Insect Circulatory System

Zoo514

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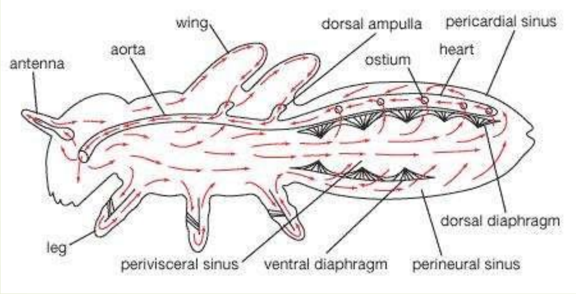
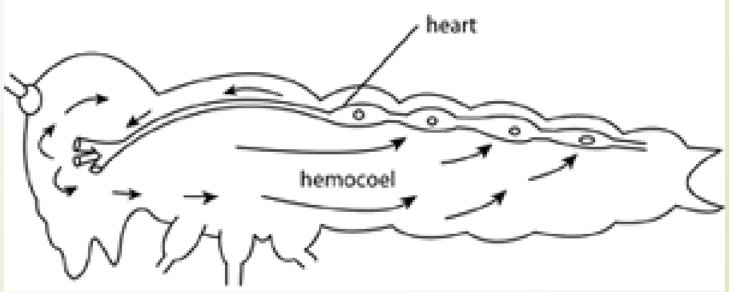
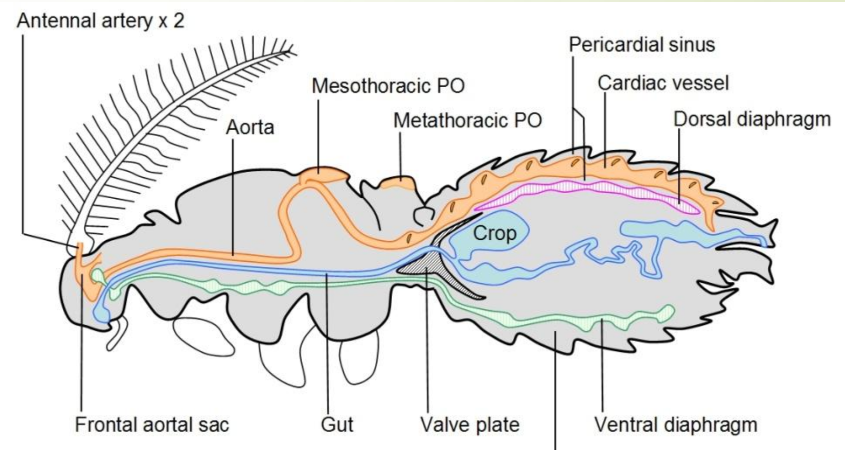
Circulatory system & Circulation in insects

- Insects have an open circulatory system , the blood (hemolymph) occupy all the body cavity (haemocoel) , the internal organs and tissues are exposed freely to it.
- The haemocoel is divided into three major sinuses by fibro– muscular septa or diaphragms; a dorsal pericardia sinus, a perivisceral sinus and a ventral perineural sinus.

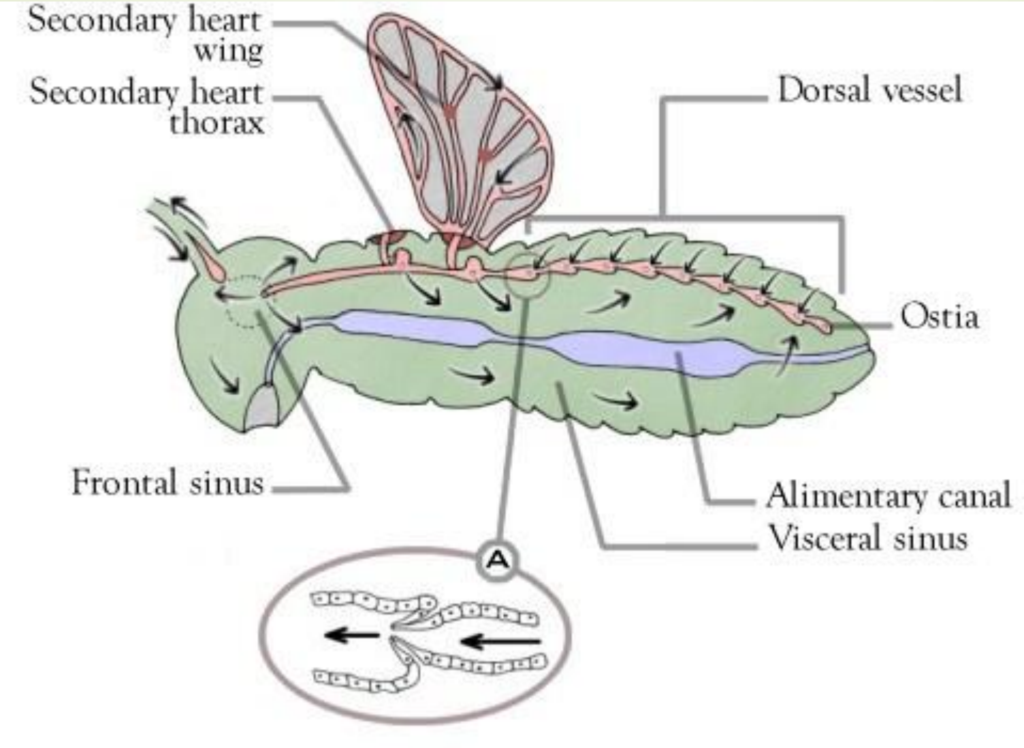


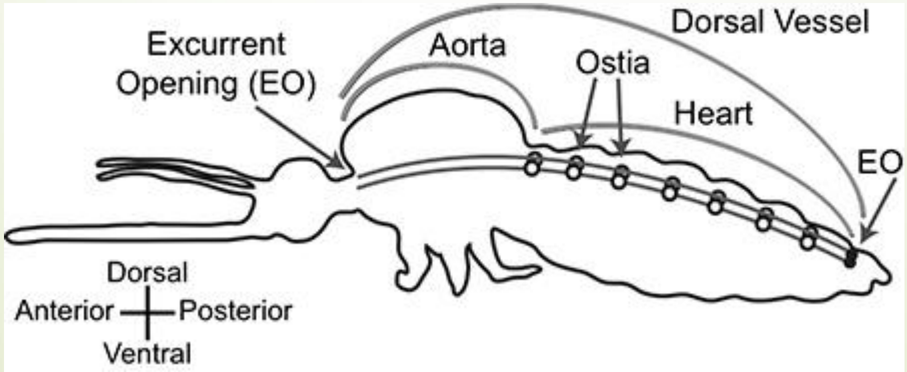


- Circulation is produced by the activity of a dorsal longitudinal blood vessel.
- The dorsal blood vessel run a long the dorsal midline and is in the form of a posterior heart and an anterior aorta.
- The dorsal vessel is open anteriorly and closed posteriorly except in larval mayfly.
- The dorsal vessel is divided into two regions: a posterior heart in which the wall of the vessel is perforated by incurrent and sometimes also by excurrent openings (ostia), and an anterior aorta which is a simple unperoforated tube.



The Insect Circulatory System

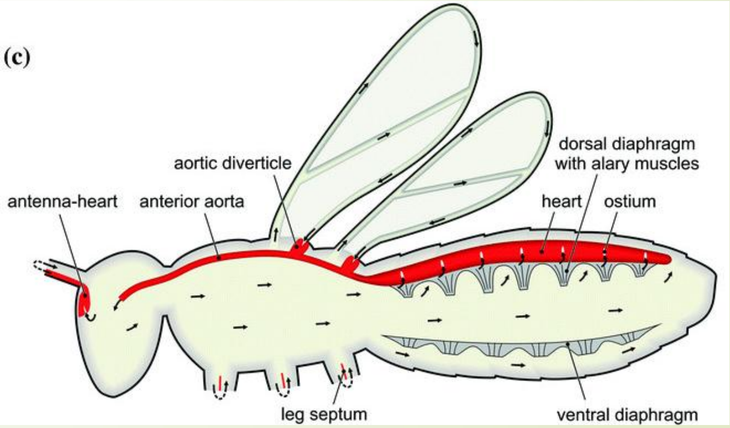

Secondary heart wing
Secondary heart thorax
Dorsal vessel
Ostia
Frontal sinus
Alimentary canal
Visceral sinus
A


Excurrent Opening (EO)
Aorta
Dorsal Vessel
Ostia
Heart
EO
Dorsal
Anterior
Posterior
Ventral

The dorsal diaphragm

- It is a septum :extends across the abdominal cavity enclosing the pericardial sinus , it contain the dorsal vessel.

-The dorsal diaphragm divides the haemocoel into the pericardial sinus and the visceral sinus.



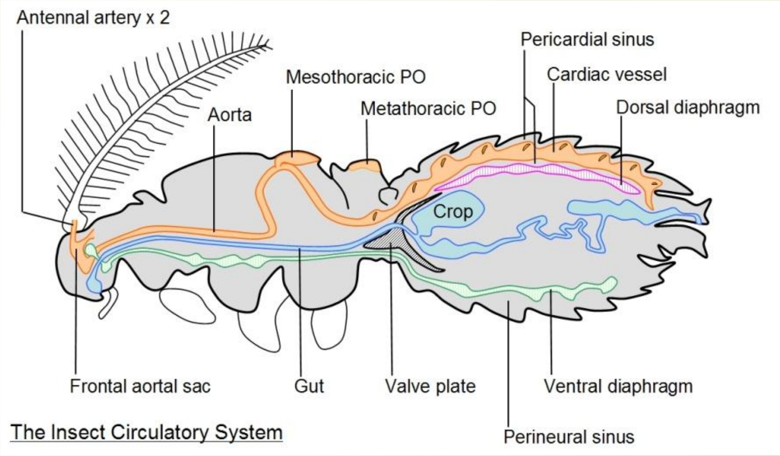
Ventral diaphragm

-The ventral diaphragm forms a continuous ventral sheath .

- It extends from the prothorax to the end of the body .

- It encloses the perineural sinus.

- The perineural sinus encloses the nerve cord.



