Indira Gandhi
National Open University
School of Sciences

MZOE-005
INSECT PHYSIOLOGY AND TOXICOLOGY

VOL

1

INSECT PHYSIOLOGY

COURSE: INSECT PHYSIOLOGY AND TOXICOLOGY

The increasing difficulty encountered in recent years in controlling insect pests has led to a searching inquiry into the conditions under which insecticides act, and the chemical, physical, and biological factors which govern their effects. Among these factors none are more important than the physiological processes which occur in the species whose control is desired. Thus insect physiology has an economic importance which makes its active development much more imperative than if it were of purely scientific interest alone. Nevertheless, few attempts have been made to apply the increasing knowledge of physiology to the problems of insect-pest control, and the accounts of such work are widely scattered in the biological literature. These conditions have made it seem worthwhile to attempt to interpret certain advances in this field in terms of their relations to insect toxicology and control.

It is desirable to study insect physiology to identify and understand systems that are unique to insects and not found in mammals, birds, reptiles or fish. If insecticides are developed that target those systems, and only those systems, there is a good chance that the insecticides will be non-toxic to other species and therefore safe to handle and safe for the environment in which they are used. For example there are some insecticides for use against fleas on domestic pets that target the pupal stage of insect development and prevent the fleas from reaching adulthood. These chemicals are of extremely low toxicity to mammals and therefore safe for the pets and their owners.

This course on Insect Physiology and Toxicology is the second elective of Entomology specialization package of fourth semester of M.Sc. in Zoology. The course comprises 2 volumes. Volume 1 deals with Insect Life Processes. It is further divided into two blocks. Block 1 consists of four units and Block 2 consists of three units of Insect Physiology. The integument is not only the characteristic feature of all the arthropods but is responsible for the great success of insects as terrestrial animals. The integument is also a central subject intimately related to a variety of applied problems such as mode of action of insecticides and water metabolism. One of the major reasons for biological success of insects in their ability to eat, digest and utilise an enormous diversity of foods. Extreme diversity can be observed in the modifications and specialization of the alimentary system of insects. Excretory physiology is of a great significance as process of elimination of nitrogen wastes takes place through various organs in different insects.

The insects have an open circulatory system as the blood bathes the internal organs diversity in the body cavity or haemocoel which is not a true coelom and the blood of insects is commonly called haemolymph. The tracheal system/respiratory system is involved is gaseous exchange in an insect with the environment. It does not use the circulatory system as the vehicle for gaseous exchange as tracheae directly transport the oxygen to different parts of the body. All the above systems are discussed in Block 1 of this course.

Block 2 discusses about the Nervous system, Endocrine system and Reproductive system of insects. The unit an Nervous system speaks about the basic functional unit of nervous system i.e., neuron. Sense organs are also discussed Bioluminescence and sound production that provide uniqueness to class Insecta; are also dealt with in this unit. Endocrine system of insects is very fascinating for learners and researchers. Hormones, like the nervous system regulate the physiological and behavioral responses of the insects. Role of various hormones secreted by major endocrine glands are discussed in the unit on Endocrine system. Insects usually reproduce sexually and show sexual dimorphism. The reproductive system of both male and female insects usually consists of a pair of glands connected to median duct that open exteriorly through a gonopore.

Volume 2 of this course is a comprehensive exploration of the evolving landscape of insect toxicology, offering a deep dive into both the foundational principles and the latest innovations shaping the field. Volume 2 has two blocks i.e. Block 3 and 4. Block 3 comprises five units and Block 4 consists of three units. Unit 8 of Block 3 begins by tracing the historical development of insect toxicology, providing a context for the modern challenges and advancements that researchers face today. Unit 8 speaks about different fields of toxicology and deliberates upon important issues of pesticide industry, market trends and different stages of pesticide registration. Unit 9 focuses on a comprehensive understanding of pesticide toxicology. The unit examines various aspects of pesticides such as their chemical nature and dose. Future, route of exposure methods for evaluating pesticide toxicity are also discussed in the Unit.

Pesticides may be classified in many ways which can provide useful information about their action, impact on humans, non targets and environment. Based on the types of target pests, mode of action, and chemistry they can be classified into various groups. Unit 10, 11 and 12 of this course will cover these groups based on their origin and chemistry. Unit 10 will explain characteriststions of organochlorines, organophosphates and carbamates and specific chemical structure of cited examples. In the same manner, Unit 11 tells about pyrethroids, rotenoids, fumigants and neonicotinoides and Unit 12 covers Group III pesticides viz. IGRs, semiochemicals, repellents and antifeedants.

Units 13 and 14 of Block 4 thoroughly examine the mechanisms by which insecticides act on insects, from their interactions at the cellular level to the complex molecular pathways involved. This detailed analysis helps learners to understand the intricate processes that underlie insecticide efficacy and the physiological impacts on target pests. The units also address critical issues related to insecticide resistance, a growing concern in pest management. By exploring the biochemical pathways and genetic factors that contribute to resistance, the text offers valuable insights into how insects adapt to chemical controls and what strategies can be employed to counteract these adaptations. Moreover, they highlight the neurotoxic effects of insecticides, which are crucial for understanding the broader implications of their use on insect nervous systems. Unit 13 appraises about classification of pesticides based on their chemical composition and target organisms. Pesticides, harmful effects on environment and health are discussed in the unit. Resistance against pesticides and management strategies to counter the resistance are also discussed here. Importance of metabolism of insecticides in insect management are discussed in Unit 14. Nonsynthetic Phase I reactions and Synthetic Phase II reactions in insecticide metabolism are also elaborated in the Unit. Microsomal and extramicrosomal metabolism of insecticides that include alkyation, epoxidation, desulfuration hydrolysis, reductive dechlorination are explained at length. The environmental fate and behaviour of insecticides are also explored, providing a nuanced perspective on how these chemicals interact with ecosystems and affect non-target organisms. Incorporating the latest technological advancements. Units 15 delves into novel approaches in insecticide delivery, such as nanotechnology, and presents cutting-edge analytical techniques that are transforming research in the field. The unit also covers the emerging role of biopesticides and molecular diagnostics in managing insect populations and resistance. Their types, chemistry, usage and delivery technology is explained in an interesting manner.

Objectives

After studying this course, you should be able to:

- explain the structure, and significance of cuticle;
- describe the process of moulting and metamorphosis alongwith its hormonal control and significance;
- describe the digestive, respiratory and excretory systems of insects and their modifications;
- explain the basic structure of circulatory system and appreciate the role of circulatory system in immunity and in thermoregulation;
- describe the morphology and anatomy of different components of insect nervous system and sense organs;
- appreciate the bioluminescence in insects and discuss the significance of bioluminescence;
- discuss about the different components of female and male reproductive system of insects and appreciate sex determination in insects;
- deliberate upon pesticide industry, market trends and demands and various stages of pesticide registration;
- explain the characteristic of organochlorines, organophosphates and carbamates;
- discuss the toxicity, mode of action and efficacy of some biopesticides and fumigants;
- list various chitin synthesis inhibitors, JH analogues, ecdysone agonists and JH antagonists;
- appreciate the action of allelochemicals, reprellents and antifeedants and, their advantages in pest control programmes; and
- enumerate the molecules and formulations that are eligible to be used as next generation pesticides.

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Block

1

INSECT LIFE PROCESSES-I

COURSE NAME: INSECT PHYSIOLOGY AND TOXICOLOGY **COURSE CODE: MZOE-005**

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BLOCK 1: INSECT LIFE PROCESSES-I

Insects are the most diverse of all the organisms on earth. Insect physiology is the study of how insects live and reproduce. This is a historic area of research that continues today. The study of insect physiology is usually divided into a system approach. The major systems are digestive, excretory, circulatory respiratory, nervous and reproductive system. These systems will be discussed in Blocks 1 and 2 of this course.

Block 1 discusses about four systems in four units. Unit 1 discusses about integumentary system or exoskeleton, a protective outer covering composed of chitinous cuticle, epidermis and basement membrane. This provides structural support, protection and a site for muscle attachment. Insects show very interesting phenomenon of moulting where integument is shed and replaced. This process allows insects to grow and develop.

Unit 2 speaks about two systems viz. digestive and excretory systems. Insect alimentary canal is a tube running from the mouth to anus. It comprises of three main regions: the foregut, midgut and hindgut where food is processed and the waste in eliminated. Malpighian tubules which are excretory organs, are attached to the hindgut. The tubules are used by insects in both excretion and osmoregulation; filtering waste products from the haemolymph and selectively reabsorbing water and ions.

Insects have an open circulatory stem, where haemolymph (insect blood) bathes the organs directly within the body cavity (haemocoel) rather than being confined to vessels like in mammals. The main circulatory organ is the dorsal vessel which functions as a heart, pumping hemolymph. Some insects have accessory pulsatile organs in their antennae, wings and legs through which hemolymph is pumped.

Insects breathe through a network of internal tubes called the tracheal system, where air enters through spiracles and diffuses oxygen directly to tissue, with carbon di-oxide exiting through the same route. Unlike humans, insects do not rely on blood to transport oxygen, instead oxygen in delivered directly to the cells though the tracheal system. Circulatory and respiratory systems are explained in detail in Units 3 and 4 of this block respectively.

Objectives

After studying this block, you should be able to:

- list the different layers of insect’s cuticle;
- describe the process of moulting alongwith its hormonal control and significance;
- describe the organs for the digestion of insects and their modifications;
- explain the structures and functions of malpighian tubules and nephrocytes;
- explain osmoregulation and adaptations in terrestrial, freshwater, and saltwater insects;
- gain insight of the basic structure and function of circulatory system;
- appreciate the role of circulatory system in immunity and in thermoregulation;
- discuss the structures of tracheae, tracheoles, and spiracles;
- describe the role of respiratory pigments in insects; and
- explain the physiology of gaseous exchange in terrestrial and aquatic insects.

UNIT 1

INTEGUMENTARY SYSTEM

Structure

1.1 INTRODUCTION

Insects are the most abundant and diverse group of animals on the earth. They have unique characteristics which provide them survival advantage over the other animals. One of these is their peculiar integument, the outermost layer which acts as an exoskeleton of insects. It not only provides them shape and support, but also gives them protection from adverse environmental conditions, prevents desiccation, and imparts strength. Apart, the integument is a rich sensory storehouse that interprets stimuli in a variety of ways. The structure of insects' integument is highly intricate and is composed of several layers. It also changes over time, particularly during development. This chapter aims to list some of the integument's remarkable and noteworthy characteristics and discusses how its features change and get modified during development.

Objectives

After studying this Unit, you should be able to:

- explain the detailed structure of an insect's integument;

- list the different layers of insect's cuticle;
- recognise the cuticular modifications;
- explain the functions of integument of an insect;
- describe the process of moulting alongwith its hormonal control and significance; and
- discuss the phenomenon of metamorphosis and hormonal control in detail.

1.2 WHAT IS INTEGUMENTARY SYSTEM

Insect integument is the outermost coating that covers the entire body of the insect. It is ectodermal in origin and creates a composite structure that builds the insect's skeleton. It provides shape and strength to the body, acts as a region for muscle attachment, and protects against mechanical or physical harm and desiccation by forming a sensory interface with the environment. It covers all the outer surfaces of the body including all the ectodermal invaginations.

1.3 STRUCTURE AND FUNCTION OF INTEGUMENT

Integument of insects is composed of three layers: the outermost cuticle, a single layer of ectodermal cells (epidermis, hypodermis) and a basement membrane.

1.3.1 Cuticle

It is a complex, non-cellular layer that is secreted by the epidermis. It forms the outermost covering of whole body and its appendages as well as lines the ectodermal invaginations, such as trachea, foregut and hindgut. Though generally believed to be non-living, the cuticle is really the site of intricate biochemical changes, some of which are controlled by enzymes. Initially, it is elastic and flexible, and in many larvae, it remains so across a large portion of the body. However, in many insects, the majority of the cuticle goes through a process called **sclerotization** during which it darkens and hardens to form somewhat rigid, tough sclerites that are separated from one another by membrane zones of soft cuticle. Such an arrangement blends flexibility and stiffness. The cuticle's protective role is complemented by its capacity to shape the insect's form, minimize desiccation, and offer a solid foundation for muscle attachment.

The cuticle can be divided into two layers: (a) the outer epicuticle and (b) the inner procuticle, both of which are compound structures (Fig. 1.1).

a) **Epicuticle:** It is a very thin layer with a complicated chemical composition and ultrastructure. Epicuticle is devoid of chitin. It is very stiff and inextensible, and folded prior to ecdysis, but its form ultimately defines the insect's overall shape. It has a surface pattern that is relatively complex. The epicuticle consists of four layers (Fig. 1.1b).

i) **Cement layer:** It is a thin and outermost layer secreted by the dermal glands. It is composed of mucopolysaccharides associated with lipids. Its primary function is to protect the insect body from external damage, and provide it overall shape and the size. It is not present in all insects, for example, honey bees lack this layer.

ii) **Wax/lipid layer:** It is a prominent layer of variable thickness. It is made up of alcohols, fatty acid esters, and long chain hydrocarbons, with hydrocarbons making 90% of the wax. It acts as a water proof layer that prevents loss of water from the body.

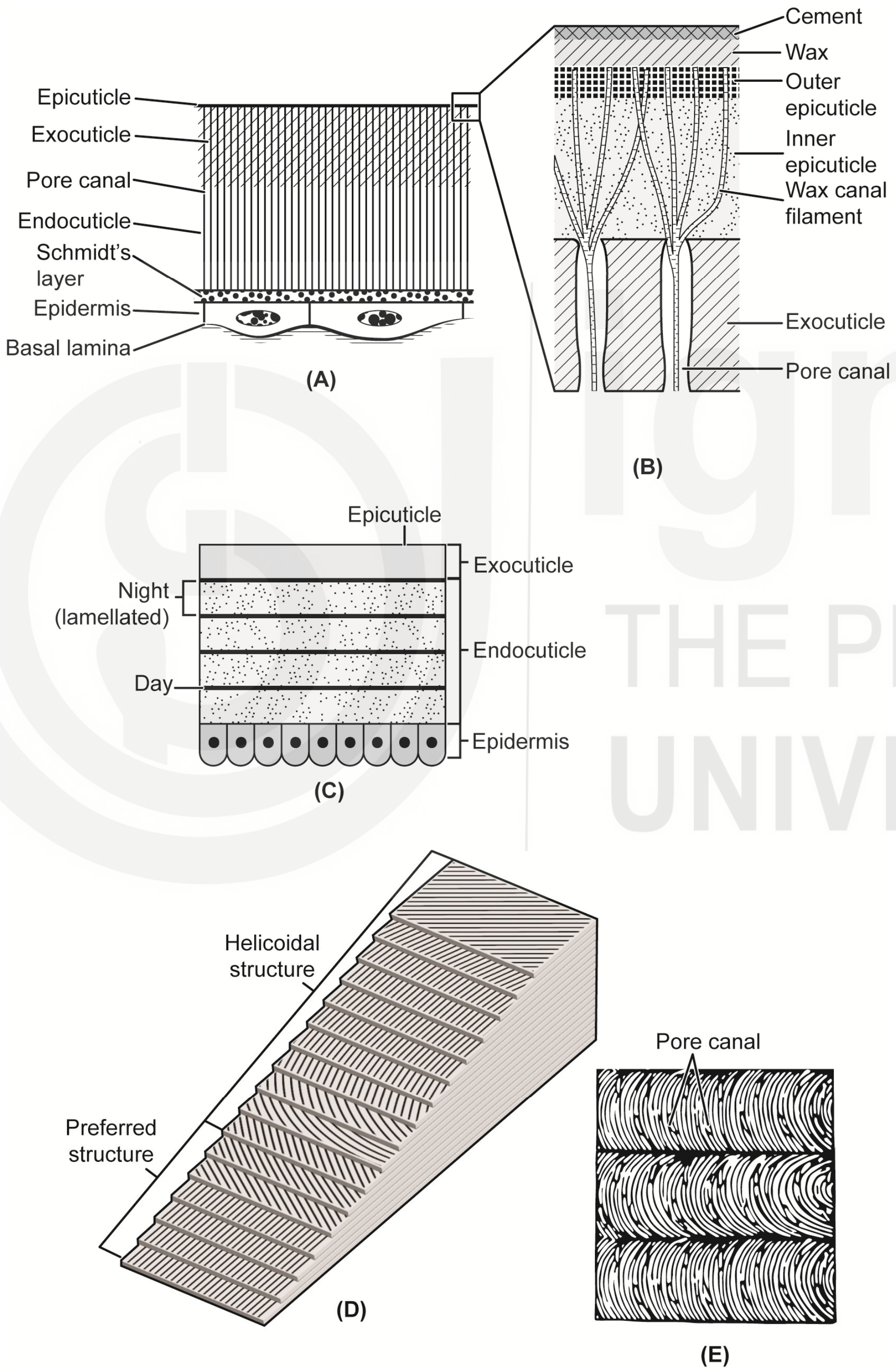


Fig. 1.1: Basic structure of the integument. (A) Section through mature integument; (B) Magnified view of the integument; (C) Daily growth layers and lamellar pattern; (D) Helicoidal and preferred structure of layers of endocuticle; (E) Transverse section of endocuticle showing parabolic effect

iii) **Cuticulin layer or the outer epicuticle layer:** It is the thin (10-15 nm) and trilaminar layer consisting of highly polymerized lipids, and probably also has a protein component. This is the first-formed layer of the new cuticle secreted at each moult protecting the procuticle from the moulting enzymes. It is inelastic in nature and limits the growth of the insect. It also acts as a selective barrier to substances during moulting.

iv) **Polyphenol layer or the inner epicuticle layer:** It is non-static and the thickest layer measuring 0.5-2.0 µm. It is present above the epidermis and consists of tanned lipoproteins. During its formation, polyphenols and phenol oxidases are present which are associated with the tanning. The layer can withstand both organic solvents and acids.

b) Procuticle: It is secreted by the epidermal cells. It makes up the majority of the integument and may not be present in the tracheoles. The procuticular components—most notably chitin (20-60%) and proteins (soluble as well as insoluble)—determine the mechanical rigidity and strength of the insect cuticle. When taken as a whole, they exhibit great compressive, tensile, flexural, and impact resistance as well as a high strength-to-weight ratio—the latter being attributed to the absence of mineral salts.

The procuticle is differentiated into an exterior exocuticle and inner endocuticle after sclerotization.

i) **Exocuticle**: It is made up of a homogenous electron dense matrix. This is hardened by sclerotization and sheds completely during the process of moulting. It mostly consists of chitin and a hard protein known as sclerotin, making the cuticle rigid and dark-coloured. The layer is absent or considerably reduced in the flexible regions, such as joints and intersegmental membranes.

ii) **Endocuticle**: It is 10-200 µm thick, unsclerotized, delicate and pale-coloured layer. It lacks the hard protein sclerotin but has more chitin than exocuticle does. The softer endocuticle can grow significantly by post ecdysial deposition and is made up of repeating layers of differently orientated protein and chitin fibres.

Mesocuticle is a transition layer between exocuticle and endocuticle. It is chemically similar to the endocuticle.

Pore canals: These are several tiny, vertical channels with diameters of less than 1µ (0.1–0.15) that cross both the exo- and endocuticles. They travel the entire length of the cuticle in a perpendicular direction from the epicuticle. These help in transporting enzymes and cuticular material to the outer procuticle and epicuticle sections. Initially they are occupied by cytoplasmic filaments from the epidermis, however later these can be filled by cuticular material. The axial filaments run a straight path through them, and they have a flat or twisted ribbon-like shape.

Chemical composition of Insect cuticle

The two main components of an insect's cuticle are a variety of proteins and the carbohydrate chitin, which makes up 25–60% of the dry weight of different cuticles.

Chitin is a polymer of high molecular weight. It is composed of anhydro-N-acetylglucosamine residues connected by β 1, 4-linkages (Fig. 1.2), though up to 10% of the residues may be deacylated. In procuticle, proteins are associated with the chitin to form glycoproteins. It only dissolves in strong mineral acids and sodium hypochlorite, but it is insoluble in water, alcohol, organic solvents, diluted acids, and concentrated alkalis.

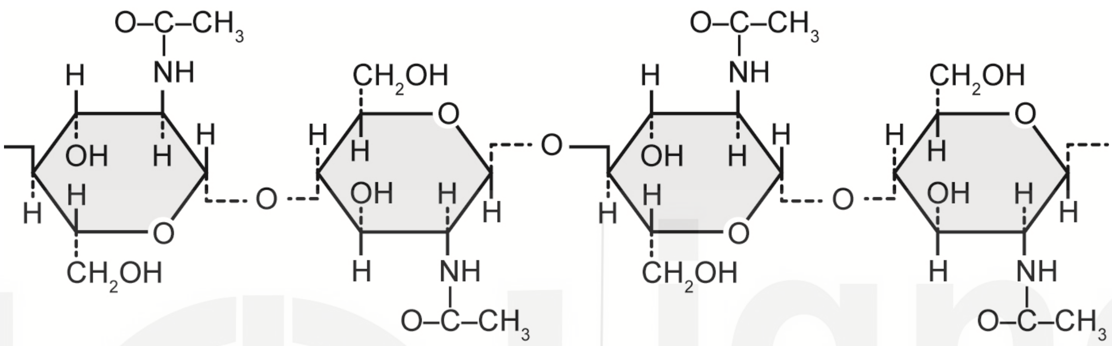


Fig. 1.2: Chemical structure of chitin

Proteins: Insect cuticle contains three kinds of proteins.

a) **Arthropodin:** This is a soluble protein found in the endocuticle. It is an untanned protein, which on sclerotization converts to sclerotin.

b) **Sclerotin:** It is the amber-colored tanned structural protein found solely in exocuticle.

c) **Resilin:** It is a colourless, rubber-like, elastic protein found in joints of insects, such as tergosternal joints, leg joints, clypeolabral joints, and wing hinge ligaments.

While making up a relatively minor portion of its weight, other cuticle components are equally important to the body. It has been stated that phenolic precursors of the quinones join amino acid chains to generate sclerotin, yet some apterygotes are devoid of these precursors and harden their cuticles by disulphide connections.

1.3.2 Epidermis

It is a unicellular continuous layer made up of polygonal cells that modify into cuboidal or columnar cells during the moulting process. These cells have a fully formed nucleus along with other cytoplasmic components. They are characterised by a microvillate surface, numerous mitochondria, smooth-surfaced endoplasmic reticulum cisternae, Golgi vesicles and cytoskeletal structures in the form of microtubules and oriented microfibers. All the epidermal cells are glandular which release cuticle as well as the enzymes needed for the generation and breakdown of old cuticle during moulting.

Certain cytoplasmic structures (**septate desmosomes**) hold adjacent epidermal cells together.Based on the functions; the epidermal cells can be differentiated into the following categories:

a) **Dermal gland cells:** These cells produce cement layer.

b) **Trichogen cells:** The trichogen cells produce shaft of hair-like trichomes or setae.

c) **Tormogen cells:** These cells form the socket at base of trichomes.

d) **Moulting gland cells:** These secrete a fluid that digests the old cuticle during moulting.

e) **Peristigmatic gland cells:** These surround the spiracles of the dipteran larvae and protect their orifice.

The epidermis is penetrated by muscle attachments. Oenocytes are derived from the epidermal cells which are probably involved in lipid metabolism. They synthesise and secrete the waxes and lipids of the cuticle. The majority of the cuticle is secreted by the epidermis, which also generates the moulting fluid. The epidermis also contains usually red or orange pigments.

1.3.3 Basement Membrane

It is a non-living, amorphous (shapeless), continuous, granular layer of integument that is produced from the degenerating epidermal cells. It forms a continuous layer at the base of the body wall and has a thickness of roughly 0.5μ. It comprises connective tissue, glycosaminoglycans (neutral mucopolysaccharides) and fibrous proteins. Just before moulting, it thickens by the deposition of more mucopolysaccharide produced from the hemocytes. The basement membrane is continuous with the muscles' sarcolemma where muscles are attached to it. Nerves, tracheoles, and chordotonal organs either pass or run through it.

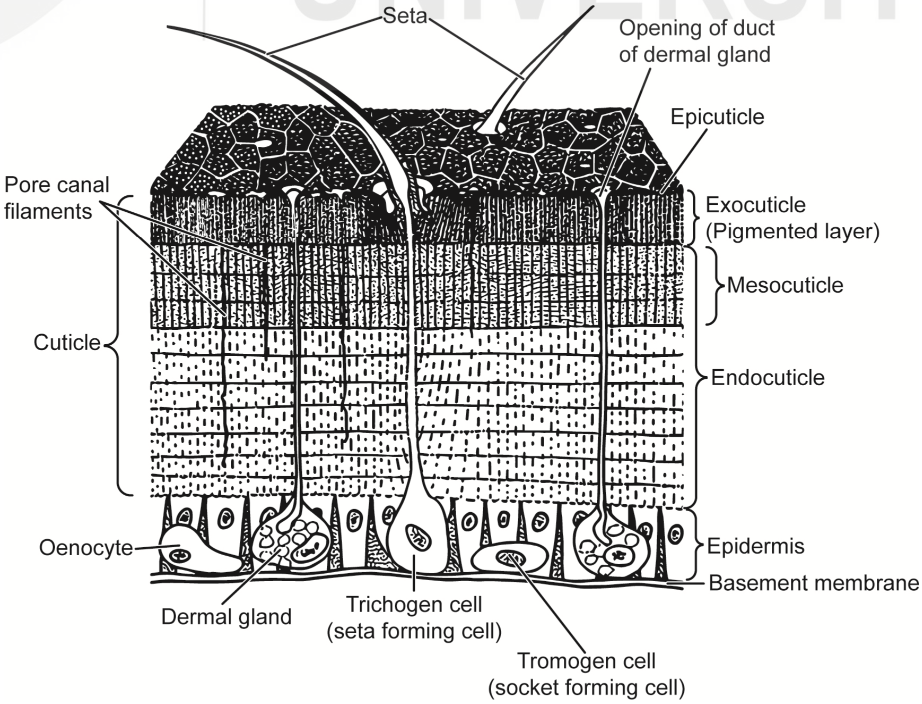


Fig. 1.3: Electron micrograph of an insect cuticle

1.4 CUTICULAR/INTEGUMENTAL MODIFICATIONS

Cuticle/Integument of insects is modified into certain internal invaginations or external outgrowths. These are as follows:

1.4.1 Cuticular Appendages

These structures comprise all cuticle outgrowths that are connected to it by a membranous joint. They originate from modified epidermal cells. These are categorized into setae and spurs.

a) **Setae**, also known as hairs, are hollow structures which arise from cup-shaped pits, or alveolus-like cup. They are produced, as extensions of exocuticle, by a single expanded hypodermal trichogen cell. The tormogen cell, as discussed in the previous section, typically produce articular membrane which attaches the seta at its base. Setae have a crucial taxonomic role and differ in various insect species (Fig. 1.4).

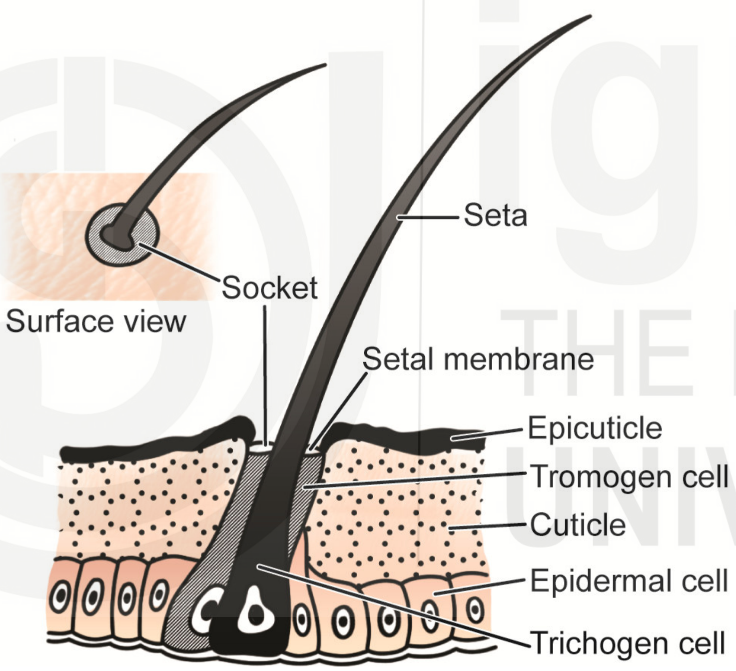


Fig. 1.4: A seta and its socket showing trichogen and tormogen cells

Various types of setae found in insects are given below.

- **Clothing hairs:** These cover the body's exterior or its appendages and usually display varying levels of specialisation. They are referred to as **plumose hairs** when they have branches that resemble threads, as in the Apoidea.

- **Bristles:** These are extremely robust and stiff setae, which are prominently displayed in certain families, like Tachinidae.

- **Scales:** Scales are essentially highly modified clothing hairs. These are a distinctive feature of Lepidoptera, Collembola, Coleoptera, Diptera, and Hymenoptera. Often transitional forms exist between the clothing hairs and the scales.

- **Glandular setae:** These are the setae that serve as the secretory exit of epidermal glands. They are regarded as **glandular bristles** if they are very stiff and robust, similar to the itchy hairs seen on some lepidopterous larvae.

- **Sensory Setae:** Often, the setae of specific body parts—especially the appendages—are modified in unique ways to acquire a sensory function. Sensory setae are associated with the neurological system.

b) **Spur:** Many insects have spurs on their legs, which are different from setae in being

1.4.2 Cuticular Processes

The exterior of the cuticle is not only sculptured in different ways, but it also has a wide range of outgrowths that are essential to its structure. They can easily be differentiated from cuticular appendages because they are firmly attached to the cuticle and lack a membranous articulation. The following are the main categories of cuticular processes.

- **Microtrichia:** Also known as fixed hairs or **aculei**, microtrichia are tiny structures that resemble hairs and are present on the wings of some Diptera. They lack the basal articulation, which gives them the appearance of extremely tiny covering hairs.

