hosts were ligated	Percentage melanotic encapsulations
	0	52.0
	6 12 18	2.7 15.2 30.5
Ligature posterior to ring gland		

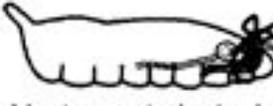
Melanotic tumor larvae	Age (Hr) when ligated	Percentage caudal melanotic tumors
	0	90.0
	60-73	0-30
Ligature posterior to ring gland		
	60-73	70-85
Ligature anterior to ring gland		

FIGURE 6. Effects of neck ligation on the ability of *Drosophila* larvae to make an immune response against eggs of *Leptopilina* (upper box) (from Nappi, 1975b). This procedure also affected tumor formation in a tumorigenic strain (lower box) (from Rizki, 1960).

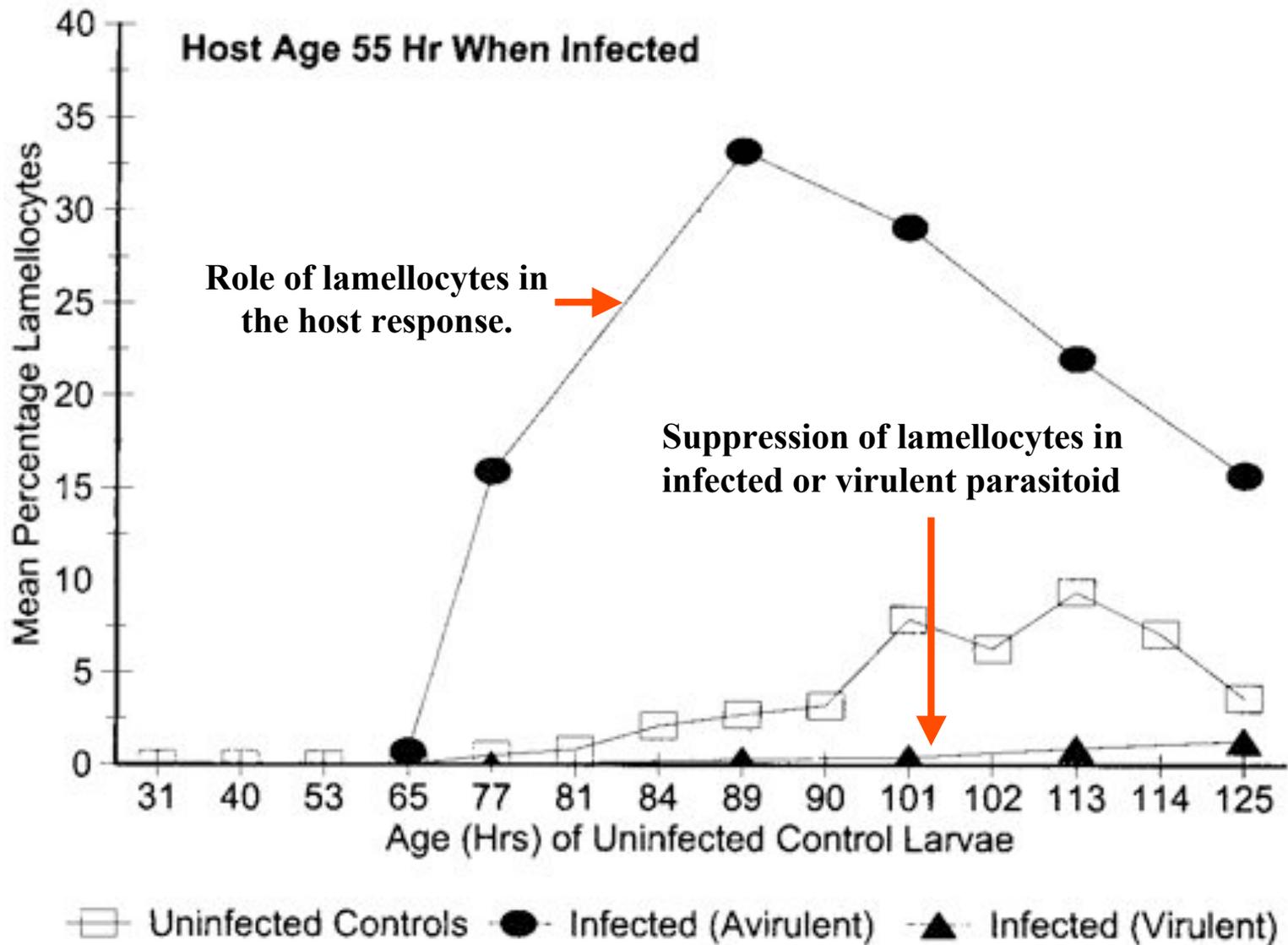


FIGURE 7. Graph showing the mean percentage of lamellocytes in uninfected control larvae of *Drosophila*, in larvae infected by the avirulent parasitoid *Ganaspis mellipes*, and in larvae infected by the virulent parasitoid *Leptopilina heterotoma* (from Nappi and Streams, 1969).

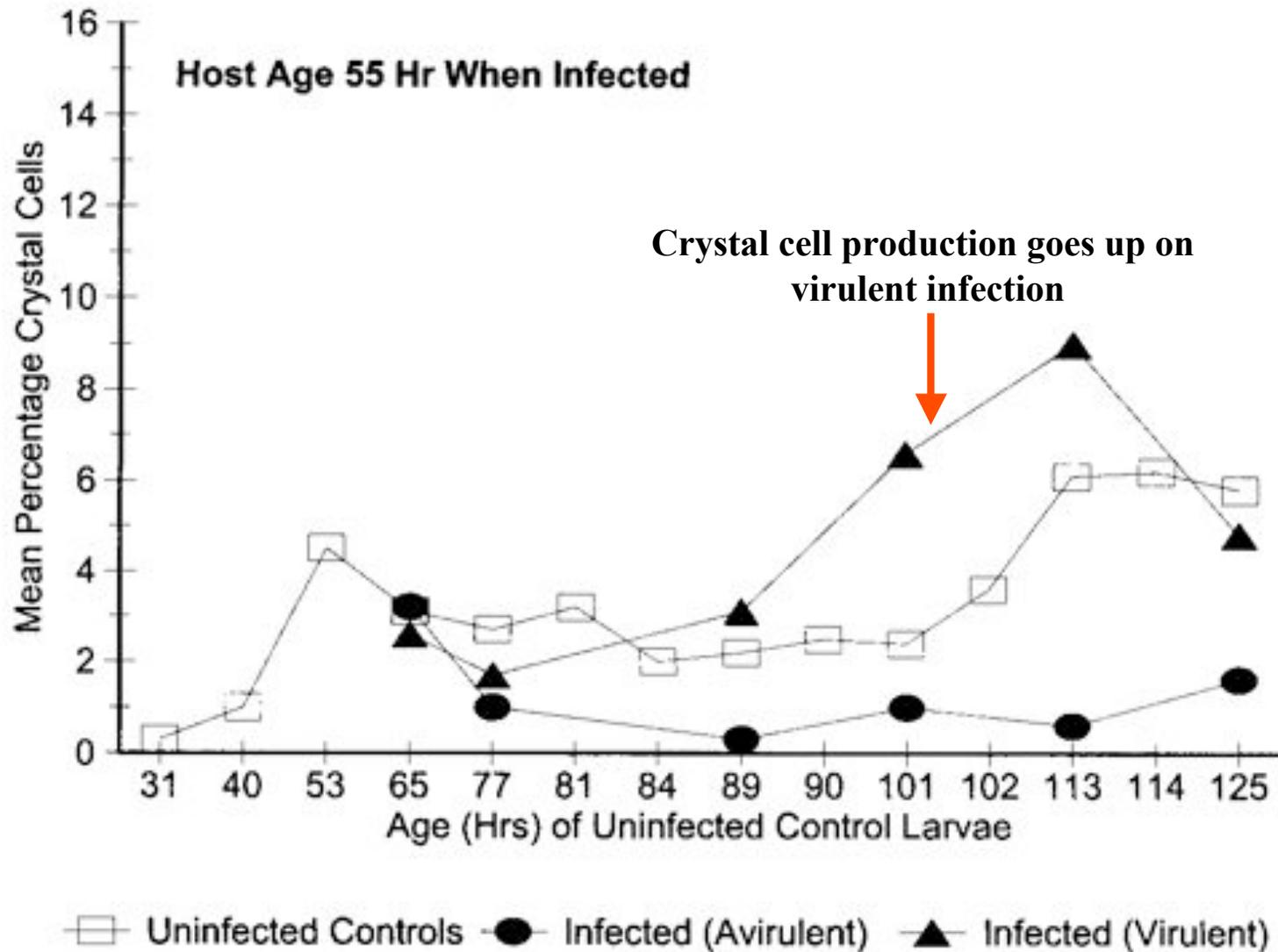


FIGURE 8. Graph showing the mean percentage of crystal cells in uninfected control larvae of *Drosophila*, in larvae infected by the avirulent parasitoid *Ganaspis mellipes*, and in larvae infected by the virulent parasitoid *Leptopilina heterotoma* (from Nappi and Streams, 1969).

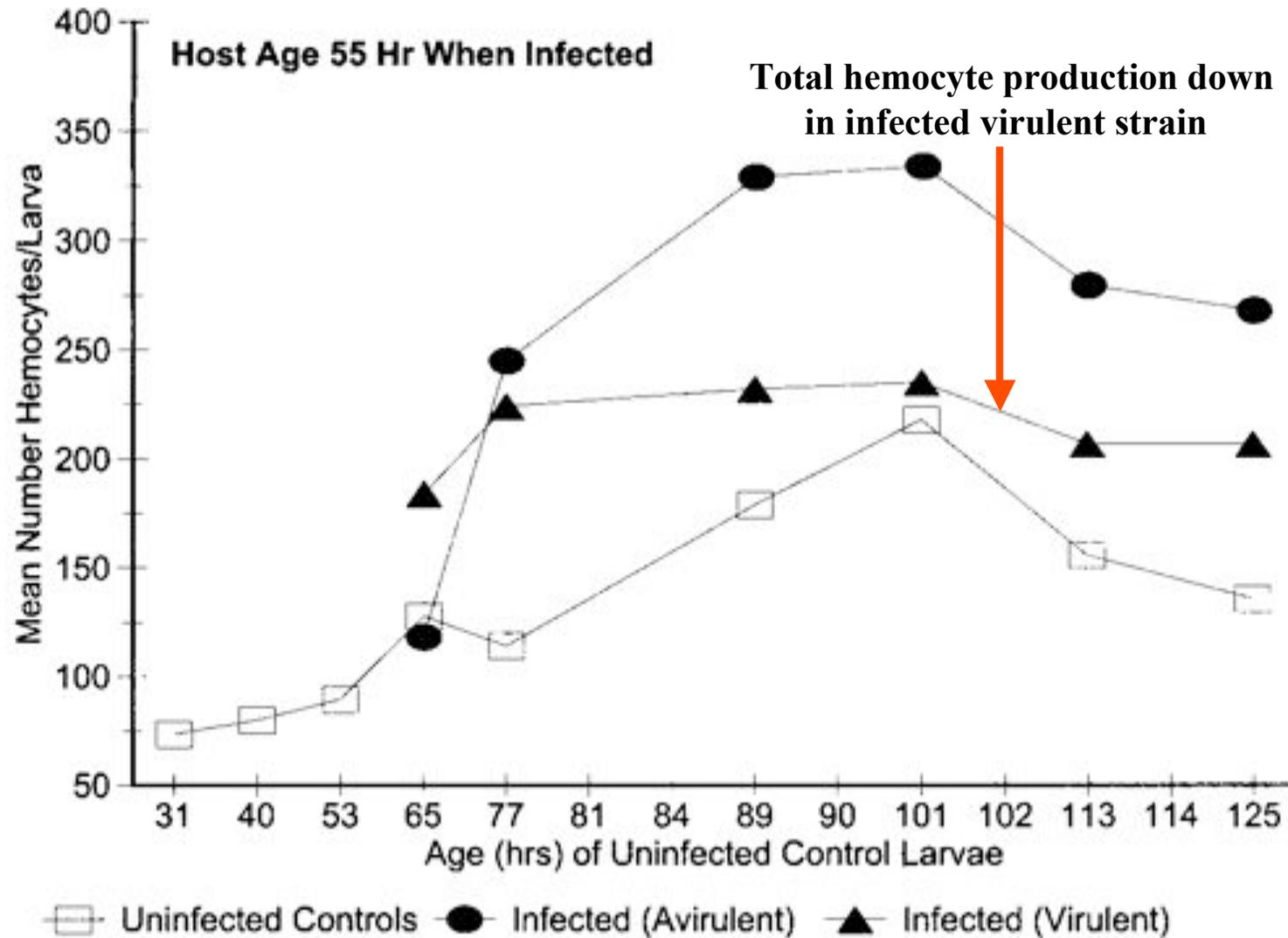
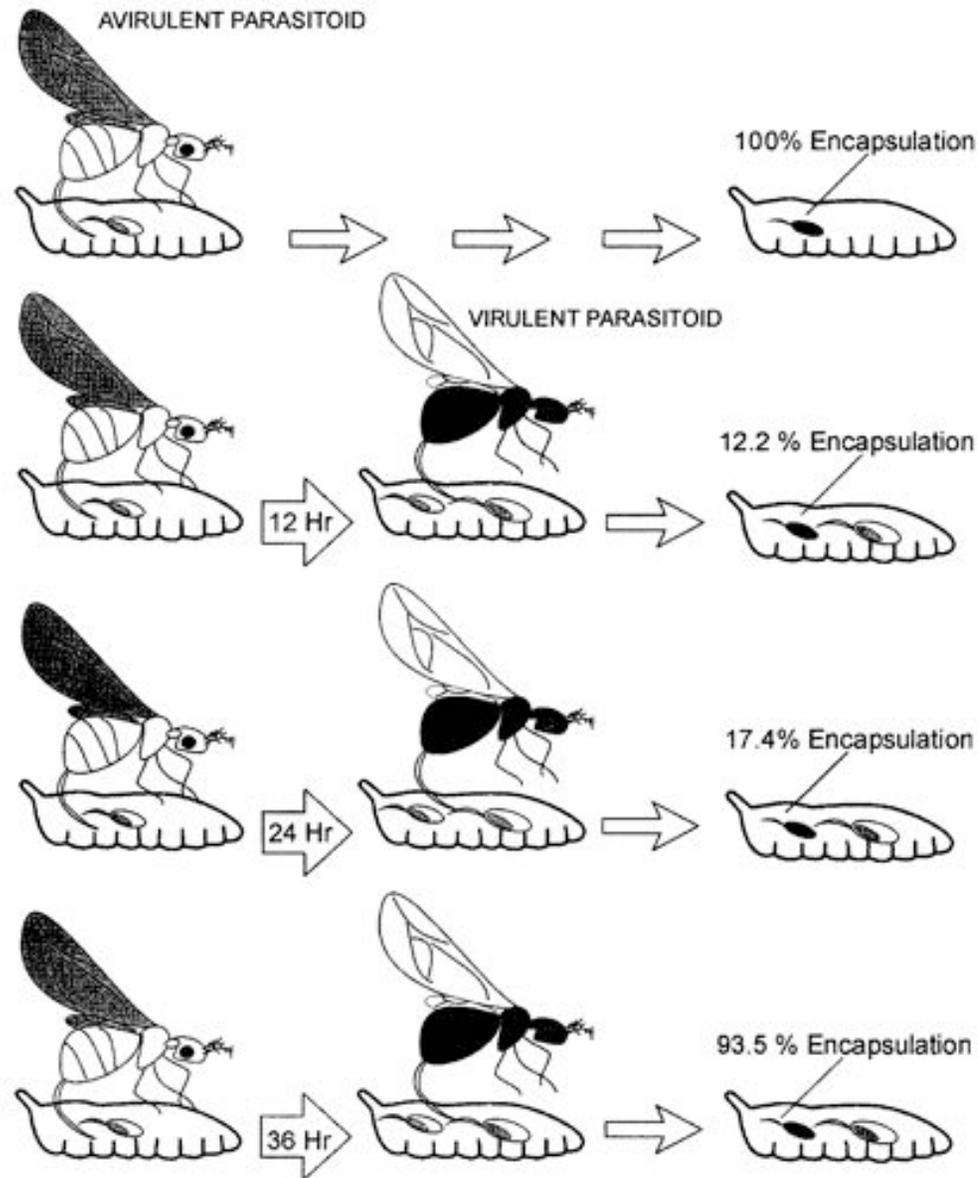
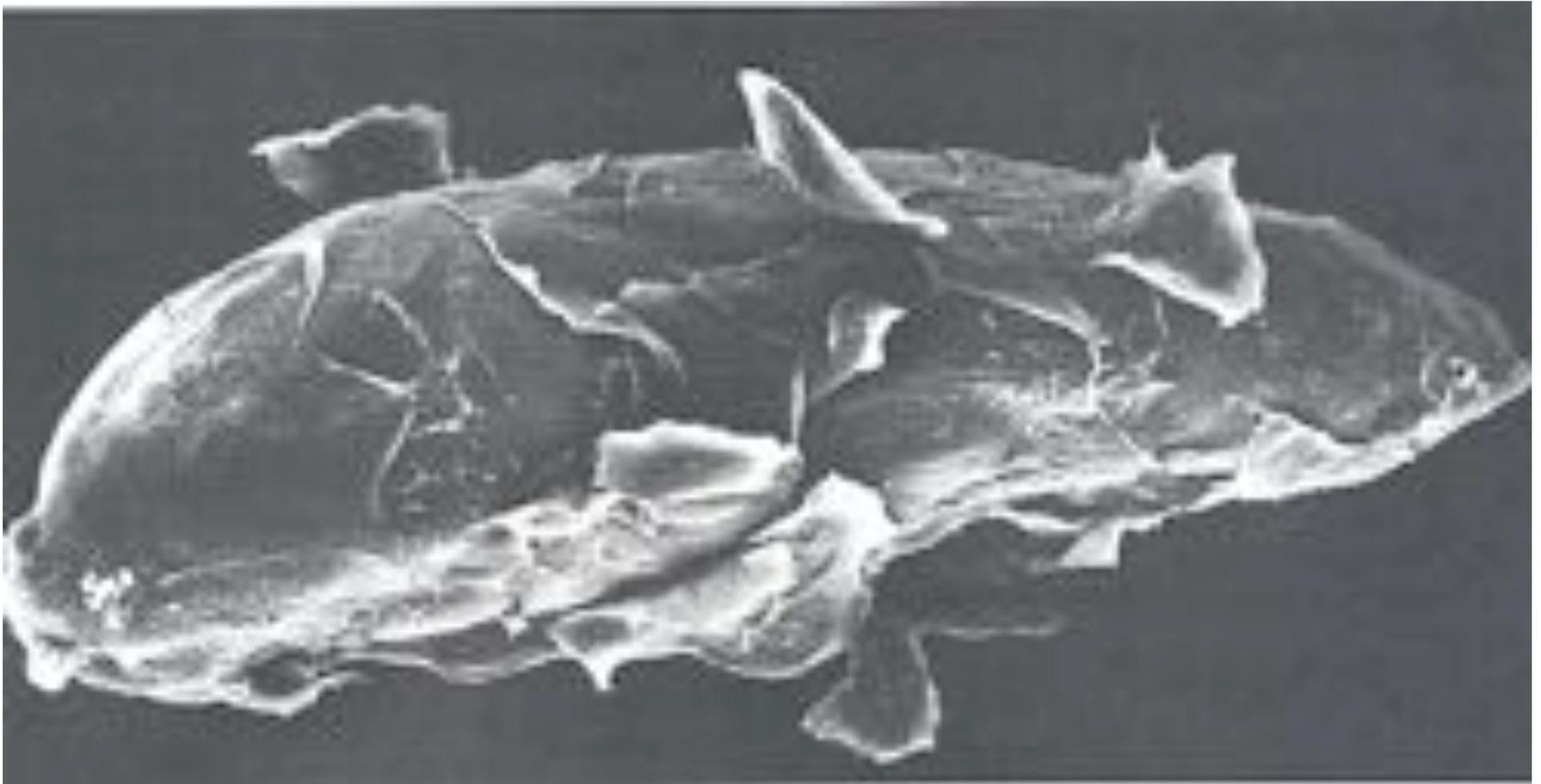