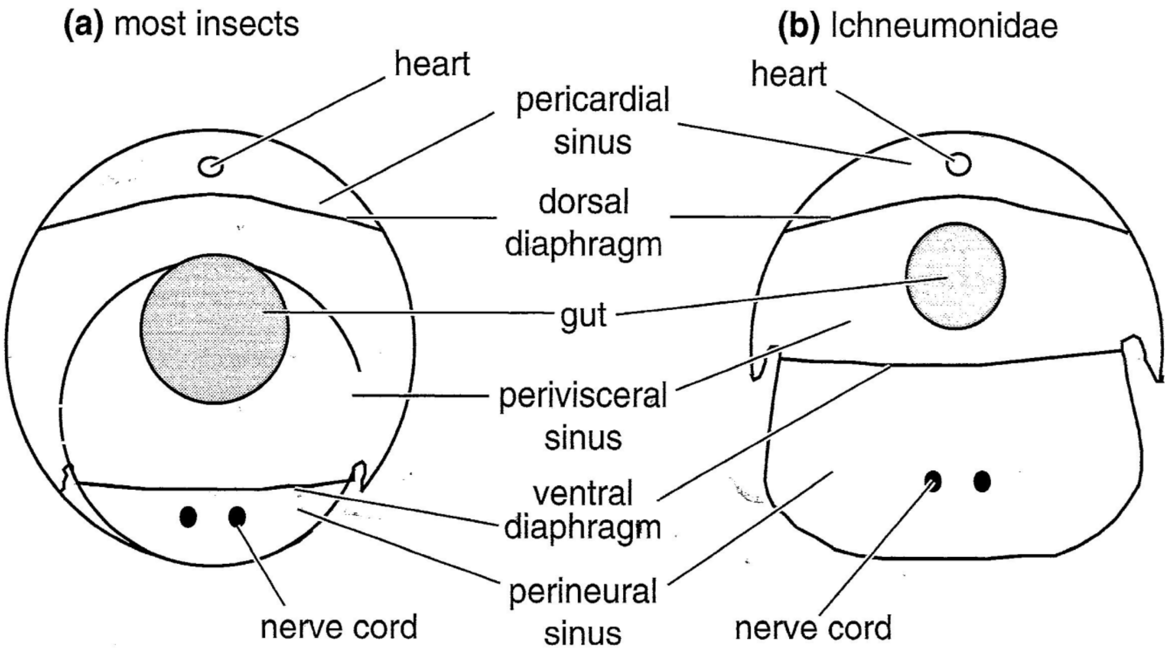
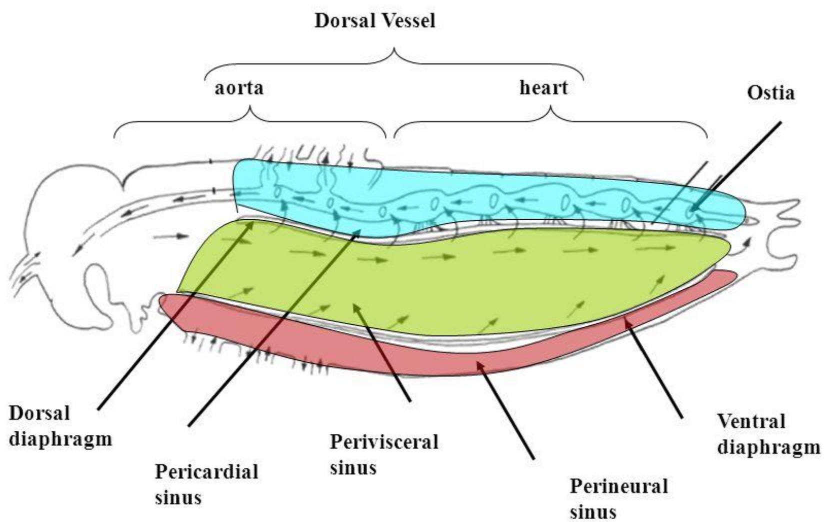
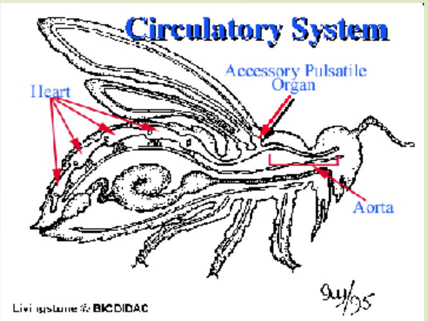


Figure 5.3 Main sinuses of the hemocoel shown in diagrammatic cross-section (after Richards, 1963).

Generalized Insect Circulatory system

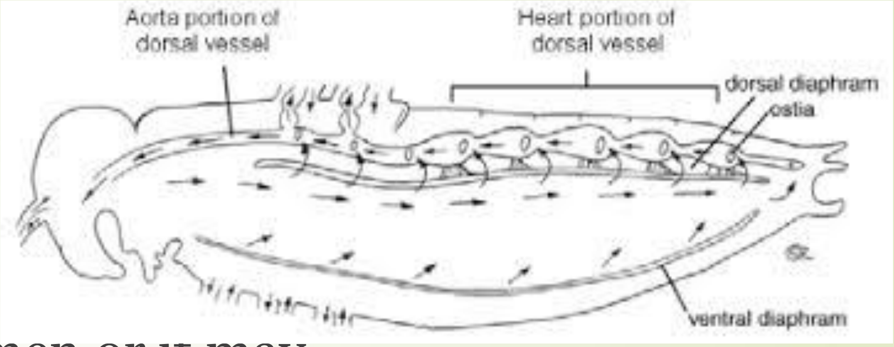


Dorsal vessel

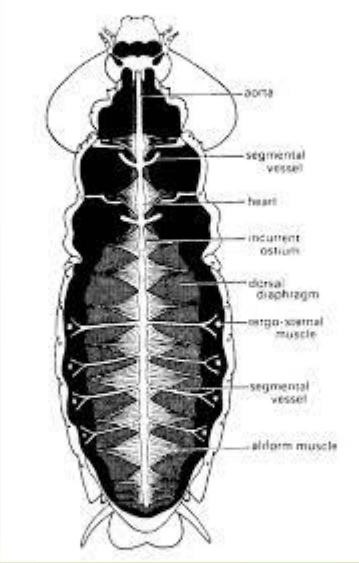


- It runs along the dorsal midline , for the whole of the body.
- It leaves the alimentary canal , just above the esophagus.
- It is divided into a posterior heart in which the wall is perforated and an anterior aorta which is a simple unperforated tube.
- It collects blood from the abdominal cavity and discharges it in the head .
- It is open anteriorly but closed posteriorly.
- The wall of the dorsal vessel is contractile. It consists of a single layer of circular and spiral muscle fibrils.

The heart

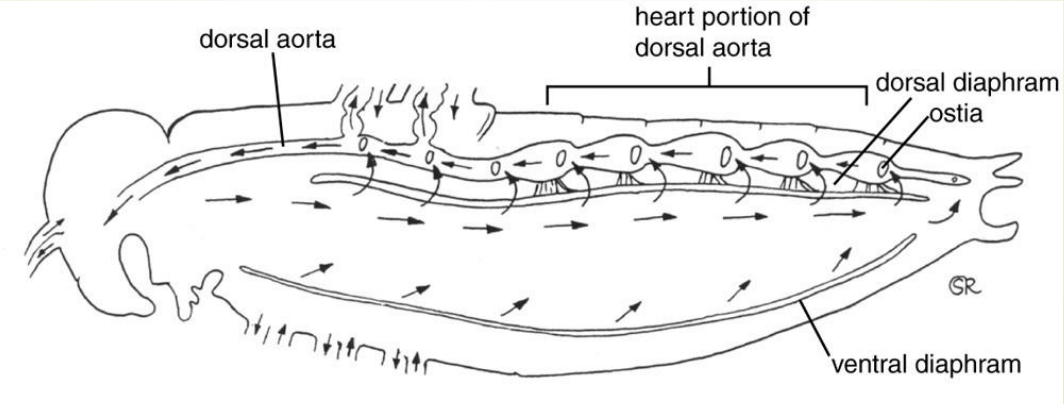


- It is often restricted to the abdomen or it may extend as far as the prothorax .
- It is often a continuous tube that is not divided into chambers, except in Orthoptera it is slightly enlarged into ampullae, these are the points where the ostia pierce the wall.
- The heart may be directly bound to the dorsal wall or suspended from it by elastic filaments.
- A pair of alary musclesare attached laterally to the walls of each chamber



Incurrent ostia

- They are vertical, slit-like openings in the lateral wall of the heart.
- The maximum number may exist are 12 pairs, 9 abdominal and 3 thoracic.
- The anterior and posterior lips of each ostium are reflexed into the heart to form a valve which permits the flow of blood into the heart at diastole (expansion of the heart), but prevents its outward passage at systole.

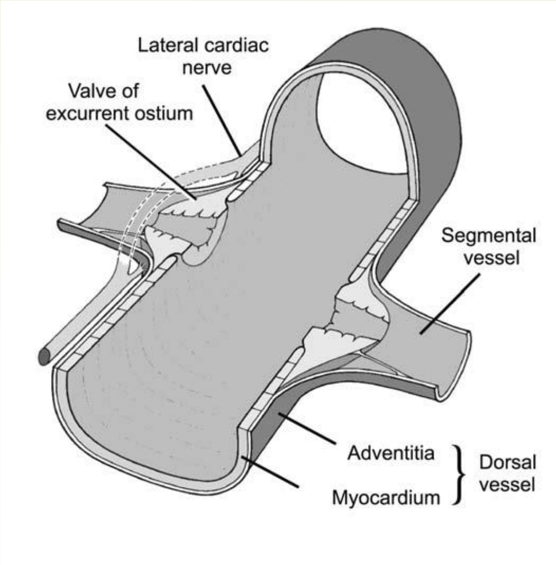


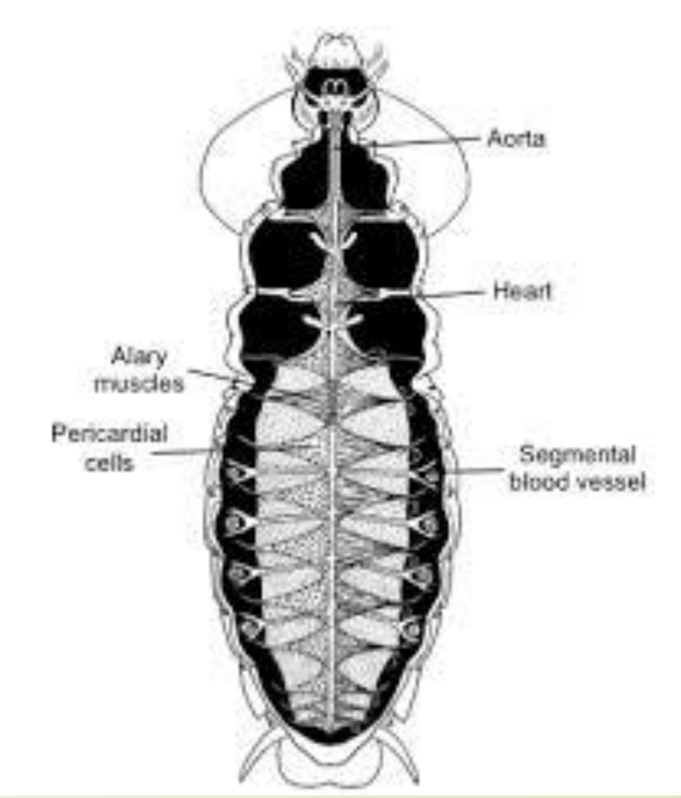
Excurrent Ostia

- These are ventro- lateral openings in the wall of the heart.
- No internal valves.
- Externally each opening is surrounded by a papilla of a spongy multinucleate cells which expand during systole, so the hemolymph is forced out, and contracts during diastole, so that entry of blood is prevented.