- **Spines:** They are cuticlular outgrowths that resemble thorns in some way. The production of spines from undifferentiated epidermal cells and their multicellular origins, typically if not always, set them apart from spine-like setae.

Apart from the aforementioned, there are numerous other cuticular processes that manifest as bigger projections, such as horns, which are specific to males of specific Coleoptera, or conical nodules and tubercles of varying shapes.

1.5 FUNCTIONS OF INTEGUMENTARY SYSTEM

The integument of an insect is highly significant and plays an important role in their successful living on the Earth. It performs the following functions in an insect.

1. The integument provides protection and preservation of the interior organs.
2. It gives the insect a shape and limits the extents of an exoskeleton.
3. It provides a base for muscle insertions and, helps in mobility and flight.
4. It is an important element in the defense of insects against various external factors, such as mechanical stresses, dry, wet, cold or hot environments. It also prevents the entry of extraneous substances like insecticides.
5. The waxy layer of the cuticle checks the loss of water and prevents desiccation.

6. Various cuticular structures provide sensory input, such as eyes, mechanoreceptors and chemoreceptors.

7. Other cuticular protuberances may help in oxygen retention, food grinding, body cleaning, etc.

8. It takes part in the transport of epidermal secretions, and acts as a chemical reservoir for the storage of metabolic wastes.

9. The coloration pattern and chemical components of the cuticle are important for thermoregulation and sometimes communication.

SAQ 1

Answer the following questions in 1-2 sentences:

i). What are the three main layers of cuticle of an insect?

ii). Name the two main components of insect cuticle.

iii). What is the function of pore canals?

iv). What are the two layers of procuticle?

1.6 MOULTING

The body wall of insects has minimal tensibility due to the non-elastic structure of cuticle. Since the cuticle cannot grow and cannot even be stretched in more stiff portions like the head capsule or appendages, it needs to be shed periodically as the insect matures, making room for a new and larger cuticle. Moulting is the process of shedding the old cuticle which involves a sequence of events. These events can be categorized into three sub-processes.

a) **Apolysis:** Separation of old cuticle from the underlying epidermis.

b) **Ecdysis:** Shedding off the old cuticle.

c) **Sclerotization:** Chemical modification of the newly formed, soft and milky white cuticle to the dark and rigid cuticle.

However, the process of moulting includes a sequence of events which are discussed here and depicted in Figure 1.5.

Process of Moulting

During moulting, the intima, or lining, of the majority of the tracheal system, the fore and hind gut, the ectodermal glands, and the efferent reproductive ducts are shed together with the overall cuticle that covers the body and its appendages externally during each moult. All of them are replenished by the underlying epidermal cells, along with hairs, scales, and cuticular sensillae.

(a) Changes in the epidermis: When an insect is ready to shed its skin, it becomes less active. The epidermal cells start dividing mitotically resulting in the net increase in the total number of cells. The increase in cell density is accompanied by the changes in the cell shape. The epidermal cells usually become columnar and microvilli degenerate resulting in the increased size of cuticle.

(b) Apolysis: The changes in the shape of epidermal cells generate tension at the cell surface which causes its separation from the cuticle probably by withdrawing the cytoplasmic filaments from the pore canals. The space created between the epidermis and cuticle is called the **exuvial or subcuticular space**.

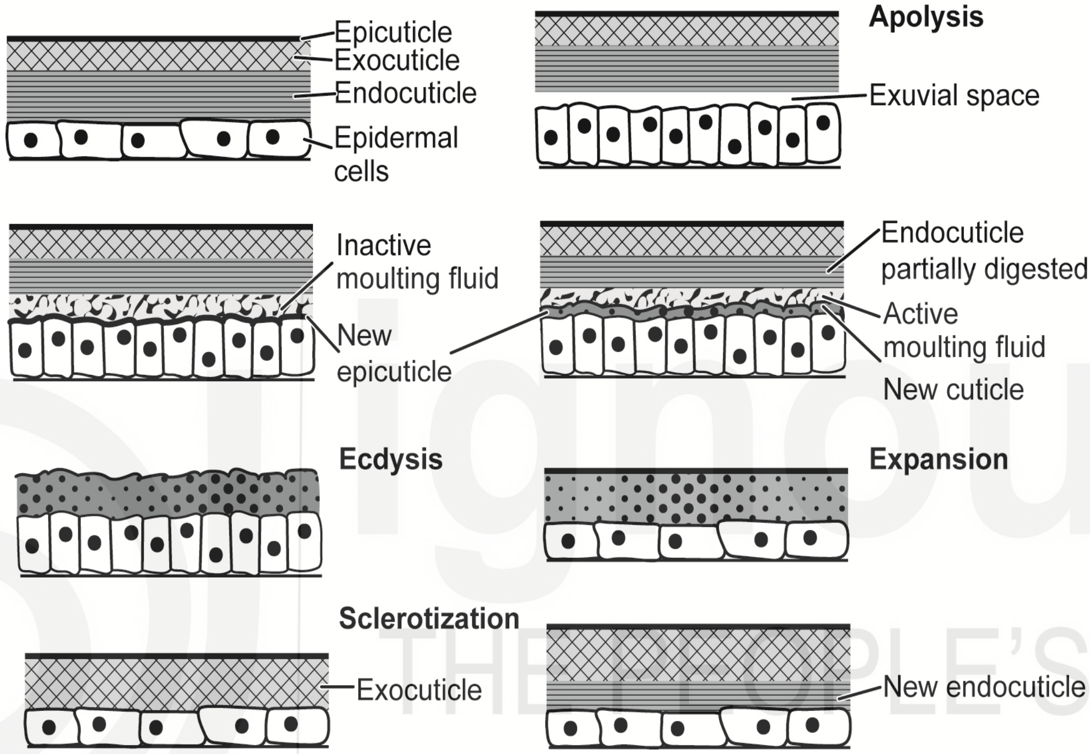


Fig. 1.5: Process of moulting in insects

(c) Secretion of moulting fluid: Before the cuticle is shed, the endocuticle is digested by enzymes secreted by the epidermal cells. This involves a mixture of enzymes including chitinases and peptidases. These enzymes are released in an inactive form and cannot digest the endocuticle. The fluid produced is called **moulting fluid**. The enzymes remain inactive until the new epicuticle is formed.

(d) Formation of the new epicuticle: The **outer epicuticle (cuticulin)** is secreted first as the sporadic patches at the tips of microvilli of the epidermal cells. The patches grow and amalgamate to form a continuous layer over the epidermis. Thereafter, the **ecdysial membrane** is produced from the inner layers of the old endocuticle and becomes sclerotized probably due to the presence of polyphenols and phenoloxidases.

Once the outer epicuticle is complete, the inner epicuticle is secreted and the apical surfaces of the epidermal cells withdraw slightly. Shortly before ecdysis, wax is secreted onto the cuticular surface. Lipids are secreted by epidermal cells, most probably oenocytes, are released in the intercellular spaces.

(e) **Digestion of old endocuticle:** The enzymes become active and digest the unsclerotized cuticle except the ecdysial membrane, which is made of the procuticle's innermost layer. These enzymes do not affect the exocuticle, muscles and nerves connected to the old cuticle which help the insect to move and receive stimuli from the environment. The products of cuticular digestion are absorbed and the cuticle becomes very thin and weak along the ecdysial lines. In other words, these lines do not have the hardened exocuticle. Most insects have an inverted Y-shape ecdysial lines along the head and prothorax.

(f) **Ecdysis:** After resorption of the moulting fluid and the products of digestion, the old cuticle consists of epicuticle and exocuticle which are separated from the new cuticle. The loosened old cuticle now breaks by the internal pressure exerted against it probably caused by increase in blood volume. It has been observed that insect swallows air or water leading to rise in the haemolymph pressure. The pumping of blood usually into the thorax exerts pressure on the old cuticle, because of which it splits along its ecdysial lines.

(g) **Production of procuticle:** New undifferentiated cuticle, sometimes called procuticle, is produced at this stage. Chitin microfibrils are produced on the epidermal cells while protein is released in the spaces between the fibrils. The zone of deposition of new cuticle is called **Schmidt's layer.**

The stage in which the insect possesses freshly formed epicuticle and procuticle as well as the old exo and epicuticle is called **pharate instar.** In other words, it is a transitional stage between apolysis and ecdysis.

(h) **Splitting of old cuticle:** Insect comes out as soon as the old cuticle splits, with head and thorax first, followed by the abdomen and appendages. During this process, insect sheds all the cuticular parts which apart from the integument, includes the lining of foregut, hindgut and major tracheae. The old cuticle shed by the insect is called an **exuviae**.

(i) **Expansion of new cuticle:** Immediately after emergence, the insect has a very soft new cuticle and is thus highly susceptible to the external pressure. Gradually, new cuticle expands, again by swallowing air or water. This results in the opening of the wrinkles and folds in the cuticle. New epicuticle can also be unfolded in response to hormone signals due to which the rigid elements of the cuticle become fixed and cannot be stretched. However, the inter-sclerite membranous regions do not expand due to tight binding of sclerites by accessory muscles. Once cuticle is expanded completely, the blood volume and thus pressure in the body reduces.

(j) **Sclerotization (hardening) of new cuticle:** Now the exocuticle becomes hard due to quinone tanning and sclerotization. During the process, chitin forms cross-links with the cuticle's protein components and causes the hardening of the newly formed cuticle. It may take several hours and probably continues at least until the endocuticle is laid down. The endocuticle in some cases is continually produced resulting in a lamellate structure. Some epidermal cells could continue to produce endocuticle for days or even weeks depending on the type of cuticle and age of insect.

SAQ 2

Answer the following questions in 1-2 sentences:

i). What is the significance of moulting?

ii). Name the different processes of moulting?

SAQ 3

Choose the correct answer from the options given below:

i). Chitin is absent in the following layer of cuticle:

a) Epicuticle only

b) Exocuticle and epicuticle

c) Endocuticle and exocuticle

d) Procuticle and epicuticle

ii). The chitin is constituted by following isomers:

a) Anhydro N-acetyl-D-glucosamine and D-glucosamine

b) D-glucosamine and D-glucose

c) D-glucosamine and N-hydroxyl-glucosamine

d) None of the above

iii). Following layer of cuticle is resistant to moulting fluid:

a) Exocuticle and procuticle

b) Exocuticle and epicuticle

c) Endocuticle

d) Procuticle

iv). Following external intergumentary processes lack basal articulation:

a) Microtrichia

b) Tibial spines

c) Macrotrichia

d) All of these

1.7 METAMORPHOSIS

One of the most defining characteristics of insects is the fact that they hatch in a state that differs from the adult's morphological state. Metamorphosis (*Gr.* Meta- “changes” + morphe- “form”) is a biological process in which insects change from one form to another and finally become adults. These changes are typically most noticeable near the conclusion of postembryonic development and are accompanied by physiological and biochemical changes.

Certain insects hatch from their eggs differently from the imago, primarily due to their immature reproductive organs and external genitalia, as well as minor morphological differences in shape, chaetotaxy (arrangement and nomenclature of bristles on the exoskeleton, important for insect taxonomy), and antennal and cerci segmentation. Apterygotes and secondary apterous exopterygotes, such as Mallophaga, Siphunculata, and female Embioptera, are examples of these insects. Although they are frequently thought to have no metamorphosis, the alterations listed above are typically enough to represent a minor metamorphosis. However, the majority of insects undergo a more significant metamorphosis, and these organisms are divided into two groups called as the hemimetabola and the holometabola.

The majority of exopterygotes are hemimetabolous insects, which undergo a straightforward metamorphosis that is frequently referred to as **direct or incomplete**.

The primary way in which the juvenile stages are different from the adult is that the wings and genitalia are only partially grown. Typically, wing rudiments are not evident during the first instar but later show up as exterior wing-pads that get bigger with each new moult. Compound eyes are virtually always present, the mouthparts have the same overall shape as in adults, and the habits of the young and adults are frequently similar. There is no pupal instar, although the juvenile stages are often called **nymphs.** The degree of metamorphosis which prevails in hemimetabolous insects varies among the different orders.

The Endopterygotes are holometabolous insects that undergo a **complete or indirect** metamorphosis. The life cycle includes a series of larval instars, with subsequent larvae typically having a close resemblance to one another but deviating significantly from the adult. Their eyes hardly ever consist of more than a few simple ocelli. Their mouthparts and feeding habits are typically different from those of an adult, and their wings are minimally represented by internal rudiments that may be submerged in epidermal sacs beneath the general body surface. Following the final larval instar is a more or less quiescent pupal instar, during which the majority of the internal and outward changes necessary for adulthood take place within the cuticle.

Hypermorphosis is the term used to describe the development of an insect that goes through two or more distinctly different larval instars. Usually, this event is accompanied by a noticeable shift in behaviour. When hypermetamorphosis occurs, the first larval instar is a campodeiform. It searches for its future food throughout this stage, and once found, it goes through a series of morphological changes to adjust to its new way of life. This phenomenon can be observed in Sterpsiptera, some endoparasitic dipterans and hymenopterans, Mantispidae (Neuroptera), etc. Table 1.1 depicts various types of metamorphosis in insects.

Table 1.1: Types of metamorphosis in insects with examples.

Type of metamorphosis	Characteristic features	Examples
Ametamorphosis	No visible metamorphosis, minor changes in form during growth, immature resembles the adult ones	Springtails, Silverfish
Gradual metamorphosis/ Hemimetamorphosis	Immatures are called as nymphs, change is gradual, different from the adult in having no wings and undeveloped genitalia, nymphs and adults reside in the same habitat	Grasshopper, Cockroaches
Complete/ Holometamorphosis	Immatures are called as larvae and pupae. Larvae are adapted for feeding only whereas adults are involved in feeding, reproduction and dispersal. Larvae and adults may or may not share the same habitat	Butterfly, Wasp
Hypermetamorphosis	When insect undergoes through two or more distinctly different larval instars. Shift in behavior	Mantispidae (Neuroptera)

SAQ 4

Choose the correct answer from the options given below:

i). Grasshoppers undergo incomplete metamorphosis while beetles undergo complete metamorphosis. Insects with complete metamorphosis are special because only they:

a) Have a resting stage

b) Are able to eat plants

c) Can fly as adults

d) None of these

ii). All of these represent life stages of an insect with incomplete metamorphosis except:

a) Egg b) Nymph

c) Pupa d) Adult

iii). Which of the following correctly lists the life stages of an insect with a complete metamorphosis?

a) Egg, pupa, cocoon, adult

b) Egg, larva, nymph, adult

c) Egg, larva, pupa, adult

d) Egg, nymph, pupa, adult

iv). Which of the following organisms goes through a complete metamorphosis?

a) Orthoptera, Odonata

b) Isoptera, Coleoptera

c) Lepidoptera, Hymenoptera

d) Diptera, Phthiraptera

v). Insects that undergo incomplete metamorphosis are:

a) Grasshoppers and cockroaches

b) Termites and praying mantises

c) Crickets and lice

d) All of the above

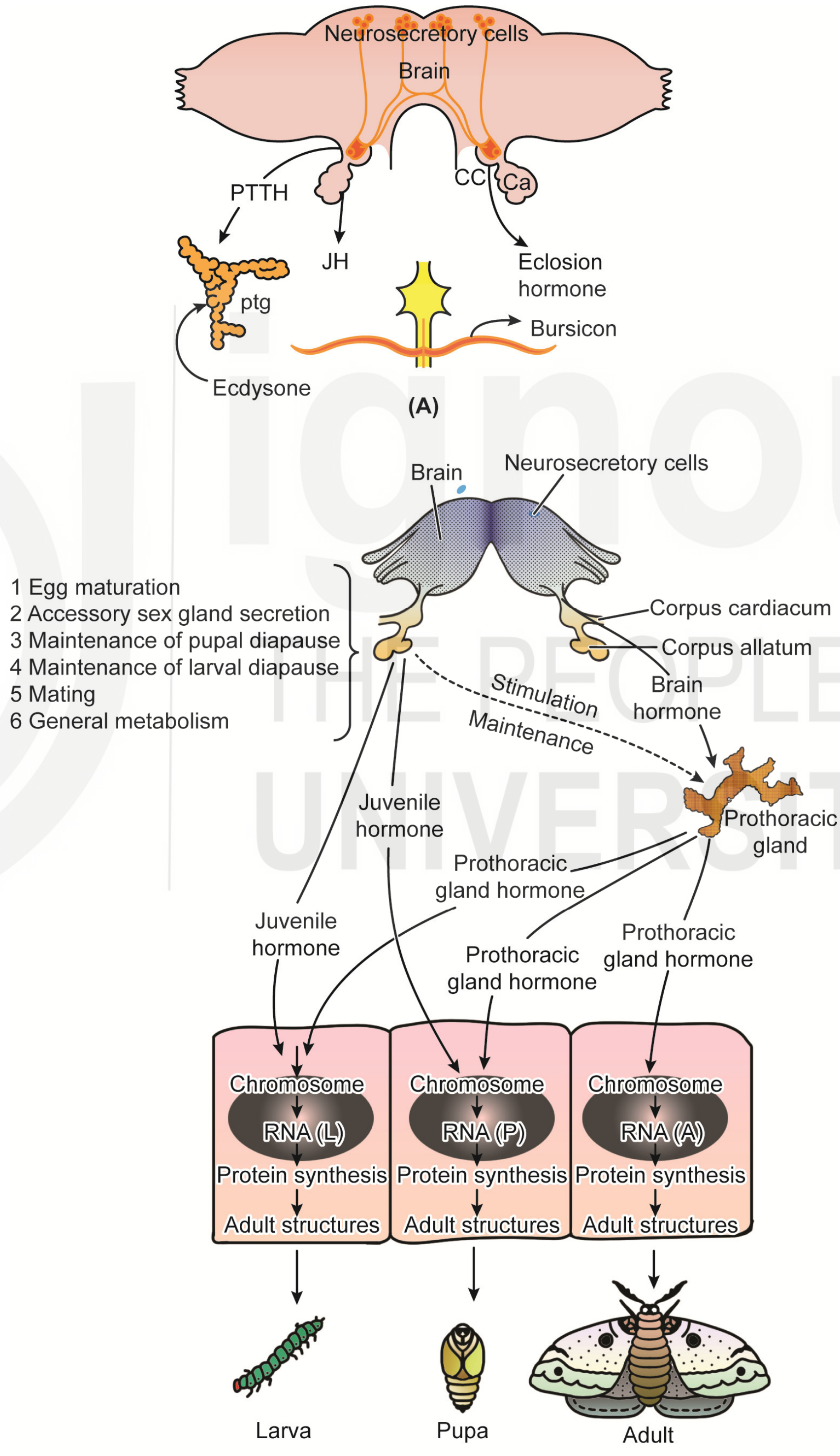
1.8 HORMONAL CONTROL OF MOULTING AND METAMORPHOSIS

Many endocrine glands in insects' anterior parts secrete interacting substances that regulate postembryonic growth and differentiation in both hemi- and homometabolous insects in essentially similar ways. Different hormones regulating the metamorphosis in insects are depicted in Table 1.2 and Figure 1.6.

a) Brain hormone or prothoracicotropic hormone (PTTH): The process of moulting begins when sensory receptors in the body wall recognise that the old exoskeleton has been replaced by internal soft tissues. This signals the well-defined neurosecretory cells i.e, median nerosecteroty cells (MNSC) in the brain to produce a polypeptide hormone, also known as **brain hormone or prothoracicotropic hormone**. This hormone travels to the **corpora cardiaca** (cc) where it is stored and maybe altered before being released into the bloodstream.

b) Ecdysone/moulting hormone: From corpora cardiaca, brain hormone is transferred to the **prothoracic glands** (ptg; an endocrine gland located in the prothorax), and stimulates the glands. The prothoracic glands convert cholesterol to α-ecdysone. Then, insect’s different tissues quickly change the α-ecdysone into 20-hydroxy-ecdysone called moulting hormone. It stimulates the epidermal cells resulting in apolysis and secretion of the new cuticle.

c) **Juvenile hormone:** During the early stages of larval development, juvenile hormone (sometimes referred to as **neotenin**) is secreted by the corpora allatum (ca) a tiny gland located behind the brain. A larval cuticle is laid down by moulting epidermal cells as long as this hormone is present in the blood and the development proceeds through the typical sequence of larval or nymphal forms. The corpora allata momentarily stops producing juvenile hormone in the last instar nymph or larva, and development is focused on development of the adult. Therefore, juvenile hormone functions as a modifying agent that promotes larval structural development and inhibits adult differentiation.



(B)

Fig. 1.6: A) Insect brain and the endocrine glands; B) Principal endocrine tissues in the silkworm moth that regulate the metamorphosis

d) **Eclosion hormone:** Eclosion hormone is a neuropeptide and has been observed in several moths. It is secreted in the MNSC of the brain. Photoperiod is believed to regulate its release. This hormone influences several aspects of pupal-adult ecdysis (eclosion), including the behaviour associated with ecdysis. Subsequent degeneration of the abdominal intersegmental muscles takes place which are used in the act of ecdysis.

e) **Bursicon or tanning hormone:** Bursicon or tanning hormone is a neurosecretory hormone secreted and/or released from a variety of tissues according to the insect species. It is commonly found in neurohaemal organs (similar to the corpora cardiaca) related with the ventral chain ganglia. **Bursicon stimulates tanning and selerotisation of the cuticle following ecdysis.**

Table 1.2.: Hormonal control of metamorphosis in insects.

Glands	Secretions
Neurosecretory cells in the brain	Brain hormone or prothoracicotropic hormone
Corpora cardiaca	Stores and releases brain hormone
Prothoracic glands	Ecdysone/ moulting hormone
Corpora allata	Juvenile hormone

SAQ 5

Answer the following questions in 1-2 sentences

i). Enumerate the types of metamorphosis in insects.

ii). What is the function of corpora cardiaca?

iii). JH and moulting hormone are secreted by which glands?

SAQ 6

Choose the correct answer from the options given below:

i). Prothoracicotropic hormone is secreted by:

a) Neurosecretory cells of brain

b) Corpus allatum

c) Corpus cardiacum

d) Thoracic glands

ii). Juvenile hormone acts upon the following:

a) Follicle cells

b) Fat body

c) Accessory reproductive gland

d) All of these

iii). Sclerotisation and melanisation of cuticle in insects are under the control of the following hormone:

a) Ecdysone b) Bursicon

c) Allatostatin d) Allatotropin

iv). Following endocrine glands are involved in the control of growth and metamorphosis in insects:

a) The median neurosecretory cells

b) The corpora cardiaca and corpora allata

c) The prothoracic glands

d) All of the above

1.9 SUMMARY

Let us summarise what you have learnt so far:

- Insect integument is the outermost coating that covers the entire body of the insect. It is ectodermal in origin.
- Insect body walls are composed of three layers: cuticle, epidermis, basement membrane.
- Cuticle is a complex, non-cellular layer that is secreted by the epidermis. It forms the outermost covering of whole body and its appendages.
- The cuticle can be divided into two layers: the inner procuticle and the outer epicuticle.
- Epicuticle is a very thin layer with a complicated chemical composition and ultrastructure, and it is devoid of chitin.
- The epicuticle consists of cement layer, wax/ lipid layer, polyphenol layer and cuticulin layer.
- Procuticle is secreted by the epidermal cells. It is made up of mainly chitin and proteins. Procuticle is divided into exocuticle and endocuticle.
- Pore canals are several tiny, vertical channels that cross both the exo and endocuticle. They are helpful in transporting enzymes and cuticular material to the procuticle and epicuticle sections.

- The two main components of an insect's cuticle are a variety of proteins and the carbohydrate chitin, which makes up 25–60% of the dry weight of different cuticles.
- Epidermis is the second layer of integument. It is a unicellular continuous layer made up of polygonal cells that change during the moulting process to become cuboidal or columnar. It is glandular in nature and releases cuticle as well as the enzymes needed for the generation and breakdown of old cuticle during moulting.
- Based on the functions they carry out; the epidermal cells differentiate into dermal glands, trichogen cells, moulting glands and peristigmatic glands.
- Basement membrane is a non-living, amorphous (shapeless), continuous, granular layer of integument that forms at the base of the body wall from degenerating epidermal cells.
- Cuticle/Integument is modified into internal invaginations or external outgrowths as cuticular appendages and cuticular processes.
- Cuticular structures comprise all cuticle outgrowths that are joined to it by a membranous joint. Setae and spurs are two types of cuticular appendages. They originate from modified epidermal cells.
- Setae or macrotrichia are hair-like hollow structures, arise from cup-shaped pits, or alveolus-like cup. The setae can be in the form of clothing hairs, bristles, scales, glandular setae and sensory setae.
- Spur are present on insect's legs. They are different from setae in being multicellular in origin.
- The cuticle's exterior is not only sculpted in different ways, but it also has a wide range of cuticular processes that are essential for its structure. They can easily be differentiated from cuticular appendages because they are firmly attached to the cuticle and lack a membranous articulation. Microtrichia and spines are two categories of cuticular processes.
- Microtrichia also known as fixed hairs or aculei, are tiny structures that resemble hair and are present on some Diptera. Their unique characteristic is the lack of a basal articulation, which gives them the appearance of extremely tiny covering hairs.
- Spines are cuticular outgrowths that resemble thorns in some way. The production of spines by undifferentiated epidermal cells and their typically, if not always, multicellular origins set them apart from spine-like setae.
- Moulting is the process of creating new cuticles beneath old ones before they shed the old cuticle. It is divided into 3 sub-processes: Apolysis, Ecdysis and Sclerotization.
- Apolysis is the separation of old cuticle from the epidermis; ecdysis is the process of shedding the old cuticle while sclerotization is the process by which the newly formed, soft, milky white cuticle turns dark and rigid after the old cuticle sheds.

- Metamorphosis (*Gr.* Meta- "changes" + morphe- "form") is a biological process in which insects change from one form to another to eventually form an adult.
- The metamorphosis can be categorized into ametamorphosis, gradual/hemimetamorphosis, complete/holometamorphosis and hypermetamorphosis.
- Metamorphosis and moulting process are under the control of hormones.
- Brain hormone is secreted by the neurosecretory cells and is also called as prothoracicotropic hormone. This hormone is stored in corpora cardiaca.
- Brain hormone stimulates prothoracic gland to secrete ecdysone/moulting hormone which impels metamorphosis to occur.Juvenile hormone is secreted by corpora allata and keeps the insect in its juvenile/immature state.
- Additionally, two other neurosecretory hormones namely bursicon and eclosion, regulate specific processes of development. Bursicon is involved in tanning the cuticle of newly emerged insects whereas eclosion hormone sets off a series of actions that include spreading the wings, ecdysial behaviour, abdominal motions, and bursicon secretion.

1.10 TERMINAL QUESTIONS

1. Discuss the structure of integument of an insect in detail. Give a well labelled diagram of the same.
2. What do you understand by cuticular modifications? Describe it with suitable examples.
3. Write about the importance of integument in insects.
4. Define moulting. How and why does it occur in insects?
5. What is metamorphosis? Discuss its types with suitable examples.
6. Name the hormones controlling moulting and metamorphosis. Describe the hormonal control of moulting and metamorphosis in detail.

1.11 ANSWERS

Self-Assessment Questions

1. i) Cuticle, epidermis and basement membrane.

   ii) Protein and chitin (carbohydrate).

   iii) These help in transporting enzymes and cuticular material to the outer procuticle and epicuticle.

   iv) Exocuticle and endocuticle.

2. i) Moulting helps insects to grow after replacing the old cuticle with new one.

   ii) Apolysis, ecdysis and sclerotization.

3. iii) a) Epicuticle only

   iv) a) Anhydro N-acetyl-D-glucosamine and D-glucosamine

   v) b) Exocuticle and epicuticle

   vi) a) Exocuticle and procuticle

4. i) a) Have a resting stage

   ii) c) Pupa

   iii) c) Egg, larva, pupa, adult

   iv) c) Lepidoptera, Hymenoptera

   v) d) All of the above

5. i) Ametamorphosis, gradual/hemimetamorphosis, complete/ holometamorphosis and hypermetamorphosis.

   ii) To store and release brain hormone.

   iii) Corpora allata and prothoracic gland respectively.

6. i) a) Neurosecretory cells of brain

   ii) d) All of these

   iii) b) Bursicon

   iv) d) All of the above

Terminal Questions

1. Refer to Section 1.3.
2. Refer to Section 1.4.
3. Refer to Section 1.5.
4. Refer to Section 1.6.
5. Refer to Section 1.7.
6. Refer to Section 1.8.

SUGGESTED READING

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UNIT 2

DIGESTIVE SYSTEM AND EXCRETORY SYSTEM

Structure

2.1 INTRODUCTION

Insects are the most diverse groups of animals on the Earth. They have developed several adaptions to live in various environmental conditions efficiently. Among several adaptations, their ability to utilize diverse food resource has made them one of the most successful life forms to survive in adverse conditions on earth. They have modified their organs of ingestion to ingest and process food in various ways. Apart from this, they have developed mechanisms to protect their gut from various toxins and microorganisms ingested with food.

Along with, another system, excretory system helps in elimination of toxins, nitrogenous and other unwanted compounds from the body. It plays an important role in homeostasis in the body by maintaining levels of salt, water and osmotic pressure in the haemolymph. Accordingly, insects have developed various excretory organs to live a successful life in different habitats.

This Unit will comprehensively discuss the structure and functions of various digestive and excretory organs of insects to understand their diverse and successful living on this Earth.

Objectives

After studying this Unit, you will be able to:

- ❖ describe the organs for the digestion of insects and their modifications;
- ❖ explain the structure of salivary glands and the role of saliva in insects;
- ❖ discuss, how the various components of the food are digested with the help of digestive enzymes, their absorption, and expulsion of undigested food;
- ❖ explain the structures and functions of malpighian tubules and nephrocytes;
- ❖ discuss formation of primary urine to maintain homeostasis; and
- ❖ explain osmoregulation and adaptations in terrestrial, freshwater, and saltwater insects.

