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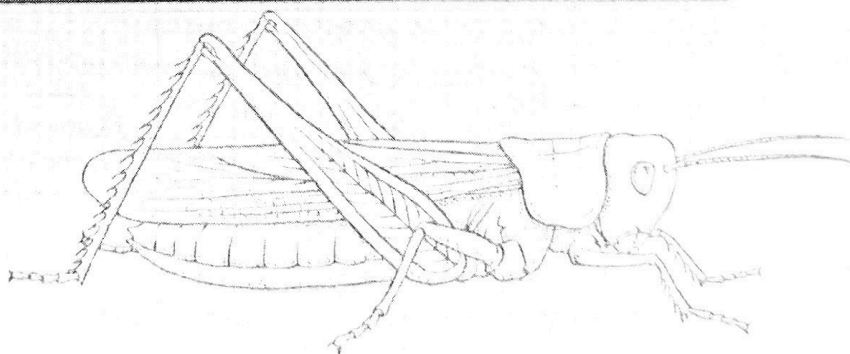
Circulatory system, blood and the immune system

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INTRODUCTION

The cells of all animals, including insects, are bathed in an extracellular fluid (ECF); and most cells of the animal exchange solutes with the ECF, not the external environment. In most animals, including insects, solute exchange between cells and ECF is facilitated by the bulk flow of ECF, powered by one-to-many pumps (including hearts). The physiological system mediating bulk flow is known as the circulatory system, the topic of this chapter. Most insects have one major pump, the dorsal vessel, and multiple accessory pumps, with the circulating portion of the ECF known as the blood or hemolymph. The insect circulatory system is open, meaning that the hemolymph flows freely around insect organs, in contrast to the closed circulatory system of vertebrates in which blood is retained within vessels. The structural organization of the insect circulatory system and the determinants of blood flow are described in Sections 5.1 and 5.2, respectively, and the composition of hemolymph is considered in Section 5.3.

Hemolymph has four key functions. It is the vehicle for transport of hormones and nutrients between tissues, and a site for storage of some nutrients and water; these two functions are considered in Section 5.3. It is also a crucially important component of the insect immune system. The humoral (non-cellular) immune function is addressed in Section 5.3 and the cellular function in Section 5.4. Other insect organs also contribute to insect immunity, notably the gut (Chapter 3), fat body (Chapter 6) and Malpighian tubules (Chapter 18).



5.1 The circulatory system

5.1.1 The dorsal vessel

The dorsal vessel has the primary role in the circulation of hemolymph around the body. In most insects it runs along the dorsal midline, just below the terga, for almost the whole length of the body (Fig. 5.1). Exceptionally, in the thorax of adult Lepidoptera and some Hymenoptera, it loops down between the longitudinal flight muscles (see Fig. 19.3). It may be bound to the dorsal body wall or suspended from it by elastic filaments. Anteriorly, it leaves the dorsal wall and is more closely associated with the alimentary canal, passing under the brain and just above the esophagus.

The dorsal vessel is open anteriorly, ending abruptly in most insects, but as an open gutter in orthopteroids. Posteriorly, it is closed, except in larval mayflies (Ephemeroptera), where three vessels diverge to the caudal filaments from the end of the heart.

The dorsal vessel is divided into two regions: a posterior heart in which the wall of the vessel is perforated by incurrent and sometimes also by excurrent openings (ostia), and an anterior aorta which is a simple, unperforated tube (Fig. 5.2). The heart is usually restricted to the abdomen, but may extend as far forwards as the prothorax in cockroaches (Blattodea). In orthopteroids it has a chambered appearance because it is slightly enlarged into ampullae at the points where the ostia pierce the wall. In the larvae of dragonflies (Odonata) and the crane fly, *Tipula*, the heart is divided into chambers by valves in front of each pair of incurrent ostia, and in *Cloeon* (Ephemeroptera) larvae, the ostial valves themselves are so long that they meet across the lumen.

The wall of the dorsal vessel is contractile and usually consists of one or two layers of muscle cells with a circular or spiral arrangement. Longitudinal muscle strands are also present; in Heteroptera they insert into the wall of the vessel anteriorly and posteriorly and do not connect with other tissues.