Segmental vessels

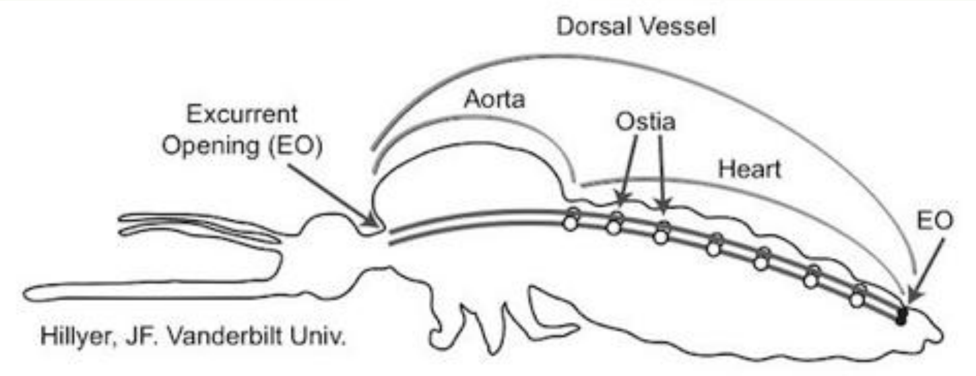
- Most Blattodea and Mantodea have no excurrent ostia, but the blood leaves the heart via segmental vessels that extend laterally.





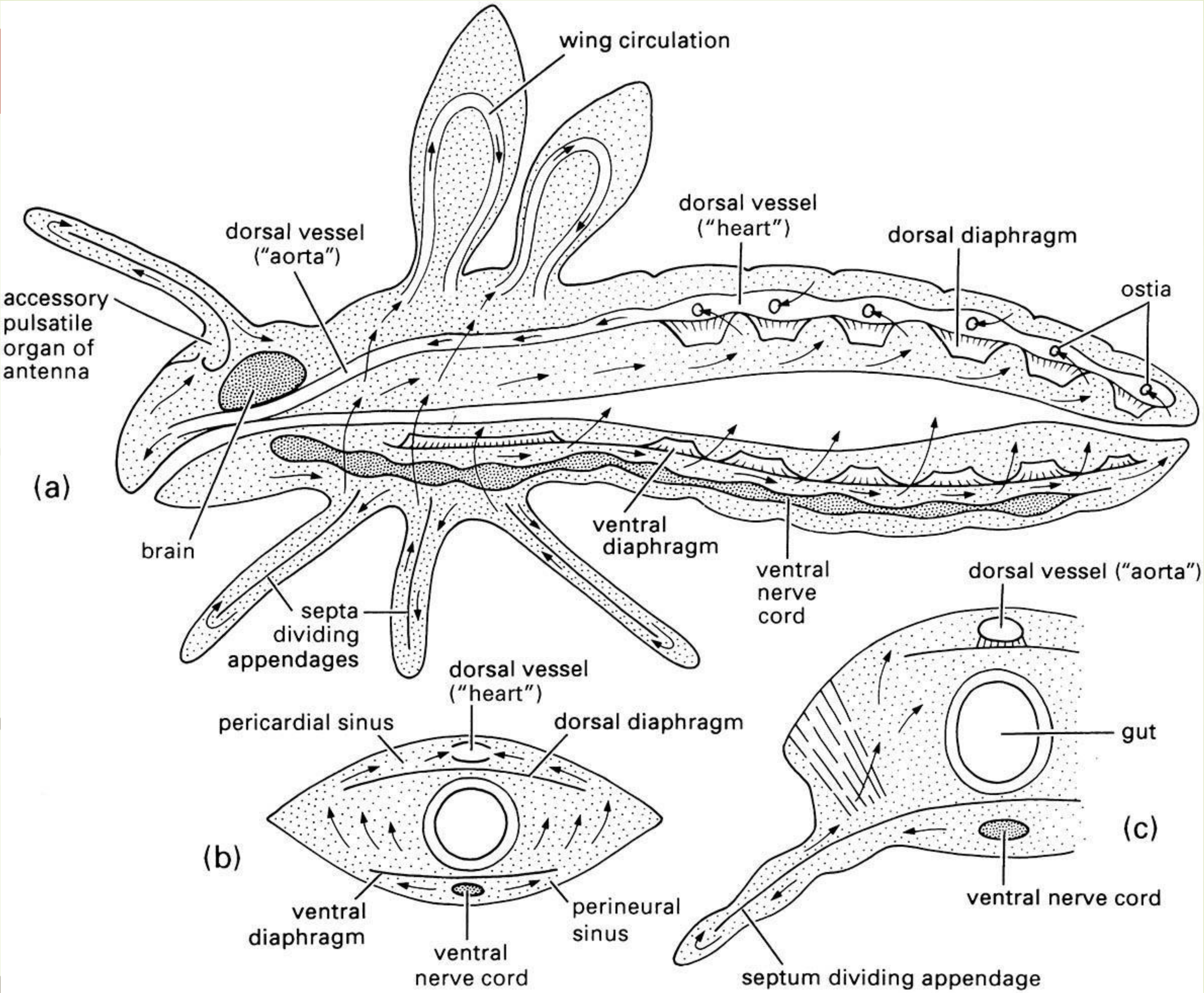
The Aorta

- It is slender anterior prolongation of the heart, it carries the blood from the heart .

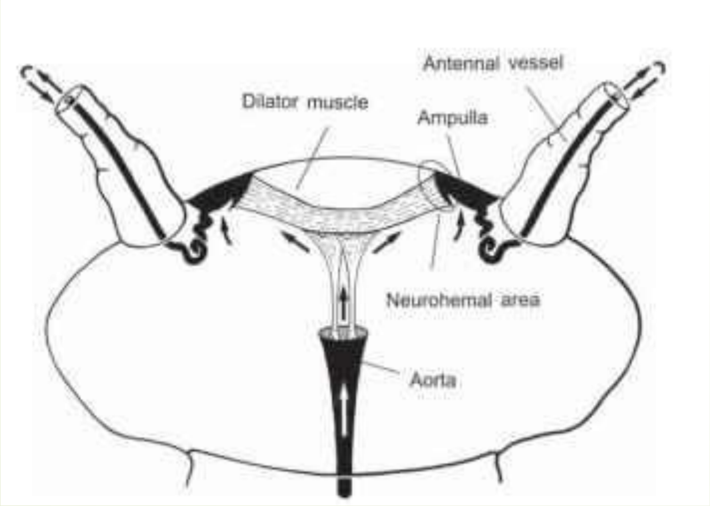


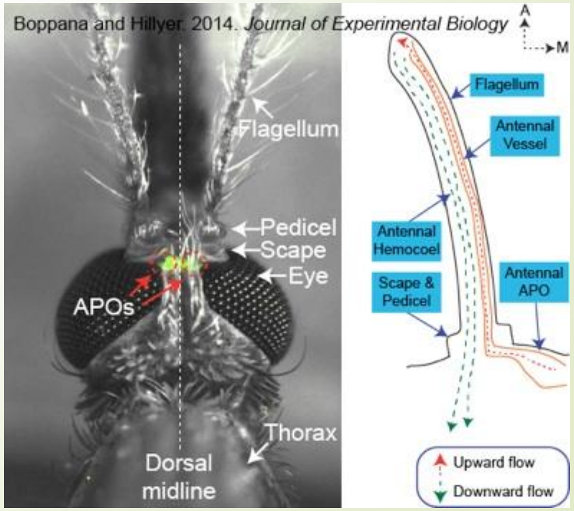
Accessory pulsatile organs

- In addition to the dorsal vessel, insects have other pulsating structures that maintain circulation through the appendages.
- A pulsatile organ drowning blood from the wings is present in both wing-bearing segments of most adult insects.
- A blood space, or reservoir, beneath the posterior part of the tergum which is largely or completely isolated from the remaining hemocoel of the thorax connects with the posterior veins of the wing via the axillary cord of the wing.
- The ventral wall of the reservoir forms a muscular pump.

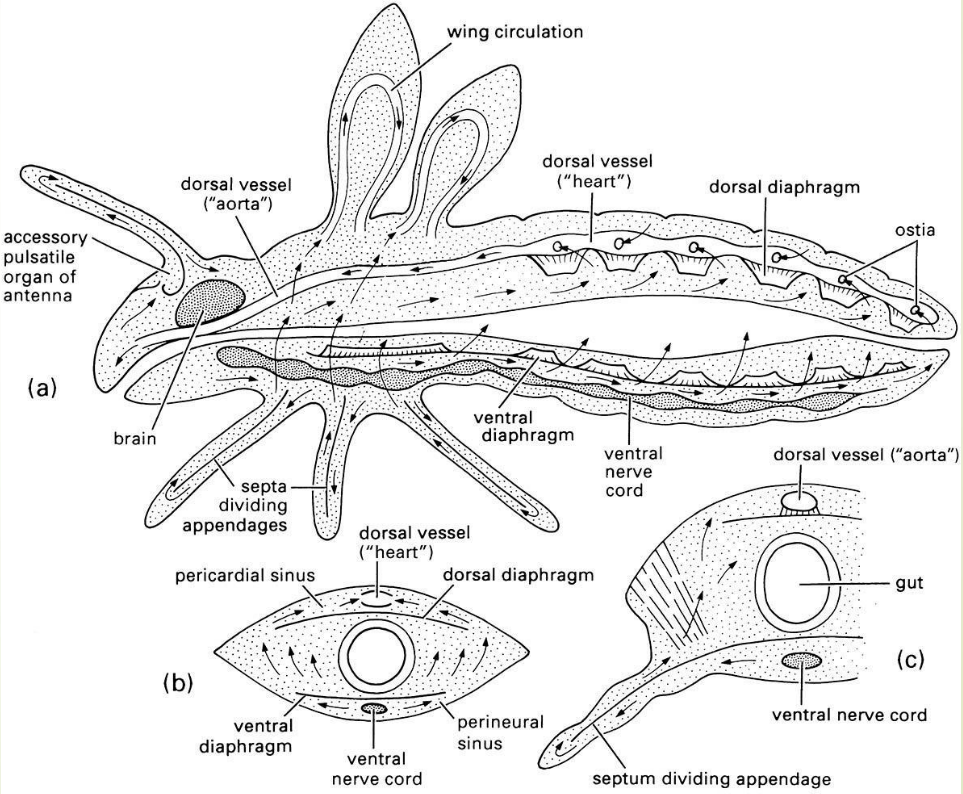

wing circulation
dorsal vessel
("aorta")
dorsal vessel
("heart")
dorsal diaphragm
ostia
accessory
pulsatile
organ of
antenna
(a)
brain
ventral
diaphragm
ventral
nerve
cord
septa
dividing
appendages
dorsal vessel
("heart")
pericardial sinus
dorsal diaphragm
(b)
ventral
diaphragm
ventral
nerve cord
perineural
sinus
dorsal vessel ("aorta")
gut
(c)
ventral nerve cord
septum dividing appendage

- A pulsatile organ is also found at the base of each antenna.
- It consists of an ampulla from which a fine tube extends almost to the tip of the antenna.
- In most insects , the ampullae have dilator muscles. Compression drives hemolymph into the antenna.