2.2 DIGESTIVE SYSTEM OF INSECTS

Insects feed on a wide range of food such as plants, seeds, wood, plant sap, blood, and even dead and decaying matter. They ingest food with the help of different mouth parts which then passes through a long and coiled alimentary canal where ingested food is digested, assimilated and absorbed in the body for obtaining energy. The energy produced is utilized for growth, development and cell repairs.

The alimentary canal of insects runs lengthwise, from mouth to anus, with differentiation and evaginations at places. The food enters through the mouth placed anteriorly and the undigested food is eliminated out through the anus located at the posterior end of the body (Fig. 2.1).

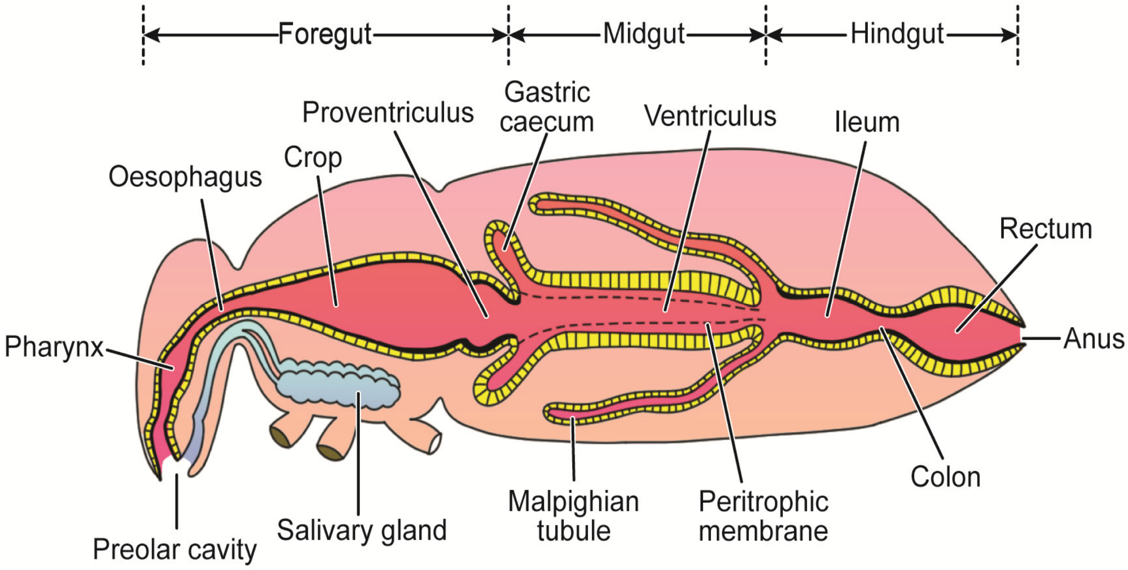

Fig. 2.1: Generalized insect alimentary canal. The cuticular lining of the foregut and hindgut are indicated by thicker black lines

2.3 GENERAL ORGANIZATION OF ALIMENTARY CANAL

The alimentary tract of insects is divided into three regions - foregut or stomodeum, midgut or mesenteron, and hindgut or proctodeum. The foregut and hindgut are invaginations of the ectoderm, and thus they are lined by the cuticle or intima which is in continuation with the cuticle of the integument and is shed at each moult. The midgut, on the other hand, has endodermal lining and is not lined by the chitin.

The mouth is the anterior most part of the alimentary canal equipped with mouth parts which include labrum, maxillae, mandibles, labium, and a median hypopharynx. The structure and function of these mouth parts differ in different insects based on their feeding habits. Mouth parts form a small mouth or preoral cavity which is also sometimes called the **cibraium.**

2.3.1 Foregut

It is the anterior most region of the alimentary canal with flattened and unsclerotized epithelial cells, and unsclerotized cuticle. It may have spines on its surface for backward movement of the food to the midgut. A layer of longitudinal and a layer of circular muscles lie above the epithelial layer above which there is a delicate sheath of connective tissue.

The foregut is differentiated in the four regions; pharynx, oesophagus, crop and proventriculus. Initial part of the foregut is known as **buccal cavity** which leads to the **pharynx.** It has well developed musculature. The cibarial muscles are located between the head capsule and the anterior wall of the pharynx. The pharynx also has muscles called the pharyngeal muscles. The cibarial and pharyngeal muscles are well-developed in fluid-feeding insects. The contraction of these muscles enlarges the volume of the pharynx. In other insects, the pharynx is the organ for ingestion and allows the passage of food.

The pharynx opens into a tubular **oesophagus**. This is well developed in holometabolous insects in comparison to the hemimetabolous insects. The food moves down by the peristaltic movement of the muscles and reaches the crop.

The **crop** is the dilated distal part of the oesophagus. In some insects such as in adult dipterans and lepidopterans, crop is the lateral diverticulum of the oesophagus (Fig. 2.2). The crop serves as a storage organ. It has a folded wall which increases its surface area. As it receives the food and gets filled, the folds get flattened to accommodate more amount of food. In a few insects, some digestion may occur in this region due to salivary enzymes or regurgitation of the midgut enzymes, for example in *Periplaneta*. In locusts, the crop is expandable because of the presence of stretch receptors that get stimulated by the hormone released from the corpora cardiaca when the food is in abundance.

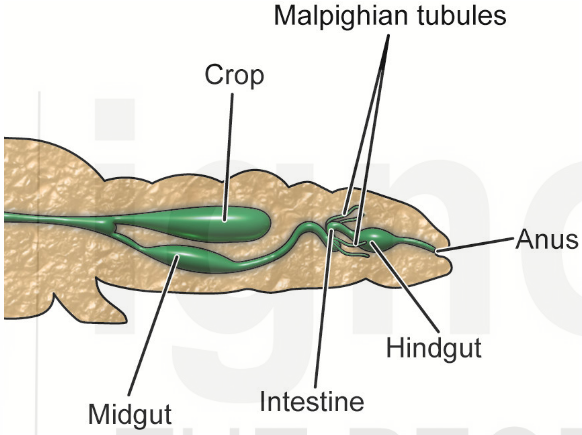

Fig. 2.2: Crop as a lateral diverticulum in butterfly

The posterior portion of the crop is the **gizzard or proventriculus,** the structure of which varies in different insects. In cockroaches, it bears tooth-like structures to grind the food particles. In honey bees, the gizzard is plate-like for separating pollen from nectar. The nectar is retained in the gizzard for regurgitation and processing for honey, while the pollen forms the bolus and moves to the midgut. In fleas, the gizzard has the spine to break the blood corpuscles as they feed on the blood of mammals. Usually, the posterior part of gizzard has a valve, called **stomodeal valve,** which projects into the lumen of midgut to regulate downward movement of the food.

2.3.2 Midgut

The midgut, also called **mesenteron**, is the site for enzymatic digestion of food and absorption of nutrients. Near its anterior end, thin finger-like projections called the **gastric caecae** arise which are usually 2-10 in number. They increase surface area for the secretion of enzymes and absorption of water from the alimentary canal. Rest of the mesenteron is called as **ventriculus,** where digestion and absorption of nutrients take place. The posterior part of the midgut comprises a **pyloric valve,** which controls the passage of food into hindgut.

The midgut epithelium has varied kinds of cells in different insects. Majority of the cells are tall and columnar with microvilli at the luminal end to raise the surface area. These are known as **enterocytes** which secrete enzymes and absorb nutrients. The epithelium also has hormone-secreting **enteroendocrine cells**, which regulate gut functions. In lepidopterans, specialized **goblet cells** are present which facilitate in the alkalinization of the gut.

Peritrophic Membrane

The midgut in majority of insects is lined by a **peritrophic membrane** secreted by the columnar epithelial cells. It is composed of chitin, sugars, and proteins. In locusts, the peritrophic membrane is multilaminar, while it is absent in some hemipterans, adult lepidopterans, ants, and fluid feeders.

The peritrophic membrane can be categorized into two types based on its production. Type I membrane is produced by all the cells of the midgut and is thus present through the length, for example in Coleoptera, Odonata, Orthoptera, and Hymenoptera. Type II membrane is secreted by the certain specialized anterior midgut cells, called **cardia**, as in Isoptera and Dermaptera.

The peritrophic membrane acts as a physical barrier between the gut contents and the midgut epithelium, and protects the delicate digestive cells from the luminal content which might contain abrasive particles, toxins, pathogens, etc. In some phytophagous insects that feed on plants, the membrane acts as a barrier against allelochemicals such as tannins, which are a major component of plants. In addition, the peritrophic membrane separates the midgut lumen into two regions; **ecto-peritrophic space** in the gut lumen and **endo-peritrophic space,** the region between the peritrophic membrane and the midgut cells (Fig. 2.3). The digestive enzymes are secreted into the ecto-peritrophic space from the midgut epithelial cells, which then diffuse into the endo-peritrophic space to carry out the process of digestion. Similarly, the digested food diffuses back to the ecto-peritrophic space to be absorbed by the midgut epithelial cells.

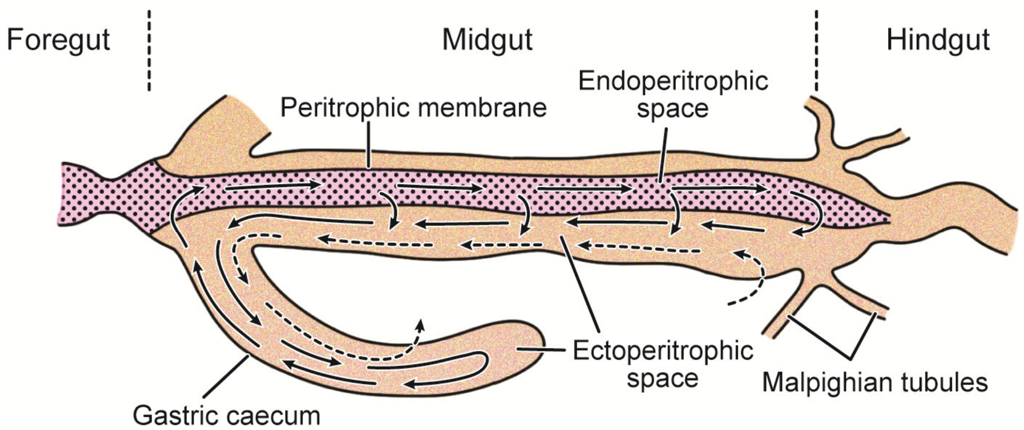

Fig. 2.3: Generalized scheme of endo-ectotrophic circulation of digestive enzymes in the midgut

Filter chamber

In some of the hemipterans, such as aphids, leafhoppers, planthoppers, and cicadas, part of the foregut, midgut, and hindgut are associated together and form a loop. As these insects are fluid feeders, the excess of water and soluble carbohydrates reach the intestine directly while protein and lipids are retained in the stomach for digestion. The excess of water moves out of the hindgut and is excreted as honeydew. In leaf hoppers and aphids, the rapid removal of water to the rectum is achieved by the anterior midgut forming a large thin-walled bladder which is closely bound to anterior hindgut and Malpighian tubules by its own basement membrane. The chamber formed within this fold is called the filter chamber (Fig. 2.4). Water passes directly from the midgut to the hindgut along an osmotic gradient and there may be no significant flow of fluid through the lumen of the gut.

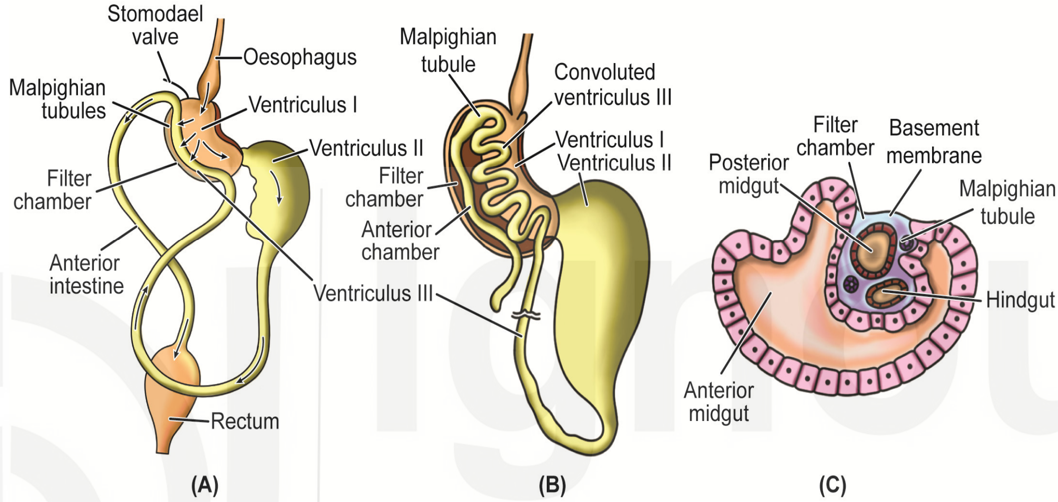

Fig. 2.4: Filter chamber in fluid-feeding insects

2.3.4 Hindgut

The hindgut is the last portion of the alimentary canal. Just like the foregut the hindgut is lined internally by the cuticle and, is shed and replaced when the insect moults. The intima of the hindgut is thin and more permeable as compared to that of the foregut. The hindgut is subdivided into three parts: pylorus, ileum and rectum.

Pylorus is the first portion of the hindgut, in which the food enters from the midgut, flow of which is under the regulation of pyloric valve. The pyloric valve also prevents the backward flow of the digested food. At the joining point of the midgut and hindgut, arise long, spaghetti-like tubules, the **Malpighian tubules**, the organs for excretion. The Malpighian tubules vary in number in insects and can be from dozen to hundreds in number, extending in the abdominal cavity. The secretions from the tubules are released into the pylorus region. When the pyloric sphincter is closed, the hindgut receives contents only from the Malpighian tubules.

Ileum is an undifferentiated and narrow tubular structure. The distal part of the ileum sometimes looks different and known as **colon.** The apical plasma membrane is folded increasing the surface area. In some insects such as termites that feed on wood containing cellulose, the ileum bears a paunch that lodges microorganisms concerned with the digestion of cellulose.

The **rectum** is an enlarged sac that is lined with thin epithelium except the area containing **rectal papillae** (pads) projecting into the lumen. The rectal papillae absorb water, salts, and amino acids from the food coming from the midgut. In some aquatic insects, the rectum has tracheal gills which act as the site of gaseous exchange. The base of the gills has chloride cells that take in chloride and other inorganic ions from the water. Rectum terminates into the anus from where the undigested food is expelled.

Functions of the hindgut include the following:

i) water absorption from urine and faeces,

ii) ion absorption from urine and faeces,

iii) cryptonephridial system for water conservation,

iv) pheromone production,

v) respiration in larval dragonflies, and

vi) modifications in structure for housing symbiotic microorganisms (e.g., termites).

2.4 SALIVARY GLANDS

The salivary glands are associated with labial segments and are therefore also known as the labial glands. These are paired and ventral to the foregut extending up to the abdomen. Saliva secreted by the salivary gland is a clear fluid. The salivary glands in most of the insects are of acinous type (acinar), while some such as lepidopterans and dipterans, have tubular type of glands.

Although there may be glands associated with the mandibles (e.g., silver fishes, termites, queen honey bee), maxillae (e.g., proturans, spring-tails), and hypopharynx (e.g., worker honey bees), salivary glands are typically associated with the labial segment. The salivary glands or labial glands (Fig. 2.5) are paired structure, lie ventral to the foregut in the head and thorax and occasionally extend posteriorly into the abdomen. Depending on the type of food eaten and the insect species involved salivary glands vary in size, shape, and the type of secretion produced. Two basic types of salivary glands exist, acinar and tubular. Orthoptera and Dictyoptera have the acinar type while Diptera, Lepidoptera, and Hymenoptera have the tubular type. In the acinar type, each acinus, bears a tiny duct that communicates with other similar ducts, eventually forming a lateral salivary duct. Lateral salivary ducts run anteriorly and merge as the common salivary duct, which empties between the base of the hypopharynx and the base of the labium. This region is called the **salivarium** and in some sucking insects forms a salivary syringe that 'injects' saliva into whatever is being pierced. The lateral salivary ducts may communicate with salivary reservoirs, as in the cockroaches. The secretory products of the salivary glands are generally clear fluids that serve a variety of functions in different insects: (i) they moisten the mouthparts and serve as a lubricant, (ii) they act as a food solvent, (iii) they serve as a medium for digestive enzymes and various anticoagulins and agglutinins, (iv) they secrete silk in larval Lepidoptera (caterpillars) and Hymenoptera (bees, wasps, and relatives), (v) they are used to 'glue' puparial cases to the substrate in certain flies, (vi) they serve for the production of toxins, and (vii) they secrete antimicrobial factors (e.g., in certain blow fly larvae).

Amylase and invertase are the most common enzymes found in saliva of insects, however, the saliva may also contain lipase and protease. Aphids secrete a pectinase that aids their mouthparts in the penetration of plant tissues. The spreading factor, hyaluronidase, which attacks a constituent of the intercellular matrix of many animals, has been found in the assassin bug.

Blood-sucking (haematophagous) insects contain various antihaemostatic (anticoagulant) agents. Current evidence, at least for mosquitoes, is that various salivary components mainly increase the chances of the female locating a blood vessel.

Production and secretion of saliva in the dragonflies, grasshoppers, and cockroaches are regulated by nervous innervation from both the stomatogastric nervous system and the subesophageal ganglion, whereas in the Diptera (e.g., the adult blow fly) these glands are controlled by an unidentified neurohormone. Salivation has been shown to be controlled by phagostimulation of external chemoreceptors on the mouthparts. This same stimulus probably also activates the salivary pump.

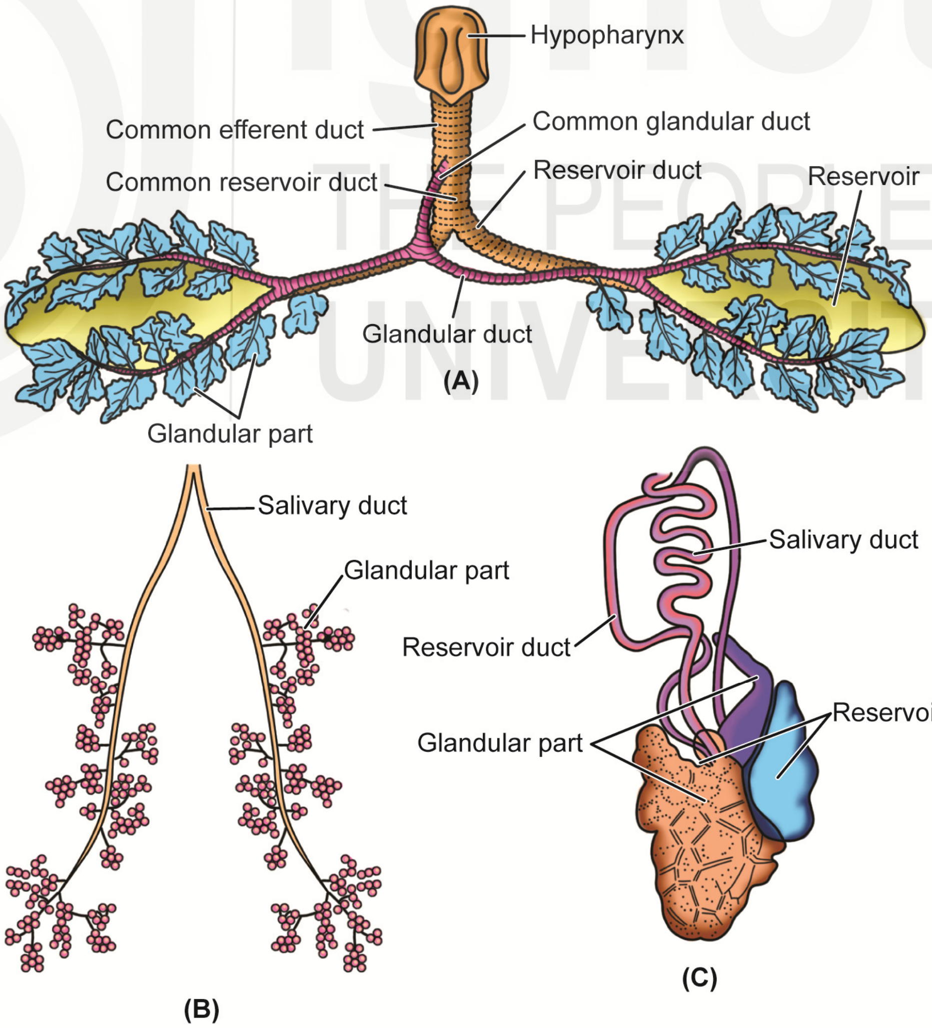

Fig. 2.5: A) Salivary glands of cockroach, B) Grasshopper and C) Red cotton bug

Functions of saliva

Saliva is a clear fluid with various functions in different insects.

- Saliva acts as a lubricant moistening the mouthparts and the food ingested by the insects.
- Saliva can also help in digestion as in some insects it has digestive enzymes. For example,
  (a) In *Acromyremex*, the leaf-cutting ant, saliva has chitinase to dissolve the chitin wall of the fungus on which these ants feed.
  (b) The saliva of cockroaches has amylase for digestion of starch.
  (c) In honeybees, saliva has invertase for digestion of sucrose.
- The saliva of blood-sucking insects such as mosquitoes, and bed bugs has anticoagulants and agglutinins which help in free flow of blood for sucking.
- Saliva of caterpillars of Lepidoptera secretes silk to spin a cocoon around.
- In hymenopterans and some bugs, the saliva produces toxins.

SAQ 1

i). Fill in the blanks:

a) The other name for foregut is ....

b) .... is the dilated distal part of the oesophagus.

c) The junction between the foregut and midgut bears thin finger-like projections ...., ...

d) The ...., ... is the lining of the midgut.

ii). State whether the given statement is 'True' or 'False'.

a) The midgut is ectodermal in origin.

b) Labial gland is the other name for the salivary gland.

c) Liquid-feeding insects have filter chambers.

d) Gastric caecae are present between the junction of the midgut and hindgut.

2.5 DIGESTION OF FOOD

Digestion of the food consumed is the process of chemical transformation of larger or macromolecules such as carbohydrates, proteins, and lipids into smaller molecules that can be absorbed through the gut wall in the presence of digestive enzymes. In insects, digestion is usually of two types.

2.5.1 Extra-intestinal Digestion

As the name suggests, the food is converted to simpler form before it is ingested. The digestive enzymes are regurgitated from the mouth onto the food before they are ingested. It primarily takes place in the fluid feeders that secrete saliva onto the food. For example, in house flies and blowflies, the digestive fluid is regurgitated onto the solid food to dissolve it, and then the liquid form of food is taken in with their sponging mouthparts. Similarly carrion beetles that feed on decaying matter first release the digestive enzymes to break down the food into simpler form and then ingest it.

2.5.2 Intestinal Digestion

Usually, in most insects, the digestion of the food occurs after it is ingested. The process of digestion involves digestive enzymes and various organs of the alimentary canal. The kind of enzymes released depends on the type of food the insect consumes. For example, insects feeding on carbohydrate-rich diet (seeds, and stored products) have more amylase enzyme while those feeding on animal products such as flesh, blood, and wool have more of proteases.

Majority of digestion takes place in the midgut though digestion can also take place in the foregut due to enzyme regurgitation. Saliva secreted by the salivary glands also plays a role in digestion inside the buccal cavity. In some, partial digestion can occur in the crop.

Factors affecting digestion of food

a) **Temperature:** The enzymatic activity rises with increase in temperature. The maximum activity takes place in the range of 35-45°C above which the enzyme gradually denatures. The activity of the digestive enzymes is negligible at temperatures lower than zero degrees.

b) **pH:** It varies along the length of the gut and ranges from 5.8 to 7.3 in the midgut. There are some exceptions such as the midgut of lepidopteran larvae having a pH of 8-10 while the pH is acidic around 3-4 in dipterans as it helps in killing the bacteria present in the diet. The enzymatic action, thus depends upon the midgut pH and is maximum within a specific range of pH, generally 6-7.

> The hindgut is slightly acidic compared to the midgut due to the presence of products from the Malpighian tubules.

c) **Redox potential:** Redox potential is defined as the tendency of a species to gain or lose electrons. In most of the insects, the gut lumen has positive redox potential. The redox potential is negative for insects that have microbes in the water or undergo fermentation. It plays an important role in biological processes as it regulates enzyme activities, removal of toxicants, supports beneficial microbes, and facilitates nutrient absorption.

Digestion of carbohydrates

The carbohydrates can be ingested by insects in the form of monosaccharides, disaccharides, and polysaccharides. All the complex carbohydrates are converted to monosaccharides; glucose or fructose; to be absorbed by the gut walls.

The common disaccharides in insect food are maltose, sucrose, trehalose and lactose; while polysaccharides include starch and cellulose in the case of plant feeders, and glycogen and chitinin in case of insects feeding on animal products.

Some of the enzymes involved in the digestion of carbohydrates are as follows.

- Amylase hydrolyses the 1,4-α-glucosidic linkages in starch to form maltose, and in glycogen resulting in the formation of glucose.
- Maltase converts maltose to glucose and invertase or sucrose digests sucrose into glucose and fructose.
- Trehalase breaks down trehalose into glucose.
- Lactose is broken down into galactose and glucose by lactase.

Cellulase is abundant in plant-feeding insects as cellulose is the major component of plant cell walls. Some insects such as termites harbor cellulose-digesting microbes in the hindgut.

> Herbivorous insects have high levels of amylase and cellulase enzymes as compared to the carnivorous insects.

Digestion of Proteins

The proteins are hydrolysed to polypeptides and peptones in the midgut with the help of trypsin-like proteases. The peptide bonds in these compounds are then broken down by endopeptidases and exopeptidases. The endopeptidases act within the protein molecules, while the exopeptidases act at the end of the chain. The carboxypeptidase acts on the -COOH end and aminopeptidases act at the chain from the -NH_2 end.

Digestion of Lipids

Lipids are hydrolysed to fatty acids and glycerol by the enzyme lipases. The digestion of lipids occurs in the proximal end of the midgut. Some insects such as wax moths have the enzyme lecithinase for the digestion of beeswax.

SAQ 2

i). Fill in the blanks:

a) All the digestion and .... takes place in the midgut.

b) Cellulase is the enzyme for digestion of ....

c) For the digestion of food range for enzymatic activity is .... to 45 degrees centigrade.

d) .... are enzymes for digestion of lipids.

ii). Match the following:

Column A		Column B	
a)	Amylase	I)	Lepidoptera larvae
b)	pH-8	II)	Before ingestion of food
c)	Extra-intestinal digestion	III)	Carbohydrates

2.6 ABSORPTION OF THE DIGESTED FOOD

Absorption of the digested food majorly takes place in the midgut and is transcellular. The food is absorbed from the epithelial gut cells and transported to the hemocoel. The products of the extra-oral cavity and the midgut digestion are absorbed through the enterocytes of the midgut, while water and inorganic ions are absorbed in the hindgut. The presence of microvilli raises the absorptive surface area, though differential membrane permeability of gut regions may affect the absorption.

2.6.1 Absorption of Carbohydrates

The carbohydrates are absorbed as monosaccharides across the midgut epithelium and transported to haemocoel. The absorption of simple sugars is often facilitated by some transporters, which vary in different insects. The conversion of glucose into trehalose also promotes absorption of sugars. In insects feeding on sugar-rich saps, such as aphids, sugars are absorbed by simple diffusion along the concentration gradient.

2.6.2 Absorption of Proteins

Digestion of proteins forms amino acids in the midgut. Some of the amino acids are reabsorbed in the hindgut. Many amino acids are absorbed into the haemolymph against the concentration gradient leading to the occurrence of large repository of free amino acids in the haemolymph.

2.6.3 Absorption of Lipids

The lipids are digested into free fatty acids, glycerol, acylglycerols and lysophospholipids. These are primarily absorbed by midgut caecae while in some insects such as hymenopterans, lipids are absorbed in the hindgut and in cockroaches, lipids are absorbed in the foregut. Wax is absorbed in phosphorylated form and cholesterol is esterized before absorption.

Emulsification of fats promotes their absorption which takes place by the formation of fatty acid-amino acid or fatty acid-glycolipid complexes. The formation of micelles of fatty acids with lysophospholipids also facilitates diffusion.

2.6.4 Absorption of Inorganic Ions

Ions from the digested food are absorbed from the midgut as well as the hindgut. Sodium and calcium ions move to the hemolymph from the gut lumen against the electrochemical gradient. Energy for such movements is provided by V-ATPase pumps in the anterior caeca and rectal cells. Potassium moves passively into the haemocoel. The chloride ions are actively removed from the rectal apical membrane cells from where they pass into the haemocoel passively.

2.6.5 Absorption of Water

Water is absorbed from the midgut as well as the hindgut. In the midgut, water is absorbed from the food, while in the hindgut water is absorbed from the faeces in the rectum. The water absorbed from the midgut enhances the efficiency of digestion and maintains the concentration gradient for the absorption of nutrients through the gut wall. The absorption of water from the gut lumen to the hemolymph depends on the sodium and potassium ions in the extracellular space between the epithelial cells. This creates a hydrostatic pressure which water is transported to the haemolymph using energy.

The absorption of water in the midgut varies in different insects. For example in cockroaches and grasshoppers, absorption of water occurs in the midgut ceca, while in blood-sucking insects it is absorbed from the anterior region of the midgut.

Rectal pads also play an important role in the reabsorption and conservation of water in terrestrial insects. The rectal pads are lined with cells that actively transport ions and water as the cells create a concentration gradient for the movement of water from the waste back into the insect body. Rest of the undigested waste is egested through the anus.