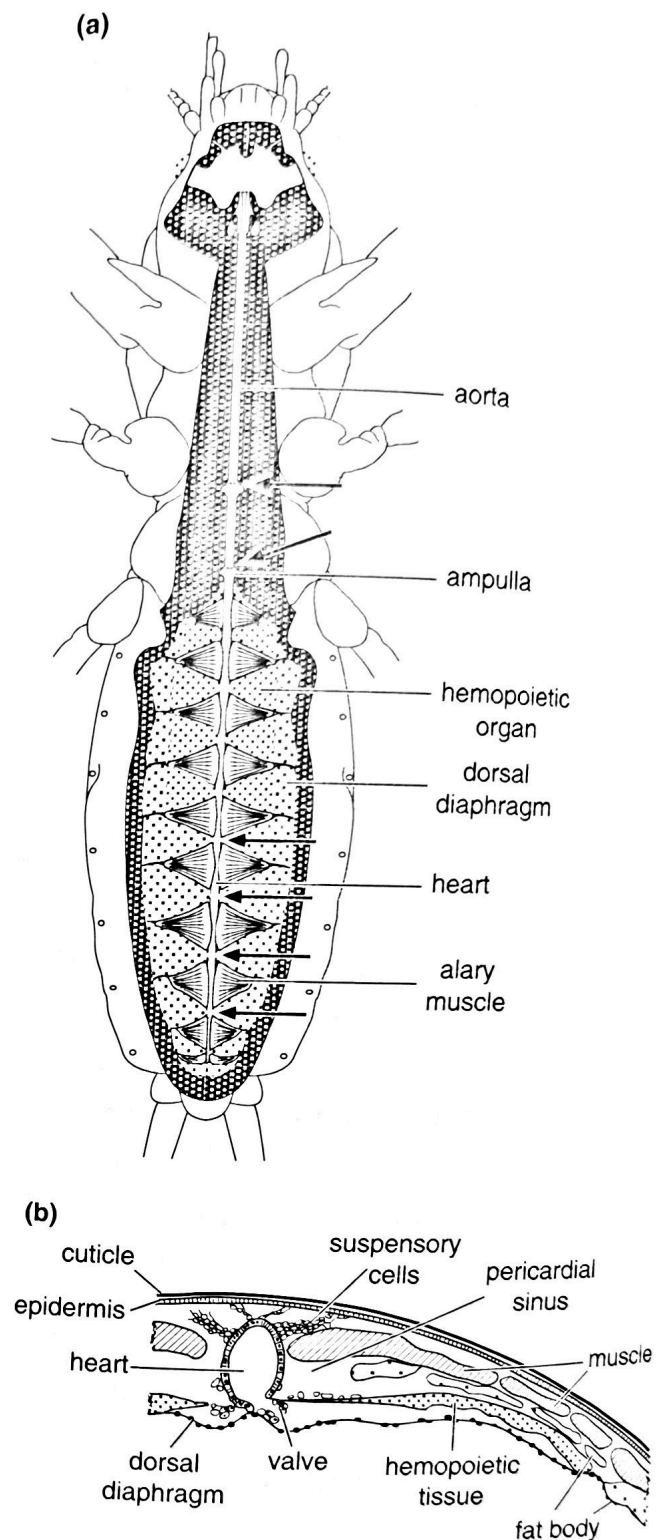


Figure 5.1 Dorsal vessel and hemopoietic organs of the mole cricket, *Gryllotalpa*, in ventral dissection. The dorsal diaphragm is continuous over the ventral wall of the heart, but is omitted from the drawing for clarity. Arrows show positions of incurrent ostia.

The muscles of the heart are sometimes oriented in many different directions, but this appearance may arise through the insertion of the alary muscles into

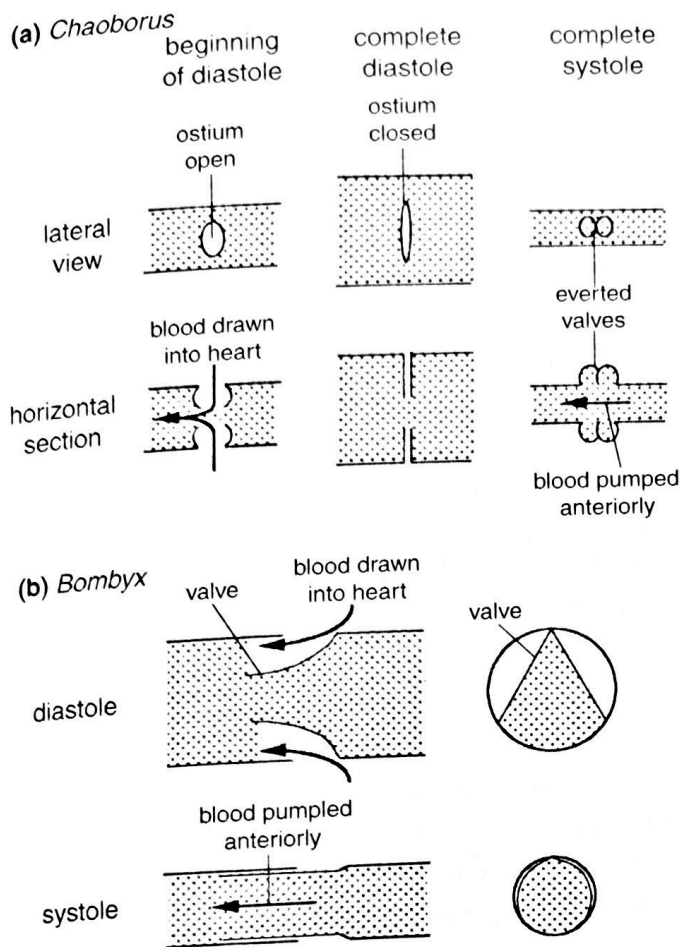


Figure 5.2 Functioning of incurrent ostia. (a) The larva of *Chaoborus*; the valves are prevented from opening outwards at systole by a unicellular thread (not shown) attached to the inside of the heart (after Wigglesworth, 1972). (b) The larva of *Bombyx*; the heart is shown in horizontal (left) and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