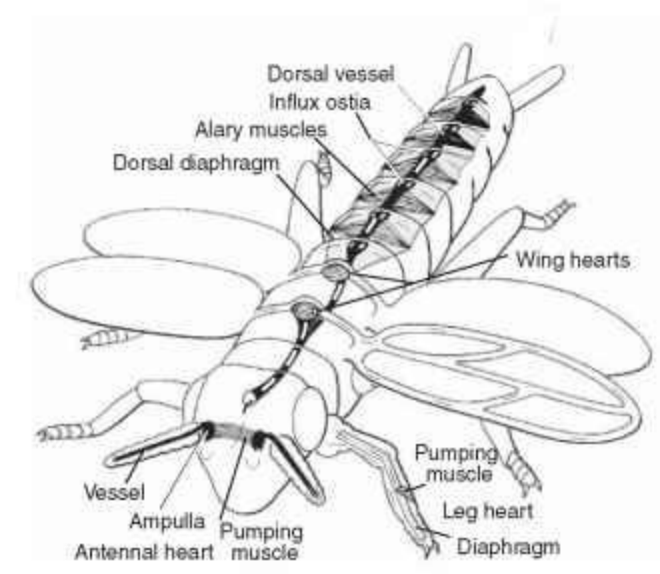


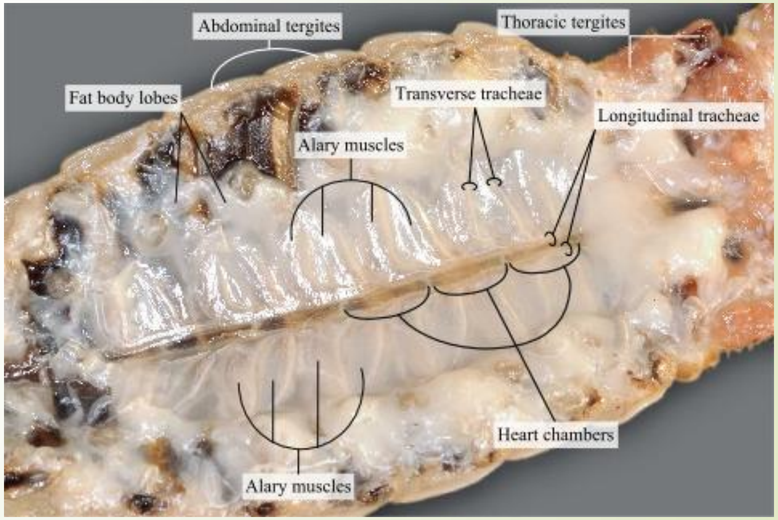
➢ Most insects have a longitudinal septum in the legs which divides the lumen into two sinuses and permits a bidirectional flow of blood within the leg.



Alary muscles

- These are muscles that are closely associated with the heart.
- In Orthoptera ten abdominal and two thoracic pairs are present.
- In other insect species the number is reduced.
- They form integral part of the dorsal diaphragm which spreads between them as connective tissue membrane .





Heartbeat

- **Systole**

  - It is the contraction phase of the heartbeat.

  -It results from the contraction of the muscles in the heart wall which starts posteriorly and spreads forward as a Wave.

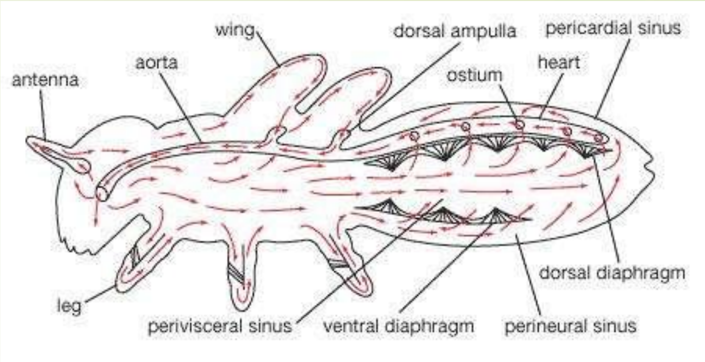
- **Diastole**

  - It is the relaxation phase of the heartbeat.

  - It results from relaxation of the muscles assisted by the elastic filaments supporting the heart.

- After diastole there is a third phase in the heart cycle known as diastasis in which the heart remains in the expanded stat.
- Frequency with which the heart contracts varies from 14beats/ minute to 150 beats/minute

Circulation



- **Normal circulation**

At systole

- The blood is pumped forwards through the heart, entering the perivisceral sinus through the anterior opening of the aorta in the head and through the excurrent ostia where these exist.
- The valves on the incurrent ostia prevent the escape of blood through these openings.
- The force of blood leaving the aorta anteriorly tends to push blood backwards in the perivisceral sinus.
- The backwards flow is aided by the movements of the dorsal diaphragm and by the inflow of blood into the heart, through the incurrent ostia, at diastole.
- Movements of the ventral diaphragm presumably help to maintain the supply of blood to the ventral nerve cord.

At diastole

- Blood is drawn into the heart through the ostia.

Circulation through the wings

- In most cases, blood is drawn in and out of the wings by the thoracic pulsatile organs. Specific veins are contributed to this function.
- In Lepidotera, blood enters the wings along all the veins and go out again during heartbeat reversal.
- The movement of blood out of the veins causes the tracheae inside them to expand so that air is drawn into the wing.
- When heartbeat starts beating forwards again, the negative pressure on the wing tracheae is removed and their elasticity causes them to contract.
- This produces a negative pressure in the hemolymph space outside the trachea and blood flows in.

- In insects lacking leg pulsatile organs, the flow of blood through the legs is thought to be maintained by pressure differences at the base.

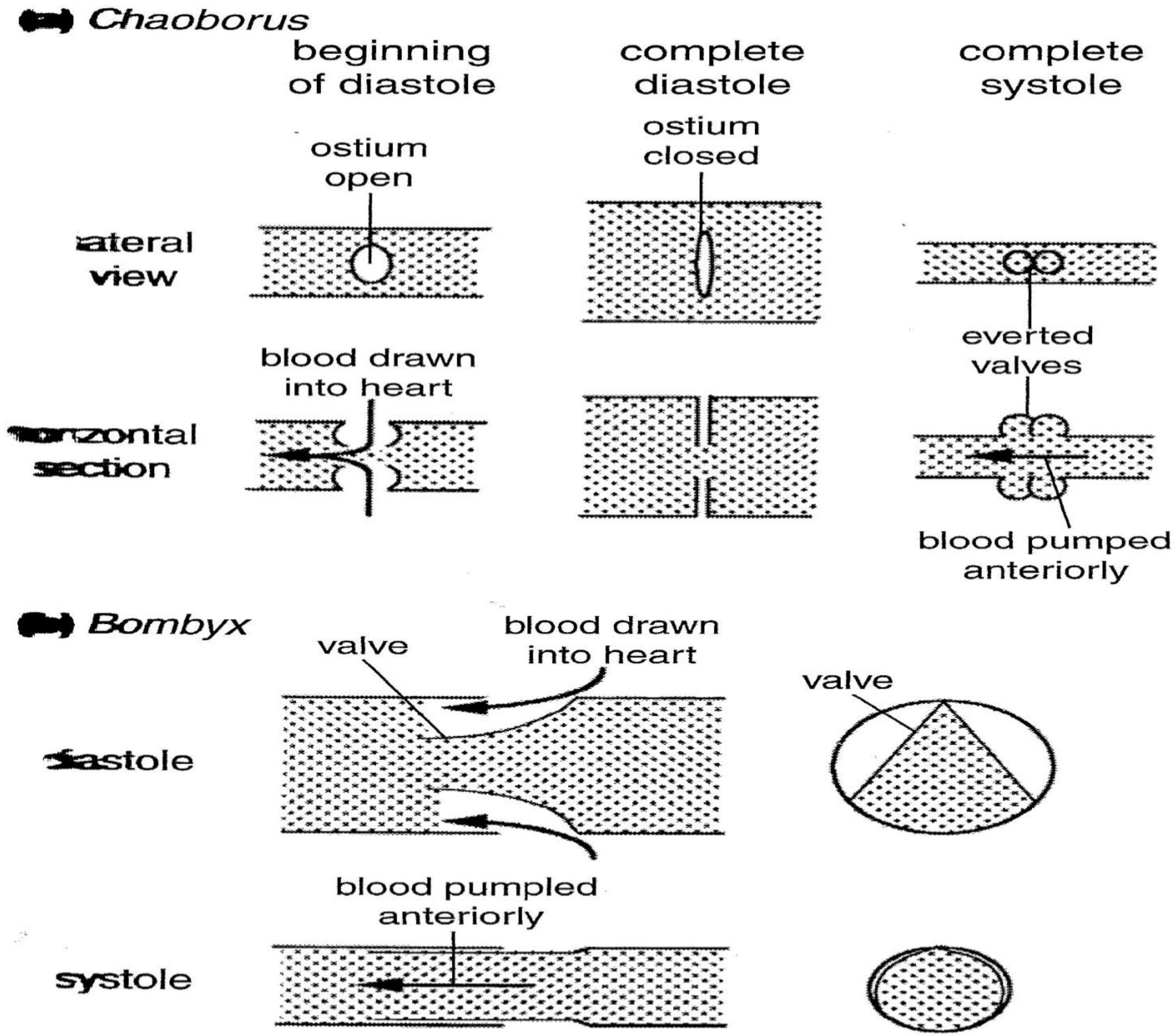


Figure 5.2 Functioning of incurrent ostia. (a) The larva of *Chaoborus*; the valves are prevented from opening outwards at systole by a unicellular thread (not shown) attached to the inside of the heart (after Wigglesworth, 1972). (b) The larva of *Bombyx*; the heart is shown in horizontal (left) and transverse (right) sections.

Factors affecting the rate of the heartbeat

- **Age and stage**. Heartbeat is faster in adult than immature stages.
- **Environmental factors** such as temperature. Activity usually stops above 45-50 ˚C and below 1-5 ˚C. Within this range, the rate is higher at higher temperatures.
- Strong movement of the gut (slow it or stop it). It is common for the heart to stop beating sometimes for a few seconds.
- It is also common for the heartbeat to undergo periodic reversal, with waves of contraction starting at the front. When this occurs, blood is forced out of the incurrent ostia.