FIGURE 9. Graph illustrating the mean numbers of hemocytes in uninfected control larvae of *Drosophila*, in larvae infected by the avirulent parasitoid *Ganaspis mellipes*, and in larvae infected by the virulent parasitoid *Leptopilina heterotoma* (data derived from Nappi and Streams, 1969).



Immune suppression of melanotic encapsulation as evidence by double infection, first by the avirulent *Ganaspis mellipes*, then at different times by the virulent *Leptolina heterotoma* (data from Streams and Greenberg, 1969).

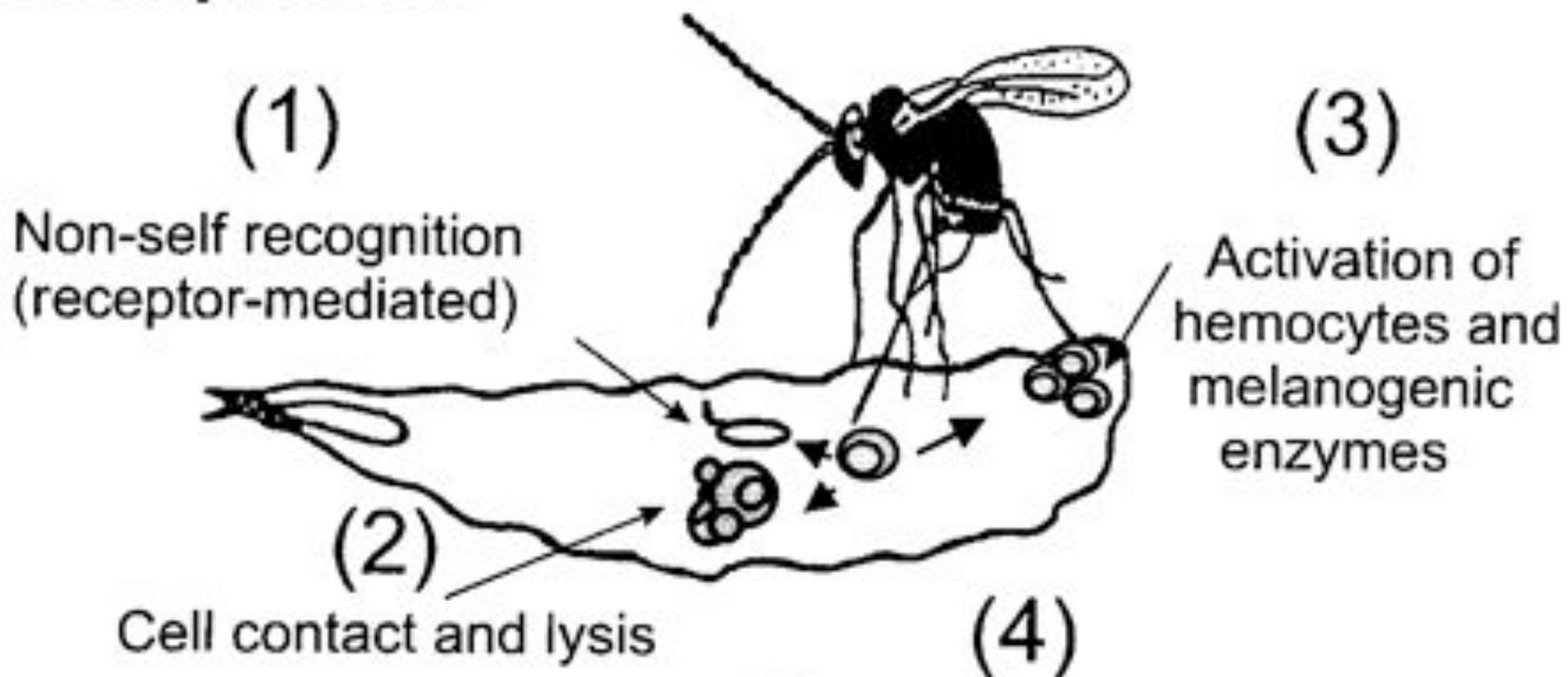
Cellular response or hemocytic encapsulation, within the hemolymph of *Drosophila* larva, around the egg of a parasitoid wasp, *Leptopilina heterotoma*. Note the large flattened hemocytes, which are called lamellocytes by Nappi, but considered as plasmatocytes by some (from Nappi)



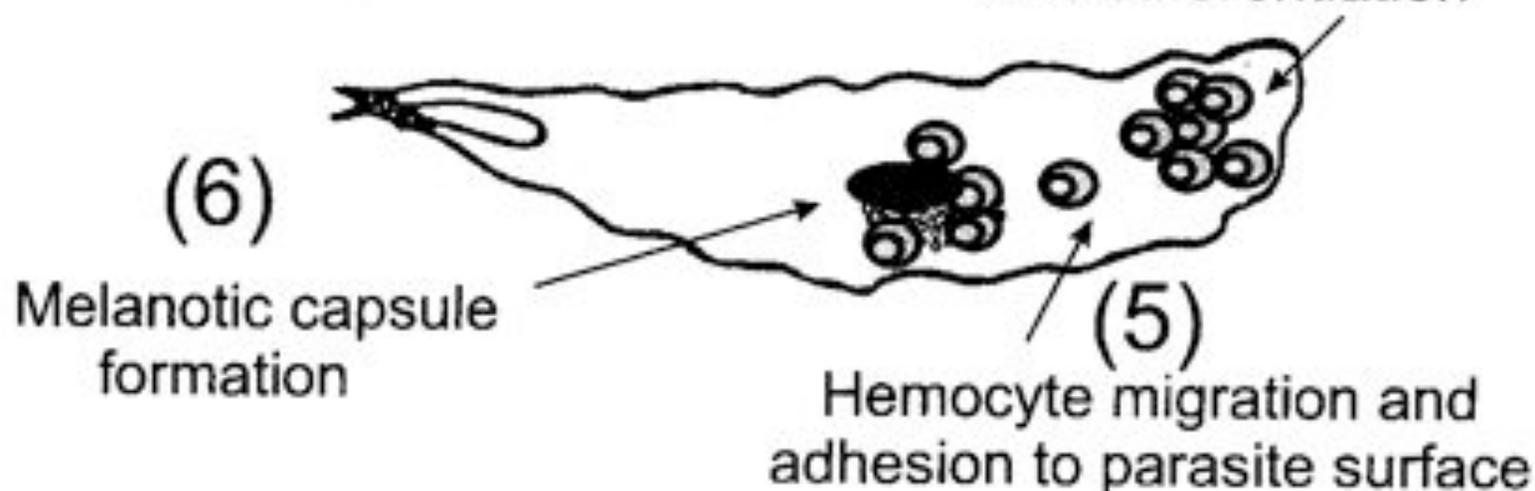
Arrow pointing to the dark, melanized and encapsulated egg of the larval parasite of *Drosophila* and now seen in the adult as an encapsulated dark (melanized) mass.



# Parasite oviposition



# Hemocyte encapsulation



# HOW DO SOME PARASITES IN THEIR NORMAL HOST AVOID BEING ENCAPSULATED?

## The Parasitic Wasp's Secret Weapon

*Parasitic wasps must develop inside living caterpillars. They survive this hostile environment by smuggling in a virus that suppresses their host's immune system.*

by Nancy E. Beckage

caterpillar will never be a moth. It burks deep inside of a tummy hidden from predators, but its end is anyway. In search for offspring, the parasitoid is on the distinctive lepidopteran victim.

Now the tiny wasp injects eggs through the caterpillar and into its body

will die as well. For the caterpillar cannot be allowed to gain the upper hand using its immune defenses. Much of the responsibility for maintaining this delicate balance falls to the wasp's viral accomplice. Like the wasp, many parasites of insect hosts have evolved associations with bacteria and viruses that help them perform their often deadly deeds.

Microbial Weapons

the University of  
Cambridge





We think that this is an unparasitized tomato hornworm but, what is going on inside?

In the photo on the right you can see the cocoons of the parasitic wasp. The larvae have crawled out and have formed the cocoons, which are white and made of silk. This caterpillar will die and never reach adulthood. Good example of biological control.





1. Wasp (*Cotesia congregata*) and polydnavirus possess related genes
2. Similarity between genes of the parasite's venom and that of the virus
3. Viral genes are incorporated into those of the wasp



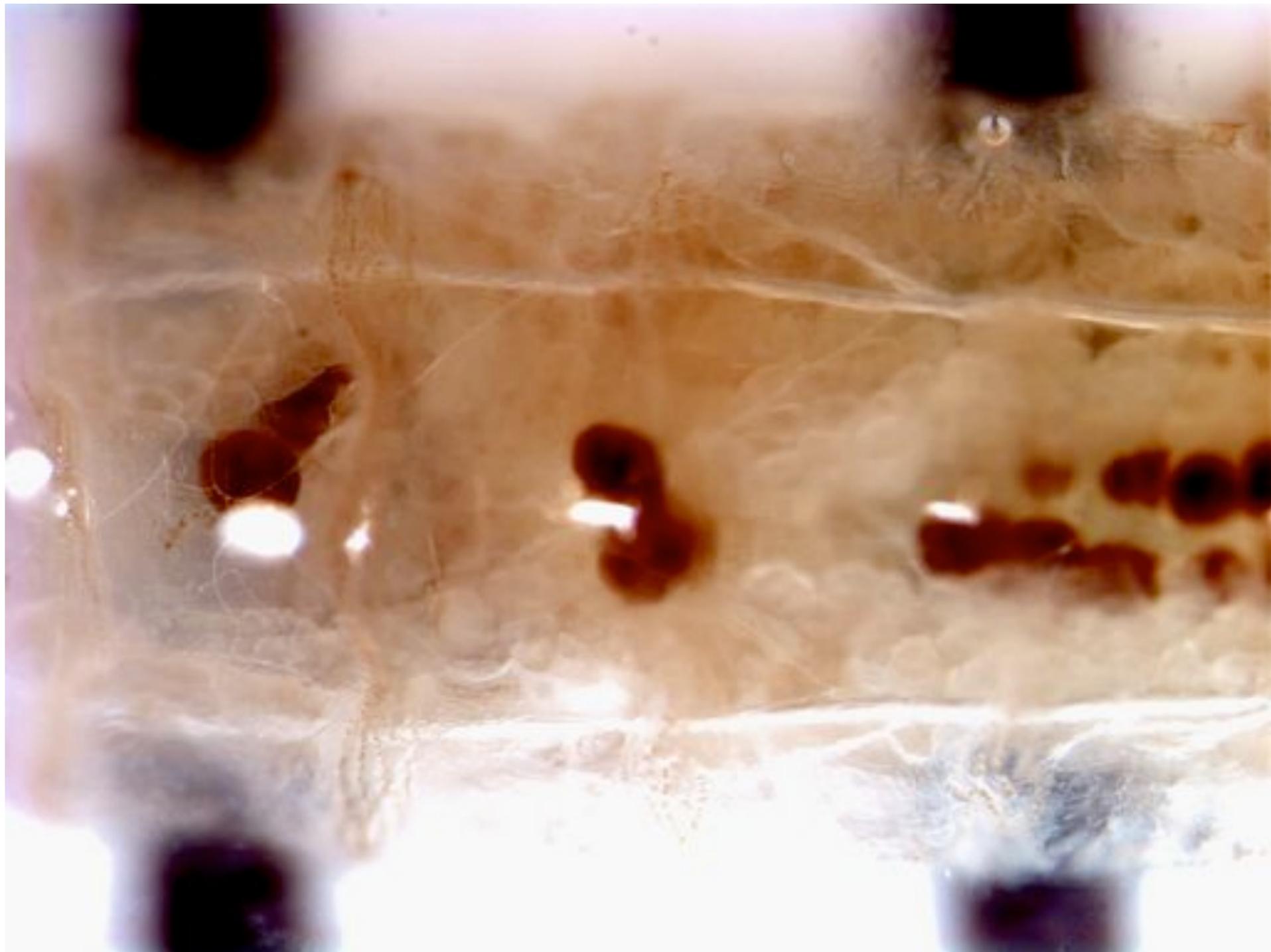


**INTERWEAVING LIFE CYCLES** reveal the relations among caterpillar, wasp and polydnavirus. The normal hornworm life cycle (left) is disrupted when a wasp injects her eggs and polydnavirus. The wasp matures and reproduces normally (blue circle), but the hornworm dies prematurely (yellow circle, right). This sabotage is orchestrated by polydnavirus, which enters and disables caterpillar cells (brown circle). Wasps inherit the virus in their chromosomes, and the virus multiplies in the developing wasp's ovaries in preparation for the next round of trouble.

Slides from John Nambu concerning the mutant he developed that would not complete metamorphosis. Several areas became melanized as if they were like Rizki's tumor forming strain.

A real problem. Nambu sent me these slides asking for help as to what is going on. You should know enough from lab. to provide some ideas.







1. Within hours of infection, the hemocytes round up failing to make contact with the eggs of the parasite
2. The granulocytes, which normally release granules that coat the egg, undergo apoptosis or cell death
3. Plasmatocytes, which normally adhere to this coating (probably serves as recognition molecules) and continue to do so until a layer surrounds the egg, which ultimately dies because of lack of both food and oxygen, also are disabled.

**GRANULOCYTES**-lay down a recognition layer on foreign object

**PLASMATOCYTES**-recognize this recognition layer and also move to and change shape, thus suggesting cytoskeletal involvement.

*Alterations in the cytoskeleton of *Heliothis virescens* hemocytes after parasitization by *Campoletis sonorensis*.* Luckhart, et. al. at Rutgers. After parasitization, the hemocytes fail to spread normally. F-actin, using FITC-phalloidin staining, showed decreased fluorescence in abnormal plasmatocytes and altered distribution of F-actin when compared to normal plasmatocytes.

**Journal of General Virology, Vol 78, 3061-3070, Copyright © 1997 by Society for General Microbiology**

**A polydnavirus-encoded protein of an endoparasitoid wasp is an immune suppressor**

**S Asgari, O Schmidt and U Theopold**

Department of Crop Protection, University of Adelaide, Glen Osmond, Australia.

The molecular mechanism by which polydnaviruses of endoparasitoid wasps disrupt cell-mediated encapsulation reactions of host insects is largely unknown. Here we show that a polydnavirus-encoded protein, produced from baculovirus and plasmid expression vectors, **prevents cell surface exposure of lectin-binding sites and microparticle formation during immune stimulation of haemocytes**. The inactivation of immune-related cellular processes by this protein was analyzed using a specific lectin and annexin V and shown to be virtually identical to polydnavirus-mediated effects on haemocytes. Cytochalasin D application has similar effects on haemocytes, suggesting that the immune suppression by the polydnavirus protein is caused by the destabilization of actin filaments. Since the exposure of cell surface glycoproteins and the formation of microparticles are part of an immune response to foreign objects or microorganisms and a prerequisite for cell-mediated encapsulation of microorganisms and parasites, the virus-encoded protein may become an important tool for the inactivation of cellular immune reactions in insects and an essential component in understanding immune suppression in parasitized host insects.

## G. NON-CELLULAR, HUMORAL IMMUNE RESPONSES

### Humoral encapsulation

In some insects, encapsulation of the parasite or foreign object does not involve the hemocytes. In these insects, strands or threads of material are produced in the hemolymph. These surround the parasite and aid in forming the capsule. Later, the melanization reaction occurs.

**Superoxide** has now been found in insects to be a very toxic molecule involved in the immune, humoral response.

The **phenoloxidase system**, normally involved in tanning and melanization, is also involved in the humoral response of insects.