the heart (see below). Muscles of the dorsal vessel have short sarcomere lengths, with A-bands about $2\mu\text{m}$ long. Each thick filament is surrounded by 9–12 thin filaments, as commonly found in visceral muscles of insects. The muscles are ensheathed inside and out by a basal lamina which also covers the nephrocytes (Section 18.8).

The incurrent ostia are vertical, slit-like openings in the lateral wall of the heart. The maximum number found in any insect is 12 pairs – nine abdominal and three thoracic. All 12 pairs are present in Blattodea and Orthoptera; many Lepidoptera have seven or eight. In aculeate Hymenoptera (bees, wasps and ants) there are only five pairs, while the housefly,

Musca, has only four. Lice (Phthiraptera) and many Heteroptera have only two or three pairs and the heart is restricted to the posterior abdominal segments.

The anterior and posterior lips of each ostium are reflexed into the heart to form a valve which permits the flow of hemolymph into the heart at diastole (expansion of the heart), but prevents its outward passage at systole (contraction of the heart) (Fig. 5.2a). During diastole the lips are forced apart by the inflowing hemolymph. When diastole is complete the lips are forced together by the pressure of hemolymph in the heart, and they remain closed throughout systole. In larvae of the phantom midge, *Chaoborus*, the valves tend to be evaginated by the pressure exerted during systole, but they are prevented from completely everting by a unicellular thread attached to the inside of the heart. In the silkworm, *Bombyx*, only the hind lip of each ostium is extended as a flap within the heart (Fig. 5.2b). During systole this is pressed against the wall of the heart and prevents the escape of hemolymph; and, when the heartbeat is reversed (see below) hemolymph flows out of the “incurrent” ostia. Excurrent ostia are restricted to the hearts of a few insect groups, including the Thysanura (silverfish), Orthoptera, Plecoptera (stoneflies) and Embioptera. In the last two orders they are unpaired, but in Orthoptera they are paired ventro-lateral openings in the heart wall and lack internal valves. Their number varies, but grasshoppers have five abdominal and two thoracic pairs. Most Blattodea and Mantodea have no excurrent ostia, but the hemolymph leaves the heart via segmental vessels that extend laterally. For example, the cockroach, *Periplaneta*, has five pairs of abdominal segmental vessels in late-stage nymphs; adults additionally have two pairs of thoracic segmental vessels. Mantids have only abdominal vessels, each of which has a muscular valve that permits only the outward flow of hemolymph. The walls of the vessels are non-muscular.

In most insects, the heart is innervated by nerves running around the body wall from the ventral

segmental ganglia. In Odonata, Blattodea, Phasmatodea, Orthoptera, larval Lepidoptera and some adult Coleoptera, branches of the segmental nerves combine to form a lateral cardiac nerve running along each side of the heart. In *Locusta*, for example, groups of neurons with cell bodies in the midline in each abdominal ganglion send axons to the heart. In addition, a pair of neurosecretory cells in the subesophageal ganglion each sends one axon forwards into the circumesophageal connective and another back along the length of the ventral nerve cord. This axon branches in each of the abdominal ganglia, sending a branch into a lateral nerve which extends dorsally to the heart. These branches contribute to the lateral cardiac nerve and have varicose terminals, typical of a neurosecretory cell, along the heart. The cells produce a FMRFamide-like peptide (Fig. 20.6). The lateral cardiac nerves also receive innervation from neurons whose somata are not in the central nervous system, but lie adjacent to the heart itself. They are called cardiac neurons. *Periplaneta* has about 32 such neurons, some of which are neurosecretory.

In the majority of holometabolous insects, the segmental nerves extend to the heart, but do not form lateral cardiac nerves. Exceptionally, the heart of some insects (e.g., *Anopheles* mosquitoes) receive no direct innervation at all, although there are segmental nerves to the alary muscles.