Control of heartbeat

- No nerve cells are associated with the heart , and the heart muscles are the seat of automation (**Myogenic beat**). Although the myogenic pattern maybe modulated naturally or hormonally.
- The alary muscles are activated by the contraction of the heart and they exert tension that modifies the heartbeat.
- The direction of a beat, from back to front or vice versa, maybe related to the distribution of blood pressure. Also it may be related to the availability of oxygen. In the absence of a good oxygen supply, the rate of heartbeat is strongly reduced.

Hemolymph

- Insect blood is known as hemolymph.
- It circulate round the body, bathing the tissue directly.
- It consists of a fluid plasma in which blood cells, hemocytes, are suspended.
- Making up usually less than 25% of an insect's body weight.
- Hemolymph water comprises 20-25% of the total body water in adult insects, but in caterpillars, the figure is close to 50%. This reflects the important hydrostatic functions of the hemolymph in these larval forms

- It transports hormones, nutrients and wastes and has a role in, osmoregulation, temperature control, Immunity, storage (water, carbohydrates and fats) and skeletal function.
- It also plays an essential part in the moulting process (McGavin 2001, Triplehorn 2005). An additional role of the hemolymph in some orders, can be that of predatory defence. It can contain unpalatable and odorous chemicals that will act as a deterrent to predators (Gullan 2005).

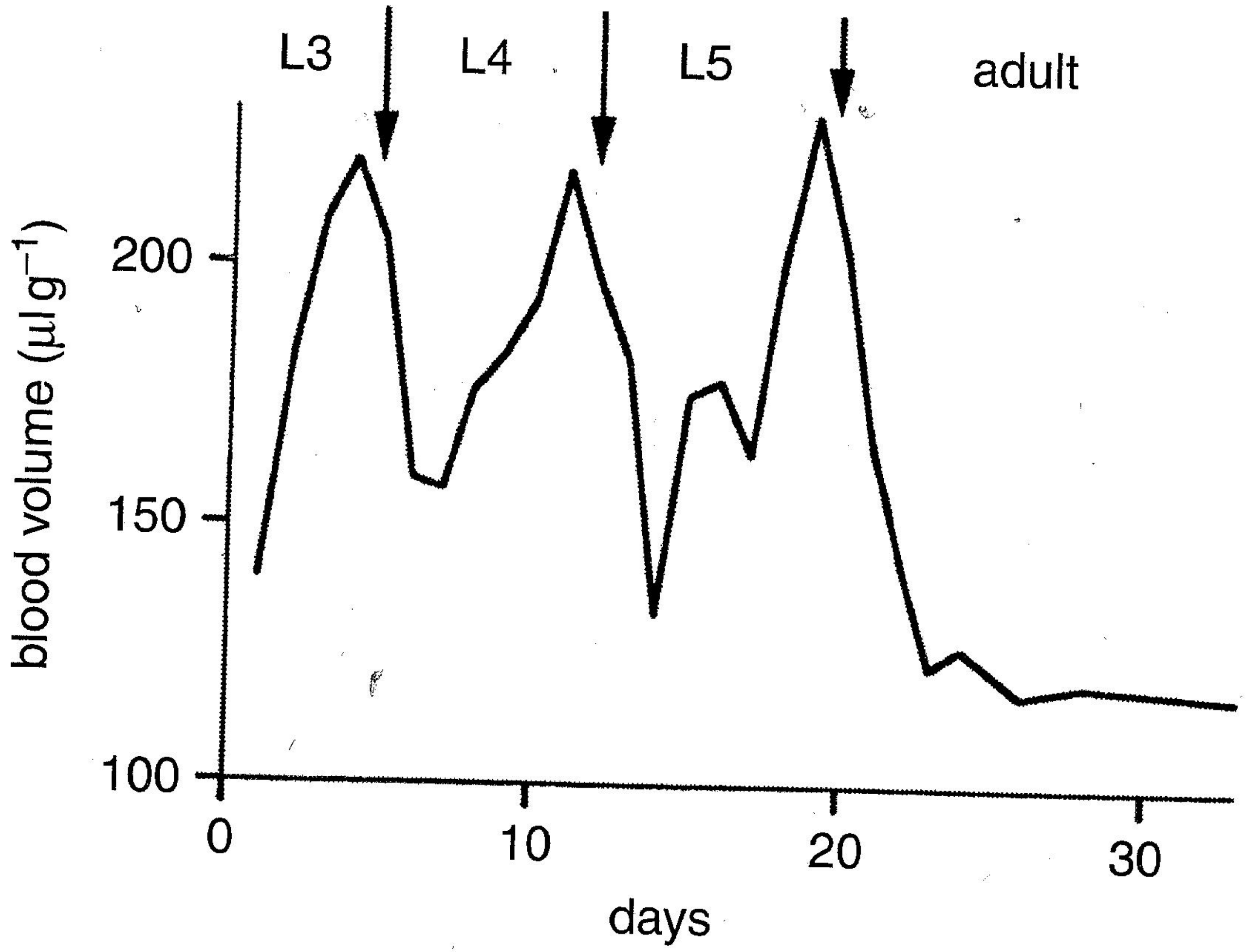


Figure 5.10 Changes in the blood volume (expressed as volume per unit weight) during the development of *Schistocerca*. L3, L4 and L5 refer to successive larval stages. Arrows indicate the time of ecdysis (after Lee, 1961).

Constituents of the plasma

- In Plasma Inorganic & Organic Constituents

➢ Inorganic

✓ anion

1- Chloride is the most abundant inorganic anion in insect blood.

- Conc. high (hemimetabolous)

- low (holometabolous) lower than 10% of total osmolar concentration.

2- Phosohates & carbonates

✓ Cations

1- Sodium Na + is the most abundant cation.

- Conc. Varies with taxonomic position & diet.
- phytophagous : low
- predators : high

2- Potassium K & Magnisium Mg

- high in phytophagous
- low in carnivorous
- Blood –sucking e.g., Cimex & Stomoxys high Na + low K ++

• Na + and K ++: in the ionized form.

• Ca & Mg : are bound to macromolecules

Variation in cation concentration are wide

- e.g., in locust : K conc, increases before moulting.
- These changes affect the behaviour of insect , since ; neuromuscular junctions are directly exposed to Hl. & low concentration in K ++ raises the muscle resting potential.
- Mg : concentration is high in phytophagous insects (as a result of its high conc. In the diet since it is a constituent of chlorophyll .
- Mg conc. Is low in Lepidoptera when the larvae stop feeding.
- It’s conc. Is low in adult than the larvae
- In aquatic insects, the ionic composition of hemolymph affected by the composition of water.

Metallic traces

- Copper (A constituent of tyrosinase)
- Iron
- Zinc
- Manganese
- These are found bound to organic complex & not found as free ions .

Organic constituent

1- Free Amino Acids (A.A.)

- Very high level of free a.a. which constituting 33 –65 % about (0.29 –2.43 gm) , most of the known amino acids are present.
- They vary both quantitatively & qualitatively from one species to another & in different stages of the same sp.
- They depend on those available in food.
- Changes in A.A. concentration at different stages in the life cycle.

2-proteins

- Numerous proteins are present in the hemolymph with a variety of functions.
- They are not all present at the same time but, there are changes through the life cycle.
- Proteins present in insect haemolymph are grouped as: storage proteins, lipid transport proteins, vitelogenins, enzymes, proteinase inhibitors, chromoproteins and a range of proteins involved in the immune response of insects.

3- Other organic constituents

- Uric acid , urea and ammonia.
- Trehalose, a disaccharide, is the most characteristics sugar found in insect hemolymph. Activity and starvation affect its conc. As it is the main source of energy
- Glucose is also often present and other carbohydrates.
- Lipids. Marked increase may occur during development or flight , where lipid in fat body are mobilized as fuel for the flight.

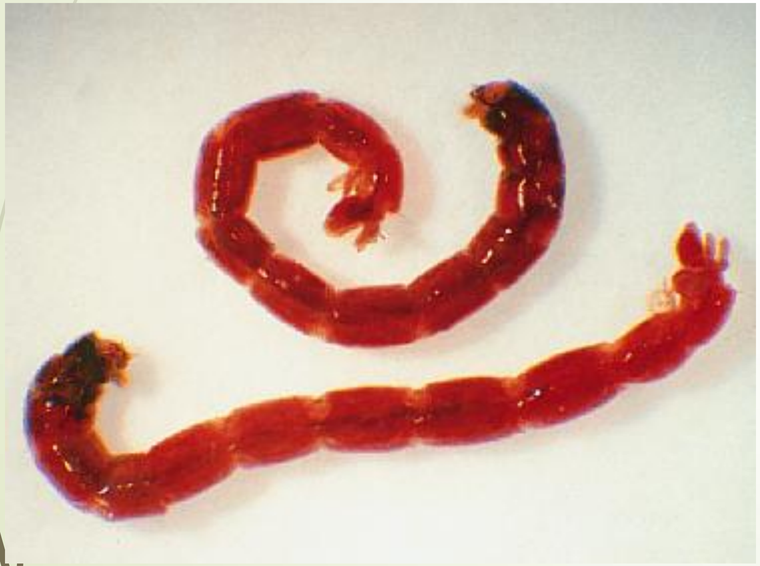
- Organic acids

i) e.g., Citrate : It is present in high concentration (vary with species and with development).

ii) Organic phosphates.

- Pigments

Respiratory pigments do not occur in insect blood. Except for :
Chironomus larvae : Haemoglobin is present in solution in plasma



Properties of the plasma

1- Osmotic pressure

- The inorganic and organic solutes present in the hemolymph contribute to its osmotic pressure. Variation in plasma osmotic component in different orders. Three categories are present:

1- Na & Cl form most of the osmolar conc.

Basic type of insect blood & most of the arthropods.

e.g., Odonata. Ephemeroptera , Homoptera, Orthoptera.

2- Cl is low relative to Na which constitute 21-48 % of total osmolar conc, and A. A. are in high conc.

e.g., Diptera , Neuroptera& most Coleoptera.

3 – A.A. accounts for about 40% of the total osmolar conc. The other substance never exceed 10 % .

e.g., Lepidoptera & Hymenoptera.

