

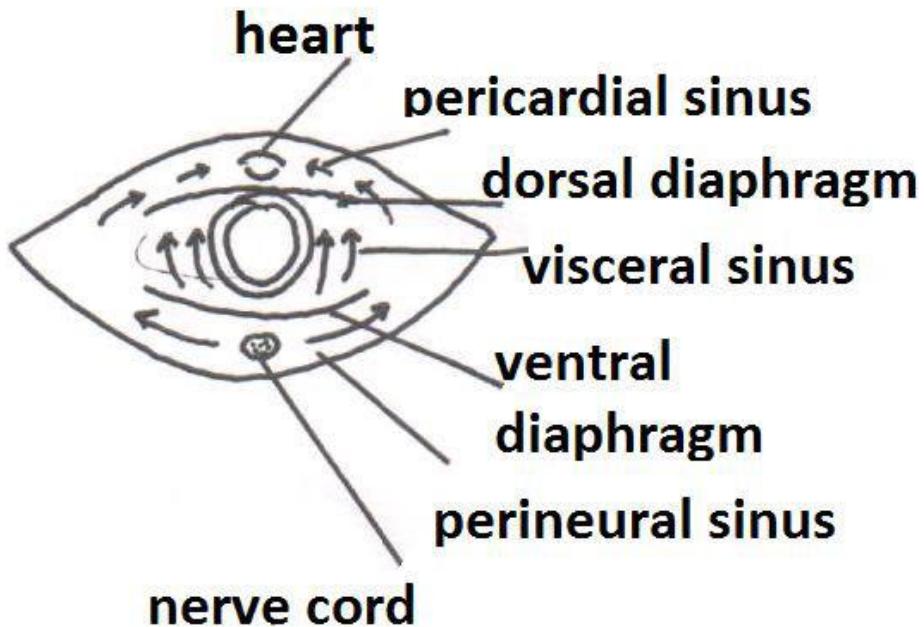
## Introduction

The insect circulatory system is at the center of most physiological processes. It delivers nutrients and hormones to cells and removes waste, also coordinates defense mechanisms, modulates heat transfer, assists in gas exchange, facilitates ecdysis, maintains homeostasis, and more (Figure 1). Unlike the “closed” circulatory system of humans, insect circulatory systems are said to be “open”, meaning that they lack a complex network of veins and arteries to help transport blood throughout the body. Instead, insect blood (called **hemolymph**) flows relatively “freely” throughout the hemocoel. Only one vessel is present in the insect circulatory system: the **dorsal vessel**. Posteriorly (in the abdominal region), the dorsal vessel acts as the **heart**, pumping hemolymph forward into the anterior region (in the head and thorax), where it acts as the **aorta** and dumps the hemolymph into the head. It flows posteriorly and is returned to the heart via **ostia**, which are small slits in the heart region of the dorsal vessel designed for hemolymph uptake.

As hemolymph moves in large spaces instead of vessels, the organism is known to possess an open type of circulatory system. The thoracic abdominal body cavity is divided into three major compartments with the help of two partitions. These partitions are called **dorsal diaphragm** placed dorsally and **ventral diaphragm** placed ventrally. Due to these diaphragms the insect body cavity is divided into three cavities

- 1- **pericardial cavity** that surrounds the dorsal **aorta**
- 2- **perivisceral cavity** that surrounds the **alimentary canal**
- 3- **perineural cavity** that surrounds the **nerve cord**.

Although insects have an open circulatory system, hemolymph does not diffuse freely throughout the hemocoel, and instead flows along distinct channel-like routes that are created by the structural organization of the internal organs and by fibromuscular septa or diaphragms. The flow is unidirectional from posterior to anterior region and having open spaces or cavities called hemocoel (Fig 1) The respiratory system is a way to distribute oxygen to all cells of the body and for excrete waste product carbon dioxide ( $\text{CO}_2$ ) in cellular respiration.