5.1.2 Sinuses and diaphragms

The hemocoel of many insects is divided into the major sinuses: a dorsal pericardial sinus; a perivisceral sinus; and a ventral perineural sinus (Fig. 5.3). The pericardial and perineural sinuses separated from the visceral sinus by the dorsal and ventral diaphragms, respectively. In most insects, the visceral sinus occupies most of the body cavity. In ichneumonids the perineural sinus is enlarged. The dorsal diaphragm is a fenestrated connective tissue membrane. It is usually incomplete laterally, so that the pericardial sinus above it is broadly continuous with the perivisceral sinus below. The lateral limits of the diaphragm are indefinite and determined by the presence of muscles, tracheae, the origins of the alary (or aliform) muscles which form an integral part of the diaphragm. Generally, the alary muscles stretch from one side of the body to the other just below the heart, but in the cecropia moth, *Hyalophora*, they are directly connected to heart muscles by intercalated discs. They usually out from a restricted point of origin on the tergum and meet in a broad zone in the midline (Fig. 5.1), sometimes, as in grasshoppers, the origin of the muscles is also broad. In most orthopteroids, only a portion of the alary muscle near the point of origin is contractile, the rest, and greater part, being made of bundles of connective tissue which branch and

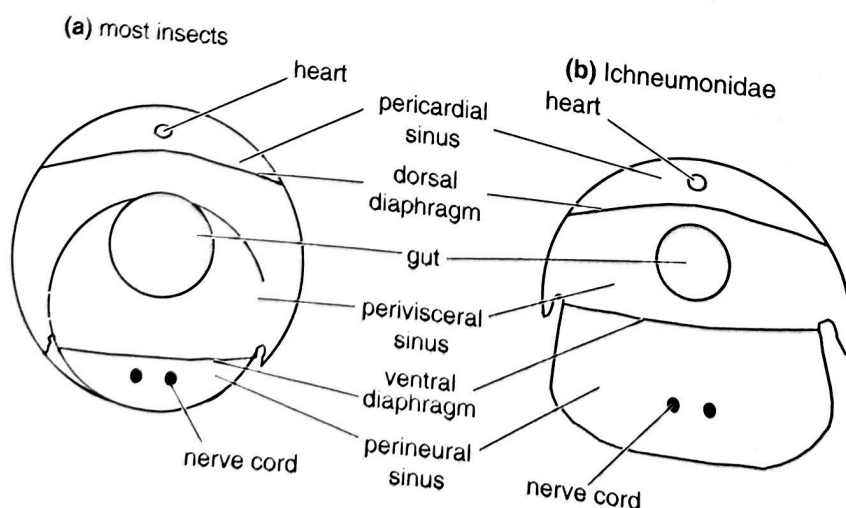


Figure 5.3 Main sinuses of the hemocoel shown in diagrammatic cross-section (after Richards, 1963).

anastomose. Some of the connective tissue fibers form a plexus which extends to the heart wall. Orthopteroids may have as many as ten abdominal and two thoracic pairs of alary muscles, but in other insects the number is reduced. Most terrestrial Heteroptera, for instance, have 4–7 pairs. The alary muscles are visceral muscles with 10–12 thin filaments to every thick filament.

The ventral diaphragm is a horizontal septum just above the nerve cord separating the perineural sinus from the main perivisceral sinus (Fig. 5.3). It is present in both larvae and adults of Odonata, Blattodea, Orthoptera, Neuroptera (lacewings) and Hymenoptera, but is only found in adults of Mecoptera (scorpion flies), Lepidoptera and nematoceros Diptera. In the Lepidoptera the nerve cord is attached to the ventral surface of the ventral diaphragm by connective tissue. When present, the diaphragm is usually restricted to the abdomen, but it extends into the thorax in some grasshoppers and Hymenoptera. The structure of the ventral diaphragm varies. For instance, in the thorax of grasshoppers it is a delicate membrane with little or no muscle, but in the abdomen it is a solid muscular sheet. Laterally it is attached to the sternites, usually at one point in each segment, resulting in broad gaps along the margins where the perivisceral and perineural sinuses are continuous. Its structure may vary with developmental stage, e.g., in *Corydalis* (Neuroptera) it forms a solid sheet in the larva, but a fenestrated membrane in the adult.

5.1.3 Accessory pulsatile organs

Insects have multiple pulsatile structures that assist the bulk flow of hemolymph, especially through the appendages, including wings, legs, antennae and, in adult females, the ovipositor.

A pulsatile organ drawing hemolymph from the wings is present in both wing-bearing segments of most adult insects, but only in the mesothorax of Diptera and Coleoptera Polyphaga. A hemolymph space, or reservoir, beneath the posterior part of the

tergum (scutellum) which is largely or completely isolated from the remaining hemocoel of the thorax connects with the posterior veins of the wing via the axillary cord of the wing. The muscular pump is formed from the ventral wall of the reservoir, which may be derived from the heart or a separate structure (Fig. 5.4). The exact form of the wing pulsatile organs varies among the different insect groups.