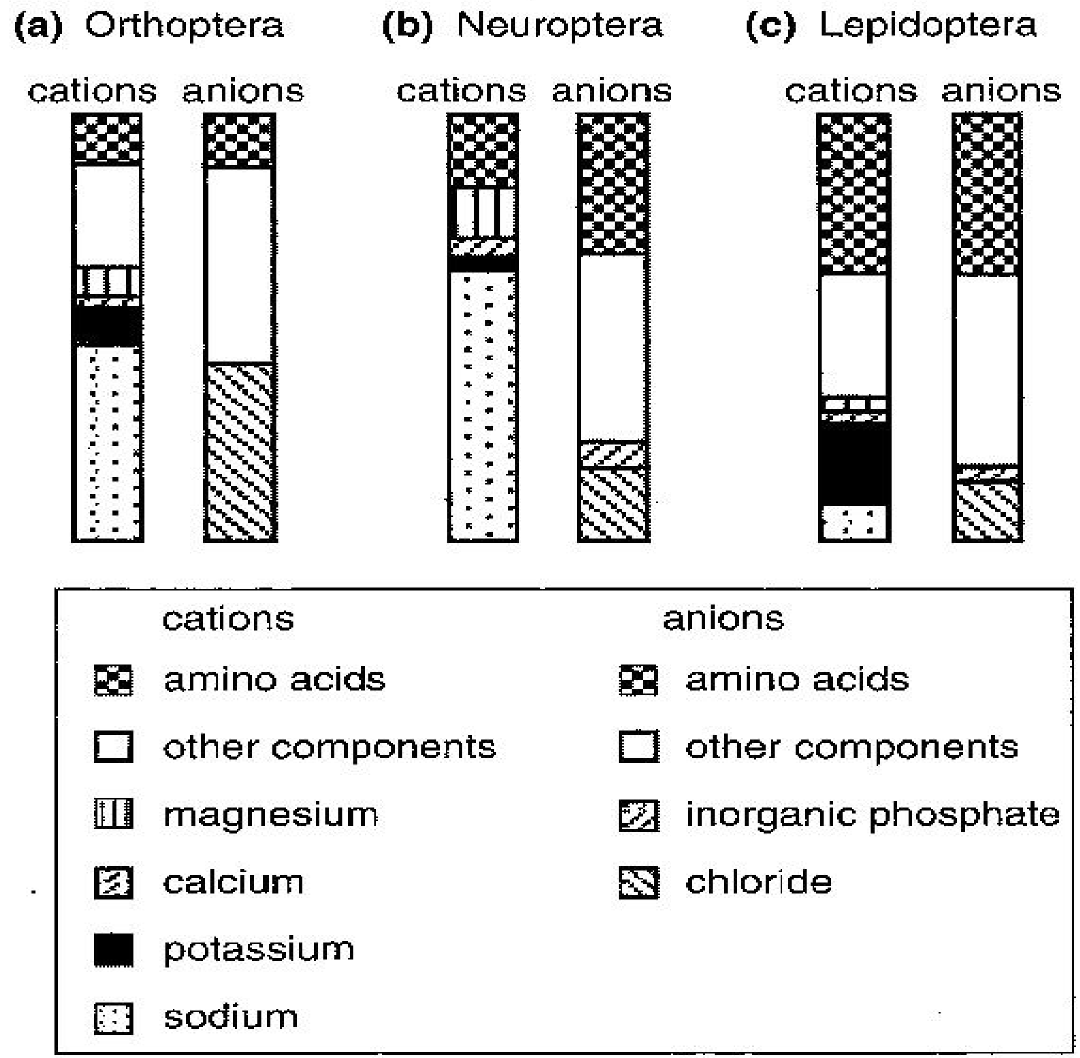


Figure 5.13 Osmotic components of the hemolymph in different groups of insects expressed as percentages of the total osmolar concentration. Each vertical column represents 50% of the total concentration (after Sutcliffe, 1963).

2- Hemolymph pH

- Definition: pH is defined as the negative logarithm of hydrogen ion concentration of a solution.
- pH= - log H+
- In most insects the blood is slightly acidic, pH 6.4 – 6.8, although slightly alkaline values have been recorded in a dragonfly larva and in the larva of midge *Chironomus*.
- Most enzymes work within a limited pH range so its control is important .
- During normal activity the blood become acidic due to liberation of acid metabolites (CO_2), this tendency is buffered by substances in the blood.

- The buffering capacity of insect blood (its ability to prevent change in pH) is low in the normal physiological range, but it increases sharply on either side of this range.
- Bicarbonates and phosphates are the most important buffers.

Functions of Plasma

1- Nutritive materials are carried out from alimentary canal and storage tissues to the sites at which they are metabolized.

2- Carrying excretory products from the place of origin to the malpighian tubules.

3- Transporting hormones from endocrine organs to the site of action.

4- The plasma contains much more CO_2 than O_2, CO_2 in solution accounts for about 20% of the total blood but the remainder is bound as bicarbonate. the greater affinity of blood to CO_2 than to O_2 is important for the cyclic release of CO_2 which occurs in some insects.

5 – Store for some substances e.g., Trehalose as a source of energy and its supply is achieved rapidly from fat body.

6- Water storage:

- An insect can maintain the level of its cell fluids if the food is dry.
- The water itself functions as a hydrostatic skeleton in forms such as larvae , where the cuticle is soft.

7- At molting the hydrostatic properties are used in expansion of appendages. (The amount of fluid present at this time influences the ultimate size of the insect. e.g., larvae of Lucilia with more moisture produce bigger adult).

8- Reflex bleeding results from the increase in hydrostatic pressure (plasma is forced through weak spots or pores in the cuticle . In species with this phenomena the blood contain some repellant substances.

9- As a result of muscular activity; there is an increase in hydrostatic properties (this is responsible for the eversion of the penis in male insects and the osmeterium (Fleshy bifurcate defensive device on the prothoracic segment in the larvae of Papilinoidae) .

Osmeterium of *Papilio xuthus* Larva.

Insect Blood Cells (Hemocytes)

- The blood or hemolymph of insects consists of a fluid plasma in which nucleated cells are suspended , These cells are sessile. Several different types of blood cells occur . Their functions include: encapsulation , nodule formation and phagocytosis which are considered as cellular immunity , wound healing and intermediate metabolism .

- Rowley and Ratcliffe (1981) and Gupta (1997, 1985, 1991) grouped insect hemocytes into six cell types:

1- Prohemocytes.

2- Plasmatocytes.

3- Granulocytes (which are probably the same as cystocytes or coagulocytes)

4- Spherulocytes.

5- Oenocytoids.

6- Adipohemocytes.

- Another group, Brehelin and Zachary (1986) make the distinction among nine types of hemocytes: prohemocytes, plasmatocytes, oenocytoids, spherule cells, thrombocytoids, and four granular hemocytes labeled GH1, GH2, GH3, and GH4.

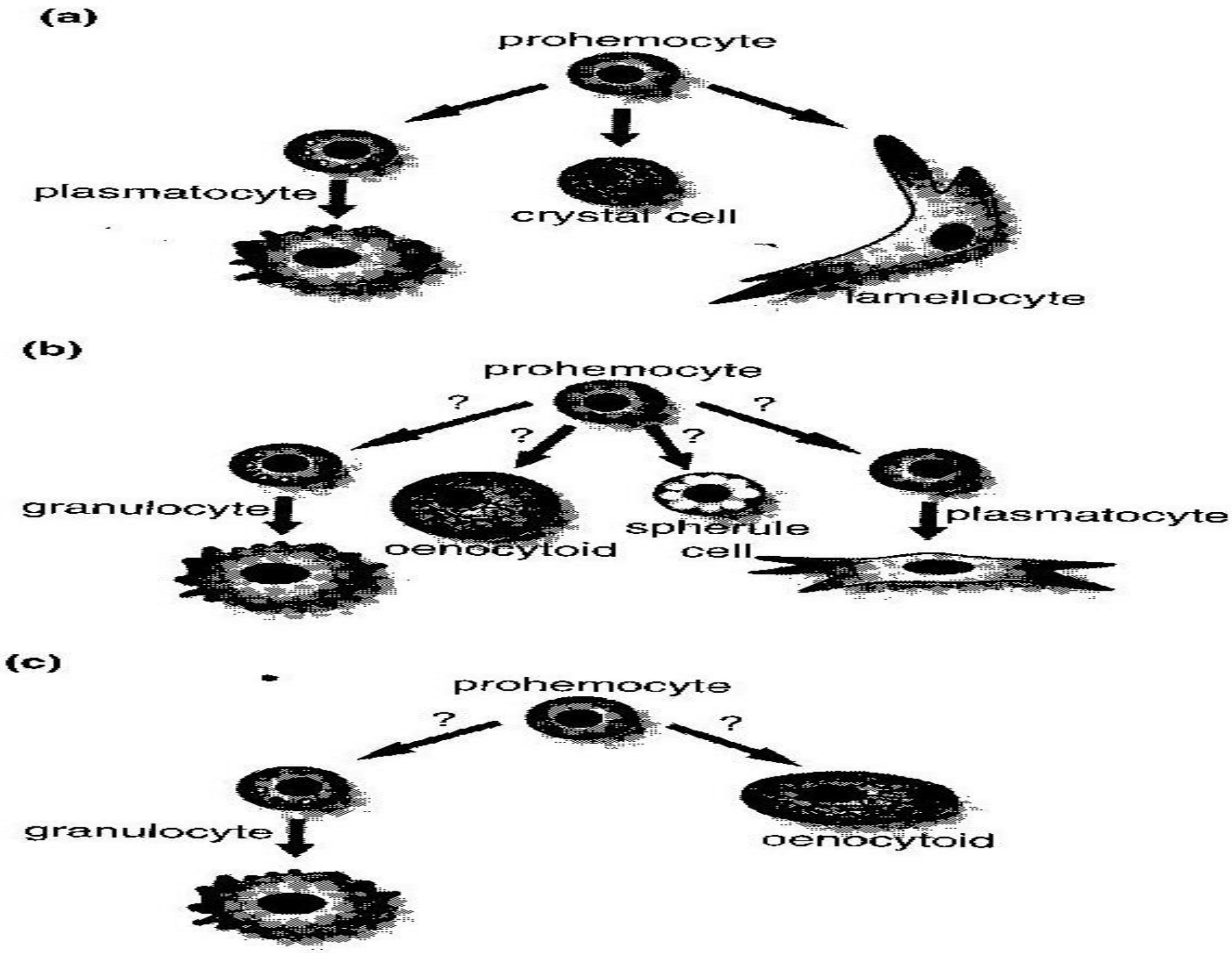
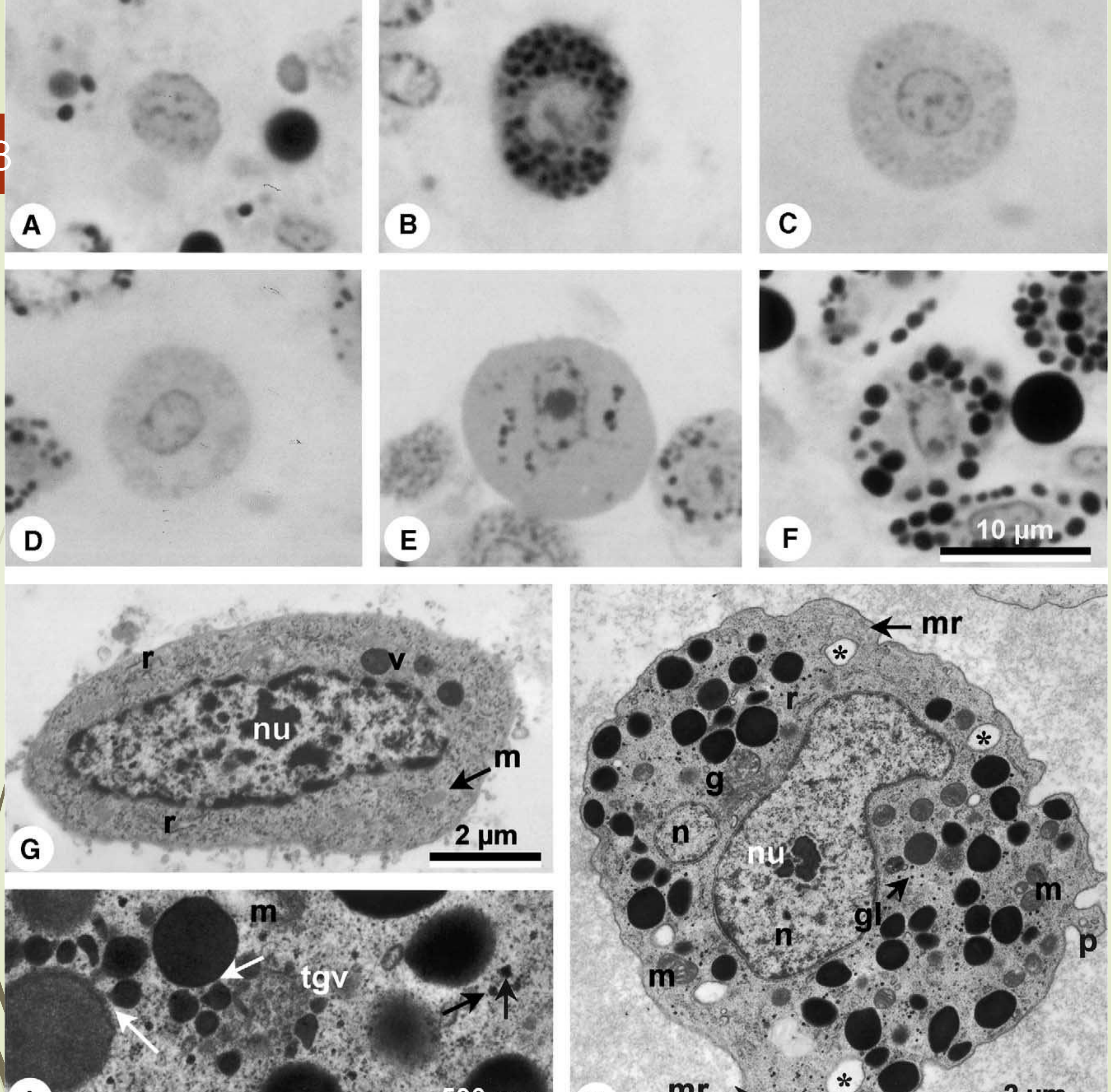