This image illustrates the electrostatic field (yellow) around an active site (red) of the enzyme superoxide dismutase, which controls oxygen toxicity by converting the superoxide radical to less dangerous forms

**Monocytes in humans are white blood cells and once they leave the bloodstream they mature into macrophages, which is one of the main phagocytic cells in the body.**

**Macrophages contain organelles that fuse to form phagocytic vesicles (phagosomes). This exposes the phagocytized microorganism to a barrage of enzymatically produced, highly reactive molecules of superoxide ( $O_2^-$ ) and hypochlorite (HOCL, the active ingredient in bleach).**

**Taken from pg. 974 of Alberts, et.al.1989. Molecular biology of the cell, 2<sup>nd</sup> edition.**

**Do superoxide molecules exist in insects and are they involved in the non-cellular response?**



Stanley, D.W. 2006. Prostaglandins and other eicosanoids in insects: biological significance. *Annual Review Of Entomology*. 51:25-44.

Stanley, D.W., Miller, J.S. 2006. Eicosanoid actions in insect cellular immune functions. *Entomologia Experimentalis et Applicata*. 119:1-13.

“One of the important growth areas in the physiology of insects is our increasing appreciation of the many information-bearing molecules and their signal transduction systems responsible for organismal integration and homeostasis.” some of these molecules are:

- 1. Ecdysteroids**
- 2. Juvenile hormones**
- 3. Hydrocarbons**
- 4. Pheromones**
- 5. Peptides**
- 6. Proteins**
- 7. Certain amino acids**
- 8. Biogenic amines**
- 9. Eicosanoids**

**EICONSANOIDS - Prostaglandins are found in all body tissues and body fluids of mammals. They generally act as signaling molecules. They get their name from studies on the human prostate gland. They were discovered by researchers using uterine muscle contraction studies and showed that some factor in the male semen caused muscle contractions of the uterus.**

**1982 Nobel Prize in Physiology and Medicine to Sir John Vane who showed that aspirin's action is by inhibiting prostaglandin synthesis.**

**Eiconsanoids -**

Arachidonic acid is one of the essential fatty acids required by most mammals. Some mammals lack the ability to—or have a very limited capacity to—convert linoleic acid into arachidonic acid, making it an essential part of their diet. Since little or no arachidonic acid is found in plants, such animals are obligatory carnivores; the cat is a common example.

# **EICOSANOIDS AS SIGNALING MOLECULES IN INSECTS**

## **1. INSECT IMMUNITY**

**To test the effect of eicosanoids in insect immunity they used tobacco hornworm larvae treated with pharmaceutical inhibitors of eicosanoid biosynthesis. These treated caterpillars were unable to clear bacterial infections from circulation. These results showed that eicosanoids mediate one or more of the insect infection-clearing reactions.**

**They are involved in the expression of two genes encoding antibacterial protein cecropin in fat body of the silkworm, *Bombyx mori*.**

- 1. ACTION ON TICK SALIVARY GLANDS**
- 2. INDUCTION OF STRESS PROTEIN IN MOSQUITO CELLS**
- 3. OVARIAN YOLK PROTEIN UPTAKE**

# Can insects express the febrile response?

Letters to Nature

*Nature* 395, 281-284 (17 September 1998) | doi:10.1038/26233; Received 7 April 1998; Accepted 9 July 1998

Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP<sub>3</sub>

Fumitaka Ushikubi<sup>1,4</sup>, Eri Segi<sup>2,4</sup>, Yukihiro Sugimoto<sup>2</sup>, Takahiko Murata<sup>1</sup>, Toshiyuki Matsuoka<sup>1</sup>, Takuya Kobayashi<sup>1</sup>, Hiroko Hizaki<sup>2</sup>, Kazuhito Tuboi<sup>2</sup>, Masato Katsuyama<sup>2</sup>, Atsushi Ichikawa<sup>2</sup>, Takashi Tanaka<sup>3</sup>, Nobuaki Yoshida<sup>3</sup> and Shuh Narumiya<sup>1</sup>

Fever, a hallmark of disease, is elicited by exogenous pyrogens, that is, cellular components, such as lipopolysaccharide (LPS), of infectious organisms, as well as by non-infectious inflammatory insults. Both stimulate the production of cytokines, such as interleukin (IL)-1, that act on the brain as endogenous pyrogens<sup>1</sup>. **Fever can be suppressed by aspirin-like anti-inflammatory drugs. As these drugs share the ability to inhibit prostaglandin biosynthesis<sup>2</sup>, it is thought that a prostaglandin is important in fever generation.** Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) may be a neural mediator of fever<sup>3</sup>, but this has been much debated<sup>1,4,5,6,7</sup>. PGE<sub>2</sub> acts by interacting with four subtypes of PGE receptor, the EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub> receptors<sup>8</sup>. Here we generate mice lacking each of these receptors by homologous recombination. Only mice lacking the EP<sub>3</sub> receptor fail to show a febrile response to PGE<sub>2</sub> and to either IL-1 or LPS. Our results establish that PGE<sub>2</sub> mediates fever generation in response to both exogenous and endogenous pyrogens by acting at the EP<sub>3</sub> receptor.

**Adamo, S.A. 1998. The specificity of behavioral fever in the cricket *Acheta domestica*. Jour. Parasitol. 83:529-533.**

**Banford, S., Thomas, M.B. and J. Langeward. 1998. Behavioral fever in the Senegalese grasshopper, *Oedaleus senegalensis*, and its implication for biological control using pathogens. Ecol. Entomol. 23: 9-14.**

**Bundey, S., S. Raymond, P. Dean, S.K. Roberts, R.J. Dillon and A.K. Charnley. 2003. Eicosanoid involvement in the regulation of behavioral fever in the desert locust, *Shistocerca gregaria*. Arch. Insect Biochem. Physiol. 52:183-192.**

## G. NON-CELLULAR, HUMORAL IMMUNE RESPONSES

### Inducible humoral antibacterial immunity

It was known for a long time that when insects were injected with bacteria that the bacteria often died. Later they found out that there was also an increase in the insect's hemolymph of some small proteins of unknown function.

peptidoglycans on cell walls of bacteria



these induce an increase in **hemolin** present in the hemolymph



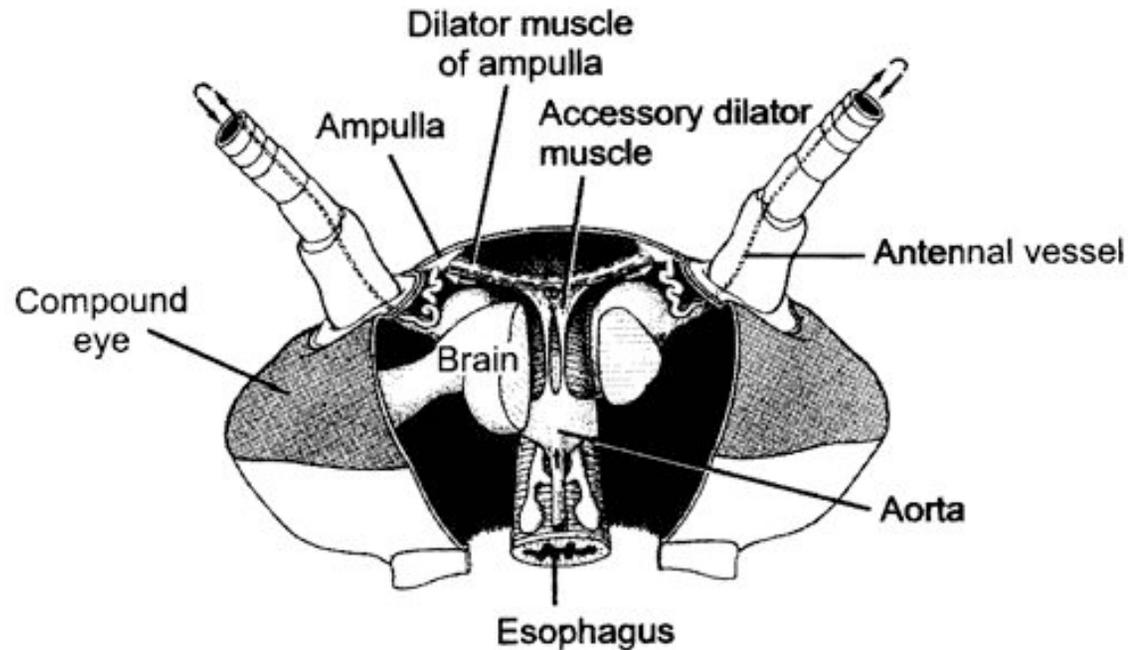
This increase in hemolin causes an increase in the production of a suite of proteins 15 different ones in *Hyalophora* and 25 in *Manduca* larvae. They are called **Cecropins** for *Cecropia* (or **defensins** for others) and are produced by the fat body. They are **antibacterial proteins**.

## H. ACCESSORY PULSATILE ORGANS

Located at the base of the:

1. Legs
2. Wings
3. Antennae

and aid in getting blood to these appendages



### **Annual Review of Entomology**

Vol. 45: 495-518 (Volume publication date January 2000)

(doi:10.1146/annurev.ento.45.1.495)

### **Animations for:**

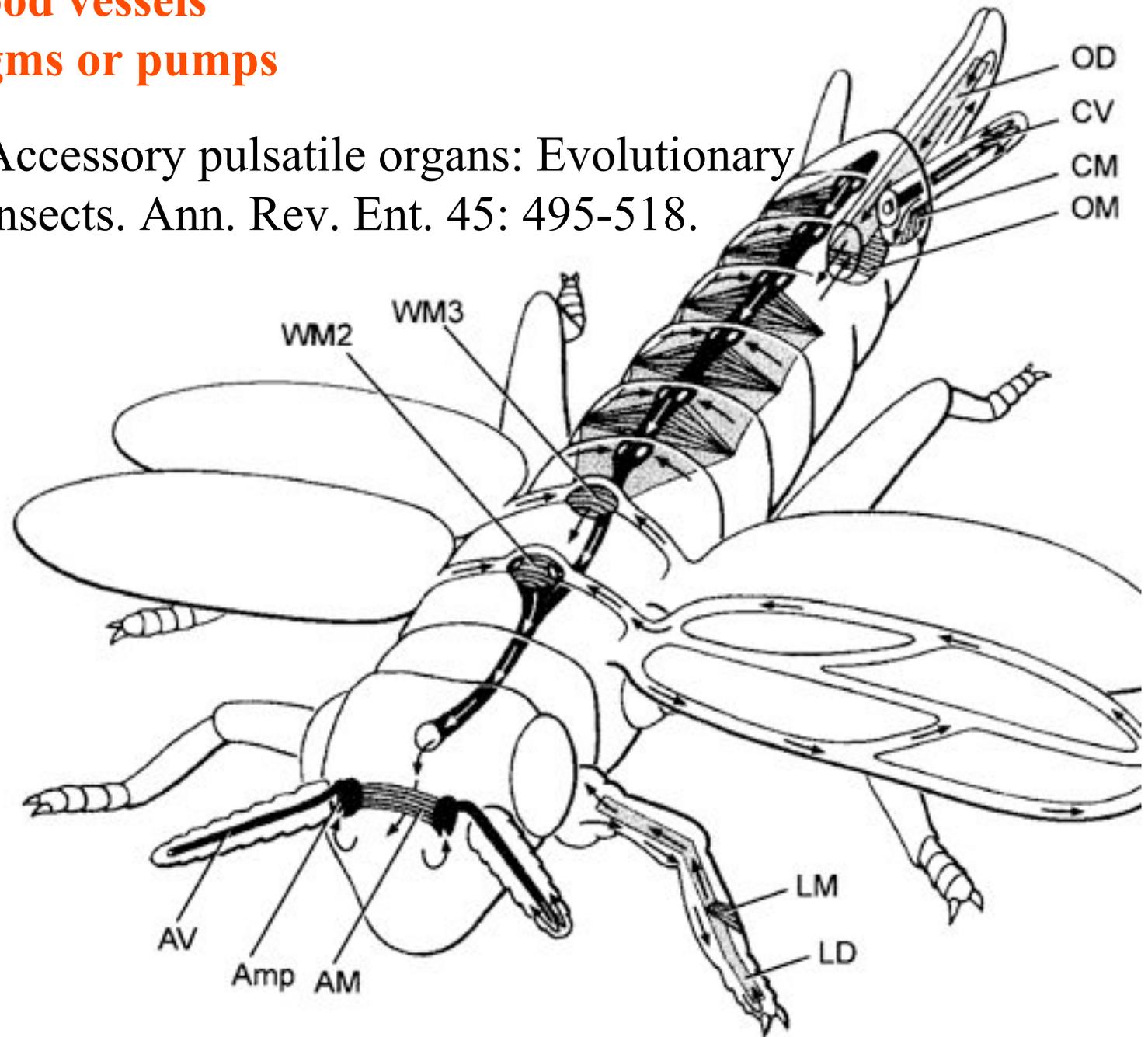
*Annual Review of Entomology*, 45:495-518

**Günther Pass, Accessory Pulsatile Organs: Evolutionary Innovations in Insects**

**Solid black=blood vessels**

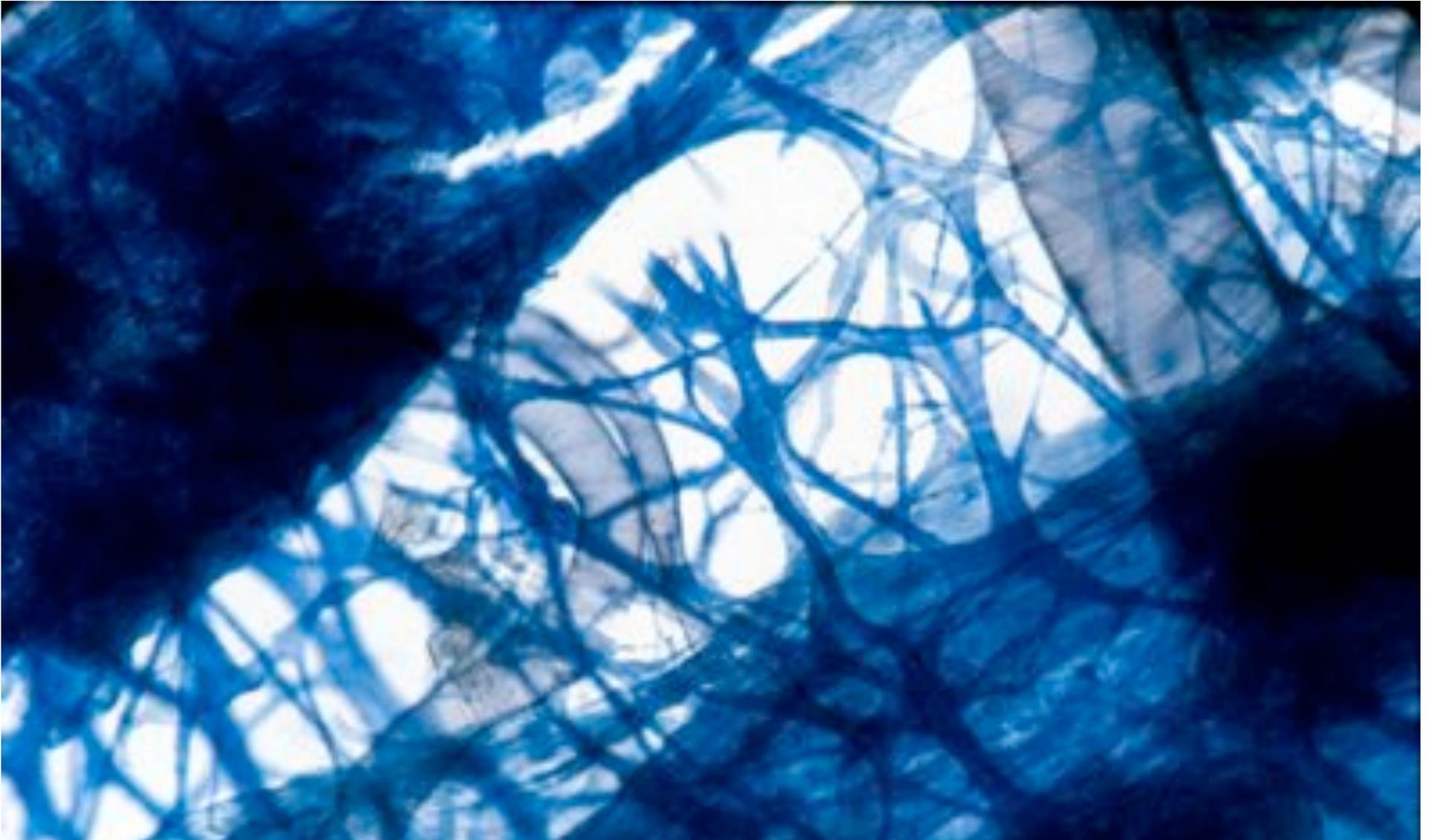
**Gray=diaphragms or pumps**

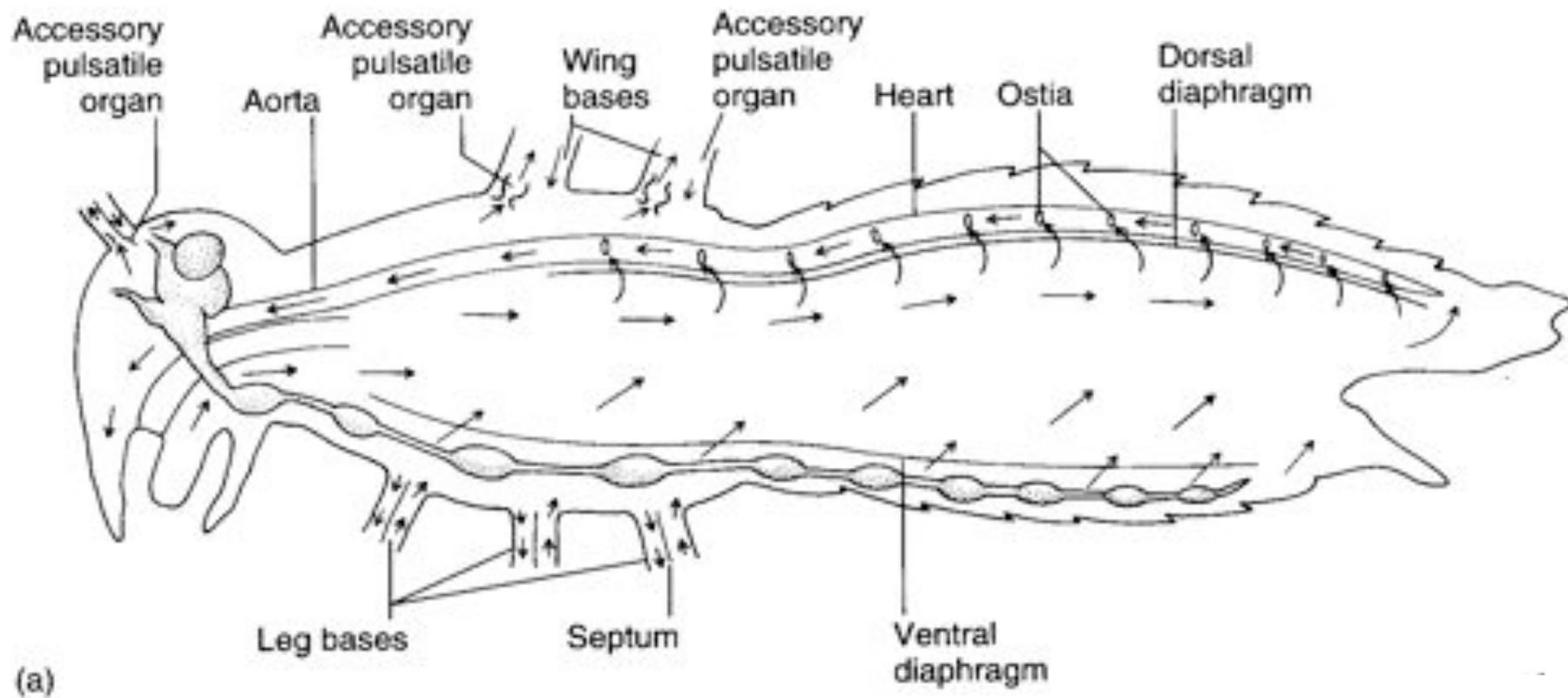
Pass, G. 2000. Accessory pulsatile organs: Evolutionary Innovations in insects. *Ann. Rev. Ent.* 45: 495-518.