Most holometabolous insects, as well as Hemiptera, have wing pulsatile organs in which a muscular diaphragm, independent of the actual heart, bounds the subscutellar reservoir on the ventral side. It is suspended from the scutellum by a number of filamentous strands (Fig. 5.4a–c). Contraction of the muscles, which are innervated from the ventral nerve cord, causes the diaphragm to flatten, drawing hemolymph from the wings. Relaxation of the muscles is associated with an upward movement of the diaphragm, presumably due to the elasticity of the suspensory strands, and hemolymph is forced into the body cavity (Fig. 5.4b,c). In hemimetabolous groups, other than Hemiptera, and in Coleoptera and Hymenoptera Symphyta, the wing pulsatile organ is an expansion or diverticulum of the dorsal vessel with a pair of incurrent ostia opening from the subscutellar reservoir. Odonata, for example, have, in each pterothoracic segment, an ampulla which connects with the aorta by a narrow vessel (Fig. 5.4d). It is suspended from the tergum by elastic ligaments, and its dorsal wall is muscular. When the muscles contract, the ampulla is compressed and hemolymph is driven into the aorta. At the same time, the volume of the subscutellar reservoir is increased and hemolymph is sucked from the wings: The posterior veins therefore deal with the afferent flow while the anterior veins carry the efferent flow. When the muscles relax, the elastic ligaments restore the shape of the ampulla so that hemolymph is sucked into it from the reservoir.

A pulsatile organ is also found at the base of each antenna. It consists of an ampulla from which a fine tube extends almost to the tip of the antenna. The ampullae of Thysanura (silverfish), Archaeognatha