Figure 5.16 Types of hemocytes and their proposed lineage relationships in selected insects. (a) *Drosophila* larvae contain three differentiated hemocyte types in circulation: phagocytic plasmatocytes; phenoloxidase (PO)-containing crystal cells; and capsule-forming lamellocytes. Plasmatocytes are rounded cells in circulation but readily bind and spread symmetrically on foreign surfaces. Each cell type derives from progenitor prohemocytes and proliferation of each cell type occurs from hemocytes already in circulation or in hematopoietic organs. (b) Hemocytes in larval lepidopterans include four differentiated hemocyte types in circulation: phagocytic granulocytes; capsule-forming plasmatocytes; spherule cells; and PO-containing


A
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- The previous figure (A–H) Light microscopy semithin sections (A–F) and transmission electron microscopy (G–H) of C. *aeruginosa hemocytes. (A) Prohemocyte* showing a high nucleus/cell ratio. (B) Granulocyte filled with the typical granules. (C) Plasmatocyte showing fine, poorly stained, granulations. (D)Coagulocyte showing nucleus with a heterochromatic aspect and the perinuclear area lacking fine granules. (E) Oenocytoid with the typical large andround profile with the central nucleus and the homogeneously stained cytoplasm with few granules. (F) Spherule cells filled with the typical large round granules. (G) Prohemocyte showing the large nucleus with an evident nucleolus (*nu), rough endoplasmic reticulum (r), small mitochondria (m) and* electron dense vesicles (*v). (H) Granulocyte presenting a lobated nucleus (n) with an evident nucleolus (nu). The cytoplasm show small mitochondria* (m), *rough endoplasmic reticulum (r), Golgi complex (g), glycogen granules (gl) and round electron lucent vesicles (asterisks). A microtubule ring (mr)* just beneath the plasma membrane and pseudopodia (*p) are present. (I) Contribution of trans Golgi vesicles (tgv) fusing towards the large immature* granules (*white arrows). Glycogen aggregates are present (black arrows*

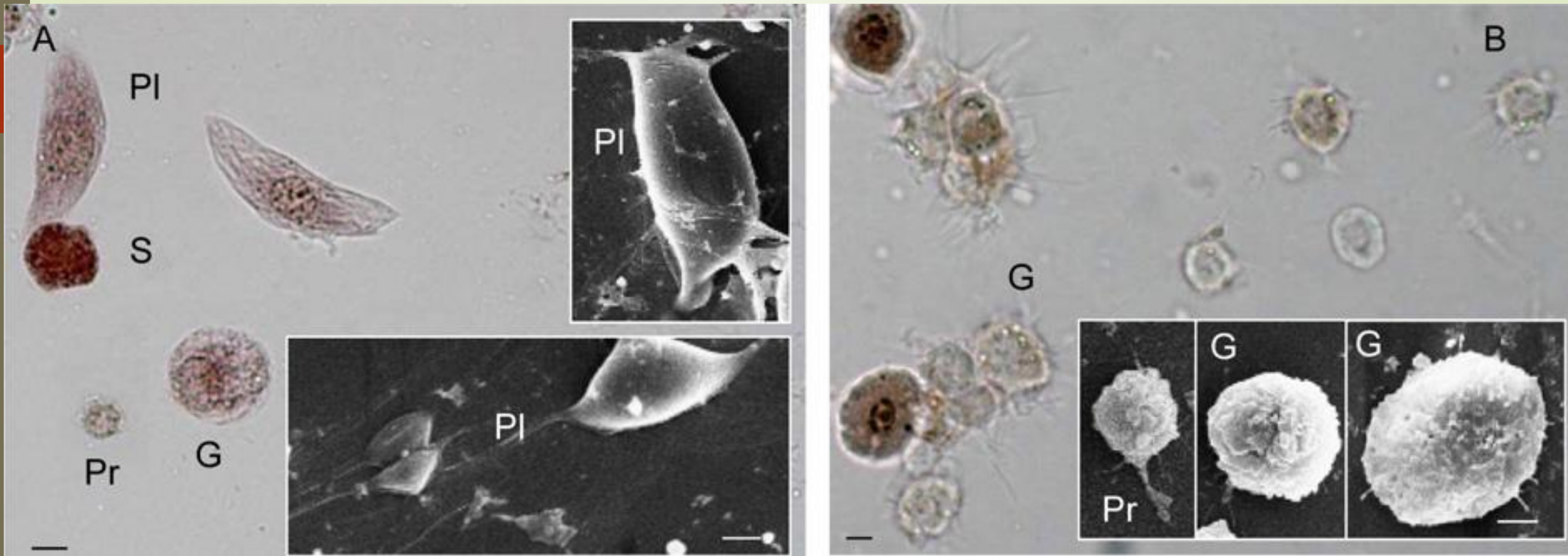
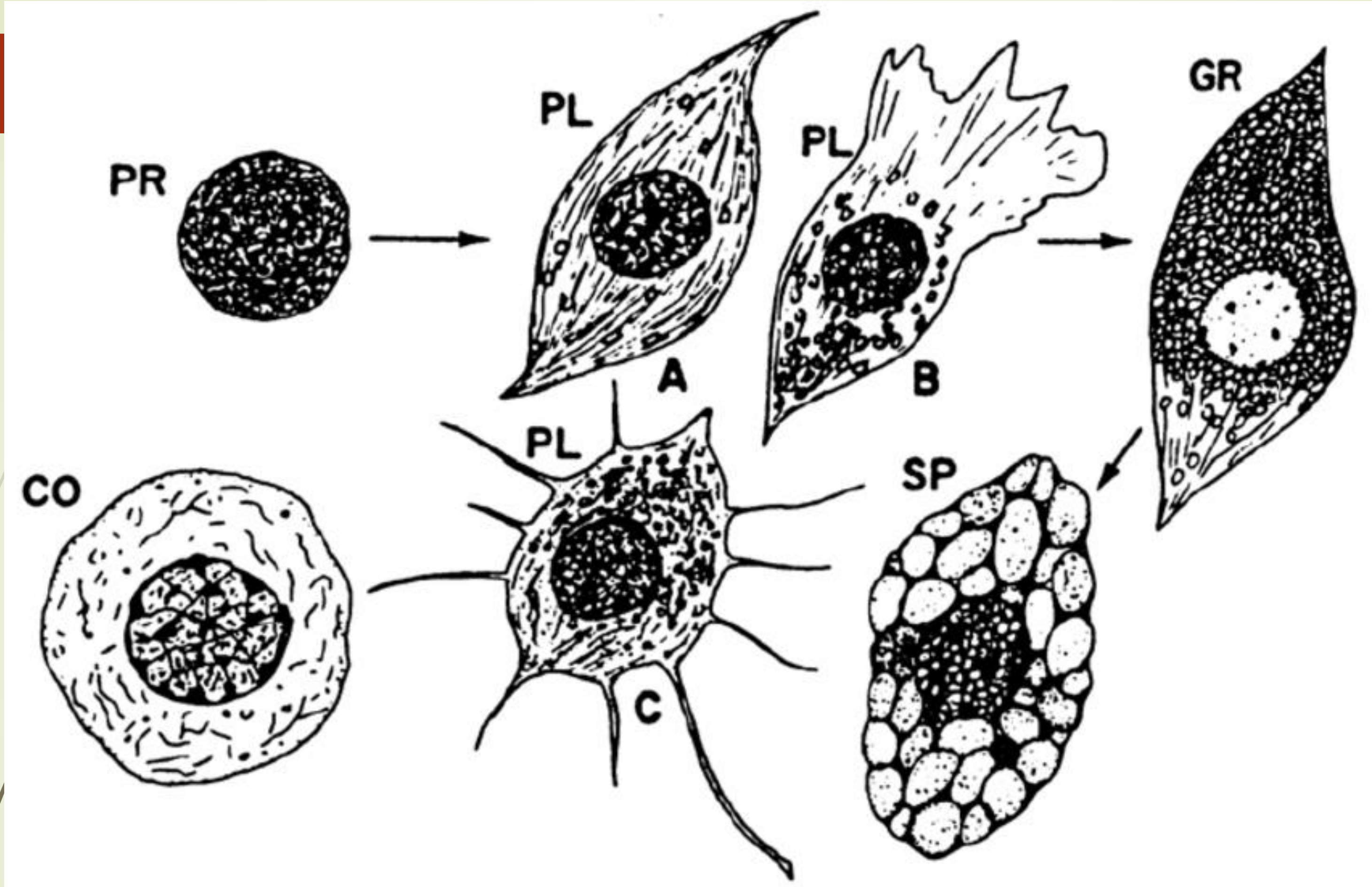


Fig: Scanning electron microscopy of *Rhynchophorus ferrugineus* larvae hemocytes

Types of Hemocytes (Ultrastructure identification)

1- Prohemocytes

- The prohemocytes are very variable in shape but usually small round cells . Nucleus fills almost the entire cytoplasm. They are characterized by undeveloped intracellular organelles .
- They rarely comprise more than 5% of the total hemocyte population.
- They are the stem cells from which most other hemocyte types are formed.