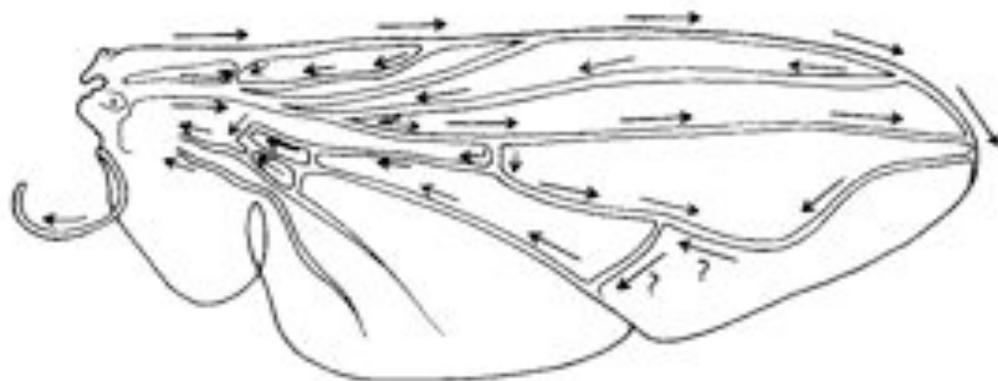
## **I. DORSAL AND VENTRAL DIAPHRAGMS**

Dorsal diaphragm of adult tabanid stained with methylene blue



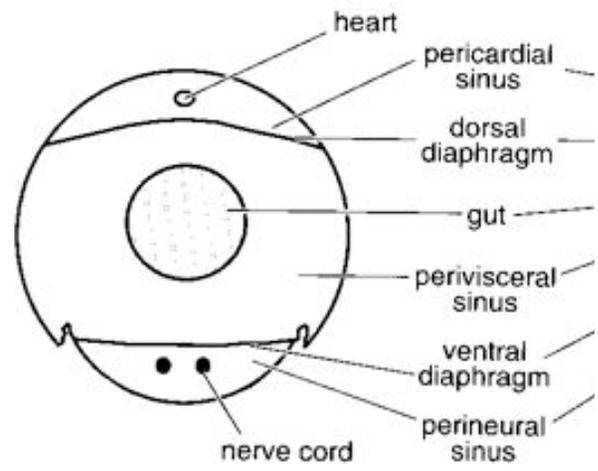


(a)



(b)

a) most insects

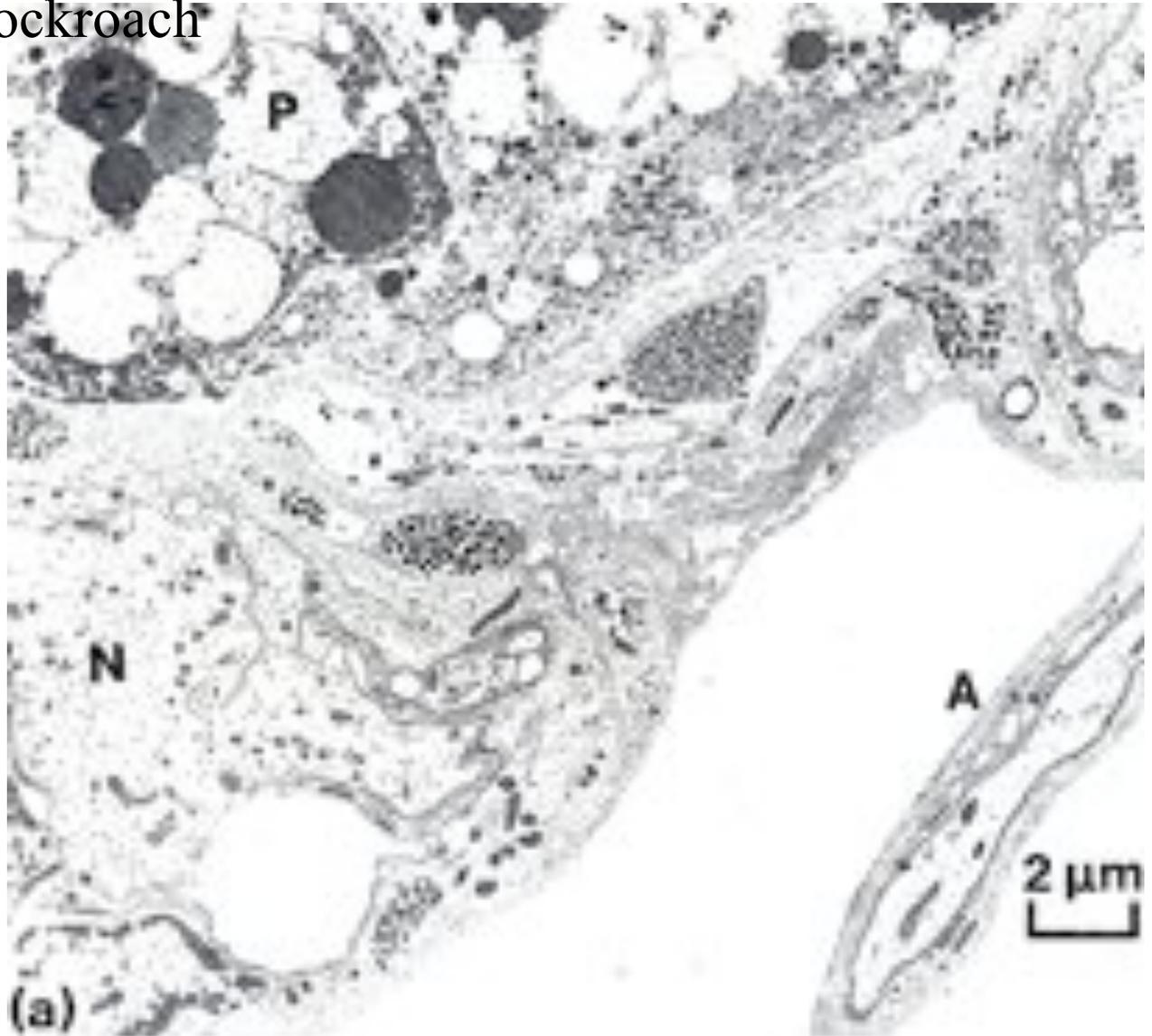


## J. NEUROHORMONAL CONTROL OF HEARTBEAT

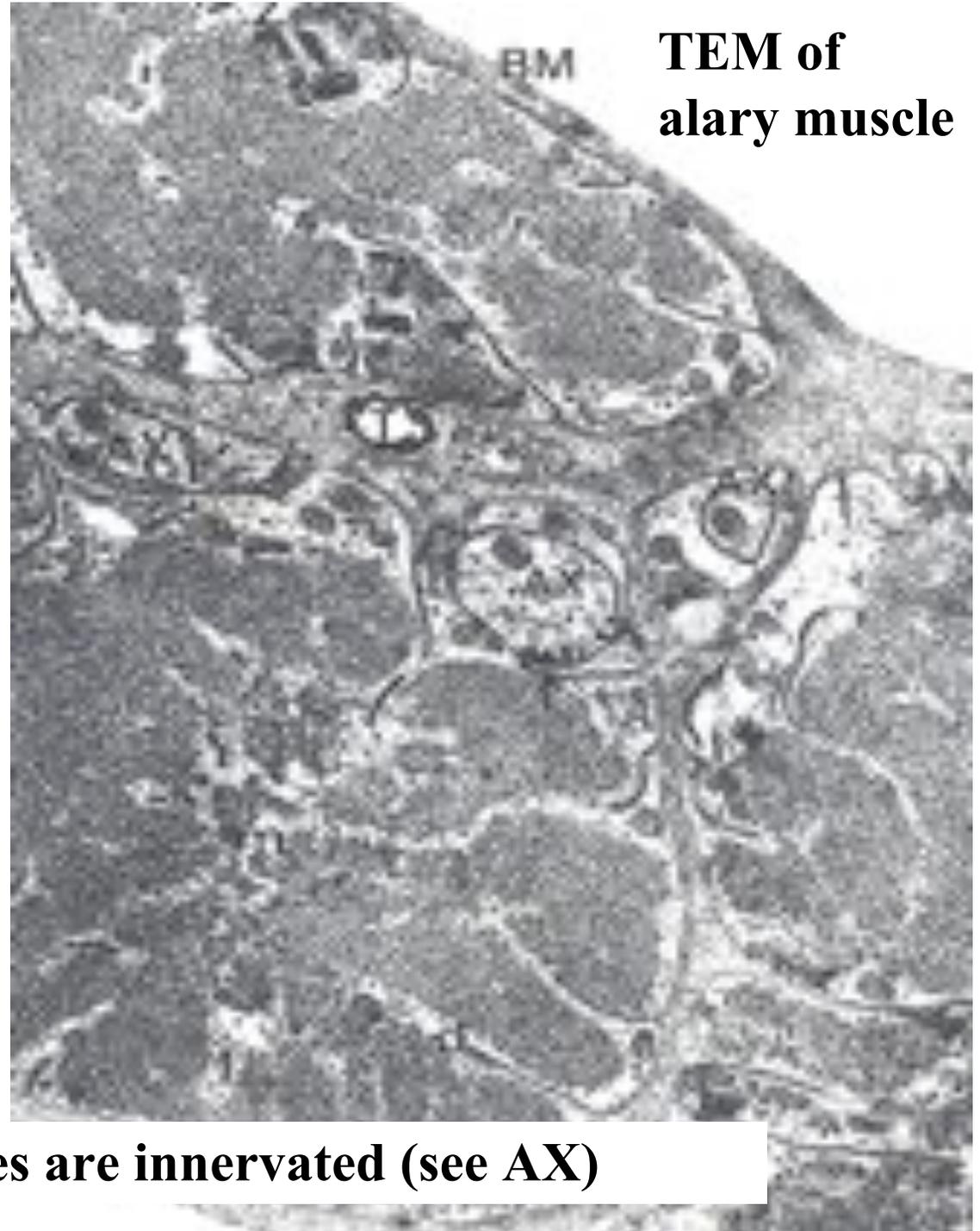
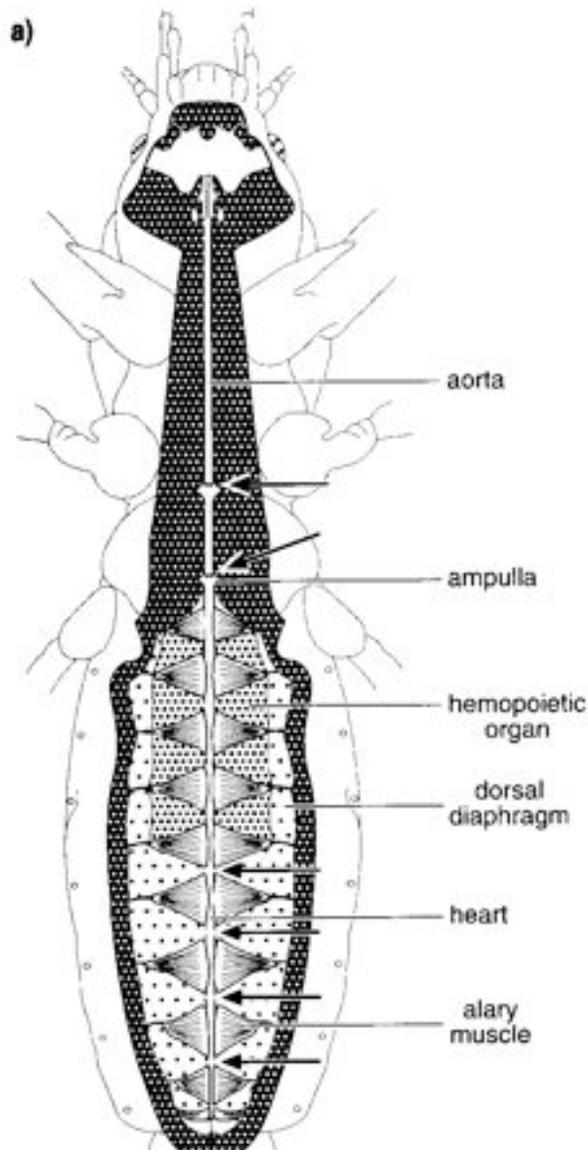
How do we know the heartbeat is influenced by neurohormones?

1. TEM
2. Extracts from the corpus cardiacum
3. Isolation of various cardioaccelerators and testing their effect using electrophysiological recordings
4. Neuropeptide D
5. Dromyosuppressin slows heartbeat

Ultrastructure of cardiac nerve in



## K. ALARY MUSCLES



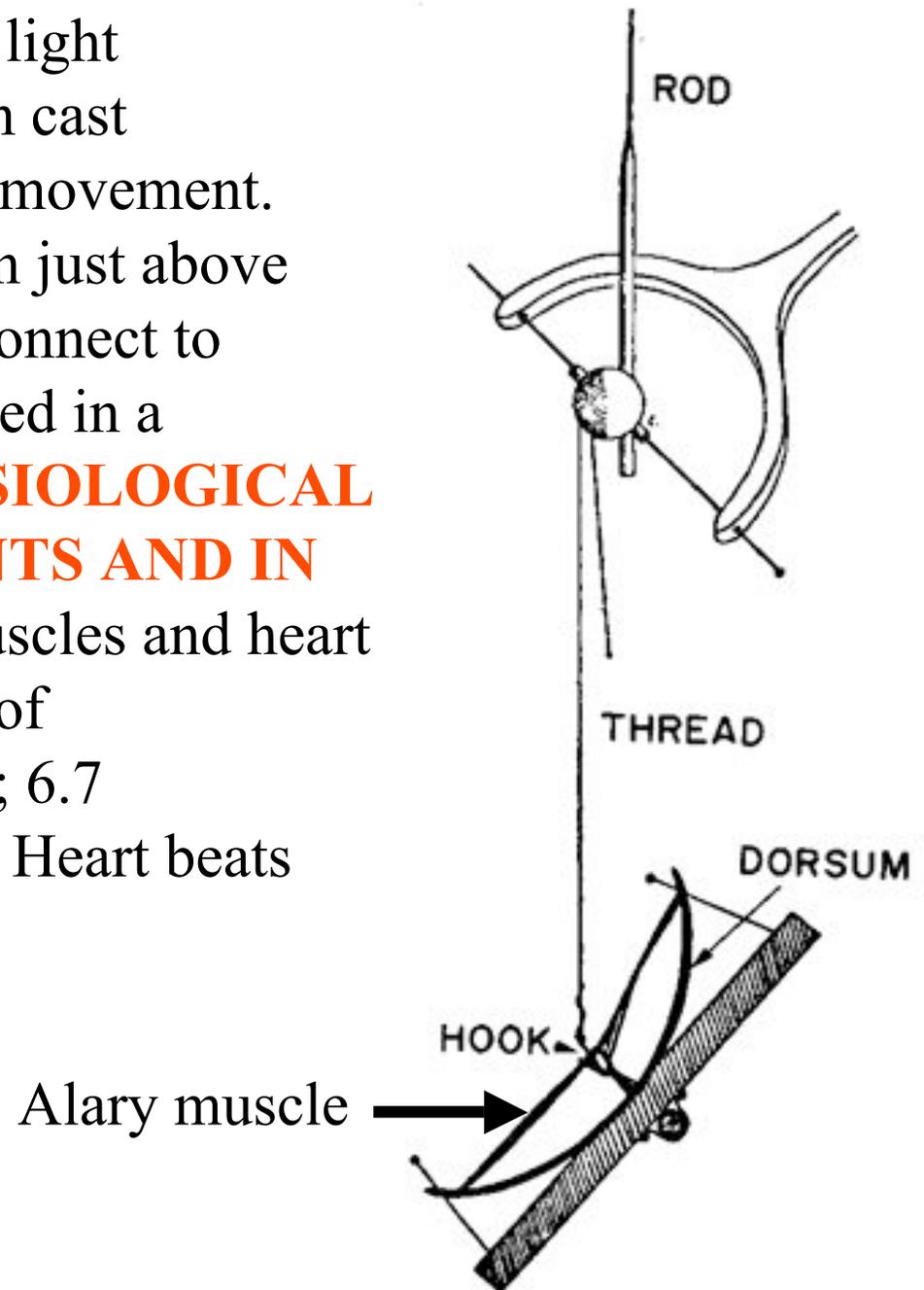
As you can see, alary muscles are innervated (see AX)

Alary muscles (number varies on the species and doesn't seem to show any correlation with group but, **more with flight**). Stronger fliers have fewer alary muscle sets in association with more longitudinal muscle sets. Sacrificed one for the other.