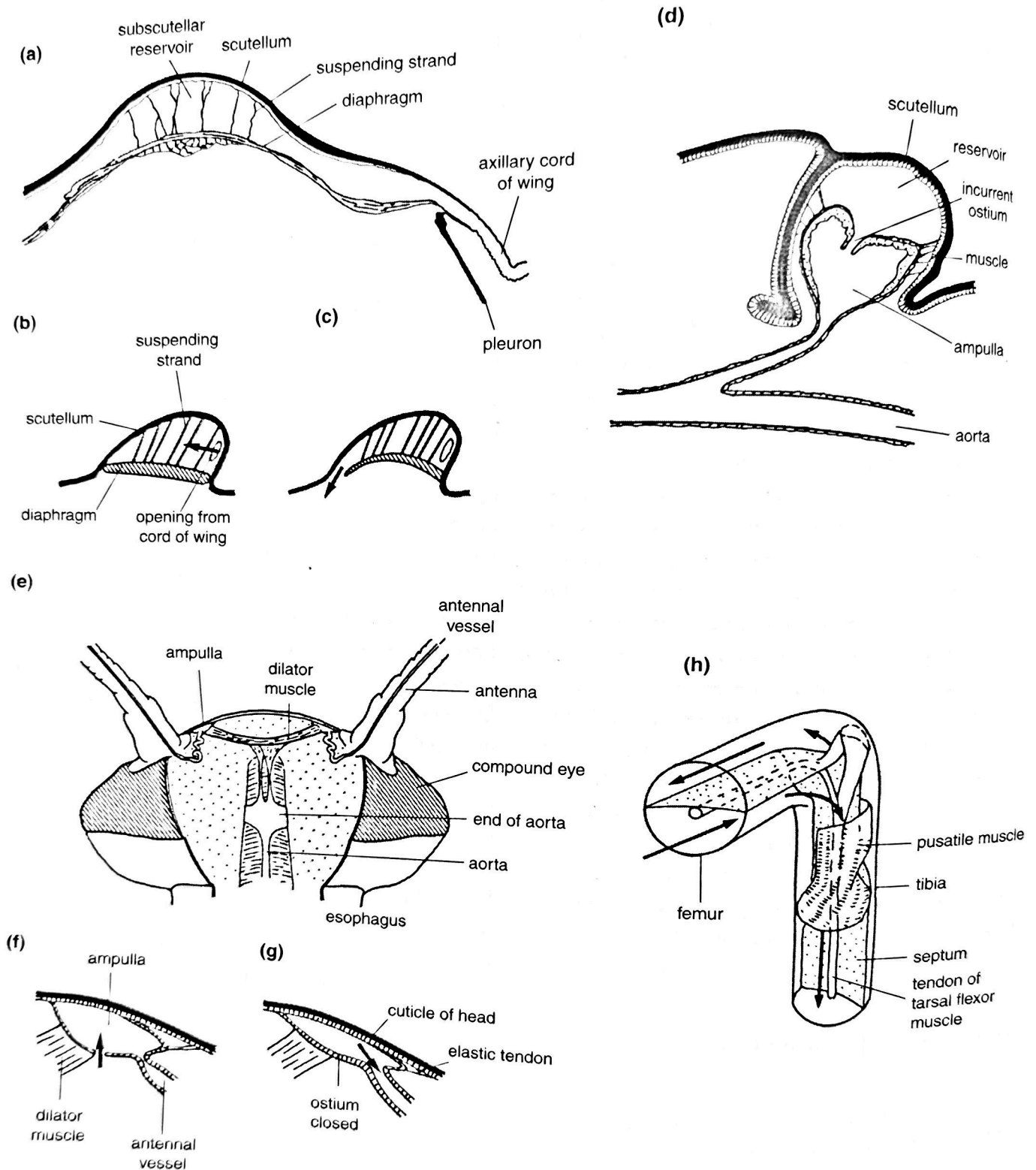


Figure 5.4 Accessory pulsatile organs. In all diagrams arrows indicate the direction of blood flow. (a)-(c) Wing heart not connected to the aorta, such as occurs in most holometabolous insects. (a) Transverse section through the thorax showing the connection of the subcutellar reservoir to the axillary cord of the wing. (b), (c) Diagrammatic longitudinal section, anterior to left. When muscles of the diaphragm contract (b), the diaphragm is flattened and blood is drawn in from the wing.

(bristletails) and some Plecoptera have no muscles, and it is presumed that in these insects the ampulla serves simply to direct the flow of hemolymph from the opening of the aorta into the antenna. In most insects, however, the ampullae have dilator muscles. Compression, which drives hemolymph into the antenna, results from the activity of elastic filaments on both sides of the wall of the ampulla. Only Dermaptera (earwigs) have compressor muscles. *Periplaneta* has a single muscle connecting the two ampullae so that when it contracts both ampullae are dilated and hemolymph flows into each one through an ostium (Fig. 5.4e–g). The contractions are myogenic in origin, but may be modulated by neural input since nerve endings do occur in the muscle. However, most of the nerve endings are concentrated in the ampulla, which appears to function as a neurohemal organ. The axons originate in the subesophageal ganglion from a dorsal unpaired median neuron and from a pair of somata placed laterally. In Lepidoptera, the aorta ends anteriorly in a sac from which the antennal vessels arise.

Most insects have a longitudinal septum in the legs which divides the lumen into two sinuses and permits a bidirectional flow of hemolymph within the leg (see below). In Hemiptera, the septum twists through 90° at the proximal end of the tibia, and there is a muscle at this point (Fig. 5.4h). When the muscle contracts, it compresses one sinus, forcing hemolymph into the thorax, and at the same time enlarges the other so that hemolymph is drawn into the leg. Its activity is probably myogenic, although it may also be subject to neural modulation.

Some insects have very long cerci in which the maintenance of hemolymph flow might require some special feature. Ephemeroptera have small, non-contractile vessels extending from the posterior end of the heart into the cerci. Plecoptera also have cercal hemolymph vessels, but these do not connect with the heart. They open directly into the perivisceral cavity, but the remainder of the cercal lumen connects with the perivisceral cavity via the lumen of the paraproct. Changes in the volume of the paraproct produced by a small muscle draw hemolymph from the outer lumen of the cercus and pump it into the perivisceral cavity. This flow draws hemolymph into the cercus through the central vessel.

5.2 Circulation

5.2.1 Movement of the hemolymph

The movement of hemolymph around the insect body has been studied by analysis of the distribution of markers injected into the body cavity or ingested by the insect. Generally, injected substances become uniformly distributed through the hemolymph within 5–20 minutes, varying with environmental conditions, especially temperature, and the physiological status of the insect.

Hemolymph circulation is maintained primarily by the dorsal vessel. In hemimetabolous and larval holometabolous insects, the hemolymph is pumped forwards through the heart at systole, entering the perivisceral sinus through the anterior opening of the aorta in the head (and through the excurrent

Caption for Figure 5.4 (cont.) When the muscles relax (c), elastic suspensory elements draw the diaphragm up and force blood out anteriorly (after Krenn and Pass, 1993). (d) Wing heart connected to the aorta as in most hemimetabolous insects. Longitudinal section of the thorax. The subcutellar reservoir connects with the axillary cord of the wing on either side (modified after Whedon, 1938). (e)–(g) Antennal pulsatile organ of cockroach. (e) General arrangement as seen from above. Top of the head cut away and brain removed. (f) Dilator muscle contracts, enlarging lumen of ampulla so that blood is drawn in from the hemocoel in the head. Lowered pressure causes constriction at the origin of the antennal vessel so that the backflow of blood from the antenna is restricted. (g) The muscle relaxes and the ampulla is flattened by the elasticity of its inner wall and the pull of the tendon. (h) Leg pulsatile organ of *Triatoma*. The ostium is closed by a valve and blood forced into the antennal vessel (after Pass, 1985). (h) Leg pulsatile organ of *Triatoma*. Contraction of the muscle compresses the blood sinus on one side of the septum and enlarges that on the other so that blood flows down the leg on one side of the septum and up on the other (after Kaufman and Davey, 1971).

ostia where these exist). The valves on the incurrent ostia prevent the escape of hemolymph through these openings. The force of hemolymph leaving the aorta anteriorly tends to push hemolymph backwards in the perivisceral sinus. The backwards flow is aided by the movements of the dorsal diaphragm and by the inflow of hemolymph into the heart, through the incurrent ostia, at diastole (Fig. 5.5a–c). Movements of the ventral diaphragm presumably help to maintain the supply of hemolymph to the ventral nerve cord.