2.7 REGULATION OF THE ALIMENTARY SYSTEM

Regulation of the alimentary system in insects involves control of food movement, control of enzyme secretion, and control of absorption. The alimentary canal is regulated in part through the action of the stomatogastric nervous system. Food is ingested by the actions of the mouthparts, cibarium, and pharynx, and is typically stored in crop. It is then released gradually, via the stomodaeal valve, into the midgut, where digestion and absorption occur. In most insects that have been studied, stretch receptors associated with the crop provide information to the brain (via the frontal ganglion) regarding crop distension and help prevent overfilling of this organ. In some insects, stretch receptors in the abdominal wall have a similar role.

The destination of ingested food may vary with the kind of food. For example, in female mosquitoes sugar meals (flower nectar) are directed to the diverticula and blood meals are directed to the midgut. Sensilia in the roof of the cibarial pump, acting via the frontal ganglion, are thought to be involved in the so-called ‘switch mechanism’. In other blood-feeding insects, such as tsetse flies, ingested blood goes to the crop first.

Control of passage of food from the crop to the midgut (rate of crop emptying) has been studied mainly in the cockroach, *Periplaneta americana.* Passage of food from the cockroach crop is inversely related to the osmotic pressure of the food, i.e., the higher the concentration of food, slower the passage. Osmotic receptors have been identified in the wall of the cockroach pharynx.

Two mechanisms for the control of enzyme secretion in the insect gut have been suggested: **Secretogogue** (a substance in the ingested material may stimulate enzyme secretion) and **hormonal**. The secretogogue control is an immediate response to food, whereas hormonal control is more related to developmental and environmental effects. Nervous control is highly unlikely because the midgut is sparsely innervated or not at all.

Absorption appears to be controlled by the availability of absorbable molecules, release of food material from the crop being so regulated that digestion and subsequent absorption occur at an optimal rate for a given circumstance.

Many insects ingest foods with very high water content. Some of these insects (e.g., butterflies and many true flies) store the dilute food in the impermeable crop and pass it gradually to the midgut. In others (e.g., many blood-feeding insects) food may go to the midgut where excess water is rapidly absorbed in the haemolymph and removal of water concentrates solid food, increasing the efficiency of digestion.

Movements of the alimentary canal (mainly foregut and hindgut) that complement the actions of the digestive enzymes and help absorption are under neural or neurosecretory control in some insects. In others, having no neural connections, gut movements are assumed to be myogenic. Hormonal stimuli may also have a great deal to do with the rate of gut movements.

SAQ 3

i). Which of the following statement is correct?

a) Extra-intestinal digestion takes place in house fly

b) Digestion does not take place in stomodeum at all

c) Digestion takes place only in mesenteron

d) Malpighain tubules are attached to the proctodeum

ii). Proventriculus is well developed among:

a) Bees b) Termites

c) Ants d) Mosquitoes

iii). In locust, the major proportion of digestion takes place in:

a) Cibarium b) Crop

c) Proventriculus d) Midgut

iv). Digested food materials are mainly absorbed in:

a) Crop b) Proventriculus

c) Mesenteron d) Proctodeum

v). Extra-intestinal digestion takes place in:

a) Mosquitoes b) Butterfly

c) Cockroaches d) House fly

2.8 EXCRETORY SYSTEM IN INSECTS

Excretion is a significant process of removal of potentially toxic materials from the body. This process takes place in two steps;

a). Indiscriminate removal of substances from the hemolymph, forming the primary urine, and

b). The reabsorption of useful compounds from the urine or excretion of excess and unwanted compounds in the body. The excretion takes place with the help of certain organs comprising the Malpighian tubules, ileum and rectum; the primary organ is Malpighian tubules. We will discuss about different excretory organs in the following subsections:

2.8.1 Malpighian Tubules

The Malpighian tubules were discovered by Marcello Malpighi. These tubules are long, thin, blindly ending and freely lying tubes that arise at the junction of the midgut and hindgut and lie in the haemocoel. The tubules are derived from the hindgut ectoderm but are not lined with cuticle. These are found in almost all insects except in collembola and aphids while are rudimentary in strepsipterans.

The number of Malpighian tubules varies in insects from 2 in coccids to about 250 in desert locusts. The length varies from 2 mm to 100 mm, and the diameter ranges from 30 µm to 100 µm. The wall of the tubules is one cell thick with microvilli on the inner side. The proximal part is absorptive with a brush border while the distal part is a secretory with a honeycomb border (Fig. 2.6). Since tubules are blindly ended, the accumulation of fluid builds up hydrostatic pressure resulting in the flow of fluid into the gut.

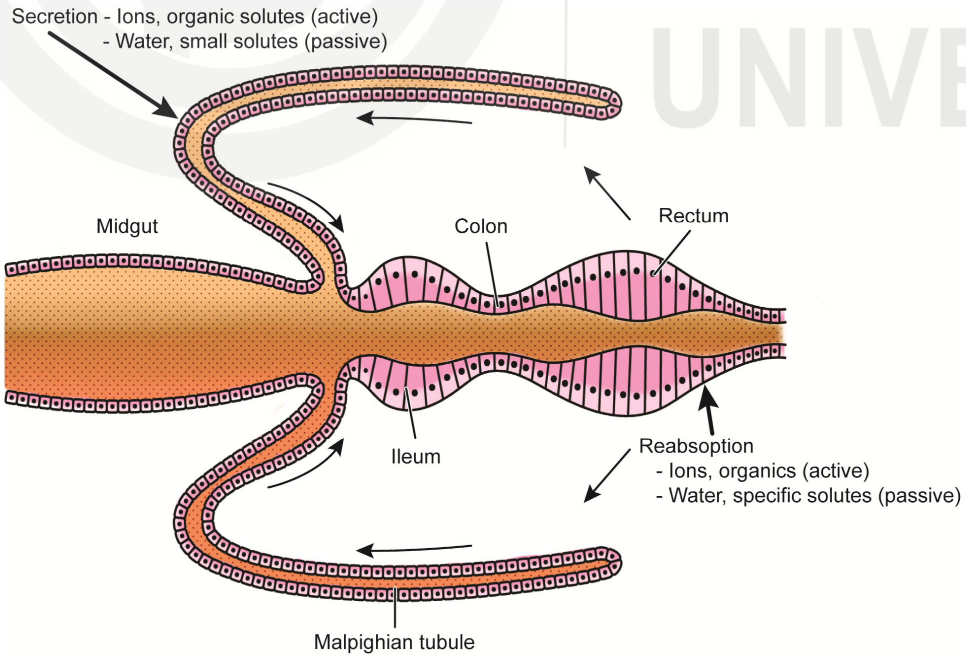

Fig. 2.6: Malpighian Tubules

Usually Malpighian tubules are made up of single type of cells, however, in some they may have different cell types or multiple kinds of regions performing different functions.

2.8.2 Nephrocytes

Nephrocytes are specialized cells for excretion present in the haemocoel. The cells were first described by Hollande (1922) and were known as **pericardial cells** due to their location along the dorsal heart. These take up compounds of high molecular weight, such as colloids and dyes, which cannot be excreted by Malpighian tubules. These are also referred to as **storage kidneys**.

Nephrocytes are large and binucleated with a deep network of infoldings of the plasma membrane. The external surface of the folds is held together by a desmosome-like structure; the cells are surrounded and held together by a basal lamina. In *Drosophila*,nephrocytes regulate hemolymph composition. These are of two types, the garland cells surrounding the oesophagus and pericardial cells in two rows around the heart (Fig. 2.7). There are three types of nephrocytes in *Bombyx* spp. namely pericardial, sub-oesophageal, and peritracheal, while in *Pediculus* they form a group on either side of the oesophagus.

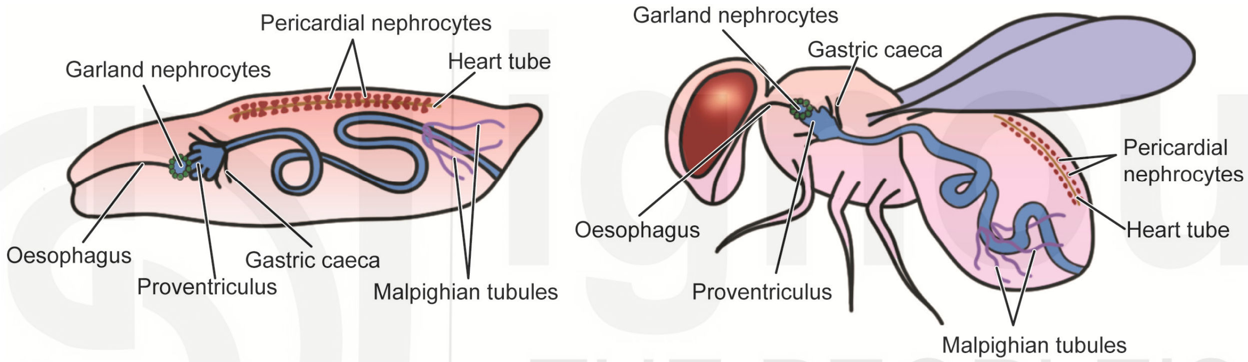

Fig. 2.7 Location of nephrocytes in *D. Melanogaster*

Nephrocytes remove circulating proteins and other toxins from the hemolymph and so may also be considered as **ultrafiltration cells**. It was proved experimentally that if these cells are destroyed it leads to systemic disruption of physiological processes in insects. The nephrocytes are compared to the nephrons of mammals, the basic structural and functional excretory unit of kidneys.

The nephrocytes are not involved in the primary urine production but help in the removal of toxicants from the hemolymph by endocytosis. The nephrocytes eliminate foreign and waste products from the blood in colloidal form and store them in their cytoplasm as **crystalloids**. These are later transported to the Malpighian tubules to be eliminated.

2.8.3 Excretion by Rectum

The malpighain tubules of *Periplaneta* do not contain uric acid, but the granules of it are found in the wall of the rectum and in the faces suggesting that the hindgut may have an excretory function. There are typically six rectal pads in *Periplaneta*, each is a longitudinal folding of cuticle containing thickened patches of epithelium and many tracheal branches. In ammoniotelic insects, ammonia passes directly into the gut without involving Malpighain tubules. In certain aquatic insects ammonia is secreted directly into the rectum.

2.8.4 Other Excretory Organs

Springtails that have no Malpighian tubules, and larvae of wasps and bees and oriental cockroaches in which Malpighian tubules do not excrete uric acid, uric acid is eliminated through other organs discussed here:

i) **Labial glands:** In springtails, labial glands are supposed to be involved in excretion which consist of an upper saccule followed by a coiled labyrinth and have a gland opening into the outlet duct (Fig. 2.8).

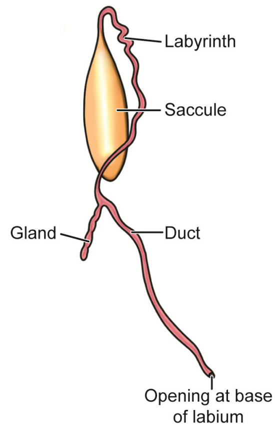

Fig. 2.8: Labial gland of a springtail

ii) **Utricular gland:** In cockroaches (*Blatta, Blatella*), uric acid is stored temporarily in the utricular glands (male accessory glands) and then is poured out over the spermatophore during copulation. Recent studies demonstrated that it provides an alternate source of nitrogen to the embryo. Also, the female's own uric acid stores could be passed onto her embryos. Thus, it is suggested that both sexes can make a parental investment in the offspring. Uric acid in embryo is hydrolysed by the enzyme uricase produced by micro-organisms (in mycetocytes of fat body) and serves as a nutritional nitrogen source.

iii) **Fat body:** In the oriental cockroaches, uric acid is also stored in the urate cells of fat body. It stores nitrogen (storage excretion) for use in the production of new tissue or that after reduction it supplies adenine for nucleoprotein synthesis. Uric acid stored in the fat body of larvae may be the end product of metabolism of the individual cells and subsequently, in pupa, it is transferred to the Malpighian tubules and excreted with meconium.

iv) **Other tissues:** Epidermis of *Rhodnius* also accumulates uric acid and during each moulting it is removed. Uric acid produced during pupal stage may also be stored in scales of wings in butterflies.

2.9 PRIMARY URINE OF INSECTS

Primary urine is the fluid produced in the Malpighian tubules to carry excreted substances to the hindgut. Fluid-feeders and aquatic insects excrete liquid urine while terrestrial insects that feed on solid food do not excrete liquid urine as it is mixed with undigested food in the rectum to egest faeces in fluid form.

Malpighian tubules are highly permeable to low-molecular-weight molecules,hence they play a significant role in active absorption and secretion of amino acids, sugars, ions, and water, etc. from the surrounding hemolymph, forming primary urine. The soluble compounds and water needed by the insects are reabsorbed in their proximal segment, hindgut or rectum maintaining the internal osmotic pressure.

Formation of primary urine

The process is initiated in the Malpighian tubules in the lumen of which the K^+ from the hemolymph reaches by active transport. The cells of Malpighian tubules secrete active solutes such as Na^+, K^+, Cl^-, and uric acid. The movement is against the concentration gradient involving transport proteins and ion pumps. The active transport of ions creates an osmotic gradient and thus water, electrolytes and nitrogenous wastes are drawn from the hemolymph into the lumen of tubules. Water enters through the process of osmosis.

The primary urine formed in the Malpighian tubules reaches the rectum, where the waste produced is concentrated by selective reabsorption of water and ions. Further reabsorption of water, Na^+ and K^+ occurs under the hormonal regulation and reaches the hemolymph. In arid conditions, water and important electrolytes can be reabsorbed depending upon the insect's requirement. It results in the elimination of dry and thick pasty excreta.

2.10 EXCRETORY PRODUCTS

In insects, the excretory products primarily are the nitrogenous wastes, produced due to protein metabolism. The toxic nitrogenous wastes can be converted and eliminated by the tubules as uric acid, allantoin, allantoic acid, urea, or ammonia. The main excretory products are as follows.

Ammonia

It is the simplest form of excretory product. Free ammonia is toxic and requires water for its elimination from the body therefore it is the main excretory product of aquatic insects. Such insects are referred to as **ammonotelic**.

Uric acid

Uric acid is excreted by terrestrial insects which live in arid conditions and need to conserve water. The water is completely absorbed from the waste produced. The uric acid is insoluble in water. Such insects are called **uricotelic**. Examples are locusts and cockroaches.

Uric acid comprises more than 80% of the total nitrogenous waste excreted by most of the insects. However, a few insects can also excrete urea, but it is a minor component. A few lepidopterans such as *Pieris,* excrete allantoin as the nitrogenous waste. Besides nitrogenous waste, Malpighian tubules can also actively excrete toxic elements of natural and chemical origin (xenobiotics) to let the insects better adapt to a wide range of nourishment.

SAQ 4

i). Choose the correct option:

a). Malpighian tubules in insects were discovered by (Marcello Malpighi/Hollande)

b). Malphighian tubules are (thin projections/thick projections)

c). The major excretory product of terrestrial insects is (uric acid/urea)

d). Dissolves in water (uric acid/ammonia) ii). Fill in the blanks:

a). ...., ... are the organs for excretion in insects.

b). The proximal part of Malpighian tubules has .... border.

c). The excretory product with no or very little water is ...., ...

d). Primary .... is produced in the Malpighian tubules.

2.11 STORAGE OR DEPOSIT EXCRETION

Sometimes the waste materials are not excreted out as urine but are retained in the body. These products do not cause any adverse effect to the insects and are known as storage or deposit excretory products. For example, uric acid which is insoluble in water can be retained in some insects and is not eliminated from the body.

(a) In the cockroach *Periplaneta americana*, uric acid accumulates in the fat body when there is more nitrogen in the food, and is utilized by the insect when nitrogen is deficient in the diet.

(b) In the case of red cotton bugs, the metabolic waste is deposited within the integument of the body wall giving a white colour.

(c) In some male insects such as, grasshoppers, crickets, and beetles these products form the outer covering of the **spermatophore**. The covering helps protect the spermatozoa from damage, and predators.

(d) In the wing scales of *Pieris* butterflies, the white colour is due to pterins, the by-product of nitrogen metabolism.

2.12 OSMOREGULATION

Insects can lose and gain water in various ways, the concentration of which needs to be maintained to make internal environment stable. Osmoregulation is a biological process that controls the concentration of water and solutes within the body of an insect. The process is important for maintaining cellular functions, and overall physiological balance. It involves the regulation of intake, retention, and excretion of water and solutes.

2.12.1 Osmoregulation in Terrestrial Insects

Terrestrial insects lose water in many ways, such as from the body and the respiratory surface as well as in excretion. Loss of water through insect cuticle varies with the extent of permeability, and temperature and humidity of surrounding air. The loss, however, is limited by the waxy layer and lipids of the epicuticle. Similarly, through respiratory surfaces are permeable and can lead to a large amount of water, the loss is reduced by the invagination of these surfaces as tracheae. The spiracles also limit the loss when they are closed. However, prolonged or frequent spiracle opening results in increased water loss. Apart from this, nitrogenous excretion is always associated with some water loss though some reabsorption of water occurs during the process.

The insect can control its water loss by regulating the relative rates of urine production and reabsorption. In other words, the loss of water is minimized through the process of osmoregulation. Similarly, insects feeding on plant juices and sap, or blood take up more liquids that is to be eliminated from the body by the process of osmoregulation.

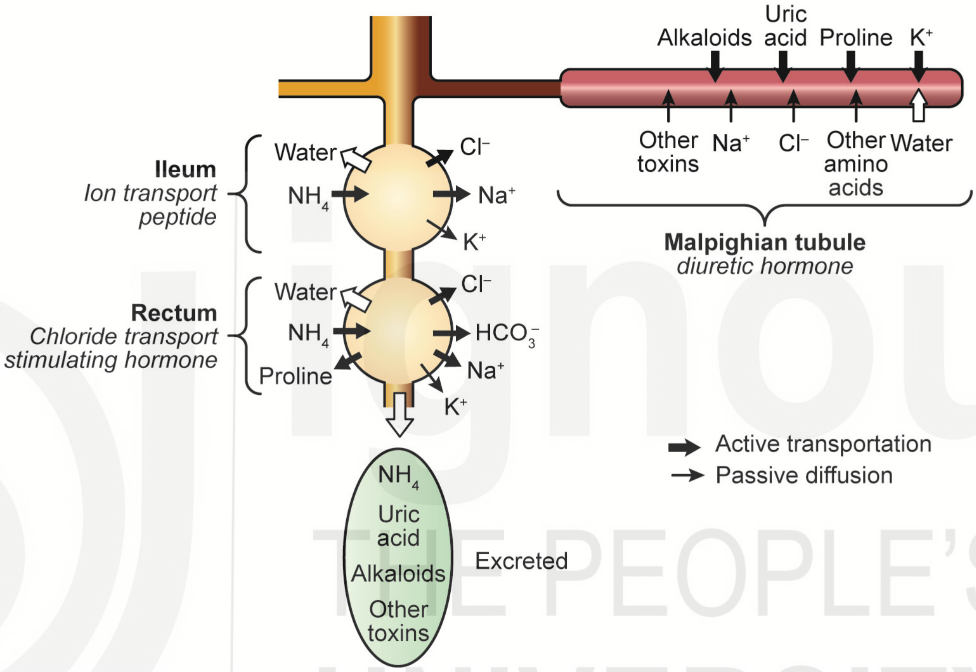

Fig. 2.9: Process of osmoregulation in *Schistocerca*

In these insects, the potassium and sodium ions move actively while the electrical equilibrium is restored by the chloride ions. The process occurs in the ileum and rectum which are the parts of the hindgut. During the process active transport of K^+ into the Malpighian tubules causes the osmotic movement of water and the solutes passively. All the solutes are recovered in the ileum and the rectum and uric acid is excreted as a semi-solid paste.

The process is mediated by hormones called the diuretic hormones in the Malpighian tubules. This can be seen in insects such as *Schistocerca, Pieris*, and *Rhodnius* (Fig. 2.9).

2.12.2 Osmoregulation in Freshwater Insects

These insects take up excess water due to osmosis, which must be expelled from the body and retain the essential ions. There is a formation of dilute primary urine as water moves into the tubules. Water reabsorption is less than terrestrial insects, and efficient reabsorption of essential inorganic ions. The urine produced is hypotonic, for example in *Aedes, Culex*, and *Chironomus* (Fig. 2.10).

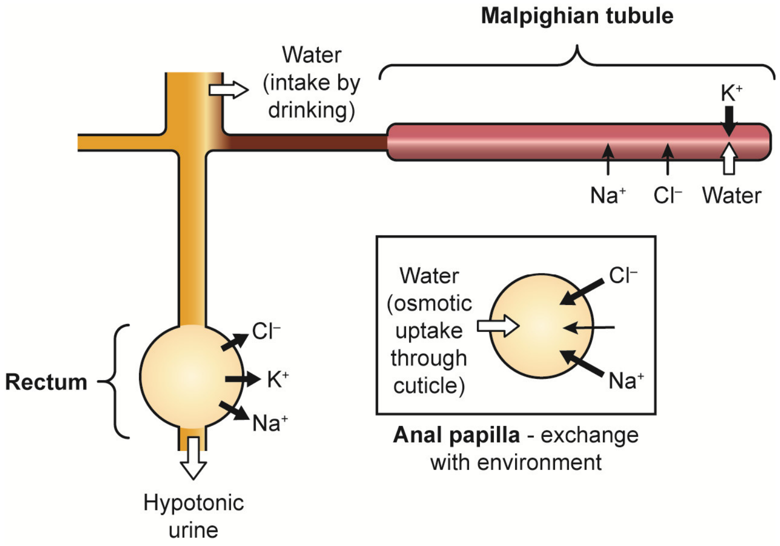

Fig. 2.10: Hypotonic urine formation

2.12.3 Osmoregulation in Saltwater Insects

These insects have a risk for dehydration due to high salinity in water as well as salts in their diet. They retain water while excreting excess salts, which is attained by extensive water reabsorption to prevent dehydration. The urine produced is hypertonic as in the case of *Ephydra,* and Caddisfly (Fig. 2.11).

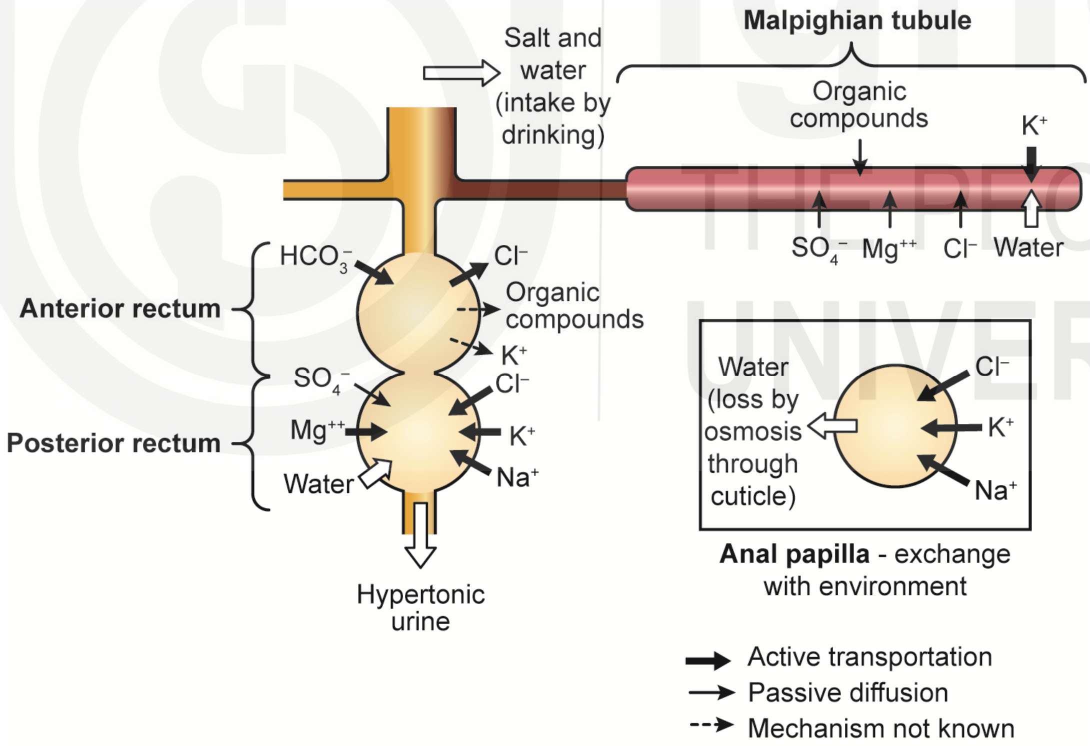

Fig. 2.11: Hypertonic urine formation

2.13 HORMONAL CONTROL OF URINE FORMATION

In insects, osmoregulation is maintained by the actions of Malpighian tubule secretion and hindgut reabsorption. Both responses are controlled by feedback of diuretic and antidiuretic hormones. These are released from corpora cardiaca in the brain, and produced in neurosecretory cells in the nervous system of the insects.

Diuretic hormones

These hormones increase the rate of urine formation by excreting excess water and salts. Three diuretic hormones have been identified in some insects; the corticotropin-releasing factor (CRF), calcitonin-like peptides, and kinins, for example, in insects belonging to the order Isoptera, Orthoptera, Coleopters, Diptera, and Lepidoptera.

All three hormones stimulate fluid secretion in Malpighian tubules. It increases the rate of Na^+ and Cl^- transport into the tubule lumen causing osmotic flow of water.

Antidiuretic hormones

These hormones reduce the rate of urine formation and promote water conservation. Insects feeding only on a liquid diet such as plant juice/sap or blood do not have antidiuretic hormones and require only the diuretic hormone as they must excrete the excess water and salts through the Malpighian tubules.

The neurotoxic insecticides interfere with the normal functioning of Malpighian tubules and the hindgut. These chemicals hamper the release of hormones that control excretion causing a disturbance in the ionic balance and water retention. This disruption leads to dehydration, accumulation of toxic wastes in the body and death of the insect.

SAQ 5

i). State whether the statement is 'True' or 'False':

a) Osmoregulation regulates water and ions in the body.

b) Diuretic hormones increase the rate of urine formation thus help in water conservation.

c) The nutrients and ions are reabsorbed from the primary urine in the rectum.

d) Hormones for urine control are released from corpora cardiaca.

ii). Answer in one word.

a). The other name for storage kidneys.

b). Urine type in freshwater insects.

c). The hormone that increases the rate of urine formation.

d). Urine type in salt water insects.

2.14 SUMMARY

Let us summarise what you have learnt so far:

- The digestive system involves the alimentary canal and associated digestive enzymes. Digestion is the process of intake of food, its assimilation and absorption to produce energy.

- The alimentary canal is a straight tube that starts from the mouth and ends at the anus and is divisible into three regions namely the foregut, midgut, and the hindgut.
- The foregut includes the buccal cavity, pharynx, crop, and gizzard. The midgut is the site for digestion and absorption of digested food. It also consists of gastric caecae and peritrophic membranes. The hind gut comprises pylorus, ileum, and rectum.
- The food is digested with the help of digestive enzymes which depends on factors like temperature, pH, and redox potential. Salivary glands are present in insects that produce saliva which performs diverse functions in different groups of insects.
- Most of the digestion occurs in the midgut. The carbohydrates are broken down into monosaccharides. Proteins are hydrolysed to peptones and polypeptides which finally form amino acids. The lipids are hydrolyzed to fatty acids and glycerol. Absorption of the digested food occurs in the midgut, while ions and water are absorbed in the hindgut.
- The insect excretory system plays an important role in the removal of metabolic wastes and osmoregulation. The excretory organs are the Malpighian tubules and the hindgut. The Malpighian tubules are located at the junction between the midgut and the hindgut. These are involved in the extraction of wastes, solutes, and water from the hemolymph.
- Primary urine is formed by releasing of K^+ and Na^+ into the lumen that creates an osmotic gradient for drawing water and solutes inside.
- The nutrients and ions are reabsorbed when primary urine reaches the hindgut. In case of terrestrial insects, the excretory product is uric acid, while ammonia in aquatic insects. The urine is hypotonic in freshwater insects and hypertonic in saltwater insects.
- The urine formation is controlled by diuretic and antidiuretic hormones. Usually, the fluid feeder insects lack antidiuretic hormone.
- Some insects have nephrocytes, which are also called the pericardial cells as they are present along the dorsal heart. The nephrocytes are compared to the nephrons of mammals, the basic structural and functional unit of kidneys.
- The nephrocytes are not involved in the primary urine production but help in the removal of toxicants from the hemolymph by endocytosis.

2.15 TERMINAL QUESTIONS

1. What is the composition of the midgut?
2. Differentiate between acinar and tubular salivary glands.
3. Name the organs of the hindgut.
4. What is extra-intestinal digestion?

5. What is a filter chamber, and how it is useful in fluid-feeding insects?
6. Give an account of the organs present in the foregut regions of insect digestive system.
7. Where is the peritrophic membrane located? How it helps in insect digestion?
8. Give an account of the digestive enzymes.
9. How are the inorganic ions absorbed in insects during digestion?
10. What are the factors affecting the process of digestion?
11. What is the primary excretory organ in insects?
12. Name the two main nitrogenous wastes in insects.
13. Name two insects that utilize storage excretory products.
14. Explain the structure and function of Malpighian tubules in insects.
15. Compare the process of osmoregulation in terrestrial and aquatic insects.
16. What are nephrocytes, and how these are important in some insects?
17. What is primary urine? How is it formed?
18. Which hormones are involved in the process of excretion? Explain briefly.

2.16 ANSWERS

Self-Assessment Questions

1. i) a) Stomodeum b) Crop
   c) Gastric Caecae d) Peritrophic Membrane

   ii) a) False b) True
   c) True d) False

2. i) a) Absorption b) Cellulose
   c) 35 d) Lipases

   ii) a) III; b) I; c) II.

3. i) a; ii) c; iii) d; iv) c; v) d.