Stimulated by both neurotransmitters and neuromodulators (DMS)

Alary muscle tissue is connected directly to the heart muscle

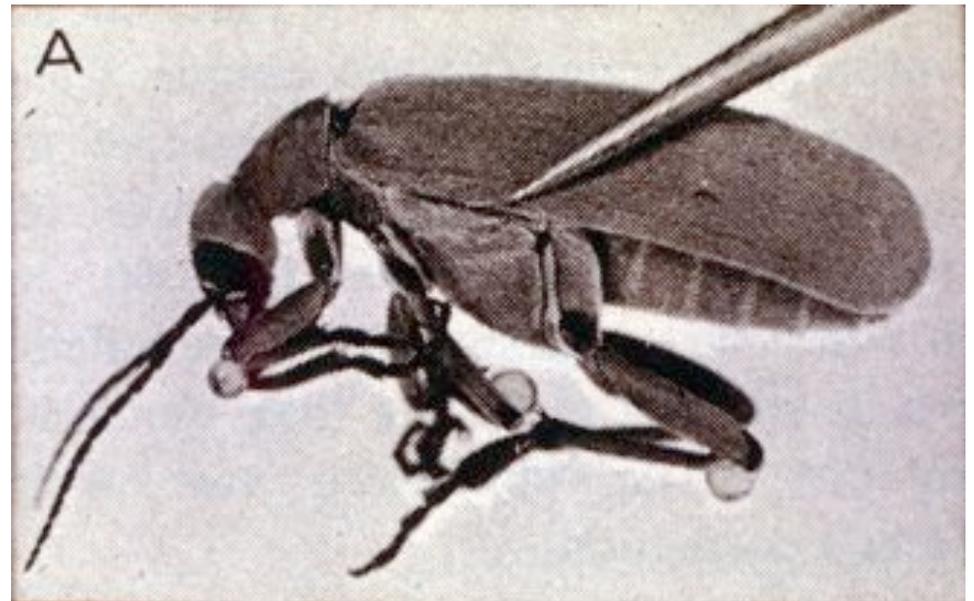
deWilde's set-up for recording the rate of contraction of the alary muscles. A light was shown on the ROD, which then cast a shadow on the wall and recorded movement. The HOOK is attached to the region just above the heart where the alary muscles connect to the heart. The whole system is bathed in a saline. **IMPORTANCE OF PHYSIOLOGICAL SALINES IN ALL EXPERIMENTS AND IN ALL INJECTIONS.** The alary muscles and heart are reported to have different rates of contraction. 80 beats/min for heart; 6.7 contractions/min for alary muscles. Heart beats faster than the alary muscles.



## L. REFLEX BLEEDING

Cantharadin produced by certain beetles, using a reflex action known as reflex bleeding (see arrows), is a powerful antifeedant compound to various predators and vesiculating compound.

Lady beetles also perform reflex bleeding when disturbed by a predator. They are also aposematically colored, thus helping the predator to learn to avoid them. They overwinter in large aggregations, thus coloration should work in a group effect to prevent feeding by the predator.



## **M. THERMOREGULATION + THE CIRCULATORY SYSTEM**

**The heat is transferred to other parts of the body via the circulatory system.**

Heat for flying

Heat for brooding eggs and larvae

Heat to kill a predator

Heat for maintaining hive temperature in winter

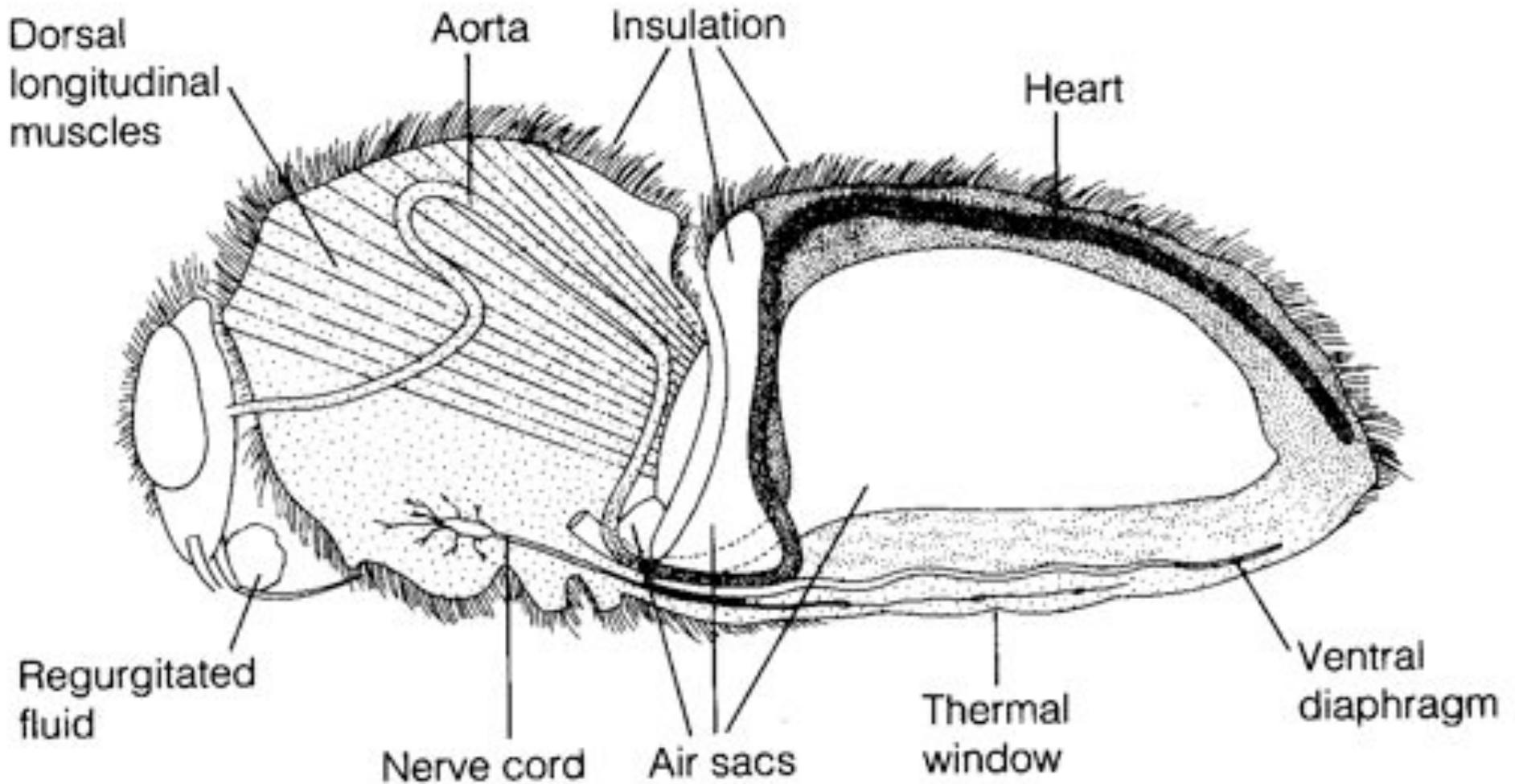
# **Muscles and shivering by bees, bumblebees, and some moths to produce cellular respiratory heat**

- 1. Increase temperature above ambient in order to fly**
- 2. Bees maintain colony temperature during winter at a constant temperature**
- 3. Bumblebees producing heat so they can incubate their eggs and/or larvae**
- 4. Japanese honeybees producing enough heat to kill the predatory wasp**

Many insects have heat exchangers that permit blood to be either heated or cooled. Some insects, like the bumblebee and wasp queens over winter in a diapause condition but, in the spring are ready to produce a brood. The queens first priority is to rear a group of helpers. Temperatures, however, in early spring may be near zero. In order to successfully hatch eggs and develop brood in temperate and arctic areas, the queen must 'incubate' the eggs and brood, just like a chicken does. To do this they have a special heat exchange unit (the dorsal blood vessel) that picks up the heat they generate by shivering (contracting of the dorsal and longitudinal flight muscles). As the blood goes through the thoracic region it picks up heat, which is then deposited into the head. This warmed blood then travels ventrally into the abdomen where there is a heat exchange 'thermal window' on the abdomen. The queen presses this area over the eggs and brood and is able to generate enough heat that they can now produce eggs and brood within a 2 week period, even at these cold temperatures.

## INCUBATION OF EGGS AND BROOD IN COLD ENVIRONMENTS

Bumblebee and heat production and transfer from Heinrich. Dorsal and longitudinal muscles produce the heat. Hot blood goes ventrally into the abdomen. Heat produced is lost through the thermal window and the cooled blood is pumped back into the heart.



Honeybee worker drinking sugar must shiver to keep warm. Sugar water in black; Thorax is at the highest temp. due to muscle shivering (in white) while the abdomen (in green) stays cool. From Heindrich



## Unusual thermal defence by a honeybee against mass attack by hornets

Masato Ono, Takeshi Igarashi, Eishi Ohno & Masami Sasaki

THE giant hornet *Vespa mandarinia japonica* (Hymenoptera: Vespidae) is the only hornet species known to have evolved *en masse* predation of other social bees and wasps. Here we show that hornets is initiated by secretion of a foraging-site marking pheromone from the van der Vecht glands (metasomal sternum VI glands) by a single foraging hornet. The lone hornet rubs the basal tuft of the terminal gastral sternite around a prey food resource, such as a honeybee colony, and the hornet nestmates then congregate and attack the marked site *en masse*. The sympatric Japanese honeybee *Apis cerana japonica* (Hymenoptera: Apidae) can detect the hornet marking pheromone, and responds by increasing the number of defenders at the nest entrance. When an invading hornet is captured by a defending bee, more than 500 other bees quickly engulf the hornet in a ball which contains isoamyl acetate. Thermography showed that the ball temperature is very high ( $\sim 47$  °C), which proves lethal to the hornet but not to the bees. Defenders patrolling the nest entrance also generate high temperatures. These findings suggest that aspects of the interaction between *V. mandarinia japonica* and *A. cerana japonica* are specifically coevolved. **letters to nature**

If a colony of 30,000 European honeybees (*Apis mellifera*) is attacked by 30 giant hornets (*Vespa mandarinia japonica*) they'd be wiped out in 3 hours. ... The defense by *Apis japonica* is to form a tight ball of living bees around the wasp and generate a lot of muscle heat—enough to kill the wasp. Temp. can be as high as about 47 degrees Centigrade.

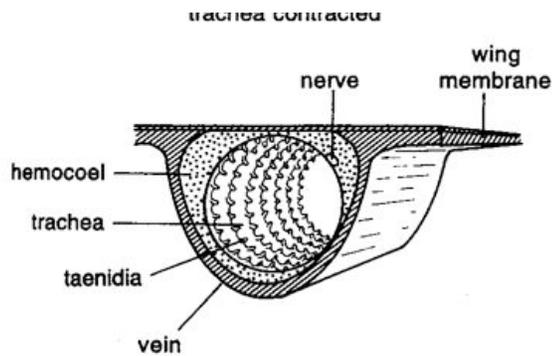
Infrared photo showing heat produced by the bees surrounding the wasp.



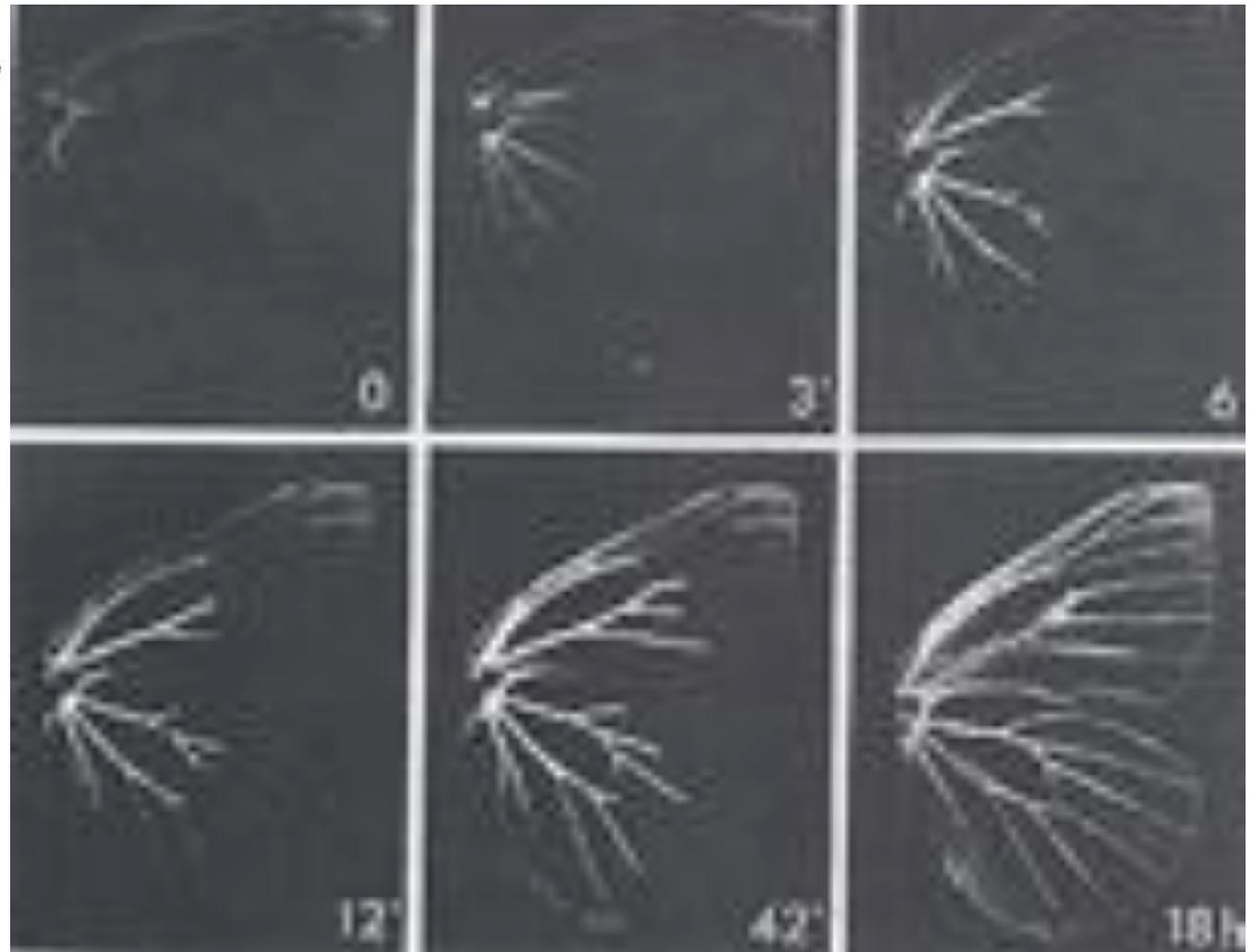
Defensive ball of *Apis japonica* with about 400 tightly aggregated bees. (Courtesy of Masao Ochi)



# Hemolymph enters the veins of the wings of insects.



Above is diagram of a cross-section of the wing and the wing vein, plus the hemocoelic space.