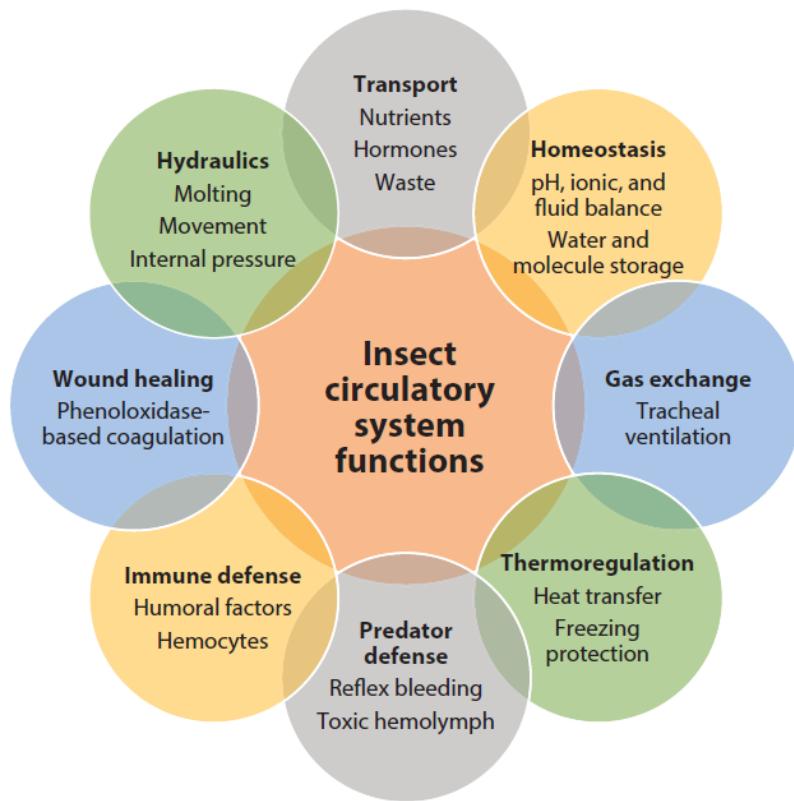


**Figure 1 Direction of circulation in T.S.**

### **Function of hemolymph**

1. Haemolymph creates hydrostatic pressure generated due to muscle contraction that helps the insect to circulate many things.
2. It also facilitates hatching, moulting and expansion of body.
3. Physical movements especially in soft-bodied larvae.
4. It also helps in reproduction like insemination and oviposition.

5. In some insects, the haemolymph aids in thermoregulation, it cool the body by conducting excess heat away from active flight muscles or it can warm the body by collecting and circulating heat absorbed while basking in the sun.



**Figure 2.** The many functions of the circulatory system of insects.

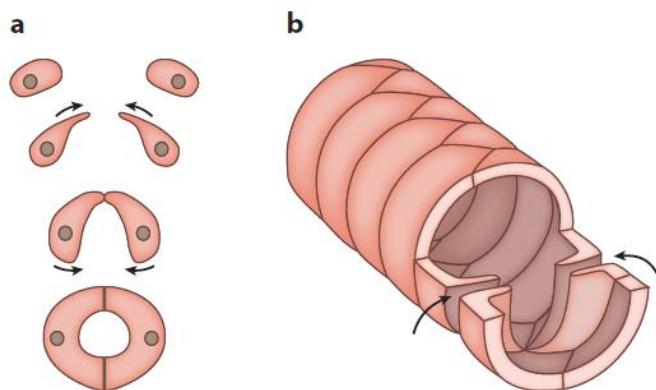
## FUNCTIONAL MORPHOLOGY OF CIRCULATORY ORGANS

### 1. The Dorsal Vessel

The dorsal vessel is a cylindrical structure that extends the entire length of the insect and is organized by serial repetition of building blocks. Each module is comprised of cardiomyocytes, a pair of alary muscles, and connective tissue. During early embryogenesis, the cardioblasts move to the dorsal midline and form two regular, opposing rows (**Figure 3 a)** The cardioblasts then extend processes that meet their contralateral counterparts in the midline, thus forming a cylindrical, spiral

arrangement that encloses a luminal space. In each segment, a pair of cardioblasts on each side of the body differentiate into specialized cells that form inflow openings called ostia. Usually, each of these ostial cardioblasts develops a flap-like extension that protrudes into the lumen of the dorsal vessel (**Figure 3b**). The ostial cells are indistinguishable from ordinary cardiomyocytes at the ultrastructural level but express a suite of specific marker. In some insects, other cardioblasts form additional flap-like intracardiac valves or muscular pads that regulate flow within the lumen of the dorsal vessel. The development of the dorsal vessel has been extensively studied in *Drosophila*, it is probably similar in all hexapods and may even represent the plesiomorphic state of the entire arthropod clade.