4. i) a) Marcello Malpighi
   b) Thin projections
   c) Uric acid
   d) Ammonia

ii) a) Malpighian Tubules

b) Brush

c) Uric acid

d) Urine

5. i) a) True; b) False; c) True; d) True.

ii) a) Nephrocytes

b) Hypotonic

c) Diuretic Hormone

d) Hypertonic

Terminal Questions

1. Refer to Subsection 2.3.2.
2. Refer to Section 2.4.
3. Refer to Subsection 2.3.3.
4. Refer to Subsection 2.5.1.
5. Refer to Subsection 2.3.2.
6. Refer to Subsection 2.3.1.
7. Refer to Subsection 2.3.2.
8. Refer to Section 2.5.
9. Refer to Subsection 2.6.4.
10. Refer to Section 2.5.
11. Refer to Section 2.8.
12. Ammonia, Uric acid.
13. Refer to Section 2.11.
14. Refer to Subsection 2.8.1.
15. Refer to Section 2.12.
16. Refer to Subsection 2.8.2.
17. Refer to Section 2.9.
18. Refer to Section 2.13.

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UNIT 3

CIRCULATORY SYSTEM

Structure

3.1 INTRODUCTION

Transporting nutrients, waste products, and gases into and out of cells is a vital aspect of life. In unicellular organisms, the body surface serves as a point of exchange with the external environment. Whereas, multicellular organisms have developed transport/circulatory systems in order to supply food and oxygen to their cells and eliminate carbon dioxide and waste products resultant of their metabolism.

The organisms have two types of circulatory systems-open and closed circulatory systems.

In an ***open circulatory system***, the blood or haemolymph flows around organs in open spaces called sinuses. Arthropods and most molluscs have an open circulatory system. Certain molluscs, all higher invertebrates, and vertebrates have far more efficient ***closed circulatory system***, in which blood is pumped by a closed network of veins, arteries, and capillaries. We will study about the basic structure and functions of circulatory system of insects in this Unit.

Objectives

After studying this Unit, you should be able to:

- ❖ explain the different components of circulatory system;
- ❖ describe the morphology of the insect circulatory system;
- ❖ gain insight of the basic structure and function of circulatory system;
- ❖ explain the structures of different haemocytes; and
- ❖ appreciate the role of circulatory system in immunity and in thermoregulation.

3.2 THE CIRCULATORY SYSTEM OF INSECTS

The circulatory system of insects is open type where blood flows freely in body cavity. Veins, arteries and capillaries are absent. Blood gets mixed with interstitial fluid. The circulatory system comprises haemocoel, heart, aorta alary muscles and accessory pulsatile organs. You will study about these components in this section.

3.2.1 Hemocoel

The body cavity of insects is called hemocoel which is filled with the blood called haemolymph. Haemocoel is divided into three sinuses - **dorsal pericardial sinus, perivisceral sinus** and **ventral perineural sinus,** by muscular **dorsal** and **ventral diaphragm** (Fig. 3.1). Dorsal diaphragm is a perforated membrane situated beneath the heart and separates the dorsal pericardial sinus from the perivisceral sinus. Ventral diaphragm situated above the nerve cord, separates the perivisceral sinus from the perineural sinus. Higher dipterans lack a ventral diaphragm but have large tracheal air sacs that partition the hemocoel so that a smaller volume of haemolymph can function more economically when a reduced weight is required for flight. Thus, dorsal or pericardial sinus, containing heart and aorta,lies between the tergum and dorsal diaphragm. The ventral or perineural sinus, containing nerve cord, lies in between the sternum and ventral diaphragm. Perivisceral sinus is the area in between dorsal and ventral diaphragms. It harbors the visceral organs like alimentary canal and gonads. In most insects, the visceral sinus occupies most of the body cavity, but in ichneumonids the perineural sinus is enlarged.

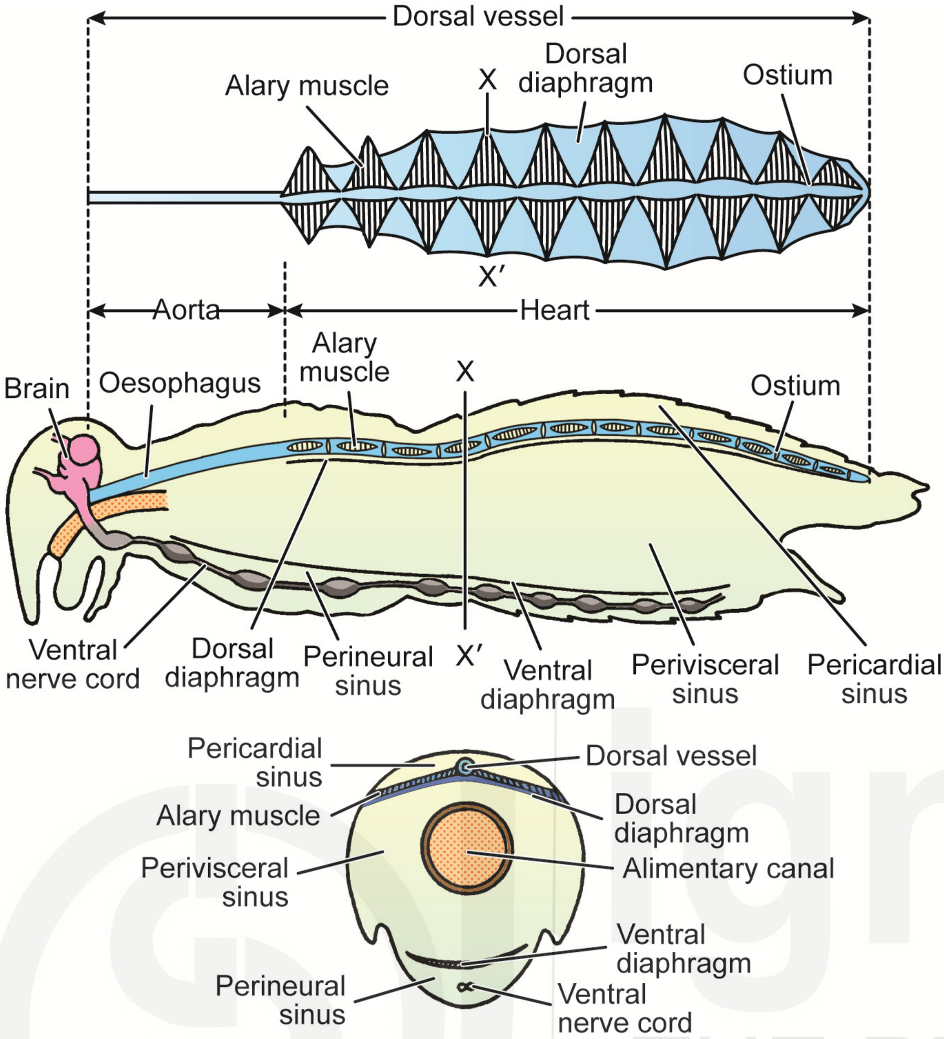


Fig. 3.1: Circulatory system of a generalized insect. X-X's in top two figures indicate cross-sectional plane of bottom figure. Transverse section of insect abdomen showing diaphragm and sinuses

3.2.2 Dorsal Blood Vessel

It is principal blood conducting organ in insects, which extends along the mid dorsal line just beneath the tergum, for almost whole length of the body, from the hind end to the head. It is open at the anterior end while closed posteriorly except in the may fly naiads. The wall is made up of 1-3 layers of contractile muscles. The dorsal blood vessel has two regions, the *anterior aorta* or *conducting vessel* which extends forward through the thorax to the head, and the *posterior heart* or *pumping organ* which is limited to the abdomen (Fig. 3.2).

Aorta: The aorta functions as principal artery and acts as a conducting vessel. It is a simple unbranched and imperforated tube that is thinner than the heart and lacks ostia (pores). It is present in the thoracic region and opens into the head near the brain (tritocerebrum). Anteriorly, sometimes it gets divided into 2 or more cephalic arteries in the head. It continues into the heart through an aortic valve. In silkworm, the aorta has dilated portions along its length.

Heart: The heart, though restricted to the abdomen in most of the insects, may extend anteriorly upto the prothorax in cockroaches. Heart consists of a number of chambers marked by constrictions. Number of chambers in heart varies in different insects, such as 12 in cockroaches, 5 in Hymenoptera, while one in Mallophaga and Anoplurans. Each heart chamber has a vertical, slit-like opening in the lateral wall, called the incurrent '**ostia**'. These allow the entry of blood from pericardial sinus into the heart. The wall of the each ostium is deflected inwards and forwards to form an *auricular valve* which prevents return flow of the hemolymph into the dorsal sinus. Each pair of auricular valve also functions as *ventricular valve* in many insects which prevents the backward flow of the blood in the heart itself. Thus, ostia permit the one-way flow of hemolymph into the dorsal vessel. The number of ostia depends upon the number of heart chambers. There may be up to three pairs of thoracic ostia and nine pairs of abdominal ostia. Control of heart beat is *neurogenic* when there is supply of nerves and *myogenic* when the nerve supply is cut off.

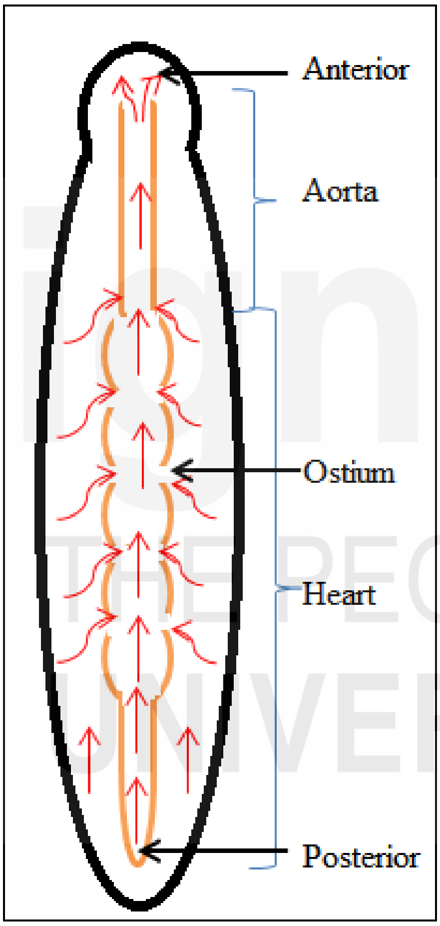


Fig. 3.2: Diagram showing flow of hemolymph in heart chambers and aorta of dorsal vessel

The incurrent ostia: The incurrent ostia, usually nine pairs in abdomen and three pairs in thorax, are vertical, slit-like openings in the heart wall (Fig. 3.3). There is a tendency of reduction in incurrent ostia so that there are only five pairs in wasps and three pairs in house flies. The valvular ostium permits the flow of blood into the heart at diastole, but prevents its outward passage at systole.

Excurrent ostia: In certain insects, e.g., grasshoppers and silverfishes there are paired (2 pairs thoracic and 5 pairs abdominal in grasshoppers) ventrol-lateral and non-valvular excurrent ostia (Fig. 3.4). These ostia expand and contract during cardiac systole and diastole, respectively to allow the blood flow from heart to pericardial or perivisceral sinuses.

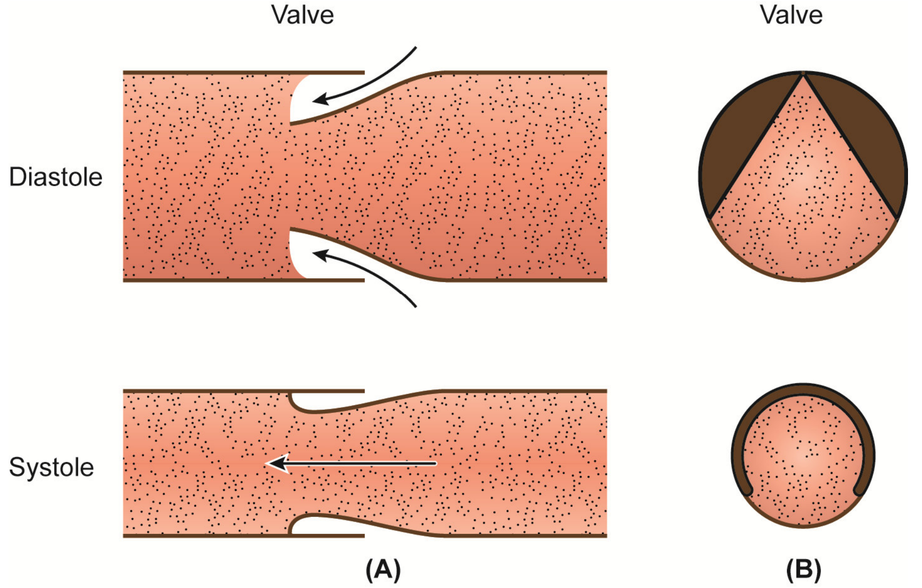


Fig. 3.3: A) Diagrammatic representation of the incurrent ostial valves seen in horizontal section of the heart. B) Seen is transverse section of the heart

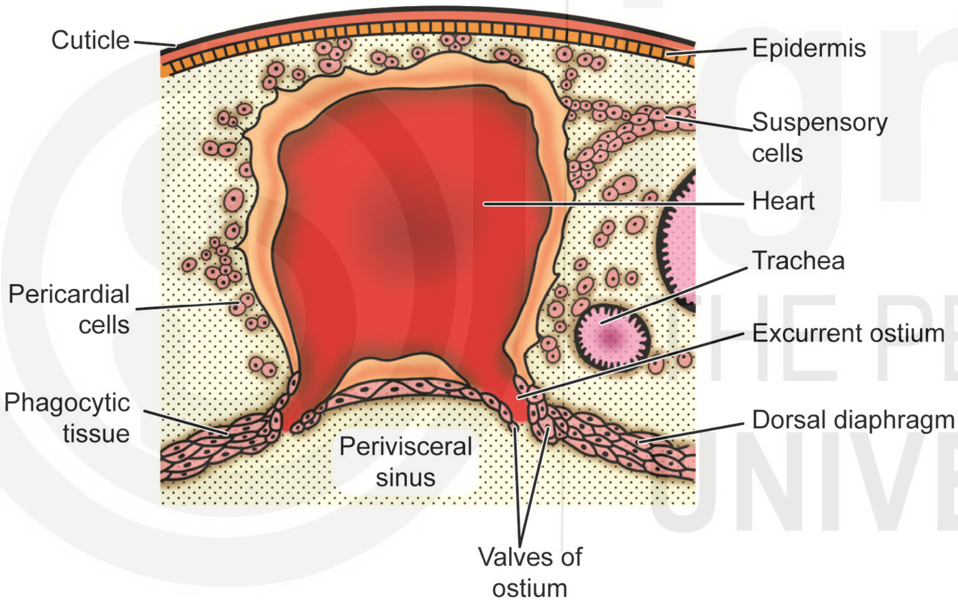


Fig. 3.4: Transverse section of the heart showing the excurrent ostia opening directly to the perivisceral sinus

The heart may be constricted between successive ostia (e.g., cockroaches), giving it a chambered look, but its lumen is nearly continuous throughout. On either side of the heart, in a segmental arrangement, are the alary muscles which are attached laterally to the body wall. Lateral segmental vessels associated with the heart have been identified in several insects, for example, many cockroach species where excurrent ostia are absent. At the origin, these vessels are valvular permitting the outward flow of blood from the heart.

3.2.3 Sinuses, Diaphragms and Alary Muscles

The haemocoel is usually separated into two and sometimes three cavities, or sinuses by one (dorsal diaphragm) or two (dorsal and ventral diaphragm) fibromuscular horizontal septa. Dorsoventral movements of both the diaphragms help in the circulation of blood.

i) **Dorsal diaphragm:** The dorsal diaphragm, or pericardial septum typically consists of two layers, which enclose the alary muscles associated with the heart. Usually it is perforated through which haemolymph can readily pass. This diaphragm divides the dorsal pericardial sinus (around the heart) from the perivisceral sinus (around the visceral organs).

ii) **Ventral diaphragm:** The ventral diaphragm is present in certain insects like dragonflies, grasshoppers and wasps. It is located just above the nerve cord and is ventral to the gut. Like the dorsal septum, it is usually perforated around its periphery. Laterally it is attached to the sternum. It separates the perivisceral sinus from the perineural sinus (around the nerve cord).

Heart mainly functions as the pumping organ and remains in the position with the help of **alary muscles**. The muscles are triangular and fan-like that are attached to the tergum of the abdomen on one side and to the dorsal diaphragm on other side. They are composed of striated fibers and project laterally from the dorsal vessel in each abdominal segment (Fig. 3.5). Although sometimes mistakenly thought to play a key role in heartbeat, the alary muscles are more properly called **muscles of the dorsal diaphragm**.

The alary muscles may be arranged in a loose network, as in Lepidoptera, or arranged like a weave surrounding the dorsal vessel, as in some Diptera. The number of alary muscles varies according to the heart chambers, such as cockroaches have 12 pairs, aculeate Hymenoptera (ants, bees and wasps) 5 pairs, honey bee 4 pairs, *Musca* (housefly) 3 pairs and *Chironomus* (midges) has 2 pairs. The alary muscles and heart are reported to have different rates of contraction; 80 beats/minute for heart while 6.7 contractions/minute for alary muscles indicating that heart beats faster than the alary muscles.

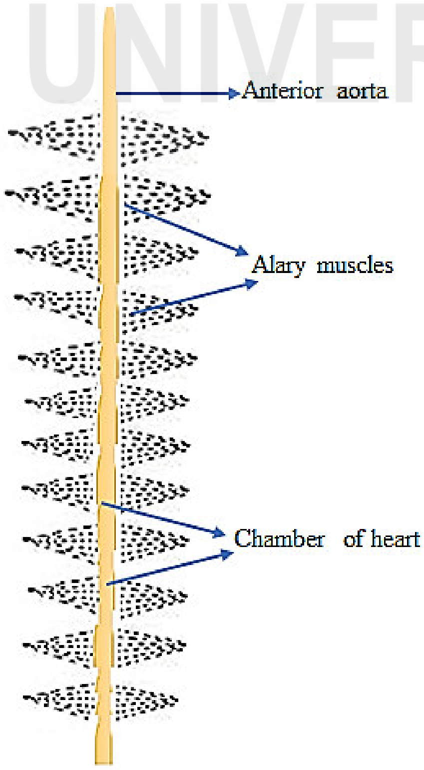


Fig. 3.5: Dorsal vessel showing alary muscles and chamber of heart

3.2.4 Accessory Pulsatile Organs (Fig. 3.6)

In addition to dorsal vessel, insects have accessory pulsatile organs (auxiliary heart) that supply hemolymph to body appendages, such as wings, legs, antennae and ovipositor (in females). These are sac-like structures situated in various regions of the body, pulsate independently of the heart and supply adequate circulation of hemolymph through respective appendages. Few examples of position of accessory pulsatory organs are:

- At the base of antenna: *Periplaneta* (cockroach) and Lepidoptera
- In legs: Hemiptera
- In wings: Lepidoptera
- In scutellum: Cyclorrhapha in Diptera

The pulsatile organ at the base of antenna consists of an ampulla from which a tube arises and extends till the antennal tip. The expansion of the pulsatile organ fills it with the hemolymph while compression drives the blood up the antennal vessel till the tip and pours blood in the hemocoel of the antennae. After that, it returns downward and exits the antennae. Similarly, the pulsatile organ at the base of the leg pumps hemolymph out and back from the leg with each contraction. The pulsatile organs situated at the base of the wings are crucial for inflating the wings in addition to providing hemolymph to the wings.

In moth, the dorsal blood vessel itself loops up to the dorsal surface of the thorax forming the pulsatile organ. Grasshoppers and cockroaches possess a small ampulla at their antennal base. The ampulla communicates the haemocoel by a valvular opening and continues as a vessel into the antenna. During ampullar expansion the blood is drawn into it from haemocoel and during contraction the blood is pumped into the antennae.

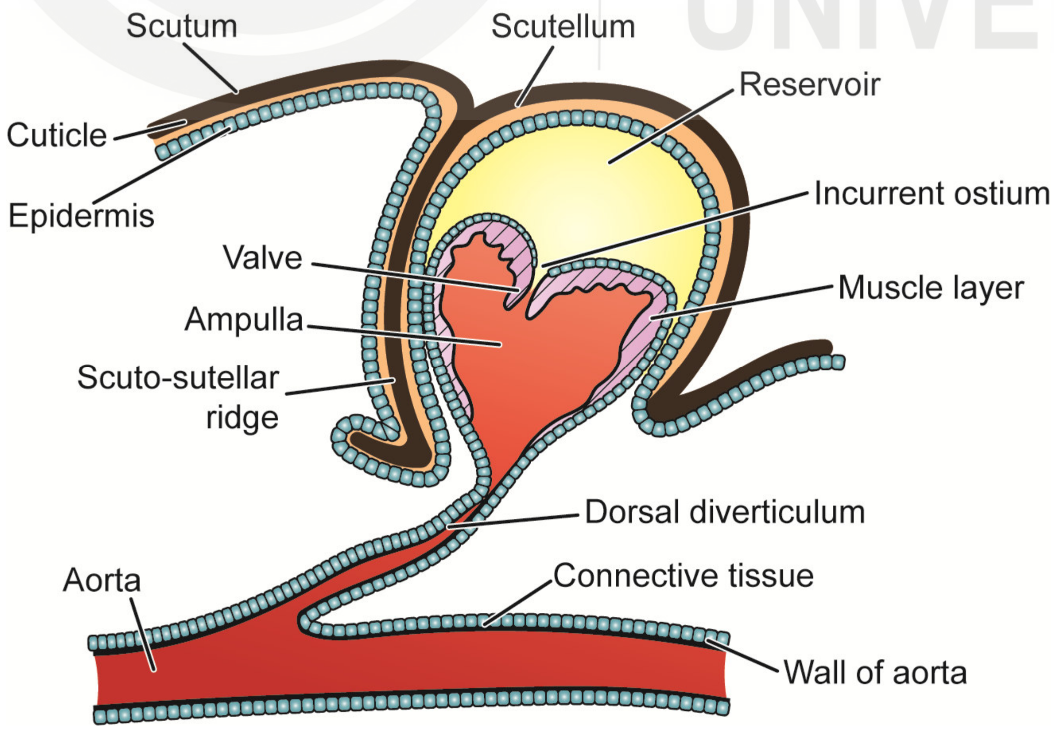


Fig. 3.6: Sagittal section of the mesothorax showing pulsatile organ

SAQ 1

i) Fill in the blanks:

a) The principal organ concerned with blood circulation is ....

b) The anterior end of dorsal vessel opens into ....

c) The ventral diaphragm is situated above ....

d) The area lying in between the tergum and dorsal diaphragm is .... sinus.

e) In insects mostly heart is restricted to ....

f) Control of heart beat is …………… when there is supply of nerves.

ii) Underline the correct answer

a) The opening of heart is known as ………………

I) Aorta II) Ostia III) Spiracles IV) Atrium

b) The heart of insect is …………….

I) Respiratory organ II) Circulating organ

III) Blood flowing organ IV) Pumping organ

c) The number of pairs of alary muscles in cockroach is …………….

I) 3 II) 12 III) 8 IV) 2

d) Heart beats ………….. the alary muscles

I) slower than II) faster than

III) lower than IV) same as

e) The anterior thoracic portion of dorsal vessel in insect is …………

I) Aorta II) Heart

III) Air sac IV) Accessory pulsatile organ

f) ……………. supplies hemolymph to body appendages.

I) Accessory pulsatile organ II) dorsal vessel

III) heart IV) aorta

3.3 MECHANISM OF CIRCULATION IN INSECTS

In phylogenetically ancestral insects, hemolymph flow in the dorsal vessel is bidirectional. During contraction, it flows in the anterior part towards the head and simultaneously in the posterior part. An intracardiac valve in the abdomen is responsible for the posterior directed flow, which supplies the long caudal appendages (cerci and terminal filum), for example in certain apterygotes and mayflies. Nonetheless, the haemolymph flow in the majority of insects is unidirectional, meaning that the dorsal channel contracts at the posterior end which then moves forward in a peristaltic wave-like manner.

Heartbeat reversal refers to the regular alteration in the direction of the contraction wave that occurs in the pupae and adults of Coleoptera, Diptera, and Lepidoptera.

Circulation of haemolymph takes place in two phases (Diastole and Systole) due to the action of the alary muscles as well as the muscles of the walls of the heart.

3.3.1 Diastole

The contraction of the tergum and the heart's associated alary muscles results in the expansion of heart. It causes the area of the pericardial sinus to shrink and the increase in heart's volume. As a result, the haemolymph in the pericardial sinus is under pressure, which forces the haemolymph to enter the heart through the *incurrent ostia* (Fig. 3.7). These incurrent ostia only permit haemolymph to enter the heart from the sinus but stop its backward flow from the heart to the sinus. **During diastole, the lips of ostium valve are forced apart causing inflow of haemolymph.**

3.3.2 Systole

During systole, the lips of ostium valve are forced together and they remain closed preventing the outflow of haemolymph. The contraction of the heart wall muscles and the expansion of the alary muscles cause the heart to contract. It puts pressure on the haemolymph inside the heart due to which it moves forward and enters the aorta (Fig. 3.7). Haemolymph enters the head through the aorta and flows back, bathing the neural cord in the perineural sinus and the visceral organs in the visceral sinus.

3.3.3 Diastasis

Diastasis is a short period of rest interval that occurs between diastole and systole.

Undulating movements of the dorsal and ventral diaphragms cause haemolymph to flow backward through the pericardial sinus and, into the visceral and perineural sinuses before entering the posterior region of the body. A portion of the blood that is circulated throughout the body enters the auxiliary pulsatile organs located at the bases of appendages such as wings, legs, and antennae. In general, the body's temperature and physiological state affect heart rate, and these factors might vary between species or even between stages of insects.

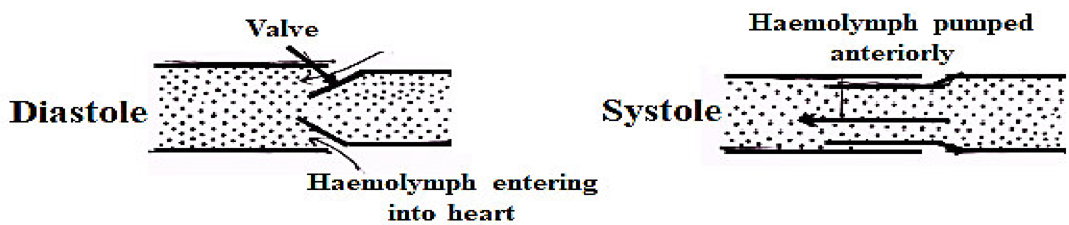


Fig. 3.7: Diagrammatic representation of haemolymph circulation in an insect

SAQ 2

Fill in the blanks:

a) Contraction of heart is called as ....

b) The blood enters the heart through ....

c) .... causes the area of the pericardial sinus to shrink and the heart's volume to grow.

d) In .... insects, haemolymph flow in the dorsal vessel is bidirectional.

e) Short rest interval that occurs between diastole and systole is known as ....

3.4 REGULATION OF HEARTBEAT

The heart of the insects usually lacks neural supply except few insects (e.g., cockroaches) where the heart is supplied with nerves from the corpora cardiaca and segmental ganglia (motor fibres). Therefore, it seems quite logical that the insect heart is myogenic. It also lacks a pacemaker. The available evidences suggest the existence of neurohormonal control of heartbeat. Both a cardioaccelerator neuropeptide and proctolin are myotropins that act on the heart. In nymphalid butterfly, during larval periods, the heartbeats alternate between forward peristalsis and backward peristalsis; but during adult emergence the heartbeat rate increases only forwardly. This forward movement facilitates the movement of the haemolymph into the wing haemocoel and helps in expansion and wing unfolding. Reflex cardiac responses to external stimuli are also probably important, for example, stimulation of either a tarsal or labellar sensillum ensued a rapid change in cardiac response of blow fly, *Calliphora*.

Several factors including intensity of activity, ambient temperature, metabolic rate, developmental stage, the presence of biologically active chemicals, insecticides, drugs etc. affect the rate and amplitude of the heartbeat in insects. Though the accessory pulsatile structures are independent of the dorsal vessel, they are influenced by the same factors. The frequency of heartbeat varies in insects from 14 beats/minute (larva of stag beetle) to 150 beats/minute in flies. The heartbeat of larva (100-130 beats/minute) is slower than the adult (150 beats/minute) in mosquitoes. The heart of younger pupae sometimes stops beating and in old pupae no beating is observed. In beetles, average blood pressure is generally quite low. In soft-bodied insects (e.g., caterpillars), tonic contractions of the body musculature raises the blood pressure so that the haemolymph maintains body shape hydrostatically.

3.5 COMPOSITION OF HAEMOLYMPH

Haemolymph is the only extracellular fluid in the insect body and makes up 5-40% of the total body weight and 15-75% volume of the insect, which varies with the sex, stage and age of the insect.Haemolymph is normally transparent but can occasionally have a green or yellowish and sometimes bluish tint. The primary oxygen transporter is haemocyanin which gives blue colour to hamolymph. Green colour of blood in insect is due to presence of insectoverdin (mixture of carotenoids and biliverdin). The colour of haemolymph may sometime depend upon the meals taken by the insects. e.g, herbivorous plant sap feeder has green colour pigment while blood sucking bugs have red pigments. The presence of haemoglobin in *Chironomus* midge, *Anisops* and *Gasterophilus* provides red colour to their blood. Specific gravity of the haemolymph varies from 1.01 to 1.06, while pH generally ranges from 6.4 to 6.8.During activity, the haemolymph becomes more acidic because of the release of carbon dioxide and acidic metabolites.

Haemolymph consists of two parts: liquid plasma or serum and blood cells or haemocytes.

3.5.1 Plasma

Plasma is composed of 85% water, inorganic salts (mostly Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}) and organic compounds (mainly carbohydrates, proteins, and lipids). The main circulating carbohydrate is trehalose, a disaccharide of glucose. The concentration of trehalose is 10-50 mM while glucose concentration is very low (<5 mM). Other carbohydrates include mannitol, inositol, hexosamines, etc.