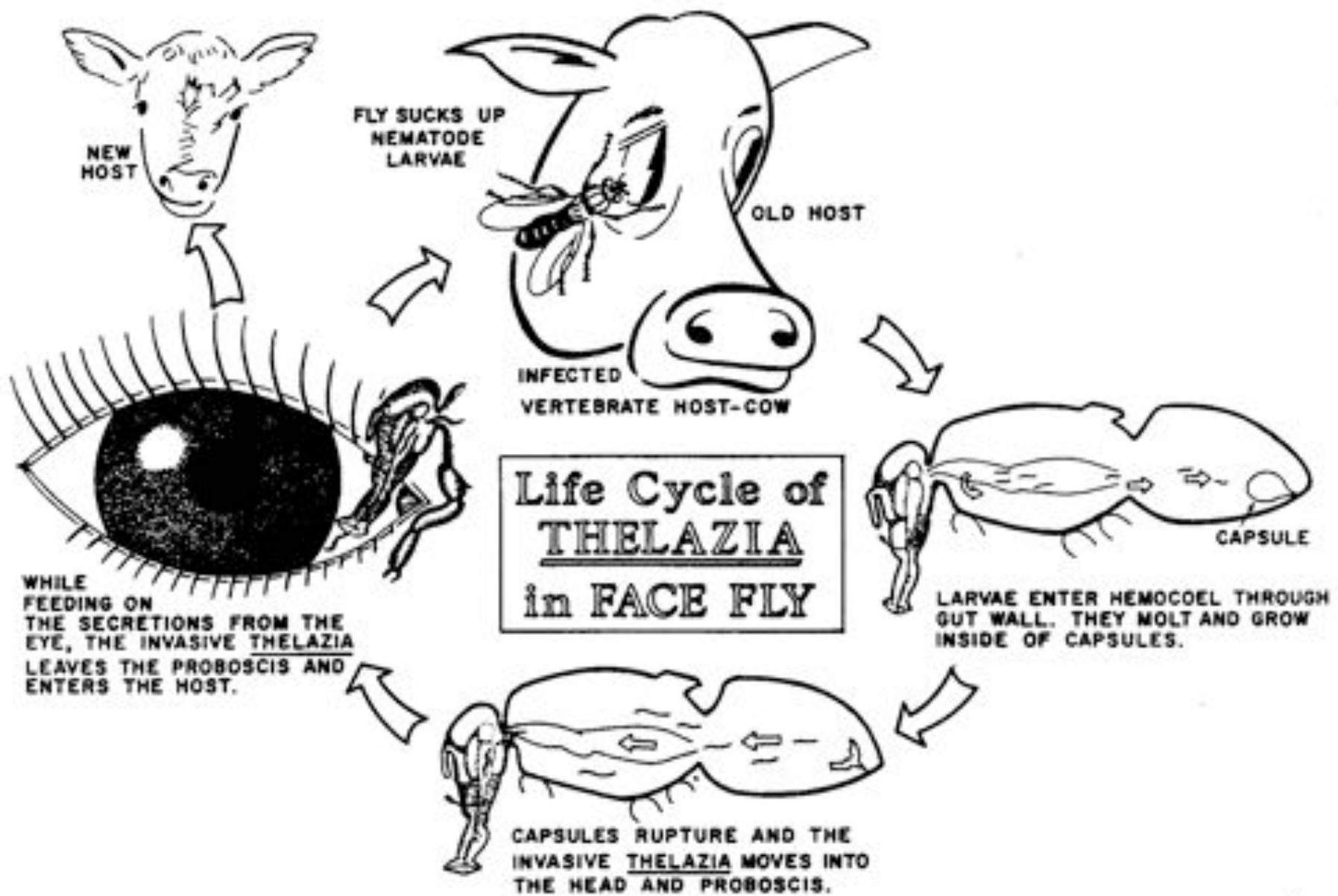
# **N. PARASITIC ADAPTATION (GIANT CELLS)**

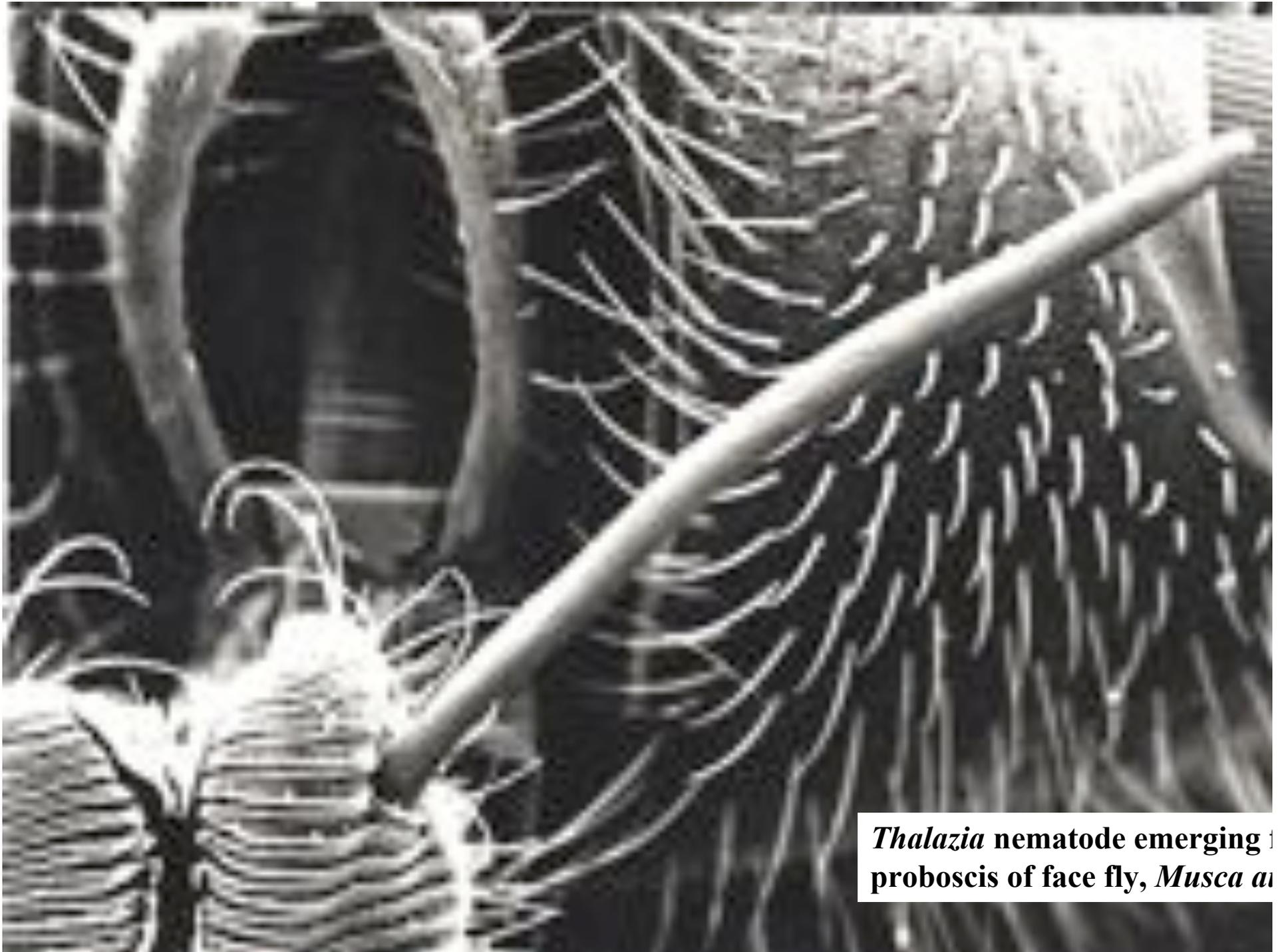
**HOW DO VARIOUS PARASITES SUCH AS NEMATODES  
AVOID THE ENCAPSULATION RESPONSE?**

**If you were a parasite and you knew what we now know about the circulatory system, how would you avoid the hemocytic response?**

1. Somehow stop the hemocytes from chemotaxis
2. Somehow stop the hemocytes from recognizing you are foreign

**BOTH 1 AND 2 HAVE BEEN DISCUSSED WITH THE HORNWORM AND THE POLYDNAVIRUS EXAMPLE OF HOW THE PARASITE MANIPULATES THE HOST AND ITS BLOODCELLS TO ITS ADVANTAGE - WHICH IS NOT TO BE ENCAPSULATED.**



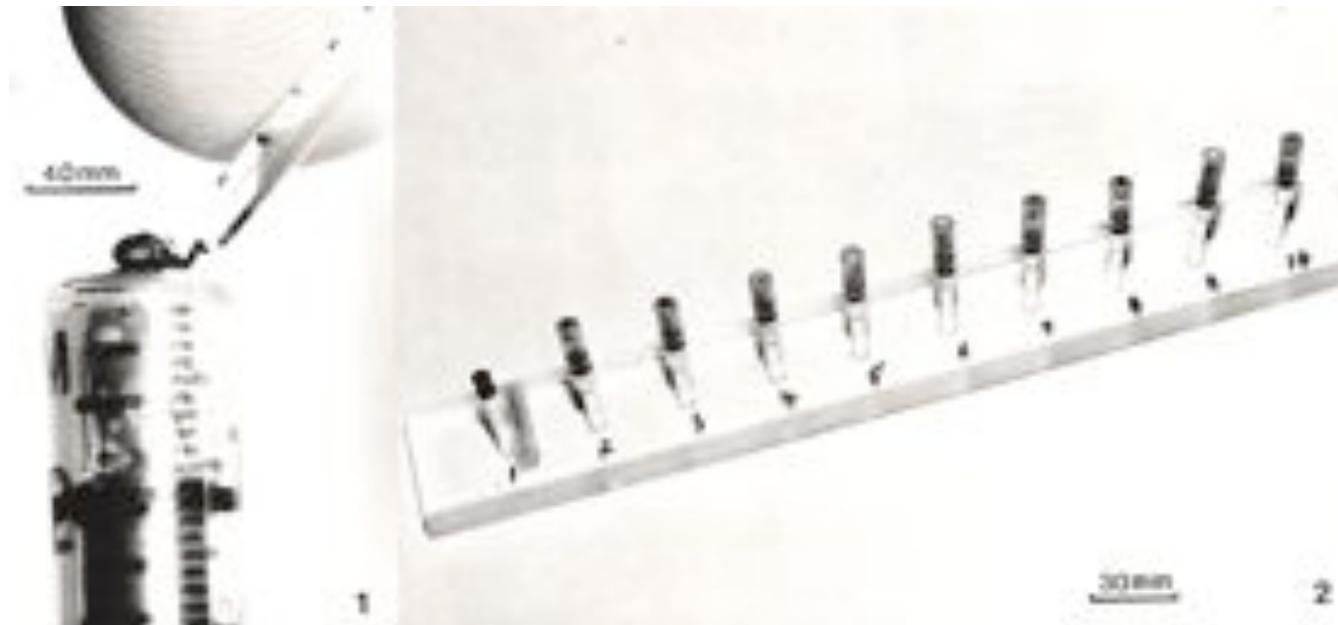


*Thalazia* nematode emerging from the proboscis of face fly, *Musca autumnalis*



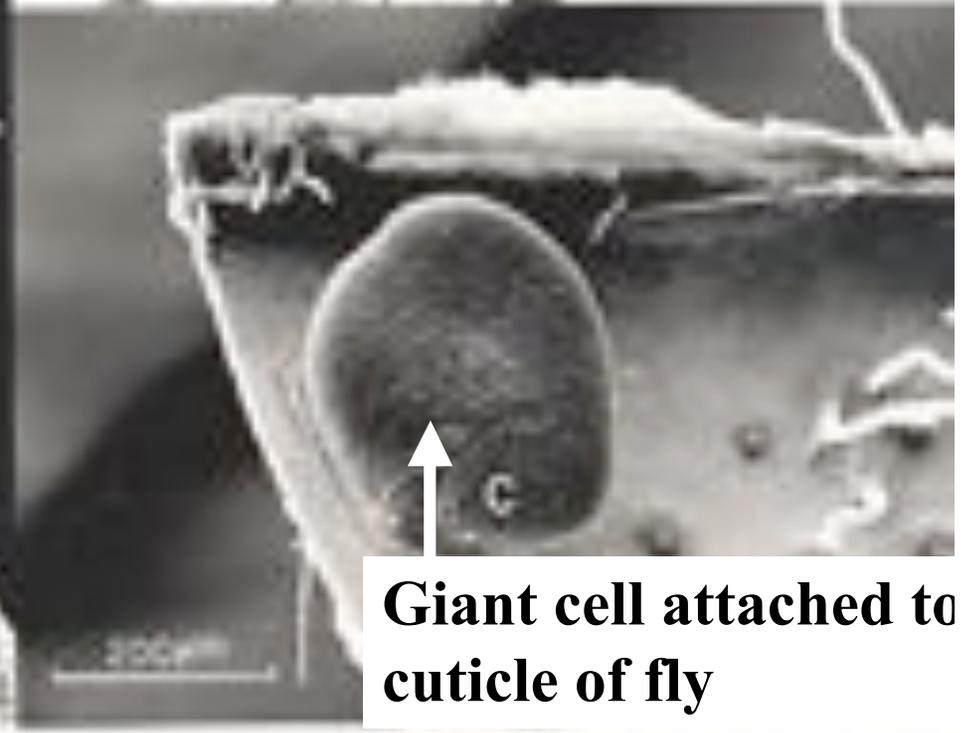
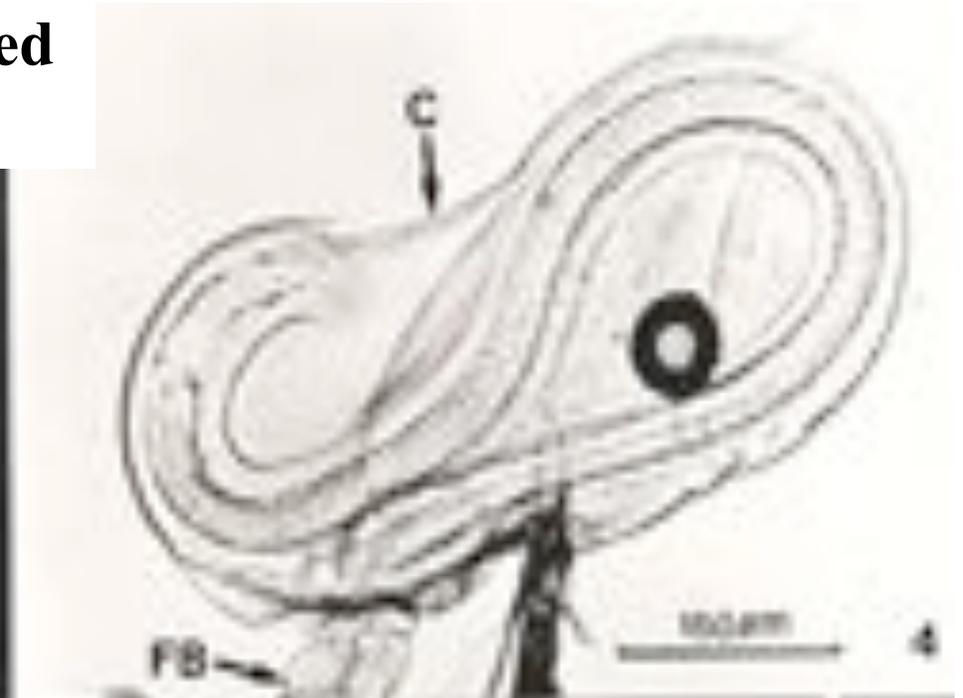
***Thelazia* nematodes are found in conjunctiva and tear ducts. Note nematode on the eye of the cow and the infection or abscess it can cause.**

In order to infect face flies, gamogenetic females are extracted from the eyes of the cow, put into a petri dish, and dissected. This causes the release of the infective stage nematodes. This is the stage that is normally found in the cow's eye and which are sucked up by the fly and then enter the fly through the midgut.