The body plan ground pattern of the hexapod dorsal vessel is a tube that is uniform in diameter along its length and contains a pair of ostia in each thoracic and abdominal segment. The anterior part, known as the aorta, has a narrow luminal diameter and lacks incurrent ostia as well as alary muscles; it is a simple conduit that extends through the thorax and into the head, where the hemolymph pours out into the hemocoel. The posterior part, known as the heart, is confined to the abdomen and has a wider luminal.



**Figure 3.** Development and structure of the dorsal vessel. (a) Consecutive stages of morphogenesis, from top to bottom. Cardioblasts become polarized and extend leading processes toward the dorsal midline until they meet their contralateral counterparts. The trailing

edges of the cardioblasts then extend posteriorly and medially and contact each other, thereby forming the lumen of the dorsal vessel. (b) The mature dorsal vessel consists of two rows of semicircular cardiomyocytes. Their staggering and contact points in the dorsal and ventral median line result in a spiral-like arrangement. The anterior front cut shows the organization of the ostial cells; the two inward directed flaps act together as a valve that allows hemolymph to enter into the vessel but hinders outward flow.

Dorsal aorta as the name suggests lie in the dorsal region of the body below the body wall. It is a longitudinal tube runs from thorax and abdomen and constitute major structural component of an insect's circulatory system. It is further divided and often constricted into 5 to 6 heart chambers separated by valves (ostia) to ensure unidirectional flow of hemolymph. In *Nymphalid* butterfly only larva has both forward and backward peristaltic movements exceptionally. It is a fragile simple tube without ostia and sometimes connected. with vertical diverticulum associated with pulsatile organs. In most insects, hemolymph flows in a direction from posterior to anterior end of the body that is from abdomen to the head.

## 2. Ostia

In heart incurrent and excurrent both types of ostia are present. Incurrent ostia consist of 9 pairs in abdomen and 3 pairs in thorax. The ostia are valvular in generalized insects like cockroach. Numbers of incurrent ostia are variable like in wasp 5 pairs and in housefly 3 pairs are present. Excurrent ostia are non-valvular, in grasshoppers, silverfishes 2 thoracic and 5 abdominal pairs are present. In cockroaches where excurrent ostia are absent certain lateral segmental vessels are associated with heart. During each diastolic phase/relaxation, the ostia open to allow inflow of hemolymph from the body cavity and during contraction of heart these ostia closes, and the hemolymph move forward.

## 3. Accessory pulsatile organs

These organs are there in mesothorax or sometimes in metathorax which are concerned with circulation of hemolymph into legs/ wings/ antenna. In some insects,

pulsatile organs are located on the base of the wings, appendages or antenna in grasshoppers and cockroaches. Pulsatile organs do not usually contract on a regular basis, but they force hemolymph out into the extremities.

#### **4. Heartbeat**

The rate of pulse is 30-200 beats/minutes. As the temperature falls or rises the heart beat vary. In larva of stag beetle heartbeat is 14 beats/minutes and in flies it is 150 beats/minutes. Heartbeat of larva is slower as compared to adult, and in older pupa no heartbeat is found. Younger larva sometimes stops beating.

#### **Control of heart**

Insect's heart is myogenic, and it lacks pacemaker. In cockroach neurogenic nerves supplied from corpora cardiac and motor fibres of segmental ganglia controls heart. Cardioaccelerator neuropeptide proctolin acts as myotropins and regulate heart. Indolalkylamine in insects is equivalent to adrenaline of higher organisms that accelerate the heartbeat.