In adult Lepidoptera, Coleoptera and Diptera, and perhaps in some other insects, the hemolymph is shunted backwards and forwards between the thorax and abdomen, rather than circulated. This is possible because the hemocoel in the two parts is separated – by a movable flap of fatty tissue in Lepidoptera or large air sacs in cyclorrhaphous Diptera – and because the heartbeat exhibits periodic reversals. As the abdomen contracts, the heart pumps hemolymph forwards into the head (Fig. 5.5d). When the heart reverses, the abdomen actively expands, drawing hemolymph past the barrier of fat (Fig. 5.5e). Movements of the hemolymph are coordinated with ventilatory movements. Any activity which tends to induce pressure differences between different parts of the body must affect the circulation, and, especially in adult Lepidoptera, Coleoptera and Diptera, there is a close functional link between ventilatory and hemolymph movements.

Many insects appear to have a well-defined, but variable circulation through the wings, although in some, apparently, circulation only occurs in the young adult. In the absence of this wing circulation, the tracheae in the wings collapse, and the wing structure becomes brittle and dry. In most cases, hemolymph is drawn out of the wings from the axillary cords by the thoracic pulsatile organs. In the German cockroach, *Blattella*, the anal veins connect with the axillary cord through channels between the upper and lower wing membranes. As hemolymph is drawn out posteriorly, it is drawn in anteriorly from a hemolymph sinus from which the major anterior veins arise (Fig. 5.6).

In insects lacking leg pulsatile organs, the flow of hemolymph through the legs is thought to be maintained by pressure differences at the base. Hemolymph passes into the posterior compartment of the legs from the perineural sinus and out into the perivisceral sinus from the anterior compartment.

5.2.2 Heartbeat

Systole, the contraction phase of the heartbeat, results from the contractions of the intrinsic muscles of the heart wall. In most insects this activity begins posteriorly and spreads forwards as a wave.

In *Periplaneta* and Orthoptera, however, the entire length of the heart contracts, usually synchronously although peristaltic contractions may sometimes occur. Diastole, the expansion phase when hemolymph enters the heart, results from relaxation of the heart muscles assisted by the elastic filaments that support the heart. In general, the alary muscles are not responsible for diastole and often contract at a lower frequency than the heart. In *Rhodnius*, however, complete diastole is dependent on the alary muscles. After diastole there is a third phase in the heart cycle known as diastasis, in which the heart remains in the expanded state (Fig. 5.7). Increases in the frequency of the heartbeat result from reductions in the period of diastasis. There is considerable within- and between-species variation in the frequency of the heartbeat. In general, the heartbeat frequency declines with increasing age and size of larval insects, and also varies with age within a larval stadium. For example, in silkworms (*Bombyx*), the rate declines from 80 beats min^{-1} in second-stage larvae to about 50 in the fifth stage, and it drops sharply just before each molt except the last (Fig. 5.8a). In the pupa, the heartbeat falls to 10–20 beats min^{-1} and remains at this low level until shortly before adult eclosion. A similar, but slightly less marked, change occurs during the development of the migratory locust, *Locusta*, but the rate of beating tends to rise before a molt (Fig. 5.8b). In Lepidoptera, a very sharp increase in the heart