The plasma has very high concentrations of amino acids (25-75 mM); their concentrations change with the physiological activity of the insect. The amino acids act as buffer in osmoregulation and substrate in protein synthesis. Among these, tyrosine, being poorly soluble, occurs as more soluble glucoside. Their levels increase just before the moult and subsequently decrease due to their use in melanisation and sclerotization of cuticle. Silkworms contain high glycine levels during production of silk. Glycine present in plasma accounts for roughly 40% of the fibroin in silk fibre.

Protein composition also varies in different insects. These play significant roles in the transport and storage of nutrients, in immunity and as enzymes. A few examples are hexamerins – the storage protein and lipophorins – the transport proteins. Insoluble diglycerides, present in large concentrations in the haemolymph, are transported by lipophorin proteins. Egg yolk proteins and vitellogenins predominate when the female is laying eggs while storage proteins predominate in larvae prior to pupation. The phenoloxidase system is an important enzyme system in insect plasma. The phenoloxidase catalyzes phenol to quinones which form melanin in insect cuticle. Another important enzyme in insect plasma is catalase which is an antioxidant and provides immunity.

Compared to vertebrate blood, haemolymph contains relatively high concentrations of amino acids, proteins, sugars, inorganic ions, low Na/K ratio and high uric acid content. It also contains hormones, allantoin, ammonia and urea. Thus, the osmotic pressure of haemolymph is often 0.7-1.2 MPa. However, it can vary according to the species, physiological condition and developmental stage of the insect.

3.5.2 Haemocytes

About 10% of haemolymph volume is made up of various cell types collectively known as haemocytes. These are involved in the wound healing, phagocytosis, and/or encapsulation of foreign bodies. Although the density of insect haemocytes varies from less than 25,000 to more than 100,000 per cubic millimetre, the amount of red blood cells, platelets, and white blood cells found in the same volume of human blood are far greater than that of insects. The haemocytes perform in various physiological functions such as development of xenobiotic resistance including invading microbes and transportation of nutrients and play an important role in melanization. Haemocytes are also indicative of growth and development.Comparative studies across different taxa suggest haemocytes are produced during two stages of insect development: embryogenesis from **head or dorsal mesoderm** and during the larval or nymphal stages in mesodermally derived **hematopoietic organs**.

The number and composition of the haemocytes varies greatly in insects according to their developmental stages and physiological activity. There is conflict over how to classify insect haemocytes. Hence, the nomenclature used to identify different cellular types varies widely among species. Nonetheless, studies on insect haemocytes indicate that most cell types and activities are similar across various insect species. There are six main morphologically distinct haemocytes, *viz*., **prohaemocytes**, **plasmatocytes**, **granular cells**, **spherule cells**, **oenocytoids** and **adipohaemocytes** (Fig. 3.8). Prohaemocytes are the progenitor of other cells.Plasmatocytes and granulocytes play primary roles in cellular defense mechanisms so these cells are also known as "immunocytes".

There are a number of synonyms for haemocytes'. For example, **plasmatocytes** have been called ameobocytes, lamellocytes, leukocytes, lymphocytes, phagocytes, giant fusiform cells and podocytes. Similarly, **granulocytes** have been described as adipohemocytes, cystocytes, coagulocytes, phagocytes, and spherulocytes. **Granulocytes** and **plasmatocytes** have been mistaken for one another as well as for other cell types. Despite the morphological differences, there is a consensus in their classification as given here:

Prohemocytes: These are the 'stem-cells' that produce other blood cells. They are the smallest of all haemocytes, mostly round and spherical in shape. Each cell has a large centrally located nucleus which is surrounded by small amount of cytoplasm. In smears stained with Giemsa, nucleus is stained pink almost filling the cell and very thin peripheral bluish cytoplasm around the nucleus.

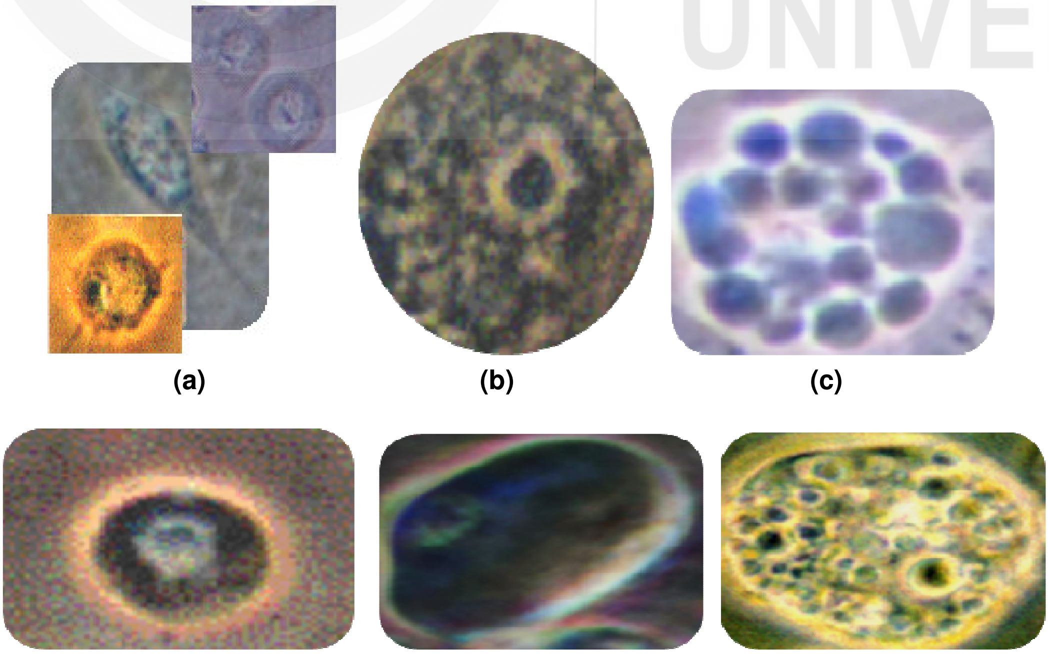
Plasmatocytes: The plasmatocytes are polymorphic (round, vermiform, fusiform, and spindle shaped) and variable in size, usually ovoid or spindle-shaped with a basophillic cytoplasm. These are strongly adherent and their spreading behaviour across foreign substances like glass slide is their most important characteristic feature. The nucleus may be round or elongated and is generally centrally located. Sometimes numerous and large vacuoles are present in the plasmatocytes.

Granulocytes: The cytoplasm of granulocytes is mostly full of dense granules. They degranulate or lyse very quickly when they come in direct contact with glass slide but granular cells never spread on glass surface like plasmatocytes. Nucleus is comparatively small to that of plasmatocytes, compact and centrally located. In smears stained with Giemsa, the cytoplasm appears pale blue with faintly pink coloured granules and nucleus is stained pink.

Spherulocytes: These are irregular in shape with variable sizes. The cytoplasm contains many small or a few large refractile spherules. The spherules are considerably larger in size than granules of granulocytes. These are weakly basophilic and stain faint blue while nucleus is stained pink. The nucleus gets concealed in the cells containing large and numerous spherules.

Oenocytoids: These are large and non-adherent cells with nucleus usually eccentric and generally small in size but bigger than that of granulocytes and spherulocytes. In smears stained with Giemsa, their cytoplasm is basophilic.

Adipohaemocytes: These are large round cells having spherical or oval-shaped lipid globules in the cytoplasm. The lipid globules often conceal the nucleus. Cytoplasm may also contain other non-lipid granules.



(a) (b) (c)

(d) (e) (f)

Fig. 3.8: (a) Prohemocytes; (b) Plasmatocytes; (c) Granulocytes; (d)Spherulocytes; (e) Oenocytoids; (f) Adipohaemocytes

SAQ 3

i) Fill in the blanks:

a) High concentrations of ……………. acids are present in the haemolymph.

b) Insects use K^+, Na^+, or Ca^{2+} along with a ……………. counter ion in their blood plasma.

c) Compared to vertebrate blood, insect contains relatively high concentrations of proteins and low concentration of ………… ratio in haemolymph.

d) Lymph glands, where haemocytes are produced are known as ……………. .

e) Prohemocytes are also known as ……………. cells.

f) Granulocytes are characterized by the presence of many intracellular ……………. .

ii) Underline the correct answer:

a) Major blood sugar of insect is

i) Glucose ii) Sucrose iii) Trehalose iv) Fructose

b) The haemocytes are……............ in origin.

i) ectodermal ii) mesodermal
iii) endodermal iv) both ectodermal and mesodermal

c) ………………. and …………… are known as immunocytes.

i) oenocytoids and granulocytes

ii) plasmatocytes and granulocytes

iii) plasmatocytes and spherulocytes

iv) coagulocytes and granulocytes

d) Humoral immunity is synonymous to …………….

i) cell free immunity ii) cellular immunity
iii) innate immunity iv) acquired immunity

e) Haemocytes are produced in …………….

i) fat bodies ii) endocrine gland
iii) lymph glands iv) accessory glands

3.6 FUNCTIONS OF HAEMOLYMPH

Haemolymph carries out the important functions in insect as discussed here:

1. ***Lubricant for tissues***: Haemolymph flows freely within the body cavity of insects where it makes direct contact with all internal tissues and organs, thus acts as lubricant.

2. ***Hydraulic medium***: Hydrostatic pressure generated by the haemolymph is used to facilitate in eclosion, moulting, expansion of body and wings after moulting, physical movements (especially in soft-bodied larvae), reproduction (e.g. insemination and oviposition), evagination of certain types of exocrine glands(osmeteria in papilionids) and maintenance of body shape, etc.

3. ***Transport medium***: Haemolymph acts as a transport medium for many molecules viz., nutrients, hormone and metabolic wastes. However, it has no oxygen transporting function like the blood of vertebrates except Chironomid larva.

4. ***Storage***: The haemolymph stores amino acids, glycerol, water and raw materials required for histogenesis.

5. ***Maintenance of osmotic pressure***: Ions, amino acids and organic acids present in the haemolymph help in maintaining osmotic pressure required for normal physiological functions.

6. ***Connective tissue formation***: Haemolymph provides lipoproteins that are necessary for the formation of the connective tissue.

7. ***Protection***: It helps in phagocytosis, encapsulation, detoxification, coagulation, and wound healing. Non cellular components like lysozymes also kill the invading bacteria.

8. ***Reflex bleeding***: In some insects, emission of blood occurs through the pores (or) slits of the cuticle/legs which mainly helps the insects for getting protection from their natural enemies. This is called *reflex bleeding*. Haemolymph that is fortified with defensive terpenoids such as cantharadin may be released outside the body through intersegmental membranes to discourage predators. Cantharadin produced by certain beetles, using reflex bleeding, is a powerful antifeedant compound to various predators and vesiculating compounds.

9. ***Thermoregulation***: The use of the haemolymph in thermoregulation of flying insects was firstly described by Bernd Heinrich. The heat is transferred to other parts of the body in bees *via* haemolymph. It provides heat for flying, for brooding eggs and larvae, to kill a predator or for maintaining hive temperature in winter. This will be discussed in detail under thermoregulation in subsequent sections.

10. ***Tidal flow of haemolymph***: Some extensive flyers, including some Diptera, Hymenoptera, and Lepidoptera, have a special circulatory condition. The amount of haemolymph is reduced to keep body weight at a minimum, and the volume is replaced by large tracheal sacs. The body

is often divided by an anatomical constriction between thorax and abdomen into two hemocoel compartments and may be additionally separated by a valve. The haemolymph in these insects is not circulated in the classical way but is transported back and forth between thorax and abdomen by heartbeat reversal. The shift in haemolymph flow causes an alternate periodic increase and decrease in haemolymph volume in both compartments with a compensatory volume change in the tracheal system. This leads to ventilation especially of the large elastic tracheal sacs. Moreover, the haemolymph in the wings oscillates in all veins simultaneously in correlation with heartbeat reversal and the resulting volume changes in the wing tracheae. This periodic exchange of air and haemolymph in these insects is known as tidal flow of haemolymph.

11. ***Immunity***: The haemolymph provides both non-cellular (humoral response) and cellular immune response. This will be discussed in detail in next section.

SAQ 4

Fill in the blanks:

i) …………………… pressure generated internally by haemolymph is used to facilitate eclosion and moulting.

ii) Haemolymph provides lipoproteins that are necessary for the formation of the …………………… tissue.

iii) Ions, amino acids and organic acids present in the haemolymph helps in maintaining ………………… pressure required for normal physiological functions.

iv) …………………… produced by certain beetles, using a reflex bleeding, is a strong antifeedant.

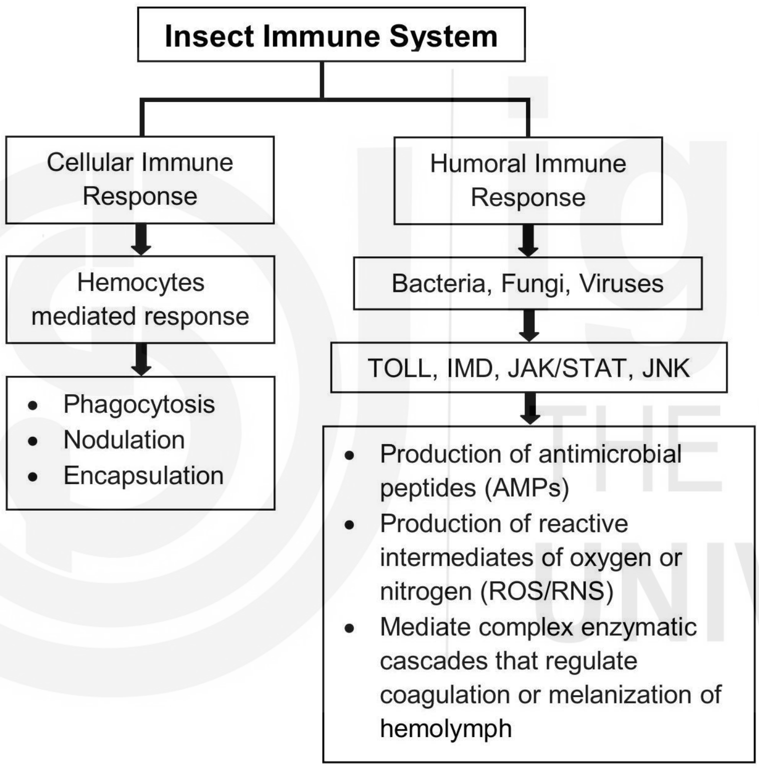
v) Haemolymph acts as ………………… for tissues and organs of insects.

3.7 INSECT IMMUNE SYSTEM

Insect haemolymph is laden with several nutrients, the composition of which varies based on the metabolic needs of active tissues. It is also the medium that carries the various hormonal signals to different tissues. Since it serves as a medium for supplying these nutrients to various organs and tissues that it bathes, it would be a great place for a pathogen to live. As a result, insects have developed various mechanisms to fight off pathogens, including an immune system. The insect immune system is mediated by the haemocytes circulating in the haemolymph and humoral responses (Box 3.1).

The defense mechanism of any organism, known as ***immunity,*** can be **innate** or **adaptive.** At the beginning of pathogenic infection, early defense mechanisms of insects include expression of antimicrobial products, recognition of microorganisms by pattern-recognition receptors (PRRs) and activation of phagocytic cells, which are collectively known as ***innate immune systems.*** In vertebrates, such as mammals, cells facilitate the recognition of microorganisms even at later times during the course of an infection with specific receptors for microbial antigens. The T- and B-lymphocytes are the cells responsible for the specific recognition of pathogenic antigens and together provide a more selective defense system, known as ***adaptive immunity*****,** which provides a much better and faster response to the same pathogen during a second challenge. *Although insects lack an adaptive immune system, they do have a powerful innate immune system for fighting infections. Insects have developed this powerful innate immune system against invading microorganisms during their evolution.*

Box 3.1: Insect Immune System.



The protection against pathogens begins primarily with certain barriers such as cuticle, gut and trachea, tissues that are difficult to be penetrated, while immune response is originated by the fat body and the haemolymph. **Fat body is the largest organ of the haemocoel and is a major site for the production and secretion of antimicrobial peptides (AMPs).** Upon infection, cellular immune responses are engaged almost immediately; while humoral responses take place several hours later. It is believed that invading microorganisms are first eliminated by haemocytes and later the humoral responses remove the microorganisms not eliminated by cells. These defense mechanisms do not work independently from each other, rather there is an effective cross-talk between humoral and cellular immunity in insects. The first step for the initiation of immune response, either humoral or cellular, is the recognition of the pathogen. This is achieved by the pattern recognition proteins/receptors (**PRPs**), that recognize and bind conserved domains (patterns) located on the pathogen surface (bacteria, fungi), which are called pathogen-associated molecular patterns (**PAMPs**).

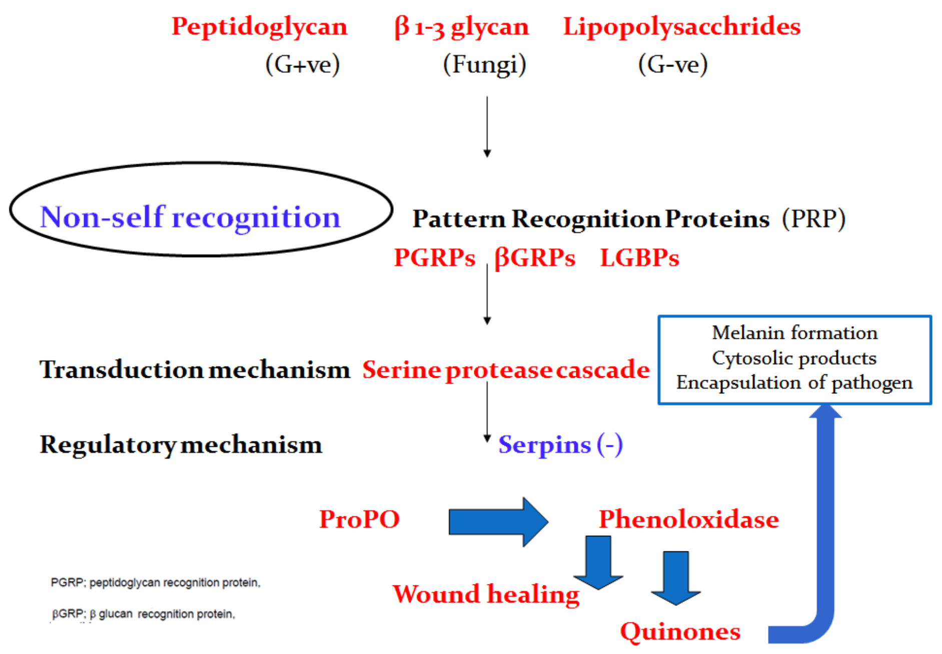
PRPs are primitive part of the immune system. They are **proteins** expressed by cells of the innate immune system to identify PAMPs, which are associated with microbial pathogens or cellular stress (e.g. lipopolysaccharides, peptidoglycans), as well as damage-associated molecular patterns (DAMPs), which are associated with cell components released during cell damage. They are also called primitive pattern recognition receptors because they are an early part of the immune system to evolve, before adaptive immunity. The most characterized **PRPs** are the type C lectins, the peptidoglycan recognizing proteins, the β-1,3-glucan proteins, the hemolin and the integrins. These proteins are present on the plasma membrane of fat body cells and haemocytes or they are soluble in the haemolymph. They bind on lipids and carbohydrates which are synthesized by microorganisms and are exposed on their surface, such as lipopolysaccharites (LPS) of Gram negative bacteria, lipoteichoic acids and peptidoglycans of Gram positive bacteria and β-1,3-glucans of fungi (Box 3.2).

3.7.1 Cellular Immune Response

This is a host defense response against the invasions of parasites, pathogens, and any foreign object that involves cells (haemocytes). If successful, the response results in:

- Phagocytosis
- Nodule formation
- Encapsulation and Melanotic encapsulation

Box 3.2: Pro-phenol Oxidase Activation Cascade.



Phagocytosis: Phagocytosis refers to the process of cellular internalization of foreign invaders. The plasmatocytes are the first line of defense against small pathogens, such as bacteria and yeast, which are phagocytosed by them. Microbes are secondarily killed by lysosomes after phagocytosis.

Nodule formation: In case of infection by large number of pathogens, the haemocytes do not consume them, instead form nodules. The pathogens aggregate and get localized by humoral compounds. They are sometimes surrounded by sclerotized protein secreted from haemocytes or the damaged cells involved in defense, resulting in melanization. Thus, nodules are aggregates of hemocytes that entrap invading microbes. This is a particularly effective means of clearing the haemolymph, and it is considered to be of greater importance than phagocytosis in clearing large doses of bacteria. There appears to be a sequence of trapping pathogens by granulocytes, followed by plasmatocytes forming a layer around the clumped melanized and dead cells. The precise sequence of events is still uncertain. The bacteria, viral polyhedrons, fungal spores and protozoans can be trapped in hardened and sclerotized nodules.

Encapsulation: When organisms or clumps of organisms are too big to be phagocytized or to form nodules, such as nematodes, then the insects evoked another immune response, referred to as encapsulation. Encapsulation is the sequesterization of these organisms or clumps with multilayered aggregates of haemocytes. In other words, it is similar to nodule formation but involves large number of haemocytes and produce larger melanized structure (*Melanized encapsulation*). The melanized capsule isolates the pathogen from the nutrients and oxygen due to which they die inside the protective case.

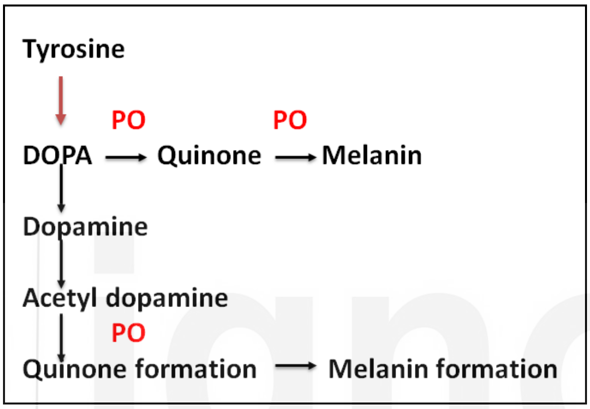
The melanization process in insects is represented by a cascade of chemical reactions catalyzed by phenoloxidase (PO). An initial stage of this process is the hydroxylation of tyrosine to 3,4dihydroxy-L-phenylalanine (DOPA) followed by the formation of insoluble polymer melanin (Box 3.3).

Coagulation: In case of any damage to the epidermis, granulocytes and oenocytoids are attracted to the sites of wounds, where they play major roles in clot formation and stop bleeding. In the area of the damage, granulocytes release material which forms gel. It gets stabilized by plasma lipophorins and plugs the wound. While the oenocytes release phenoloxidase that sclerotize and form a plug sealing the break in the cuticle. Plasmatocytes also migrate in the area of the wound, mop up debris by phagocytosis and link to each other forming a continuous tissue.

3.7.2 Non-cellular or Humoral Immune Response

As mentioned earlier, once a microorganism is detected by PRRs, a series of signaling molecules is activated inside cells to instruct them for different responses. These molecules follow particular signaling pathways. The humoral immune responses mainly involve the release of AMPs by the fat-body, *via* the Toll signaling, the immune deficiency (Imd), and the JAK-STAT pathways. *Gram-positive bacteria and fungi predominantly induce the Toll signaling pathway, whereas Gram-negative bacteria activate the Imd pathway.* The humoral immune response is based on the products of characterized immune genes induced by microbial infection and encode antimicrobial peptides such as cecropins, attacin, lysozyme, defencins, dipteracin etc., which are synthesized predominantly in fat body and released into haemolymph. Haemocytes and, epithelial layers of the integument and the gut are also sites for the synthesis of such molecules. These genes are either not expressed or/are constitutively expressed at a low rate prior to infection. In addition, humoral immune responses include activation of enzymic cascades that regulate coagulation and melanization of haemolymph, and production of reactive oxygen and nitrogen species (Box 3.1 and Box 3.2).

Box 3.3: Pathway: Melanin formation during an immune response.



SAQ 5

i) Fill in the blanks:

a) Fat body is responsible for the synthesis of ……………….. .

b) Pattern recognition proteins are expressed by cells of the innate immune system to identify ……………….. .

c) Activation of enzymic cascades that regulate coagulation and melanization of haemolymph is a part of …………………. defense response.

d) Granulocytes and …………………… are attracted to sites of wound for coagulating haemolymph to stop bleeding.

e) The melanization process in insects is represented by a cascade of chemical reactions catalyzed by ……………….. .

ii) Underline the correct answer:

a) Bacterium is ………………… in phagocytotic vesicle.

I) Fused II) Engulfed III) Absorbed IV) None of these

b) ………………… are the primary phagocytotic cells.

I) Granulocytes II) Plasmatocytes
III) Spherulocytes IV) Coagulocytes

c) Immune response to large-sized foreign objects leads to …………

I) Nodule formation II) Phagocytosis
III) Encapsulation IV) Melanization

d) Which of the following is *not* a component of an insect's defense against infection?

I) a protective exoskeleton

II) activation of natural killer cells

III) phagocytosis by hemocytes

IV) production of antimicrobial peptides

e) All the components of the insect immune system originate from.

I) Ectoderm II) Endoderm III) Mesoderm IV) All of these

3.8 THERMOREGULATION

Insects are *poikilotherms* (ectotherm) and their body temperature varies within a wide range, usually as a result of variation in the environmental temperature. However, insects have the ability to maintain a stable temperature (either above or below ambient temperature), at least in a portion of their bodies by physiological or behavioural mean, which is called *temperature regulation*, or *thermoregulation*. Though several insects are *ectotherms* (in which their heat source is primarily from the environment), some are *endotherms* (that can produce heat internally by biochemical processes). These endothermic insects are better described as *regional heterotherms* because they are not uniformly endothermic. When heat is being produced, different temperatures are maintained in different parts of their bodies, for example, moths generate heat in their thorax prior to flight but the abdomen remains relatively cool.

The majority of flying insects produce more heat than other animals because of their higher rates of metabolism based on body weight. However, small body size of insect typically prevents noticeable endothermy due to high rates of cooling. Small flying flies' body temperature is probably close to ambient temperature, while flying butterflies and locusts have body temperatures 5° to 10℃ higher than the ambient temperature.The first evidence for insect thermoregulation in flight came from experiments in moths demonstrating that dissipation of heat occurs *via* haemolymph movement from the thorax to the abdomen. The heart of these moths makes a loop through the center of the thorax facilitating heat exchange and converting the abdomen into both a heat sink and a heat radiator that helps the flying insect in maintaining a stable thoracic temperature under different ambient temperature conditions. It was believed that heat regulation was only achieved by varying heat loss until evidence for varying heat production was observed in honeybees. Many moths and bumblebees are insulated with scales and hair, and their metabolism during flight can cause the temperature of the flight muscles to increase 20° to 30℃ above ambient temperature.

It's interesting to note that insects, due to their size and insulation, retain the most heat in their thorax during flight as they need the highest muscle temperature to sustain their ability to fly. While the maximum temperature fluctuates across a relatively small range of 40° to 45°C, the minimum muscle temperature required for flight varies greatly between species. Because of this, insects that must produce high muscle temperatures in order to fly need to keep their thoracic temperature within a very small range. Certain large moths may remain active in a wide range of ambient temperatures due to active heat loss from the thorax to the abdomen, which keeps the flight motor from overheating. In a similar manner, bumblebees carry heat into their abdomen from their flight muscles while incubating their brood by abdominal contact.

The "flight" muscles are frequently engaged in patterns during this "shivering" that differ from those during flight. Rather than on the wings, the muscles contract mainly against each other. Nevertheless, rather than the patterns of activation, the action potential frequency is the primary determinant of the rate of heat production during shivering and flight. If the larger insects didn't actively warm up their flight muscles before taking off, many of them would stay on the ground. Male tettigoniid grasshoppers raise their thoracic temperature before they sing. Nocturnal dung beetles, increase their ball-making and ball-rolling velocity when their thoracic temperature increase as dung is a valuable resource for these beetles, allowing them to find mates and feed their larvae. Japanese honeybees produce enough heat to kill the predatory wasp. Furthermore, some social Hymenoptera deliberately contract their "flight" muscles in order to generate heat both before take off and during nest temperature maintenance.

For certain insects that visit flowers, thermoregulation plays a crucial role in their energetics of foraging. They can visit more flowers in a given amount of time if their muscle temperature is higher. Bees may expend comparatively high amounts of energy for thermoregulation during periods of abundant food availability. Bumblebees often use up energy at rates comparable to those they need during flight when they shiver to keep their body temperatures up during the brief moments they are perched on flowers (and in the nest).

3.9 SUMMARY

Let us summarise whatever you have learnt so far:

- Insect has an open circulatory system which consists of the haemocoel, filled with haemolymph. It is divided into three sinuses by muscular dorsal and ventral diaphragms.
- The dorsal vessel is the principal blood conducting organ in insects, extending along the mid dorsal line just beneath the integument from the hind end through the thorax to the head.
- It has two regions: the anterior aorta and the posterior heart consisting of many chambers. The alary muscles project laterally from the dorsal vessel in each abdominal segment.