#### **Composition of hemolymph**

Hemolymph or blood flows in aorta, small vessels in open channels in insects. The body cavity/blood sinus is divided into three compartments pericardial surrounding heart, perivisceral surrounding alimentary canal and perineural sinus around nervous system by two thin sheets of muscles or membrane known as the dorsal and ventral diaphragms. The dorsal diaphragm is formed by alary muscles of the heart and related structures separating the pericardial sinus from the perivisceral sinus. The ventral diaphragm separates the perivisceral sinus from the perineural sinus in the same way. Hemolymph is composed of plasma and haemocytes. In insects 170 $\mu$ l of hemolymph contains 7-20 million of circulating cells.

## **Plasma**

Major portion about 90% of insect hemolymph is plasma and carries 5-40% of total body weight. It is a watery fluid containing 85% water, usually clear colorless fluid, but sometimes green, yellow, or brown in color. It is slightly acidic in pH and consist of almost all amino acids. In comparison to vertebrate blood, insects have high concentrations of amino acids, proteins, sugars (glucose in honeybees), uric acid, pigments, and inorganic ions. Hemolymph is a dynamic fluid that changes with diet, environmental factors, or life stages. For example; In carnivores they have high concentration of Mg<sup>+</sup> and K<sup>+</sup>, In herbivores high Na<sup>+</sup>, in terrestrial insects high protein, amino acids and uric acids. In aquatic insects high allantion, allantoic acid, NH<sub>3</sub>, urea is there. Trehalose is a major blood sugar in most insects which is a non-reducing dimer of glucose. In certain insects' blood sugar may be glucose, fructose or ribose depending upon their food sources . Hemolymph also contains sorbitol or glycerol which is a cryoprotectants or antifreezing agents in the plasma to prevent it from freezing during the winters and fight against cold stress. Lipophorin is a lipoprotein that functions to transport fatty acids, cholesterol, carotenoids, xenobiotics, and hydrocarbons. Tyrosin plays important role in sclerotization of cuticle previously explained and proline acts as a flight energy source.

## **Hemocytes**

Left 10% of hemolymph volume is made up of various cell collectively known as hemocytes. All types of cells occur in haemopoietic organs present in developing stages and adults in exopterygotes and these organs are absent in endopterygotes adult. Different types of cells are as follows:

1. Prohaemocytes are like archaeocytes of sponges that give rise to all other cells. They are spherical in shape having large nucleus and quite RNA rich.
2. Plasmacytocytes are of variable shapes with vacuolated cytoplasm. It is most abundant of all and phagocytic in nature.

3. Granulocytes are the largest and phagocytic in nature like plasmacytocytes. They have granulated and acidophilic cytoplasm.

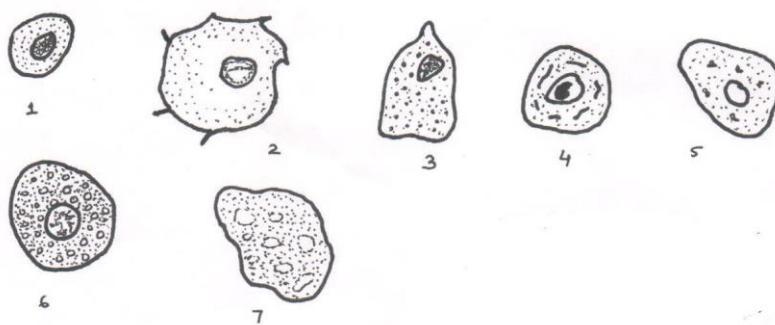
4. **Oenocytoids** are special cells present in some Coleopterans, Dipterans, Lepidopterans and Hemipterans having large and rounded nucleus eccentric in position, but they are not derived from prohaemocytes.

5. Coagulocytes/ cystocytes having scattered granules and helps in coagulation.

6. Spherules may be spherical, oval or spindle shaped with spherules present in cytoplasm. They are present in only Diptera and Lepidoptera.

**Porhaemocytes, plasmacytocytes and granulocytes are present in all types of insects.** Total number of cells varies and depends upon species, developmental stage and physiological state of insect (Fig. 5). Number of haemocytes increases with instars development, decreases first in early pupal stage and increases in later pupal stage, then decreased in adult stage.