An illustration of the most common types of hemocytes from insect hemolymph. PR, prohemocyte; PL, plasmatocyte; GR, granulocyte; SP, spherulocyte; CO, coagulocyte. Several different shapes of plasmatocytes are shown in A, B, and C. The arrows indicate transformations of cells that are believed to occur (according to Woodring, 1985).

2) Plasmatocytes (phagocytes)

- Plasmatocytes are variable in shape usually round and oval .
- They are the most abundant cell types. with a centrally placed, spherical nucleus surrounded by well vacuolated cytoplasm.
- Plasmatocytes are characterized by large nucleus containing a large nucleolus.
- They possess well developed Golgi apparatus and endoplasmic reticulum as well as many lysosomes.
- The cells are capable of amoeboid movement and are phagocytic.
- They are involved in phagocytosis and encapsulation of foreign organisms invading insect so they are responsible for most of the cellular immunity of the insect .

3) Granulocytes

- Round or disc-shaped, with a relatively small nucleus surrounded by cytoplasm, well developed granular rough endoplasmic reticulum and Golgi apparatus.
- They discharge their contents on the surfaces of intruding organisms as early part of the defense response.
- They probably derived from plasmatocytes.

4) Spherulocytes

- They are characterized by the large, refractile spherules which may occupy 90% of the cytoplasm.
- They are not common although they are found in most of the studied species.
- Their function is unknown.

5- Oenocytoids

- Mainly occur in Lepidoptera.
- They exhibit little development of rough endoplasmic reticulum or Golgi complexes, but they have a complex array of microtubules.
- Their function is unknown.

6- Adipohemocytes

- They are characterized by containing lipid droplets.
- They contain well developed endoplasmic reticulum and Golgi complexes.

Many other hemocyte types are present and they vary according to the different insect species and also to the type of investigation

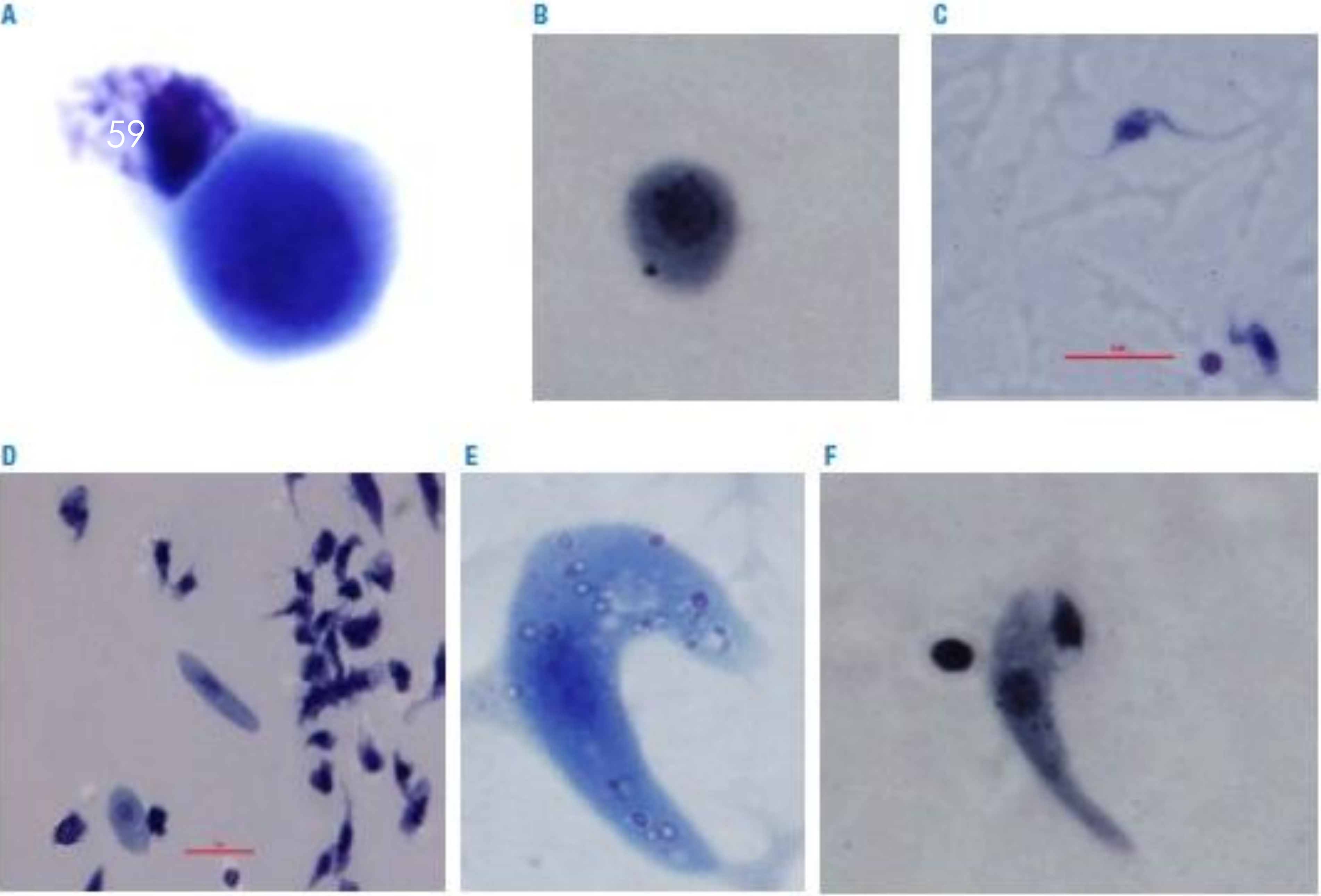


Figure 2. Hemocytes observed in *R. prolixus* and *R. robustus* hemolymph (100X), Giemsa staining. A,B: Prohemocytes; C,D: Plasmatocytes (C), cluster of plasmatocytes with two granulocytes (D); E,F: Granulocytes.

Functions of Hemocytes

1- Phagocytosis

- The most common function of hemocytes is phagocytosis of foreign particles, microorganisms and tissue debris that are comparatively smaller in size than the cell.
- Many cells are capable of phagocytosis but plasmatocytes and granular hemocyets are the most important.
- Endocytosis, especially phagocytosis, is important in both metamorphosis and defense against disease, as well as in routine cleaning up of dead or damaged cells.

2- Wound healing and Coagulation

- The hemocytes collect at the site of injuries, forming a plug which helps to seal the wound, proliferating and removing the dead tissues and discard it.
- The blood of various insects when collected in glass capillaries, usually clots within 10 – 15 minutes.

- On the other hand the blood of other insect species never clots or coagulates.
- In Cockroaches , the apparent coagulum is merely a clump of hemocytes; the blood cells become round, develop thread – like pseudopodia, and agglutinate to form a plug which is the essential factor of the clot. When blood cells coagulates due to the presence of hyaline hemocytes, these cells extrude thread – like pseudopodial expansions which fuse to form a meshwork in which the other cells are entangled.

3- Nodule formation

- This occurs in response to invasion of the body by large quantities of particulate matter (especially bacterial aggregates) that cannot be removed effectively by phagocytosis.
- This process includes two phases:

First: Within minutes of the foreign matter arriving in the body cavity, it is surrounded either by granulocytes or by specific plasma proteins and by material discharged from coagulocytes.

- This initial layer, which is often melanized includes "plasmatocyte-spreading peptide" that induces plasmatocytes to aggregate and adhere to a foreign surface

- In the second phase, which takes several hours to complete, plasmatocytes surround the melanized core, become flattened, and form a multicellular sheath comparable to that seen after encapsulation (Strand, 2002).

4- Encapsulation

- Encapsulation is essentially nodule formation on a larger scale.
- After the invader is initially coated with a thin layer of granulocytes or plasma proteins, layers of plasmatocytes surround it (Nappi, 1975; Lavine and Strand, 2001, 2002)
- This reaction on the part of hemocytes forms an insect's most important defense mechanism against metazoan endoparasites such as nematodes and insects.

- An encapsulated organism almost always dies, as a result of starvation, asphyxiation, and perhaps poisoning by quinones, antibacterial peptides, hydrogen peroxide and nitric oxide.

5- Connective tissue formation

- All tissues and organs of insects are separated from the body cavity by connective tissue membranes and basement membranes.
- Blood cells usually collect around foreign bodies and form capsules. Usually the cellular character of these capsules persists, but sometimes the nuclei disappear and a capsule of connective tissue is produced. The hemocytes are concerned in connective tissue formation.

6- Metabolic and Homeostatic Functions

- Hemocytes have been implicated in a variety of metabolic and homeostatic functions.
- Cells have been shown to contain, for example, glycogen, mucopolysaccharide, lipid, and protein, it has been suggested that hemocytes are important in storage of nutrients and their distribution to growing tissues, formation of connective tissue, synthesis of chitin, and maintenance of hemolymph sugar level.

- Cells might be important in hemolymph amino acid homeostasis.
- Certain hemocytes (spherule cells in Diptera, oenocytoids in other insects) contain enzymes for metabolism of tyrosine, derivatives of which are important in tanning and/or darkening of cuticle.

Numbers of Hemocytes

- Small insects have many fewer hemocytes than large insects.
- Number of hemocytes are variable between species and in different life stage in the same species.
- Count of the number of cells per unit volume of hemolymph (usually called total hemocyte count) may not reflect the total number of hemocytes in circulation because the blood volume varies.

Hemocyte profile

- The relative abundance of different types of hemocytes (called the hemocyte profile or differential hemocyte count) is not constant.
- Plasmatocytes and granulocytes are usually the most abundant, often comprising about 80% of the total hemocyte population.
- There number varied again with different life stage and physiological state.