- In addition to the dorsal vessel, insects have accessory pulsatile organs (auxiliary hearts) that supply haemolymph to body appendages. In most insects, haemolymph flow is unidirectional, with the dorsal channel contracting at the posterior end and moving forward in a peristaltic wave.
- Circulation of haemolymph occurs in two phases (Diastole and Systole) due to the contraction and expansion of alary muscles and heart wall muscles.
- Haemolymph, the only extracellular fluid in the insect body, makes up 5-40% of the total body weight and 15-75% volume. It is transparent and can have a green, yellowish, or bluish tint depending on the insect's sex, stage, and age.
- Haemolymph consists of liquid plasma or serum and blood cells or haemocytes, produced in lymph glands or haemopoietic organs. There are six main morphologically distinct haemocytes: prohaemocytes, plasmatocytes, granular cells, spherule cells, oenocytoids, and adipohaemocytes.
- Plasma is composed of 85% water, inorganic salts, and organic compounds, with high concentrations of amino acids, proteins, sugars, inorganic ions, low Na/K ratio, and high uric acid content.
- Haemolymph is a vital component of insects acting as a lubricant for tissues; a hydraulic medium; a transport medium for nutrients, hormones, and metabolic wastes; and a storage medium. It also provides lipoproteins for connective tissue formation and protection through reflex bleeding. Haemolymph can help in thermoregulation by conducting excess heat away from active flight muscles or by collecting and circulating heat absorbed while basking in the sun.
- Insects have evolved cellular and molecular defence mechanisms against microbial infections, known as immunity. Insects lack an adaptive immune system but have a powerful innate immune system for fighting infections. The protection against pathogens begins with certain tissues such as the cuticle, gut, and trachea, while immune response originates from the fat body and the haemolymph.
- There are two components of the insect immune system: cellular defense response and humoral defense response. Cellular defense response results in phagocytosis, nodule formation, and encapsulation. Humoral defense response is based on the products of characterized immune genes induced by microbial infection, which encode antimicrobial peptides such as cecropins, attacin, lysozyme, defencins, and dipteracin.

3.10 TERMINAL QUESTIONS

1. What is the difference between closed and open circulatory system?
2. Write short notes on the following:
    a) Haemocoel
    b) Alary muscles

c) Dorsal vessel

d) Accessory pulsatile organs

e) Thermoregulation

f) Cellular immune response

3. What is dorsal aorta? Write about mechanism of circulation in insects.

4. What is haemolymph? Discuss in detail about the components or functions of haemolymph.

5. What is immunity? Write about cellular and humoral immunity.

3.11 ANSWERS

Self-Assessment Questions

1. i) a) dorsal vessel; b) head; c) nerve cord; d) pericardial sinus; e) abdomen; f) neurogenic.

   ii) a) II; b) IV; c) II; d) II; e) I; f) I.

2. a) systole; b) ostia; c) diastole; d) phylogenetically ancestral; e) diastasis.

3. i) a) amino; b) Cl^-; c) Na/K; d) hematopoietic organs; e) stem; f) granules.

   ii) a) iii; b) ii; c) ii; d) i; e) iii.

4. i) Hydrostatic; ii) connective; iii) osmotic; iv) cantharidin; v) lubricant.

5. i) a) mesoderm; b) pathogen-associated molecular patterns; c) humoral; d) oenocytoid; e) phenoloxidase.

   ii) a) II; b) II; c) III; d) II; e) III.

Terminal Questions

1. Refer to Section 3.1.

2. a) Refer to Subsection 3.2.1.

   b) Refer to Subsection 3.2.3.

   c) Refer to Subsection 3.2.2.

   d) Refer to Subsection 3.2.4.

   e) Refer to Section 3.8 Refer to Subsection 3.2.1.

   f) Refer to Subsection 3.7.1.

3. Refer to Sections 3.2 and 3.3.

4. Refer to Sections 3.5 and 3.6.

5. Refer to Section 3.7.

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UNIT 4

RESPIRATORY SYSTEM

Structure

4.1 INTRODUCTION

Majority of the eukaryotic animals depend on oxygen to liberate energy from the food resources they consume. They do it by oxidizing carbohydrates, amino acids, and fatty acids to produce ATP in their mitochondria. Since mitochondria are cellular organelles, oxygen needs to be delivered to the cells from the external environment. As a byproduct of mitochondrial oxidation, carbon dioxide is liberated, which has to be eliminated from the cells. This process of obtaining oxygen from the external environment and eliminating carbon dioxide accumulated in the cells back into the environment is called respiration.

Oxygen and carbon dioxide in solution are capable of diffusion down a concentration gradient. In the case of unicellular and small multicellular organisms, diffusion alone is sufficient for the exchange of gases. However, in the case of larger organisms such simple diffusion is insufficient to meet their respiratory requirements. Hence, they have various structures and organs for this purpose. For example, reptiles, birds, and mammals have lungs and associated structures; fishes and crustaceans have gills; spiders have book lungs; and insects have trachea. In this Unit, you will know about various respiratory structures found in insects.

Objectives

After studying this Unit, you should be able to:

- explain various systems of respiration in insects,
- discuss the structures of tracheae, tracheoles ,and spiracles,
- describe the role of respiratory pigments in insects,
- distinguish respiratory mechanisms of terrestrial insects from aquatic insects,
- appreciate the diversity of gaseous exchange strategies in aquatic insects,
- depict the structural details of various types of gills and plastrons, and
- explain the physiology of gaseous exchange in terrestrial and aquatic insects.

4.2 RESPIRATORY SYSTEM IN INSECTS

Insects are adapted to both terrestrial and aquatic habitats, while some insects are endoparasites. Consequently, their respiratory systems also show adaptive modifications. The majority of the insects have a system of air-filled internal tubes, known as the **tracheal system**, as their respiratory structures. Many apterygote insects belonging to the orders Protura and Collembola lack a tracheal system altogether. Similarly, the tracheal system is absent in some dipteran and hymenopteran endoparasites. The tracheal system in most of the insects (all terrestrial insects and some aquatic insects) opens to the exterior through small openings called **spiracles** (Fig. 4.1). Those aquatic insects without spiracles exchange gases with the tracheal system through thin cuticle. Larval forms of the majority of aquatic insects have gills, in addition to the tracheal system. Common examples are mayfly nymphs (Order Ephemeroptera) and dragonfly nymphs (Order Odonata).Some aquatic insects use air bubbles and plastrons as supplementary respiratory structures.

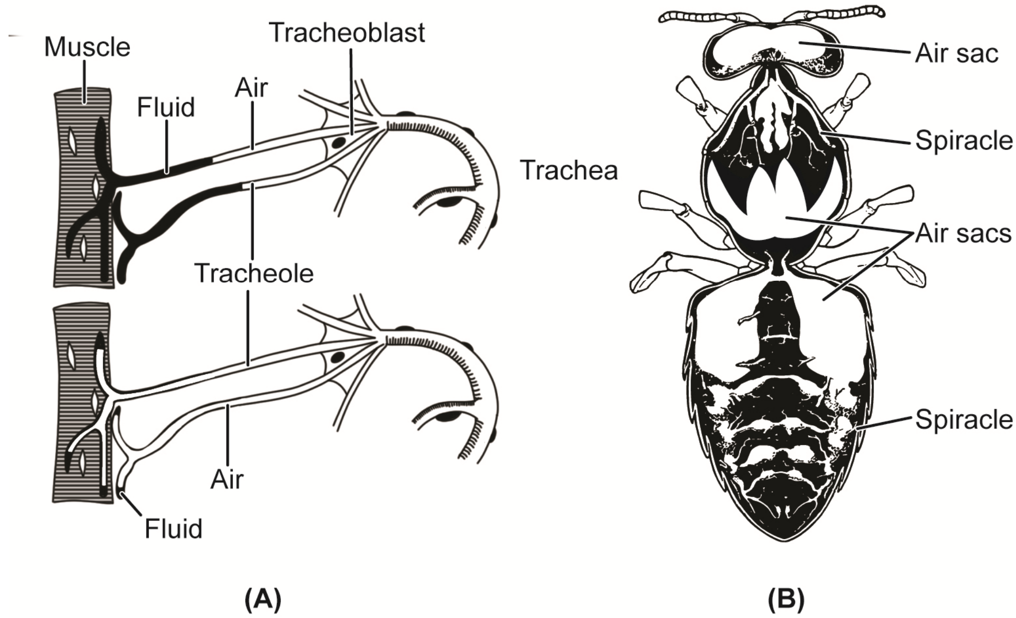

Fig. 4.1: A) Tracheoles in close contact with muscles and B) Air sacs in the honey bee

4.3 TRACHEAL SYSTEM

The tracheal system in insects consists of two types of tubes, the larger tracheae and the smaller tracheoles. Both the tubes are ectodermal structures. Structurally there are a few differences between them.

4.3.1 Structure and Function of Trachea

Tracheae are formed by the invagination of the ectoderm and run inwards from the spiracles. As they run inwards, tracheae branch into finer tubules, the smallest of which are approximately 2 µm in diameter. While the smallest branches are made up of single cells, larger ones are multicellular. The interior of the tracheae is lined by a cuticular intima, which consists of an outer epicuticle and an inner layer of either protein or chitin. The intima is modified into a spiral thickening known as **taenidium** (plural taenidia) and runs along each trachea.

While some tracheae remain expanded always, others are collapsible providing ventilation. The main function of tracheae is to transport air from the spiracles if they are present or from the permeable cuticle to the tracheoles. Since their walls are thick, they do not serve the function of delivering oxygen to the cells directly. However, they are thought to assist in the transport of carbon dioxide in the opposite direction because they are permeable to carbon dioxide.

During moulting, the cuticular lining of the tracheae is shed and replaced by a new cuticle. In some insects, the tracheae are expanded as **air sacs** which help in ventilation. They are either devoid of taenidia or have poorly developed taenidia. The tracheae from neighbouring spiracles very often (in the majority of insects studied) join to form longitudinal trunks running the entire length of the body. Hence, there is a lateral trunk on either side of the body connecting the spiracles. The lateral trunks are very often connected by transverse commissures in each segment of the insect (Fig. 4.2). Longitudinal trunks are often found along the gut, on either side of the heart, and also nerve cord. From these longitudinal trunks tracheae branch to the nearby tissues..

4.3.2 Structure and Functions of Tracheoles

Compared with tracheae, tracheoles are much finer tubules. They are formed as extensions of tracheolar cells and are found originating at various points along the length of the tracheae. Tracheoles are air-filled tubes and are 0.03 to less than 2.0µm in diameter. They are formed in specialized cells derived from the epidermal cells lining of the tracheae known as **tracheolar cells.**

Similar to tracheae, they are also lined with intima but the tracheolar intima lacks the underlining layer of protein/chitin. Though the taenidial ridges are formed from the intima they are without protein/chitin matrix. Tracheoles are the major sites of gas exchange in the tracheal system. Due to the thin walls and high surface-to-volume ratio, they have very high diffusing capacity. Unlike tracheae, some tracheoles retain their cuticular lining during moulting. The number of tracheoles varies from tissue to tissue. It is believed that the tissues with higher metabolic demands have more tracheoles. For example, flight muscles have the highest number of tracheoles.

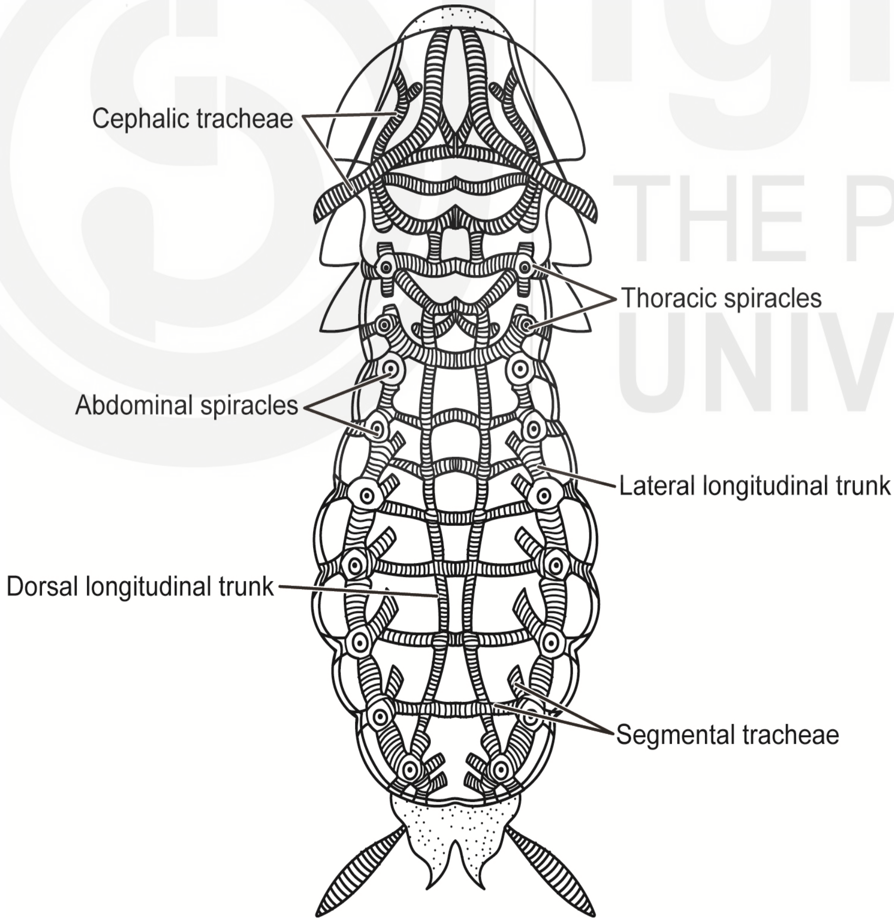

Fig 4.2: Tracheal system of Cockroach

In newly hatched insects, the tracheal system is filled with a liquid. This liquid is later replaced by gas. This process of replacement of liquid by gas is known as **pneumatization.**

4.3.3 Structure and Function of Spiracles

Spiracles are the gatekeepers of the tracheal system. They are small openings found on the lateral side of thoracic and abdominal segments. One segment never possesses more than one pair of spiracles. In an ideal situation, there are two pairs of spiracles on thoracic segments and eight on abdominal segments. However, the number tends to vary from one (mosquito larvae)to ten (dragonfly nymphs).

1. **Number and distribution of spiracles:** With the exceptions of some dipluran insects, the maximum number of spiracles found in insects in ten pairs, two thoracic and 8 abdominal. On the basis of the number and distribution of spiracles, the respiratory system is classified as:

    a) ***Polypneustic*****:** At least 8 pairs of functional spiracles. *Holopneustic* – 10 pairs of spiracles (1 mesothoracic, 1 metathoracic and 8 abdominal), e.g., cockroaches; *Peripneustic* – 9 pairs of spiracles (1 mesothoracic, 8 abdominal), e.g., some fly larvae; *Hemipneustic* – 8 pairs of spiracles (1 mesothoracic, 7 abdominal), e.g., some fly larvae.

    b) ***Oligopneustic*****:** 1 or 2 pairs of functional spiracles. *Amphipneustic* – 2 pairs of spiracles (1 mesothoracic, 1 post abdominal), e.g., larvae of moth flies; *Metapneustic* – 1 pair of functional spiracles (post abdominal), e.g., mosquito larvae; *Propneustic* – 1 pair of spiracles (mesothoracic), e.g., dipteran pupae.

    c) ***Apneustic*****:** No functional spiracles, e.g., chironomid larvae.

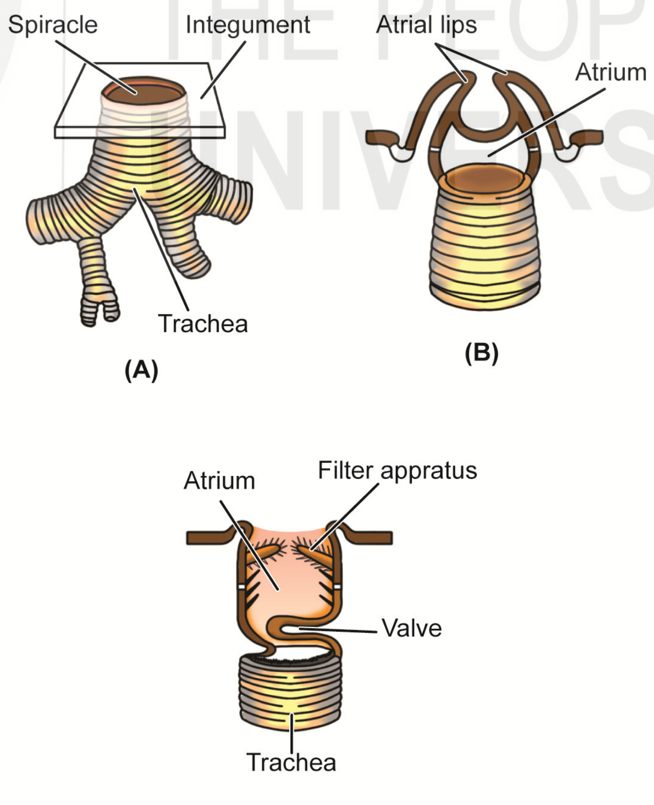

Fig. 4.3: Types of spiracles. A) Simple non-atriate type, B) Atriate type with lip closure mechanism and C) Atriate type with filter apparatus and valve closing mechanism

2. **Types of the spiracles:** The spiracles are of two basic types: simple and atriate. The simple type of spiracle (Fig. 4.3 A) is only an opening to the tracheal system. The atriate type is formed as a result of the invagination of the primitive spiracular opening. Thus, in the fully developed atriate spiracle (Fig. 4.3 B, C) the tracheal opening lies at the bottom of a spiracular chamber, or atrium. In this type the external opening is called as the atrial aperture or orifice.

3. **Structure and closing/opening of spiracles:** The tracheal system readily allows the passage of water and due to this the insects may lose water very rapidly. To prevent the loss of water, the insects have evolved various types of spiracular closing mechanisms. Principally two types of closing mechanisms are observed, lip type (folds of the integument form opposing lips, Fig. 4.3 B) and valvular type (two movable valves lie at the inner end of the atrium, Fig. 4.3 C). Irrespective of the type of mechanism, closure is carried out by contraction of the associated muscles (Fig. 4.4). Most often the atrial wall is lined with tiny hair, which form a felt chamber that filters out dust (Fig. 4.5). In flies, beetles and moths the spiracle is covered by a sieve plate having large numbers of fine pores that not only prevents the entry of dust but also the water (in aquatic insects) in the tracheal system. The spiracle is also associated with certain glandular tissue that secretes lubricants for the movable parts of the spiracular closure mechanism. The lubricants may prevent water from entering the tracheae, and may improve the seal of the closure mechanism.

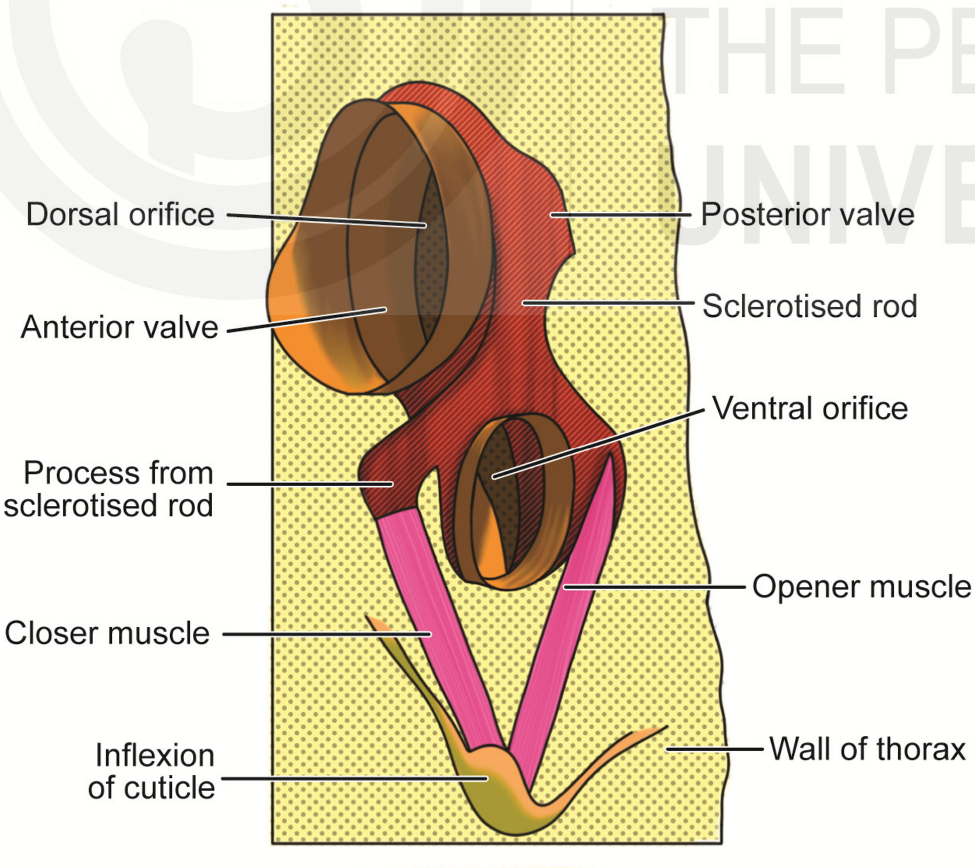

Fig. 4.4: Interior view of the first thoracic spiracle of locust

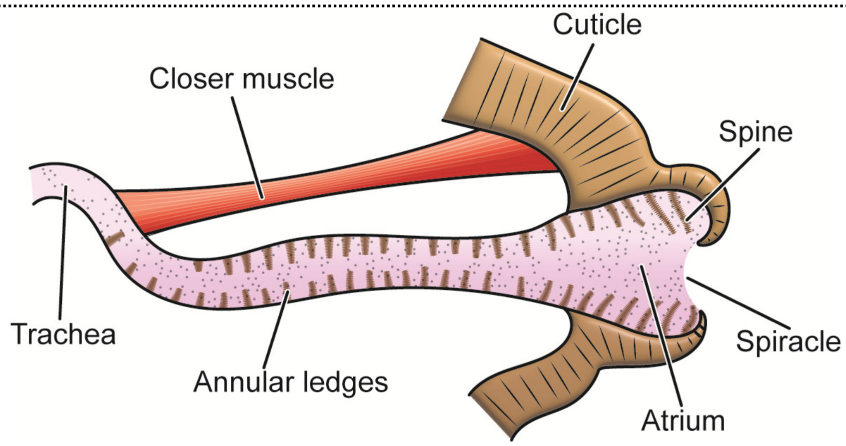

Fig. 4.5: Longitudinal section of the spiracle of a louse showing the dust catching spines

Regulation of respiration: Spiracles play an important role in the regulation of respiration by their opening and closing movements. The concentration of oxygen in the tracheal air and that of carbon dioxide in the tissues influences the opening and closing of spiracles.

Low oxygen concentration and high carbon dioxide concentration lead to the opening of spiracles for a longer time. This is believed to be due to the modification of motor discharge to the spiracular muscles from the ganglia of the ventral nerve cord under the influence of the concentrations of gases. Besides the changes in gas concentrations, other conditions like starvation, injury, water balance, and temperature also influence spiracular movements and hence respiration.

SAQ 1

i) Fill in the blanks by choosing the correct word from the parentheses.

a) Tracheae in insects are the ingrowths of ……………….. . (Ectoderm/Mesoderm)

b) If an insect has 9 pairs of spiracles, it is an example of …………… respiratory system. (Holopneustic/Heteropneustic)

c) Atrium is a part of ……………….. . (Tracheae /Spiracle)

ii) State whether the following statements are 'True' or 'False'.

a) Tracheae are present between the spiracle and tracheoles.

b) The cuticular lining of tracheoles is never retained during moulting.

c) The tracheal system is absent in some proturans.

4.4 TYPES OF THE TRACHEAL SYSTEM

Except most collembolans, many proturans, and certain endoparasitic wasp larvae, other insects possess tracheae. Tracheae along with spiracles, air sacs, and tracheoles compose the respiratory or ventilatory system.

The organization of tracheae may be comparatively simple, as in some springtails in which tracheae arise from each spiracle, but do not connect to any other tracheae. However, tracheal organization in most insects is more complex (Fig. 4.6 A). There is typically a pair of lateral longitudinal trunks into which the spiracles open, a similar pair of dorsal longitudinal trunks, and often a pair of ventral longitudinal trunks. The dorsal, lateral, and ventral trunks are connected by more or less dorso-ventrally oriented tracheae, and the longitudinal trunks on either side are connected by transverse tracheal commissures (Fig. 4.6 B).

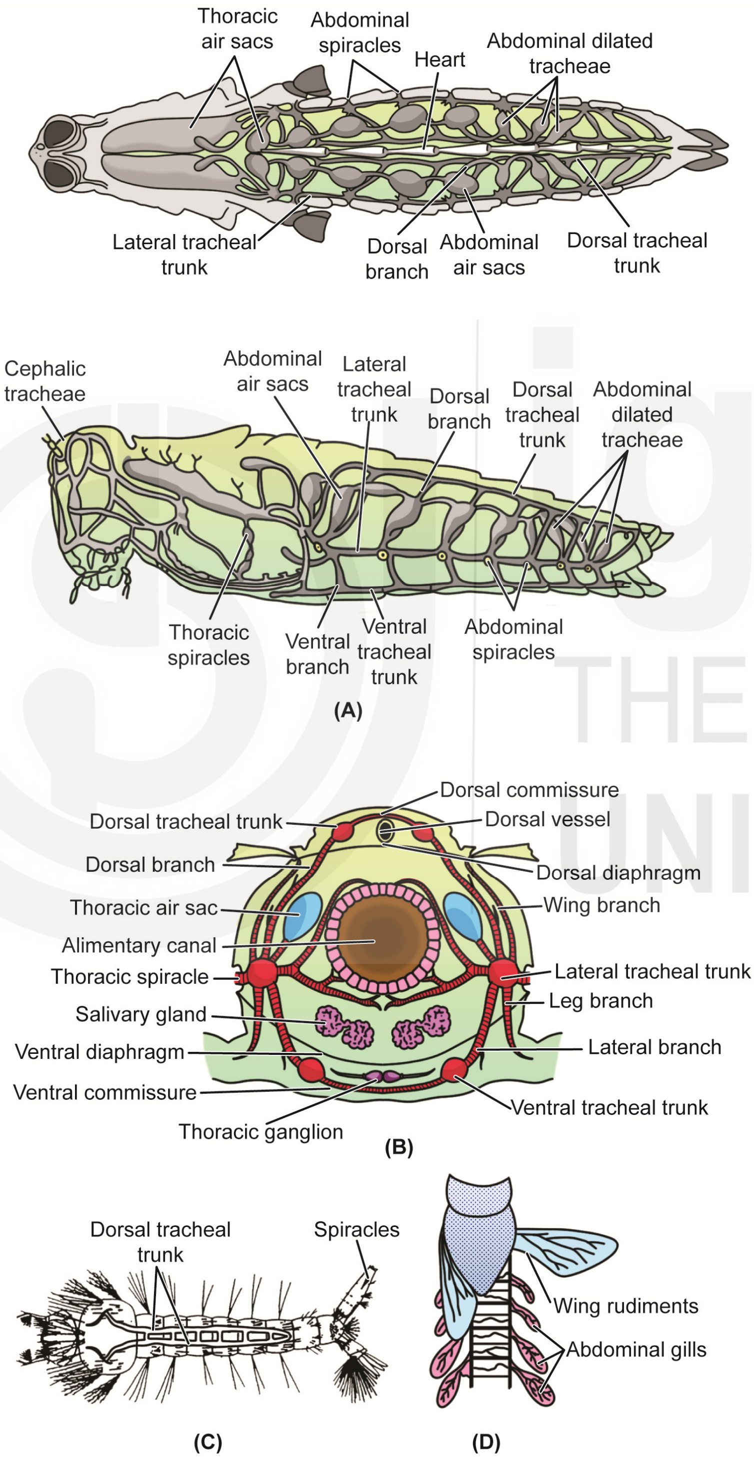

Fig. 4.6: Representative types of tracheal system. A) Dorsal and lateral views of the open type, e.g., grasshopper, B) Cross-section of the thorax showing the major tracheal branches, C) Open type with two spiracles, e.g., mosquito larva, and D) Closed type with no functional spiracles, e.g., mayfly nymph

Although the basic pattern of tracheation is genetically determined, new tracheae and tracheoles can be induced to develop if an insect is reared in an atmosphere with a very low oxygen content. New tracheae and tracheoles do not develop between successive moults, but on demand changes in the distribution can occur at the time of moulting.

Major tracheal branching patterns are species-specific and are often very similar among members of a given family or order. Based on the presence or absence and functional or non-functional nature of spiracles, there are principally two types of tracheal systems, open and closed, with a variety of modifications within each type.

1. **The open tracheal system:** Most of the insects have open tracheal system which is characterised by the presence of one or more pairs of functional spiracles (Fig. 4.6 A, C).

2. **The closed tracheal system:** Many aquatic and endoparasitic insect larvae do not possess functional spiracles and the gaseous exchange occurs directly through the integument, e.g., *Chironomus* larva, mayfly nymph (Fig. 4.6 D).

4.5 RESPIRATORY PIGMENTS IN INSECTS

Respiratory pigments are metalloproteins in animal blood that carry oxygen and thus enhance the efficiency of oxygen transport. The major respiratory pigments in animals are different types of haemoglobin, haemocyanin, and haemerythrin. Hemoglobin is the principal respiratory pigment in vertebrates. It is also found in some nematodes, annelids, crustaceans, and even in some insects. Since the role of blood as a gas transport medium in insects is negligible, the role of respiratory pigments is also not very significant. However, many insects have respiratory pigments either in their blood or in cells. The larvae of *Chironomous* and *Gastrophilus* (Order: Diptera) have haemoglobin in their blood and cells, respectively, which helps them to extract oxygen efficiently from the media containing an extremely low concentration of oxygen. Haemoglobin of both these insects has very high affinities to oxygen compared to that of vertebrates. For example, the haemoglobin of *Chironomus* larvae has two haeme groups, and is 50% saturated with oxygen at less than 0.1 kPa, compared with more than 3 kPa in vertebrates.

Most of the insects have **intracellular hemoglobin** (inside the tracheal cells and fat body). This haemoglobin does not bind to oxygen and thus, its function remains unclear. Many lower insects have haemocyanin in their blood, such as in Collembola, Dermaptera, Orthoptera, Phasmatodea, Mantodea, Isoptera and Blattaria. In the stonefly, *Perla marginata*, haemocyanin accounts for about 25% of the haemolymph proteins and is 50% saturated with oxygen at about 1 kPa, suggesting its use in both oxygen transport and storage.