The hydraulic (liquid) properties of lymph are important. The hydrostatic pressure generated internally by muscle contraction is used to facilitate hatching, moulting, expansion of body and wings after moulting, physical movements (especially in soft-bodied larvae), reproduction (e.g., insemination and oviposition), and evagination of certain types of exocrine glands. In some insects, the lymph aids in thermoregulation: it can help cool the body by conducting excess heat away from active flight muscles or it can warm the body by collecting and circulating heat absorbed while basking in the sun.



**Figure 4. Different types of haemocytes 1. Prohaemocytes 2. Plasmacytocyte**

**3. Granular haemocyte 4. Oenocyte 5. Cytocyte 6. Spherule 7. Adipocyte**

## **Insect immunity against pathogens**

Insects combat infection by mounting powerful immune responses that are mediated by hemocytes, the fat body, the midgut, the salivary glands and other tissues. Foreign organisms that have entered the body of an insect are recognized by the immune system when pathogen-associated molecular patterns bind host-derived pattern recognition receptors.

The primary immune cells in insects are the hemocytes. These cells, which are present in the hemocoel, drive cellular immune processes such as phagocytosis, and produce humoral immune factors that lead to pathogen killing via lysis or melanization. Hemocyte populations can be divided using two, non-exclusive criteria: their spatial state and their functional properties. From a spatial perspective, hemocytes either circulate with the hemolymph, in which case they are called circulating hemocytes, or are attached to tissues, in which case they are called sessile hemocytes. The distinction between circulating and sessile hemocytes is strictly spatial, and sessile hemocytes can release from their point of attachment and enter circulation, and circulating hemocytes can attach to tissues and become sessile.

The fat body, the midgut and the salivary glands are also important drivers of the insect immune response. The fat body, which is composed of loosely associated cells that are rich in lipids and glycogen, lines the integument of the hemocoel. Besides functioning in energy storage and the synthesis of the vitellogenin precursors required to produce eggs, the fat body

produces and secretes antimicrobial peptides with lytic activity as well as additional components of the humoral immune response.

## Circulatory System

- **Open circulatory system**
  - In Insects and other Arthropods
  - Haemolymph is flowing freely within body cavities
  - **Haemolymph**
    - Watery yellowish-green colour.
    - No haemoglobin (except few aquatic midges).
    - Is not responsible for the transmission of oxygen
    - Haemocyte (25000 - 100,000) in mm<sup>3</sup>.
- **Closed circulatory system**
  - In humans and other vertebrates
  - Blood is contained within vessels (arteries, veins, capillaries and heart)
  - **Blood**
    - Red colour.
    - Contain haemoglobin.
    - Responsible for oxygen transmission.
    - 5 million RBC, 300,000 platelets & 7000 WBC in mm<sup>3</sup>.

**Figure 5.** Comparison between circulatory system of insects & human beings.

# Circulatory System

- **Functions of the circulatory system**

- Responsible for movement of nutrients, salts, hormones, and metabolic wastes throughout the insect's body.
- Defense: e.g., encapsulates and destroys parasites or invaders.
- Seals off wounds through a clotting reaction.
- Haemolymph hydrostatic pressure is used to facilitate hatching, molting, physical movements (larvae), reproduction (e.g. insemination and oviposition).
- Haemolymph aid in thermoregulation >cooling & warming the body

**Figure 6.** The functions of circulatory system

## Summary

The circulatory system includes contractile dorsal aorta, heart, ostia, sinuses and hemolymph. The blood or haemolymph is made of plasma and cells performing different functions and make up less than 25% of an insect's body weight. The main function of hemolymph, is to transports hormones, nutrients and wastes and is important for osmoregulation, temperature control, immunity and storage. It also plays an essential part in the moulting process, predatory defence by chemicals that deter predators.

Body fluids enter through unidirectional way through valved ostia which are openings situated on the aorta. Flow of the hemolymph occurs by peristaltic contraction, originating at the posterior end which pumps the hemolymph forwards into the dorsal vessel. The hemolymph is circulated to the appendages with the help of accessory pulsatile organs found at the base of the antennae or wings or legs.