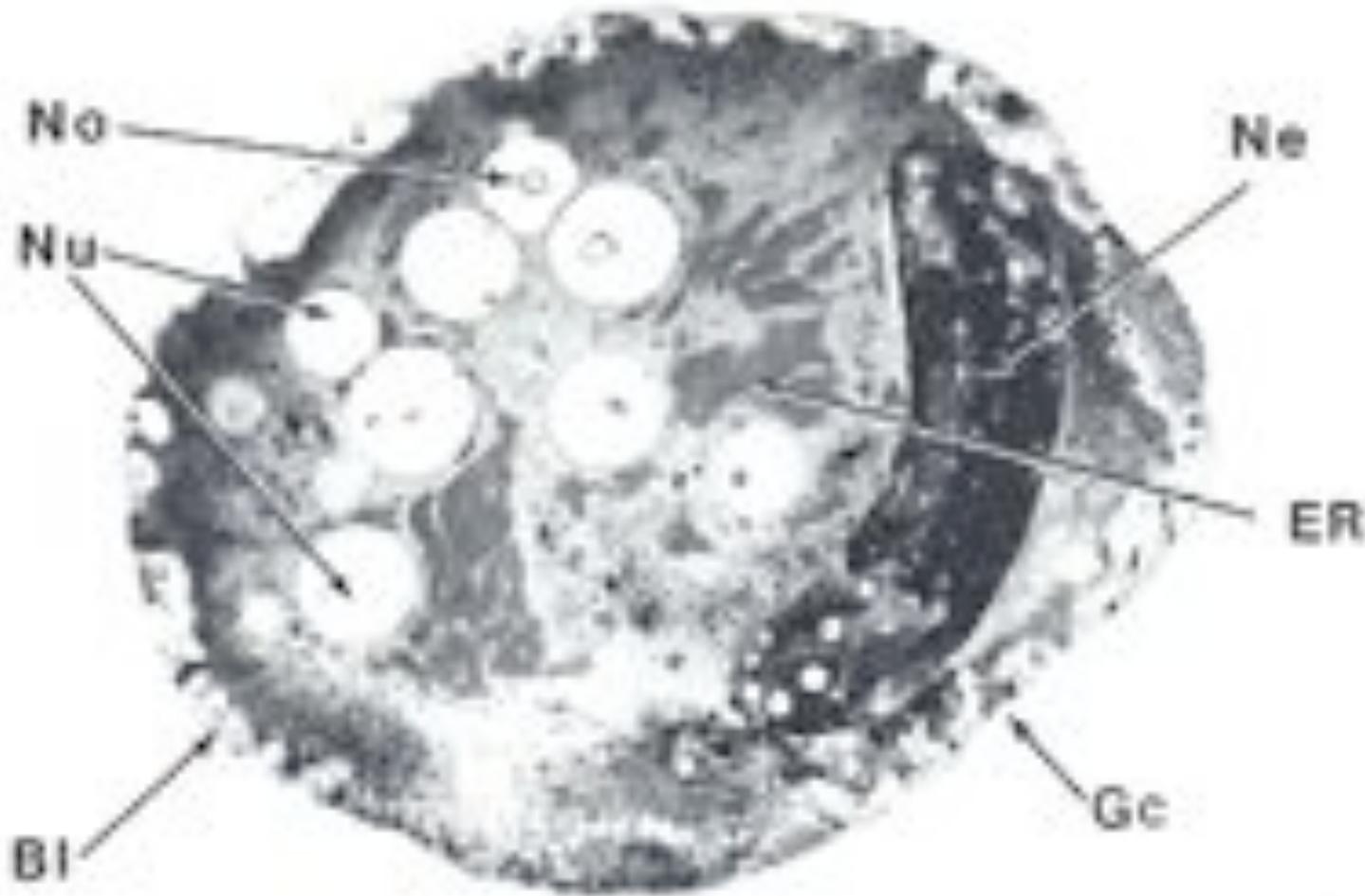


← Giant cell attached to fat body

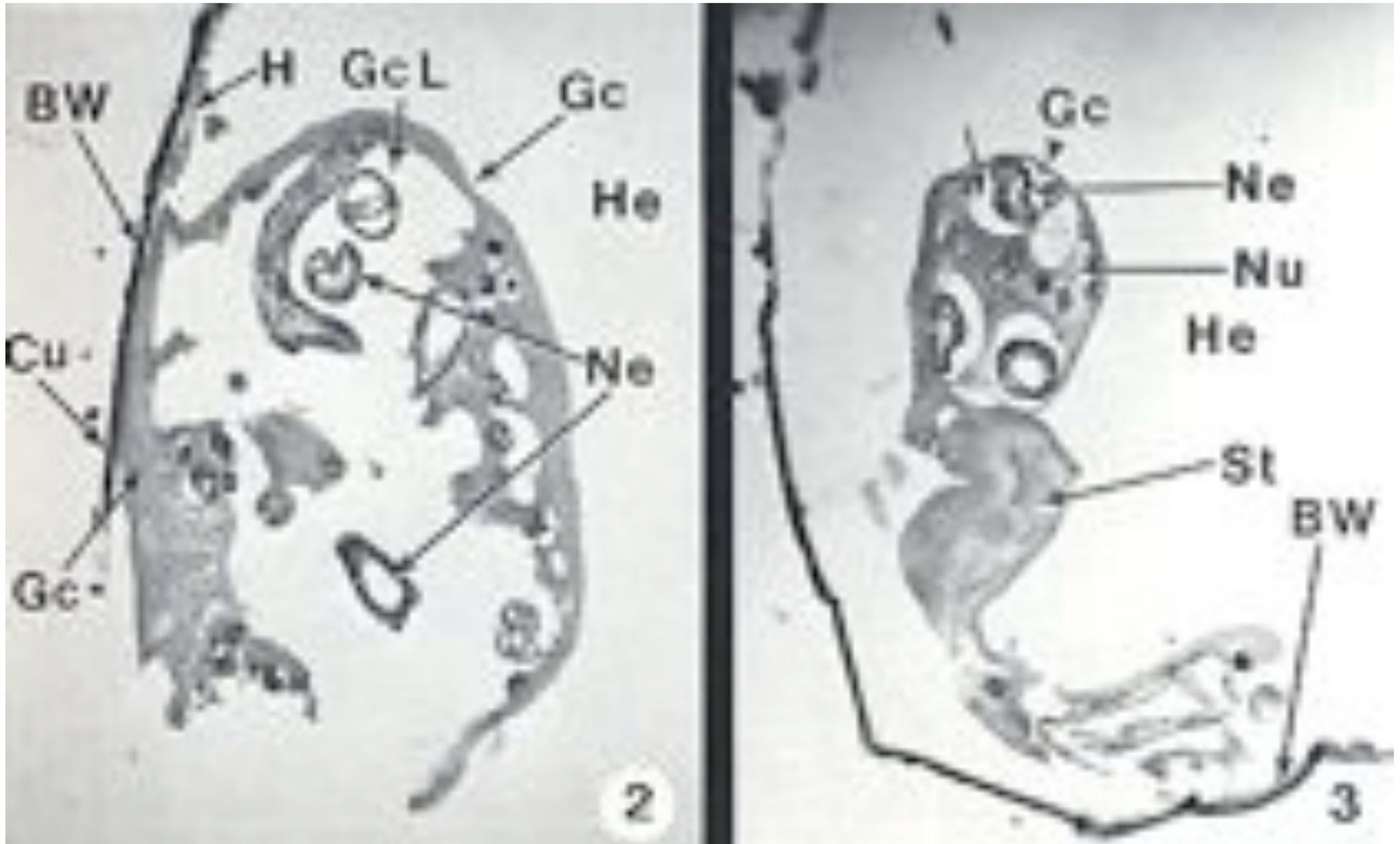


↑ Giant cell attached to cuticle of fly

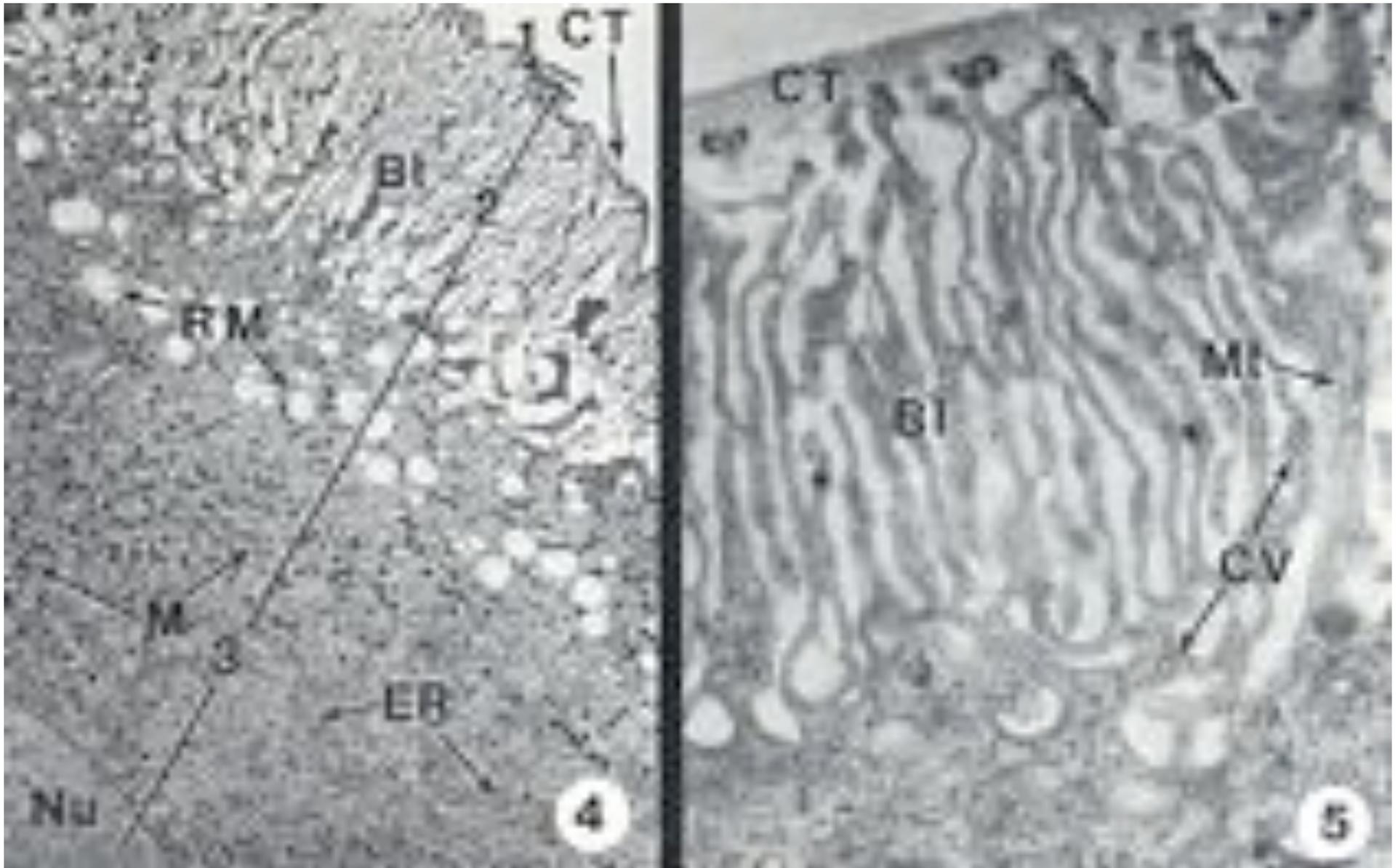
Histological, light micrograph of a giant cell (Gc). Note the nematode (Ne), the nuclei (Nu), the nucleoli (No), endoplasmic reticulum (ER), and basal lamina (Bl).



Light micrographic, histological section of a giant cell (Gc) attached to the cuticle of the face fly. He=hemolymph; GcL=giant cell lumen, Cu=cuticle of fly, BW=fly body wall, Ne=nematode, Nu=nuclei

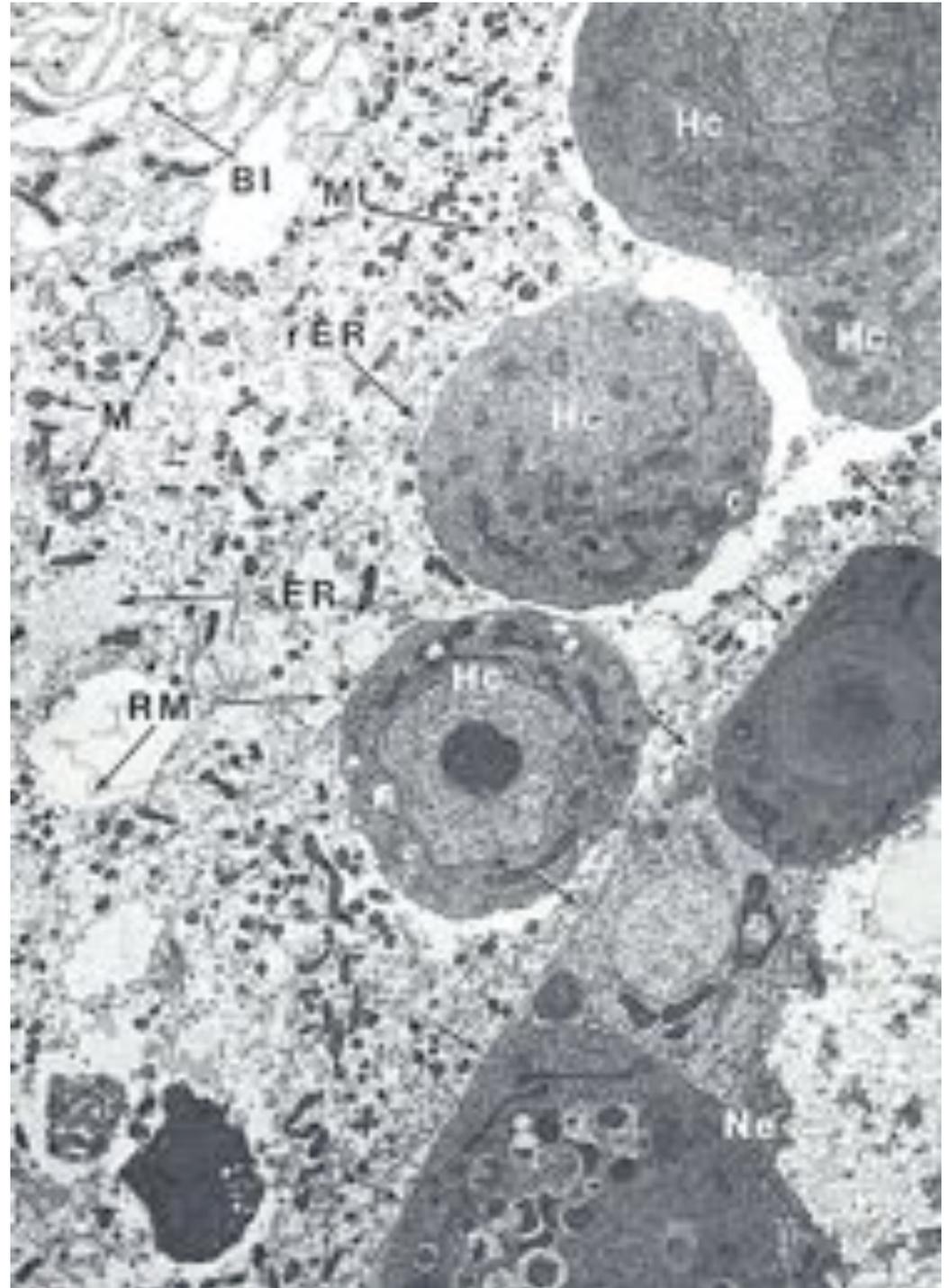


TEM micrograph of a giant cell showing CT=connective tissue, BL= Basal lamella, M=mitochondria, Nu=nucleus, ER=endoplasmic reticulum Mt=microtubules; CV=coated vesicles. Arrows show attachment to CT



TEM of a giant cell showing Hc=host hemocytes inside the capsule, Ne=nematode. The important thing to note is that there are a lot of cell organelles.

**Where did they come from?**



Somehow, the nematode enters some host cells, possibly fat body for those that are attached to the fat body or epithelial cells for those that are attached to the fly cuticle, and causes these cells to rupture and release their organelles. Eventually other cells are recruited into the giant cell, release their organelles and then they organize themselves into what looks like a normal cell. This organization aids the nematode in getting nutrition, getting rid of waste and preventing the host hemocytic response. **How does it do that?**

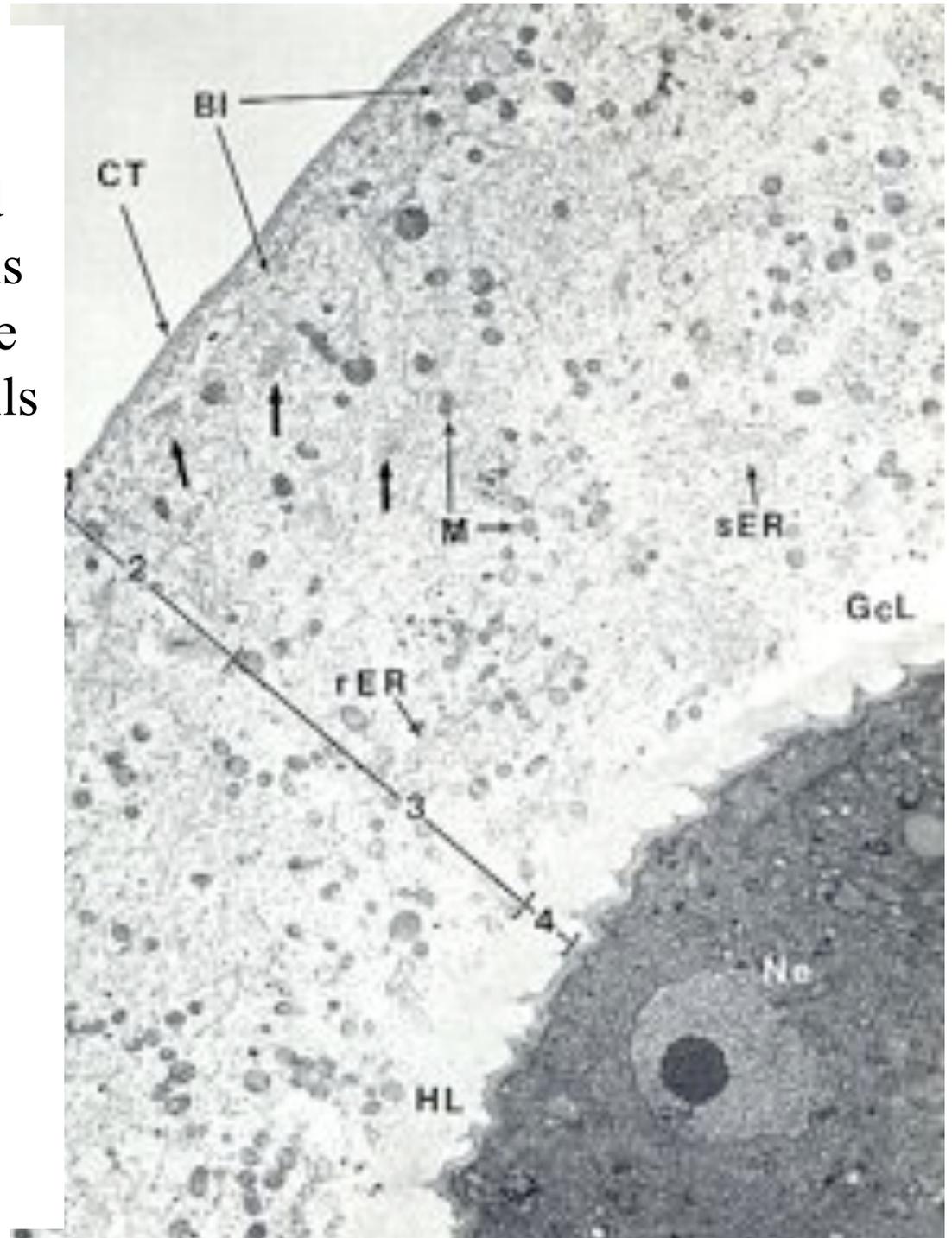


TABLE 2. Comparisons of the organelles and other cellular components found in giant cells surrounding *Thelazia* spp. and hemocytic capsules produced by insects

Organelle or cellular component	Giant cell	Hemocytic capsule	Reference*
Hypertrophied nuclei	+	→ -	
Mitochondria	+	+	3,4
Free ribosomes	+	+	2
Smooth endoplasmic reticulum	+	→ -	
Rough endoplasmic reticulum	+	+	2
Annulate lamellae	+	→ -	
Microtubules	+	+	1,7
Microfilaments	-	+	2
Golgi complex	+	+	3
Cell junctions	-	+	1,6,8
Cytolysosomes	-	+	3,4,5,7
Outer connective tissue layer	+	-	
Plaques associated with outer layer	+	-	
Disintegrating membranes found inside	+	-	
Basal infolding of outer layer	+	-	

\*References: 1, Baerwald 1979; 2, Brehélin *et al.* 1975; 3, Ennesser and Nappi 1984; 4, Grimstone *et al.* 1967; 5, Nappi 1977; 6, Poinar *et al.* 1968; 7, Rowley and Ratcliffe 1981; 8, Zachary *et al.* 1975.