4.6 GASEOUS EXCHANGE IN INSECTS

Gaseous exchange is the exchange of respiratory gases between the external environment and mitochondria of an organism. Though the majority of insects have tracheal systems as their gaseous exchange systems, there are several modifications in the processes of gaseous exchange according to the environment in which they live. Two major groups of insects with adaptive modifications in their gaseous exchange systems are terrestrial insects and aquatic insects. In this section, we will learn about the diversity of gaseous exchange systems in terrestrial and aquatic insects. Detailed physiology of gaseous exchange will be discussed in the next section.

4.6.1 Gaseous Exchange in Terrestrial Insects

Terrestrial insects could be either with a tracheal system or without one. In the absence of a tracheal system gaseous exchange takes place between the external environment and the haemolymph of the insect through the thin cuticle of the body. In insects with a tracheal system, the transport of oxygen from the environment to the mitochondria takes place in two phases. In the first phase, the atmospheric oxygen enters the tracheal system through the spiracles, moves through the tracheae and tracheoles, and finally reaches the terminal part of the tracheoles. In the second stage oxygen from the tracheoles reaches the mitochondria through the cytoplasm. The former is known as **air-tube transport** and the latter is **tissue-diffusion**. The movement of carbon dioxide takes place in the reverse direction. Insects use two different mechanisms for gaseous exchange-**diffusion** and **convection**. The details will be discussed under the physiology of gaseous exchange.

4.6.2 Gaseous Exchange in Aquatic Insects

Aquatic insects exhibit several diverse mechanisms of gaseous exchange. Based on the source of oxygen we can classify aquatic insects into two broad categories. Those that obtain oxygen directly from the air (Fig. 4.7) and those that depend on dissolved oxygen in water. Examples are mosquito larvae and mayfly nymphs, respectively.

Fig. 4.7: Respiration of a mosquito larva through abdominal siphon

I. Aquatic insects acquiring oxygen directly from the air

Aquatic insects can obtain oxygen either from the atmosphere or from the air-filled aerenchyma of aquatic plants. Most of the insects fall into the former category. The cuticle of aquatic insects may possess hydrofuge properties, which prevents the entry of water into the spiracles. The hydrofuge property may be present in whole cuticle or may be restricted to the region around the spiracles. For example, in larvae of diptera, the area around the spiracles may be oily due to secretion by the perispiracular glands. Sometimes, the hydrofuge properties around the spiracle are associated with hairs, such as in *Notonecta* or valves, such as in mosquito larvae. Hydrofuge hair piles are small, hydrophobic micro hairs. These close when the larvae are in water while open when larvae visit the surface.

(a) **Obtaining air from aerenchyma of plants:** Larvae of the mosquito *Mansonia* obtain oxygen from the aerenchyma in the roots of aquatic plants like *Pistia* and *Eicchornia* (Fig. 4.8). Other examples are larvae of *Donacia* (Coleoptera) and *Chrysogaster* (Diptera), and larvae and pupae of *Notiphila* (Diptera). These larvae have their spiracles on the tip of their spine like abdominal siphons, whereas pupae have them on the thoracic horns.

(b) **Obtaining air from atmosphere:** Most of the insects that obtain oxygen from the air visit the water surface periodically and get oxygen through their spiracles. However, a few of them like the larvae of *Eristalis,* have semi-permanent connections with the air above water so that they need not visit the surface. It has a telescopic terminal siphon which can extend up to 6 cm or more. The larva resides in the bottom mud but takes air through the spiracles.

(c) **Gas exchange *via* air bubble:** Some insects carry an air-filled bubble with them so that they can survive even after the store of air in the tracheal system gets exhausted. These insects open their spiracles into the bubble and obtain oxygen. Some insects carry the air bubble beneath the elytra (*Dytiscus*) or it is held by long hydrofuge hair piles under the wings (*Notonecta*). Sometimes these air bubbles function as physical gills rather than just air storage structures. The mechanism of such physical gills will be discussed later. Besides air bubbles, some insects store thin films of air held by small bristles over the dorsal surface of the forewing (*Notonecta*). In addition, some insects also have large haemoglobin cells inside abdominal spiracles. These cells contain haemoglobin with loosely associated oxygen (*Anisops*). *Anisops* also has ventral and sub-elytral air stores.

Fig. 4.8: *Mansonia* larvae acquiring oxygen from aerenchyma of aquatic plant root

An air bubble acts as a temporary gill, the efficiency of which depends on the oxygen content of the adjacent water. In oxygen-deficient water, the gas tends to pass out of the bubble and will be lost to the insect. Hence, a bubble will be more effective if the oxygen tension of the outside water is high.

II. Aquatic insects acquiring oxygen from water

To obtain dissolved oxygen in the water aquatic insects employ various strategies. The important ones are **cutaneous diffusion**, **gills**, and **plastron**.

a) **Cutaneous diffusion:** In all aquatic insects, some amount of oxygen diffuses through their cuticle. In some larval forms, this is the only mechanism for gaseous exchange. The prerequisites for cutaneous diffusion are the permeability of the cuticle to gases and an oxygen gradient between the external aquatic environment and the tissues of the insects. Oxygen present in a higher concentration in the water diffuses through the permeable cuticle to the cells of the insects with a lower oxygen concentration (Example: *Aphelocheirus* larvae) or the hemolymph (Examples: *Simulium* and *Chironomu*s larvae). Details of diffusion will be discussed in the next section.

b) **Respiration through Gills:** In larger insects, mere diffusion is insufficient to meet the oxygen demand and hence requires additional respiratory mechanisms, especially when oxygen tension in the water is low. Such insects may have closed tracheal system (non-functional spiracles) and obtain oxygen in the trachea through diffusion from the surrounding water which then rapidly diffuses to the tissues. However, gills are the most important structures that augment ventilation. Gills can be either **external** or **internal**. Three types of gills *viz*., **tracheal gills, spiracular gills,** and **blood gills** have been recognized in the larval forms of aquatic insects.

Tracheal gills: The gills covered by very thin cuticle with a network of tracheoles immediately beneath, are known as **tracheal gills**. These are leaf-like (lamellate) as in damselfly nymphs (Fig. 4.9) or filamentous structures as in Caddis fly larvae, and are well supplied with tracheae and tracheoles.

Tracheal gills are found in most of the aquatic larvae and pupae. They could be either external or internal. The position of the external gills varies from insect to insect; caudal in damsel fly, and abdominal in caddis fly. However, the majority of insects have abdominal gills. Internal gills are found in dragonfly nymphs (Order Odonata). These gills are situated in the **branchial chamber** or **branchial basket** (anterior part of the rectum). Water is drawn in and forced out through the anus by the contraction and relaxation of the dorso-ventral muscles in the posterior abdominal segments. The interval between inspiration and expiration varies according to the demand for oxygen.

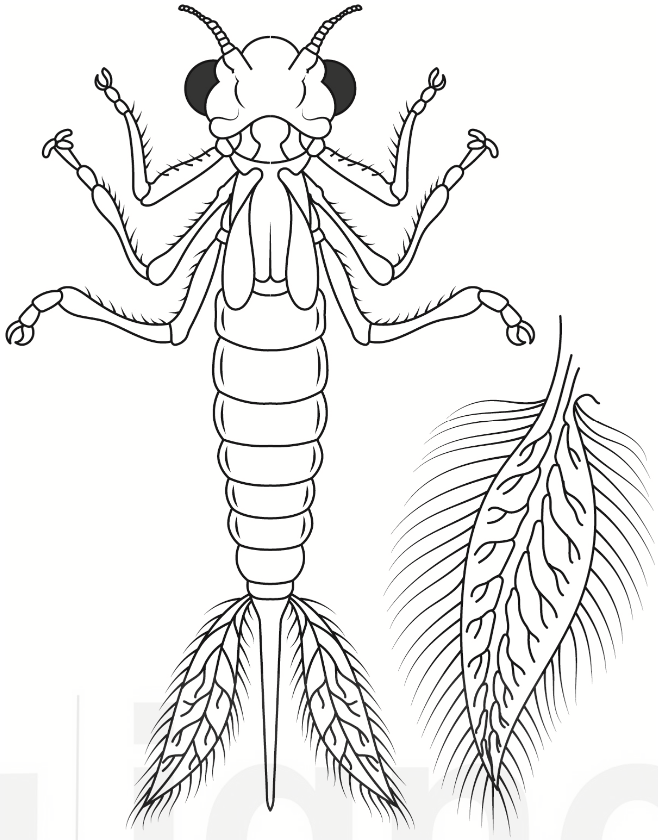

Fig. 4.9: Damselfly nymph

Spiracular gills: These are modified spiracles.Spiracles are drawn out to form long processes. Such gills are found in pupae of several insect families like Psephenidae and Torridincolidae (Coleoptera); Tipulidae, Blephariceridae, Deuterophlebiidae, Simuliidae, and Empididae (Diptera). The spiracular gills are provided with a plastron (discussed below). They are capable of functioning in water as well as outside water.

Blood gills: As the name suggests they contain blood. Tracheae are absent but a few tracheoles may be present. They have a tubular, finger-like structure. Some of them are eversible. They are present in the larvae of many Trichopterans and Chironomids (Diptera).

c) **Plastron respiration:** A plastron is a thin film of air held by some aquatic insects permanently on the outside of the body providing an air-water interface for gaseous exchange. Tracheae of the insects open into the plastron and oxygen can pass directly to the tissues. Plastrons are small in size and do not change their volume during the course of gaseous exchange. Besides, plastrons do not store air but function as gills. The plastrons are held in position by specialized hydrofuge structures, which can resist pressure changes during various activities of the insects. These hydrofuge structures could be a close pile of hairs arranged variously in different insects.

Some insects have very efficient and stable plastrons (Example: adult *Aphelocheirus*) so that they need not come to the water surface at all. Their spiracles open into a series of hair-lined(to prevent water entry) radiating canals in the cuticle which connect with the plastron *via* small pores. Plastrons in several other insects are less efficient. In such insects, plastrons are supplemented by another less permanent structure called **macroplastrons**. A macroplastron has a thick layer of air and is held by longer hairs than those of plastrons (*Hydrophilus*), or by the erection of the plastron hairs (*Elmis*), and is located outside the plastrons. They store air as well as function as physical gills. Unlike plastrons, their volume is not the same always. As the air is utilized by the insects as they submerge, the size of the macroplastron also diminishes.

SAQ 2

i) Fill in the blanks by choosing the correct word from the parentheses.

a) *Chironomous* larvae have ……………… in their blood. (Haemocyanin/Haemoglobin)

b) *Mansonia* larvae obtain oxygen from ……………… (Atmosphere/ Aerenchyma)

c) Oxygen from terminal arterioles moves to cells by ……………… (Diffusion/Convection)

ii) Answer the following questions in one or two sentences.

a) What are tracheal gills?

b) Define a plastron.

c) What are the functions of air bubbles in aquatic insects?

d) What are spiracular gills?

4.7 PHYSIOLOGY OF GASEOUS EXCHANGE

The physiology of gaseous exchange in insects with a tracheal system is different from those without a tracheal system or a liquid-filled tracheal system.

4.7.1 Insects without a Tracheal System or with a Fluid-Filled Tracheal System

As we discussed earlier, there are insects without a tracheal system. Similarly, during certain life stages, the tracheal system is filled with a liquid. In such insects, the respiratory physiology is very simple. Oxygen enters the body fluids directly from the external environment by diffusion. The rate of diffusion of a gas depends on the area available for diffusion, the distance over which diffusion has to occur, the diffusion constant of the gas in a particular medium (air or water), and the concentration gradient of the gas. The relationship is expressed by the equation:

$$J = A \times D \times \Delta C/L,$$

where, J = rate of diffusion, A = area, D= diffusion constant, Δ C= concentration gradient, and L = distance.

The diffusion constant varies with temperature, molecular size, and the medium. It increases with an increase in temperature and a decrease in molecular size. It is 10000 times larger in air than in water. Diffusion is very effective over short distances (up to 1 mm), and hence sufficient for small insects. The permeability of CO_2 is 36 times more than O_2 because of its high solubility. Hence, it travels quickly and is easily eliminated from the tissues to the external environment. This means a system capable of delivering sufficient oxygen to the tissues from the environment will also be capable of eliminating carbon dioxide in the reverse direction.

4.7.2 Insects with Tracheal System

It is convenient to discuss the respiratory physiology of insects with tracheal systems under two broad headings i.e. terrestrial insects and aquatic insects.

I. Terrestrial insects

Terrestrial insects employ two mechanisms for gaseous exchange-**diffusion and convection**.

a) **Diffusion:** As discussed above, diffusion is very effective over distances less than 1 mm. The distance among the terminal tracheoles and the mitochondria in the cells of insects is usually less than 1 mm (ranges from less than 20 µm to 50 µm), and hence diffusion is sufficient for the effective movement of oxygen between these two. Similarly, CO_2 is also diffused out of the tissues without much difficulty as mentioned in the above section. However, as the distance increases between the oxygen source and the tissues, diffusion becomes less effective. In larger insects, the distance between the spiracle and the terminal tracheoles is always more than 1 mm so diffusion alone will not be sufficient for the movement of air. Hence, in addition to diffusion, they use convective ventilation of the tracheal system.

b) **Convection:** Convection is defined as the bulk flow of fluid, driven by a pressure gradient, instead of a concentration gradient as in diffusion. Besides its better efficiency in gaseous exchange, convection has the added advantage of reducing the loss of water. It also helps in that the cells of different parts of the body have similar oxygen and carbon dioxide levels. Hence, not only large insects but even small insects utilize this respiratory strategy. Ventilation by convection occurs through discrete pumping movements. In some insects, the entire gaseous exchange through the spiracles is by convection alone (Locusts), whereas in some others both convection and diffusion are used.

Mechanism of convection

As discussed above, for convection to be operational, there should be pressure gradients in different parts of the tracheal system. It is generated either by the compression of tracheae or the air sacs associated with the tracheal system. **All tracheae are not compressible. Examples of compressible tracheae are longitudinal tracheae and secondary and tertiary tracheae found in the head and thorax of carabid beetles.** Compression of these tracheae produces tidal ventilation. In addition, compression of large air sacs produces further extensive convection. Compression of tracheae and air sacs is produced by various methods. Some of the mechanisms are discussed here:

A. **Abdominal pumping:** Alternative compression and relaxation of the abdomen lead to an increase and decrease of volume and haemolymph pressure in succession. While compression is an active process, relaxation is a passive process. Compression leads to expiration, and relaxation results in inspiration. Different strategies are employed by different insects to change the abdominal volume (Fig. 4.10). It may be by the up and down movement of the tergum (Heteroptera and Coleoptera), up and down movement of both terga and sterna (Odonata, Orthoptera, Hymenoptera, and Diptera), or movement of terga, sterna, and pleura (Lepidoptera).

B. **Haemolymph transfer:** In some lepidopterans and dipterans, ventilation is produced by transferring hemolymph between the abdomen and thorax. When the heart pumps forward, it draws haemolymph from the abdomen and transfers to the thorax and head. This would result in the expansion of air sacs in the abdomen and compression of those in the head and thorax. As a result, air is drawn into the abdominal tracheae through the abdominal spiracles and forced out through the thoracic spiracles. These changes are reversed when the heart pumps backward, and air is sucked through the thoracic spiracles, and forced out through the abdominal spiracles. In some insects, airflow is tidal, which means air moves in and out through the same spiracles (some species of cockroaches and carabid beetles). In several other insects, the air is moved inward through one set of spiracles and outward through another set (some species of cockroaches and locusts).

C. **Ventilation of the head:** Besides the ventilation of the thorax and abdomen, insects employ various strategies for the ventilation of the head. Protraction and retraction of the head on the prothorax (neck ventilation), movement of the prothorax on mesothorax (prothorax ventilation), and pumping of the proboscis during flight are some of the important strategies.

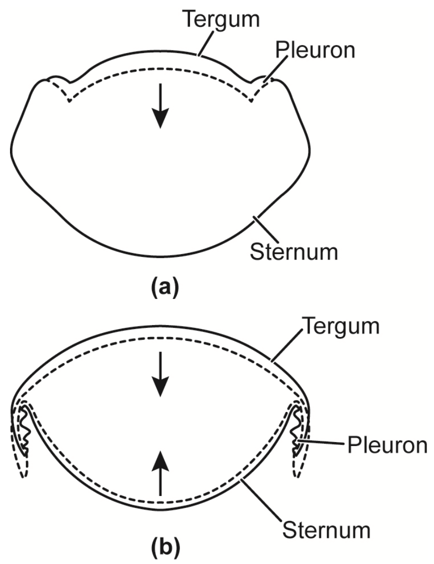

Fig. 4.10: Abdominal pumping by the up and down movement of (a) tergum (b) tergum, sternum, and pleuron

Variations in ventilation

In the above sections, we have discussed the mechanisms of gaseous exchange in terrestrial insects under normal conditions. There could be deviations from the normal processes under specialized conditions. These conditions could be inactive periods, low temperatures, pupal stage, or flight. Under the former three conditions, some insects show **Discontinuous Gas Exchange** (DSG) which means that gas exchange takes place in two stages.

(a) In the first stage, all spiracles are closed and no gaseous exchange takes place.

(b) In the second stage, all spiracles are open simultaneously and entry of oxygen and emission of carbon dioxide takes place in discrete bursts.

It has been hypothesized that DGE helps in reducing water loss, facilitating underground gas exchange and reducing oxidative damage.

A higher metabolic rate demands a higher oxygen requirement. To meet this demand more efficient ventilatory mechanisms are required. Walking, jumping, and flying require more energy and hence higher metabolic rates. Among these activities the most energy-intensive activity is flight. Insects employ various strategies to meet the higher oxygen demands of the flight muscles. Some of the strategies are thoracic pumping (*Schistocerca*), abdominal pumping (Hymenoptera, Diptera), pattern of spiracular opening (*Schistocerca*), and unidirectional airflow (hawk moths).

In thoracic pumping, the notal sclerites are raised and lowered resulting in large volume changes in the extra muscular air sacs of the pterothoracic tracheal system. Besides, the intramuscular air sacs are compressed by the changes in the diameters of the shortening muscles. In some insects, for example, *Schistocerca*, the pattern of the opening of spiracles changes during flight. Spiracles open and close rhythmically synchronised with abdominal ventilation resulting in a flow of air *via* the head to the rest of the central nervous system. Hawk moths maintain a unidirectional airstream by inspiration through the first spiracles and expiration through the second spiracles. They generate this type of directed airflow using the flight apparatus and abdominal up-and-down movements while inspiration is prevented through the posterior thoracic spiracles.

II. Aquatic insects

Respiratory physiology in aquatic insects with tracheal systems which obtain oxygen directly from the air is the same as that of the terrestrial insects. However, those aquatic insects that obtain oxygen from water have different respiratory strategies. In aquatic insects, the tracheal systems are closed and gases are exchanged through tracheal gills and physical gills (bubbles and plastron). They depend on the diffusion of oxygen from the water layer adjacent to their respiratory structures. As the respiratory process progresses, oxygen gets depleted in the adjacent layer of water. For diffusion to happen, a high concentration of oxygen in the water is needed.

Insects employ various strategies to maintain such a sufficient concentration of oxygen. Very often this is done by ventilatory movements by the insects that renew the adjacent layer of water. This may be by the movement of the gills (nymph of *Ephemera*), or by pushing water over the respiratory surface on the abdomen (nymph of *Cleon*). Damsel and dragonfly nymphs pump water in and out of their recta to achieve the same. In fast-moving water, such ventilatory movements are not necessary.

a) **Gaseous exchange using air bubbles:** As mentioned earlier, some insects use air bubbles as physical gills. They obtain air in the bubble from the atmosphere by reaching the water surface. When this insect dives, the gases in its air store (Oxygen = 21%; Nitrogen = 79%) are in equilibrium with the gases dissolved in the water. Within a short period of diving, the level of oxygen is reduced as a result of respiration, and the proportion of nitrogen is increased. This results in the loss of equilibrium of gases between the bubble and the surrounding water and leads to the passage of oxygen into the bubble from the surrounding water.

b) **Gaseous exchange using plastrons:** Plastrons also function as physical gills. As the oxygen get depleted in the plastron, oxygen from the surrounding water diffuses into the plastron. Hence, the insect can obtain oxygen throughout its life using the plastron. The advantage of plastron over air bubbles is that they are permanent and are not collapsible.

SAQ 3

i) Fill in the blanks by choosing the correct word from the parentheses.

   a) Rate of diffusion increases with ……………… in distance. (Increase/Decrease)

   b) In convection, flow of fluid is driven by …………….. gradient. (Pressure/ Concentration)

   c) A plastron contains ……………… . (Water/Air)

ii) Answer the following questions in one or two sentences.

   a) How is pressure gradient produced in the tracheal system?

   b) How do the odonate nymphs renew the respiratory water in their rectal gills?

   c) What are the differences between air bubbles and plastrons?

4.8 GASEOUS EXCHANGE IN ENDOPARASITIC INSECTS

Discussion on insect respiration will not be complete without mentioning respiration in endoparasitic insects. Larval forms of many insects lead an endoparasitic life on other insects. They are either with or without a tracheal system. The tracheal system could be either open or closed. Many of those having an open tracheal system establish a connection with the atmosphere to obtain oxygen (Dipterans and Hymenopterans). Those with a closed tracheal system depend on their thin cuticle for the diffusion of oxygen. The endoparasites without a tracheal system obtain oxygen directly into the haemolymph by diffusion.

Endoparasitic insects may obtain their oxygen directly from the air outside the host or by diffusion through the cuticle from the surrounding host tissues. In many ichneumonid and braconid (Hymenoptera) larvae, the tracheal system of the first instar is liquid-filled and, even when it becomes gas-filled, the spiracles remain closed until the last instar. Thus, these insects and the young larvae of most parasitic Diptera depend entirely on cutaneous diffusion. In braconid larvae the hindgut is everted through the anus to form a caudal vesicle. This is variously developed in different species, but in some, such as Apanteles, it is relatively thin-walled and closely associated with the heart (Fig. 4.11) so that oxygen passing in it is quickly carried around the body. In these insects the vesicles are responsible for about one-third of the total gaseous exchange.

When the tracheal system becomes air-filled, networks of tracheoles may develop immediately beneath the cuticle, thus facilitating the diffusion of gases away from the surface. In Cryptochaetum iceryae (Diptera), a parasite of scale insects, there are two caudal filaments, which in the third-instar larva are ten times as long as the body and are packed with tracheae. These filaments often get entangled with the host tracheae and so provide an easy path for oxygen transfer.

Other insects, and particularly older, actively growing larvae with greater oxygen requirements, connect with the outside air by penetrating the host's body wall or respiratory system. The majority of these insects use the posterior spiracles to obtain their oxygen. Chalcidid (Hymenoptera) larvae are connected to the outside from the first instar onwards by the hollow egg pedicel, which projects through the host's body wall. The posterior spiracles of the larva open into the funnel-shaped inner end of the pedicel and so make contact with the outside air. Many tachinid (Diptera) larvae that are parasitic in other insects tap the host's tracheal supply or pierce its body wall from within using their posterior spiracles. The host epidermis is stimulated to grow and spreads around the larva, almost completely enclosing it, and secreting a thin, cuticular membrane over its surface. The larva of Melinda (Diptera), parasitic in snails, respires by sticking its posterior spiracles out through the respiratory opening of the snail.

Parasites of vertebrates also often use atmospheric air. The larva of Cordylobia (Diptera) bores into the skin and produces a local swelling, but it always retains an opening to the outside into which the posterior spiracles are thrust. Similarly in the larva of the warble fly, *Hypoderma*, the warble opens to the outside, but here the larva bores its way out to the surface from within the host's tissues.

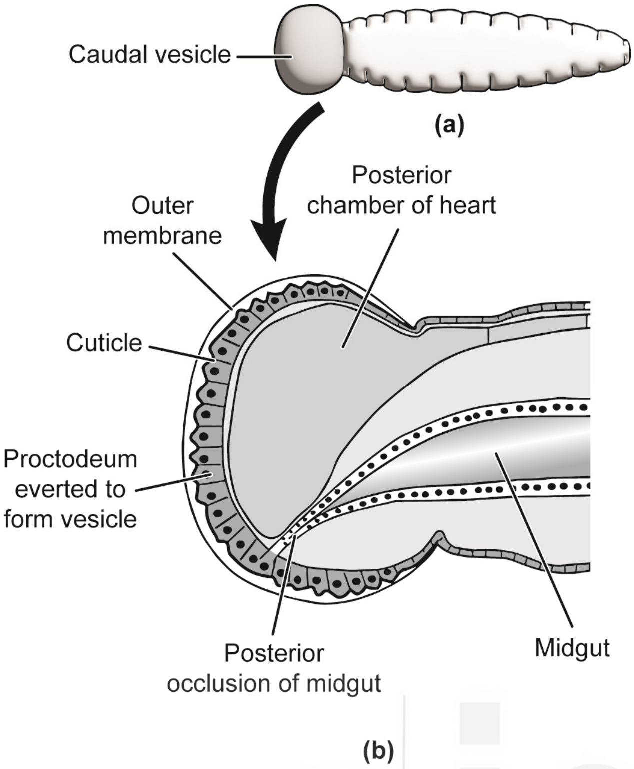

Fig. 4.11: Caudal vesicle of a parasitic hymenopteran larva (Apanteles) (from Wigglesworth, 1965). (a) Whole larva showing the caudal vesicle. (b) Longitudinal section of caudal vesicle.

4.9 SUMMARY

Let us summarise whatever you have learnt so far:

- The majority of insects have a tracheal system which serves as the respiratory organ; however, a few insect groups are without tracheae.
- The tracheal system consists of two types of internal, air-filled tubes, the larger tracheae and the smaller tracheoles. They are of ectodermal origin. Tracheae are lined with spiral thickenings, taenidia to prevent their collapse.
- Tracheal systems of all terrestrial insects and a few aquatic insects open to the outside through small openings known as spiracles.
- Spiracles are situated on either side of the mesothorax, metathorax, and abdominal segments.
- The number of spiracles varies from 0 to 10. The respiratory system with 10 spiracles is known as holopneustic; with 1-9 is known as heteropneustic; and those without any functional spiracles are known as apneustic.
- Terrestrial insects lacking a tracheal system depend on cutaneous respiration to meet their oxygen demand.
- Some insects possess respiratory pigments; haemoglobin and hemocyanin in either their blood or cells. Very little is known about the respiratory function of hemoglobin in insects.

- Aquatic insects may or may not have a tracheal system. Those without a tracheal system obtain their oxygen through cutaneous diffusion.
- Aquatic insects obtain their oxygen either directly from the air through siphon or depend on dissolved oxygen in water. Some insect larvae obtain oxygen from the aerenchyma of aquatic plants. Still others store air in air bubbles which sometimes function as physical gills.
- Aquatic insects that obtain oxygen from water may have gills and plastrons to augment respiration.
- There are three types of gills, viz., tracheal gills, spiracular gills, and blood gills. Most of the larval forms of aquatic insects possess tracheal gills.
- Plastrons are extremely thin layers of air held close to the body by specialized hairs called hydrofugepiles. Their volume remains constant and they function as physical gills.
- The gaseous exchange in terrestrial insects with tracheal systems depends on two processes- diffusion and convection. Since diffusion is very effective over short distances, small insects do not need any other mechanism. In the case of larger insects, diffusion is sufficient for delivering oxygen from the terminal tracheoles to the cells. However, for the transport of oxygen from the spiracles to the terminal tracheoles, convection movement is used.
- Some aquatic insects with gills maintain sufficient oxygen by renewing the adjacent layer of water by employing various modes of ventilatory movements.
- Plastrons and air bubbles which function as physical gills obtain oxygen by maintaining an oxygen gradient between the external environment and the interior.
- Endoparasitic insects with open tracheal systems establish a connection with the atmosphere; those with closed tracheal systems obtain oxygen by diffusion through the thin cuticle, and those without a tracheal system obtain oxygen directly to the haemolymph by diffusion.

4.10 TERMINAL QUESTIONS

1. Describe the structure of tracheae in insects and add a note on its function.
2. Distinguish between tracheae and tracheoles.
3. Write a note on the structure and function of spiracles in insect respiration.
4. Classify tracheal systems in insects.
5. Discuss the significance of respiratory pigments in insect respiration.
6. Discuss the physiology of gaseous exchange in terrestrial insects.

7. Comment on ‘Discontinuous Gas Exchange’.

8. Discuss various respiratory methods employed by aquatic insects.

9. “Some aquatic insects employ ventilatory movement to assist respiration”. Comment.

10. Briefly discuss gaseous exchange in endoparasitic insects.

4.11 ANSWERS

Self-Assessments Questions

1. i) a) Ectoderm; b) Heteropneustic; c) Spiracle.

   ii) a) True; b) False; c) True.

2. i) a) Haemoglobin; b) Aerenchyma; c) Diffusion.

   ii) a) Tracheal gills are leaf-like or filamentous outgrowths of ectoderm in aquatic insect larvae that are well supplied with tracheae and tracheoles.

   b) A plastron is a thin film of air held by some aquatic insects permanently on the outside of the body.

   c) Air bubbles are used to store air and are used when the insects are submerged. In some cases, they also function as physical gills.

   d) Spiracular gills are modified spiracles that are drawn out to form long processes. Such gills are found in the pupae of some aquatic insects.

3. i) a) Decrease; b) Pressure; c) Air.

   ii) a) It is generated either by the compression of tracheae or the air sacs associated with the tracheal system.

   b) Odonate nymphs pump water in and out of their recta to renew the respiratory water.

   c) Air bubbles are larger in size. Their volume decreases as the insect uses air for respiration. Plastrons are very thin and are permanent structures. Their volume does not change during respiration.

Terminal Questions

1. Refer to Subsection 4.3.1.

2. Refer to Subsections 4.3.1 and 4.3.2.

3. Refer to Subsection 4.3.3.

4. Refer to Section 4.4.

5. Refer to Section 4.5.
6. Refer to Subsection 4.6.1.
7. Refer to Subsection 4.6.2.
8. Refer to Subsection 4.6.2.
9. Refer to Subsection 4.6.2.
10. Refer to Section 4.8.