**Most parasites/parasitoids place their eggs into the hemocoel of the host. They do this using their ovipositor and sensing the internal environment of the hemolymph. Very few parasites/parasitoids attack the adults. This may be due to a couple of things:**

- 1. Adult cuticle is more difficult to penetrate and**
- 2. The hemocytic response of adults has not been studied that well but suggests that they have fewer hemocytes.**
- 3. Adults may live a short time compared to the immatures, thus shorter time to develop**



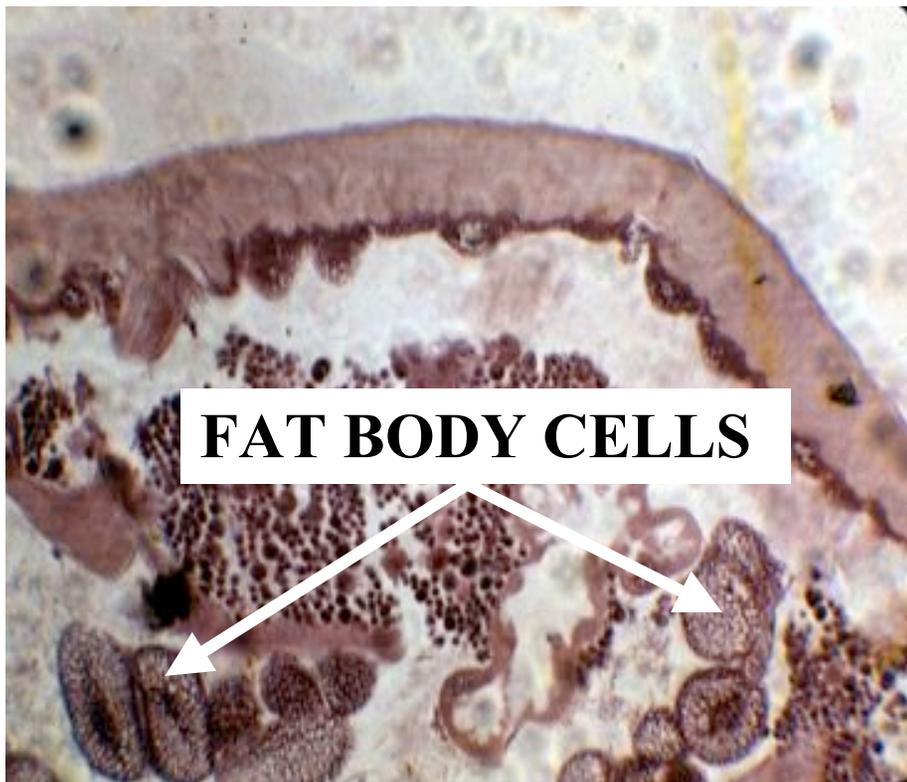
**Tachinid eggs laid on adult Japanese beetle**

# OTHER CELLS ASSOCIATED WITH THE CIRCULATORY SYSTEM

1. Fat body cells
2. Pericardial cells
3. Oenocytes

Fat body cells are involved in:

1. Intermediary metabolism (glycogen to glucose; glycerol production)
2. Contain MFO or cytochrome P450 enzymes (similar to vertebrate liver)
3. Take precursors from the hemolymph and produce the female specific protein or vitellogenin and put it into the hemolymph
4. Takes wastes out of hemolymph and produces uric acid
5. Production of antibacterial proteins known as Cecropins
6. Hemoglobin synthesis in chironomids



# Hemoglobin-

**Hemoglobin synthesis occurs in the fat body of larval midges (Chironimidae).**

**Go to the website below to see some excellent photos of the larvae  
<http://www.wdaweb.com/bloodworms.htm>**



## 2. Pericardial cells (Nephrocytes)

Provides for a blood filtration system.

These cells

**recognize,**

**absorb,**

**and clear** from the hemolymph

**macromolecules** via the

process of endocytosis. They are called

pericardial cells because they are around (or

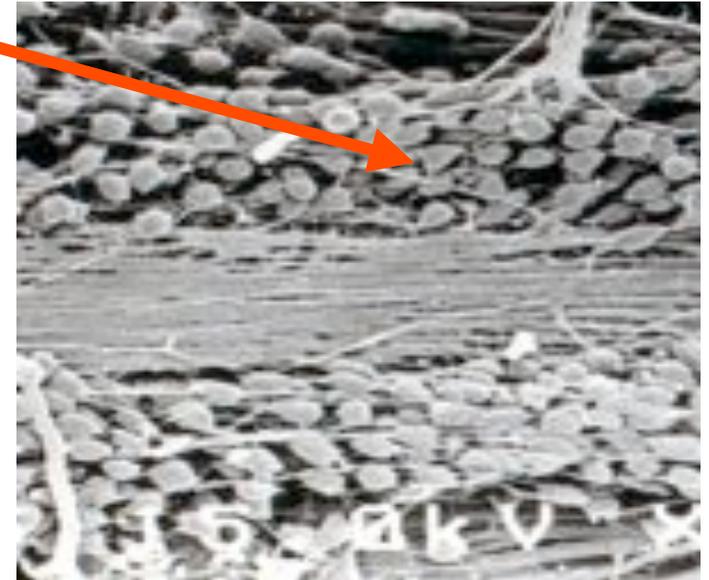
Peri, which means around) the heart (see the SEM).

In some clear aquatic insects these cells appear green

because of the clearance system removing the

chlorophyll molecule from the hemolymph and depositing

it in the pericardial cells.

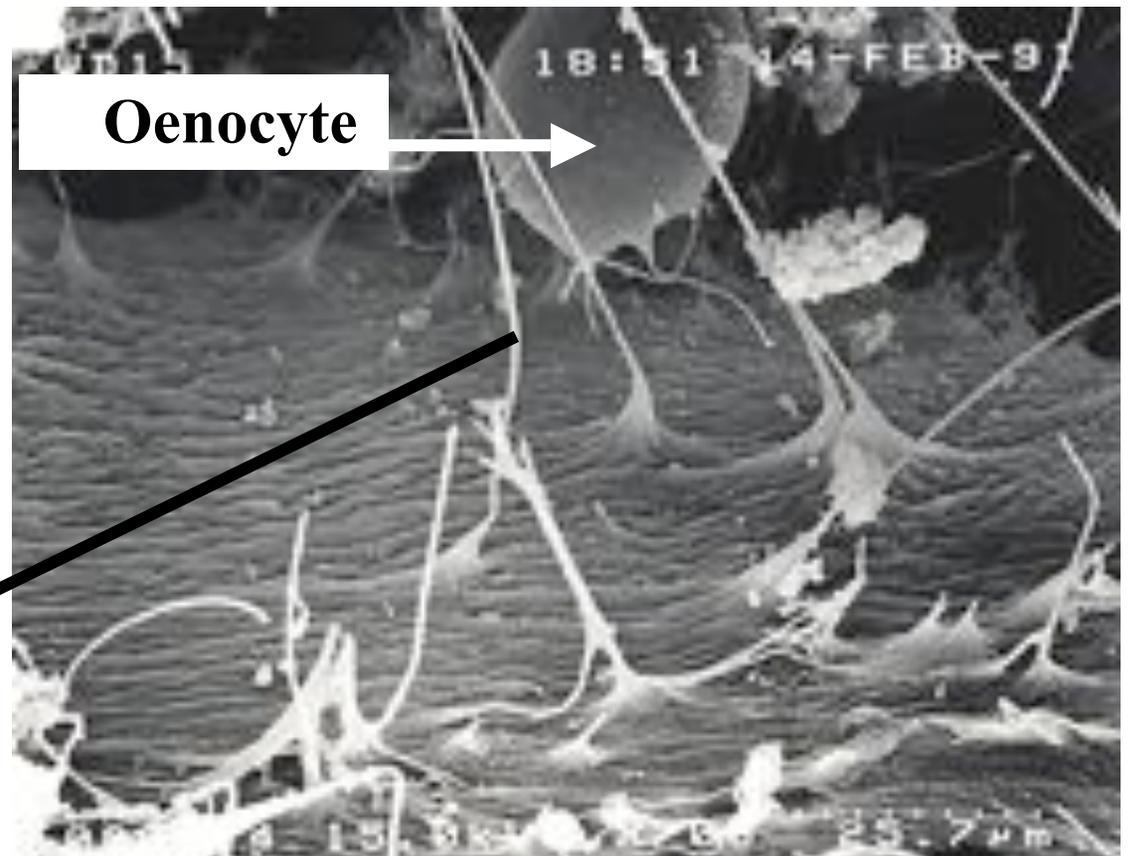


### 3. Oenocytes

Oenocytes are derived from epidermal cells, usually found near the cuticle and are very large cells. They function in the following ways:

1. Synthesize the cuticular hydrocarbons
2. Synthesize ecdysone in some insects

Where oenocytes are in direct contact (see photo to the right) with the cuticle, Wigglesworth says they transport lipids to the epidermis via cytoplasmic strands (see these in photo)



## O. CYTOCHROME P450 ENZYMES IN HEMOLYMPH

Terriere, L.C. 1984. Induction of detoxification enzymes in insects. *Ann. Rev. Entomol.* 29: 71-88.

Induction is the process where a chemical stimulus enhances the activity of the detoxification system by the production of additional enzymes.

MFO's or mixed function oxidases or the P450 enzymes are found in various tissues and aid the insect in detoxifying various toxins from those naturally occurring in plants to various pesticides.

Produced in

1. Midgut cells
2. Fat body and released into the hemolymph

## PHAGOCYTOTIC TISSUE

Phagocytotic tissue may also be involved in hemopoiesis.

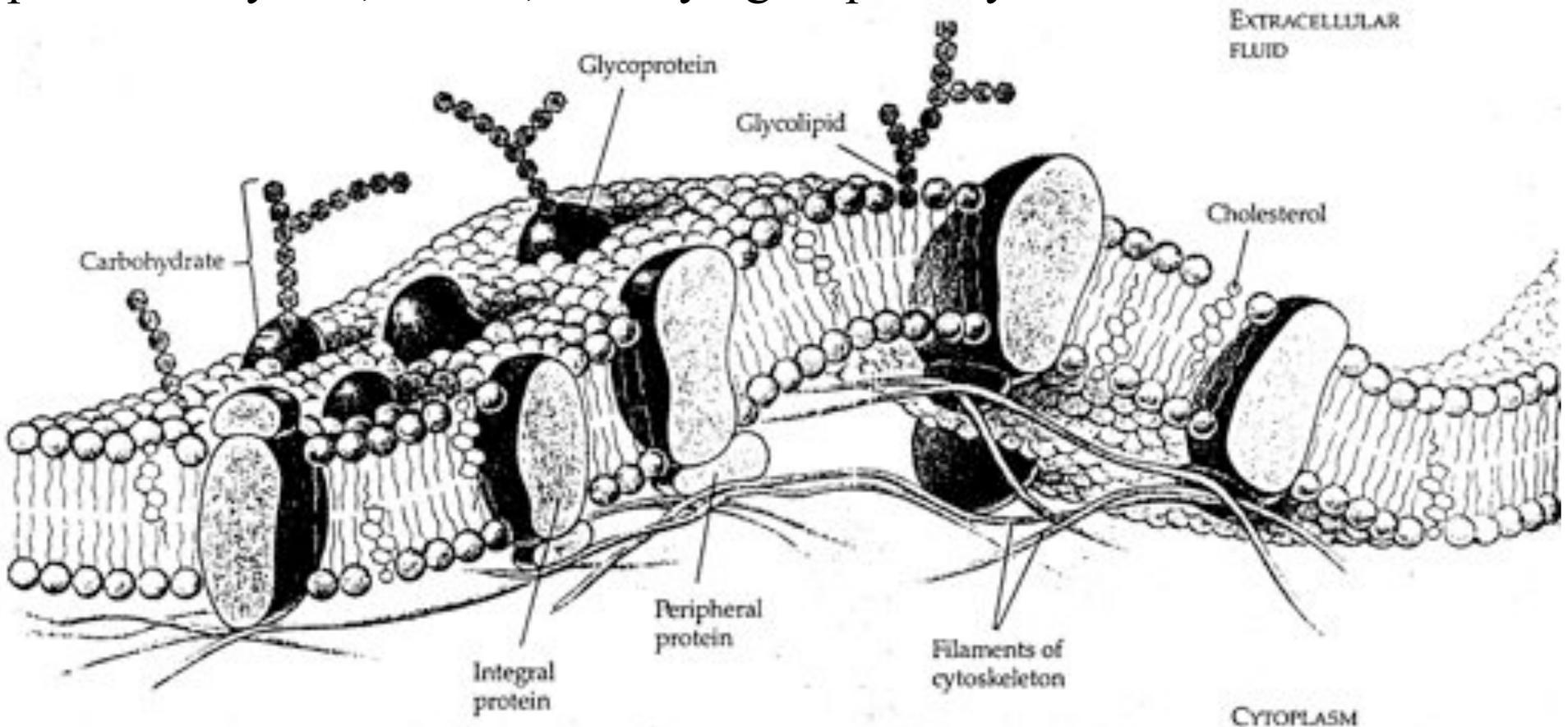
Four pairs of phagocytotic organs of *Gryllotalpa hexadactyla* on the sides of the heart 24 hrs. after injection with trypan blue. Note extensive tracheation of the dorsal diaphragm.

This may be similar to the tissue we saw in the blowfly *Phormia* larva on the sides of the heart that Lowne (1850's) called lymphoid tissue.



## How do safer or insecticidal soaps work?

Cause inhibition of enzymes involved with metabolism, then blockage of respiration and finally cell disruption and leakage of the contents. Acts on the cell membranes initially. Fatty acid pesticides interact with the lipoprotein matrix of the cell membranes, disrupting their semi-permeability and, in turn, destroying respiratory functions.



Genetic manipulation of populations of vectors to encourage their refractoriness (i.e., ability to encapsulate parasites or phagocytize them). As far as I know, however, this response is not due to a single gene but is under the control of multigenes.

**The EMBO Journal Vol. 16 No. 20 pp. 6114-6119, 1997**

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***Plasmodium* activates the innate immune response of *Anopheles gambiae* mosquitoes**

**Adam M. Richman<sup>1</sup>, George Dimopoulos, Douglas Seeley and Fotis C. Kafatos**

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Parassitologia. 1999 Sep;41(1-3):181-4.

## **Genetic basis of encapsulation response in *Anopheles gambiae*.**

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The phenomenon of encapsulation of invading organisms is widespread in insects. Co-evolution has produced an intricate balance between the immune responses of the host and immune-suppressive (or immune-evading) properties of the parasite. Genome-wide genetic mapping revealed different loci in Anopheline mosquitoes were involved in melanotic encapsulation of different malaria parasites. Certain isolates of human malaria parasites can still suppress or avoid the immune response from refractory mosquitoes. Similar interactions with parasitoids were observed in *Drosophila melanogaster*. Species-specific encapsulation locus was identified for two parasitoids, respectively, and virulent strain of parasitoid can suppress the immune system of an otherwise resistant fruitfly. It is believed that the encapsulation loci in both mosquitoes and fruitfly may encode gene products that function at the early stages of parasite/parasitoid recognition or immediate signaling events. **Future research on membrane receptor molecules and their roles in insect immunity will yield interesting insights into mosquito-parasite interactions.**

## **Quantitative Trait Loci for Refractoriness of *Anopheles gambiae* to *Plasmodium cynomolgi* B**

**Liangbiao Zheng, Anton J. Cornel, Rui Wang, Holger Erfle, Hartmut Voss, Wilhelm Ansorge, Fotis C. Kafatos, Frank H. Collins \***

The severity of the malaria pandemic in the tropics is aggravated by the ongoing spread of parasite resistance to antimalarial drugs and mosquito resistance to insecticides. A strain of *Anopheles gambiae*, normally a major vector for human malaria in Africa, can encapsulate and kill the malaria parasites within a melanin-rich capsule in the mosquito midgut. Genetic mapping revealed one major and two minor quantitative trait loci (QTLs) for this encapsulation reaction. Understanding such antiparasite mechanisms in mosquitoes may lead to new strategies for malaria control.

**From: Science, vol. 276 (18 April 1997), pgs. 425-428,**

# **Basic and Applied Aspects of the Circulatory System**

## **BASIC**

- 1. Cellular recognition and chemotaxis of hemocytes**
- 2. Identifying and understanding how the various antibacterial proteins work**
- 3. Identification and working on various cardioaccelerators or cardioinhibitors**

## **APPLIED**

- 1. Induction of MFO's**
- 2. Encouraging hemocytic response to parasites in arthropod vectors such as mosquitoes**
- 3. Preventing hemocytic response to parasite in biological control agents. Ex. Spray program in Canada against the webworm. Initially the parasite was successful but was a shift to a host population that could encapsulate the parasite's egg.**

**The ADAM metalloprotease Kuzbanian is crucial for proper heart formation in *Drosophila melanogaster*.** [Albrecht S](#), [Wang S](#), [Holz A](#), [Bergter A](#), [Paululat A](#). Universitat Osnabruck, Fachbereich Biologie/Chemie, Zoologie, Barbarastrasse 11, D-49069 Osnabruck, Germany. We have screened a collection of EMS mutagenized fly lines in order to identify genes involved in cardiogenesis. In the present work, we have studied a group of alleles exhibiting a hypertrophic heart. Our analysis revealed that the ADAM protein (A Disintegrin And Metalloprotease) Kuzbanian, which is the functional homologue of the vertebrate ADAM10, is crucial for proper heart formation. ADAMs are a family of transmembrane proteins that play a critical role during the proteolytic conversion (shedding) of membrane bound proteins to soluble forms. Enzymes harboring a sheddase function recently became candidates for causing several congenital diseases, like distinct forms of the Alzheimer disease. ADAMs play also a pivotal role during heart formation and vascularisation in vertebrates, therefore mutations in ADAM genes potentially could cause congenital heart defects in humans. **In *Drosophila*, the zygotic loss of an active form of the Kuzbanian protein results in a dramatic excess of cardiomyocytes, accompanied by a loss of pericardial cells.** Our data presented herein suggest that Kuzbanian acts during lateral inhibition within the cardiac primordium. Furthermore we discuss a second function of Kuzbanian in heart cell morphogenesis.

PMID: 16713197 [PubMed - indexed for MEDLINE]