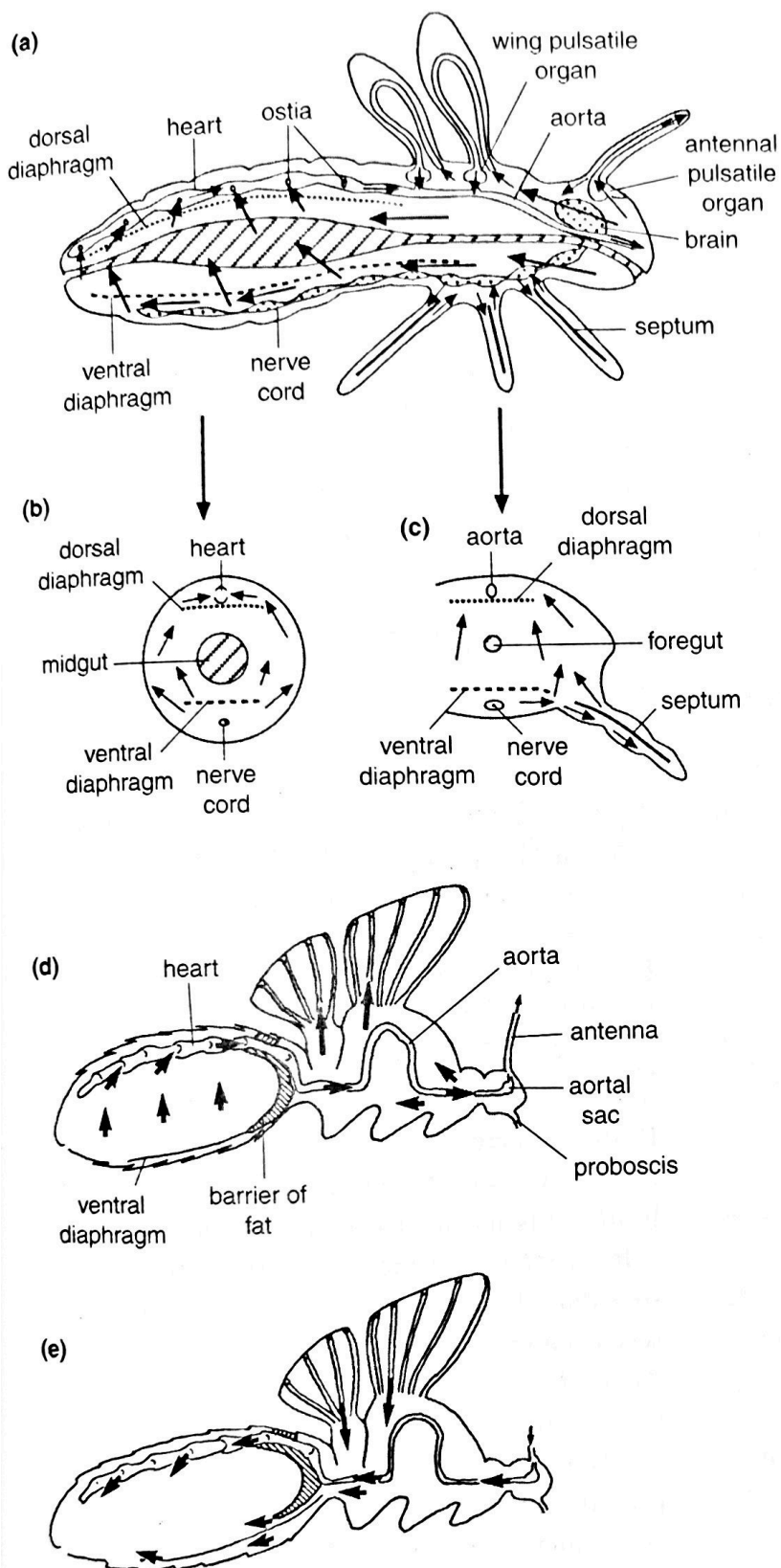


Figure 5.5 Blood circulation. (a)–(c) An insect with a fully developed circulatory system. Arrows indicate the course of the circulation. (a) Longitudinal section; (b) transverse section of abdomen; (c) transverse section of thorax. (d),(e) An insect in which the blood oscillates between the thorax and abdomen. (d) Abdominal contraction with the heart beating forwards pushes blood into the anterior regions of the insect; (e) abdominal expansion with the heart beating backwards draws blood into the abdomen (after Wasserthal, 1980).

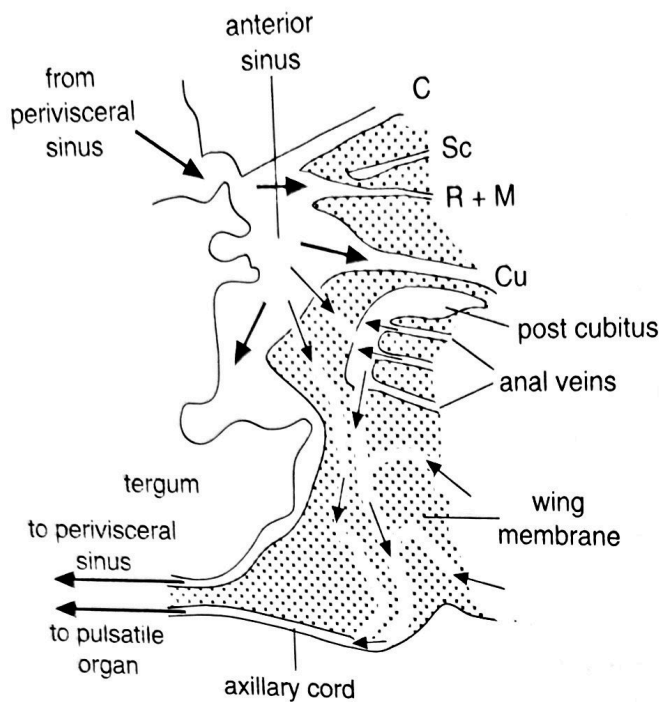


Figure 5.6 Blood circulation in the base of the forewing of *Blattella*. Areas in which the two membranes of the wing are fused together are shaded. Well-defined channels, such as veins, are outlined by a solid line, less definite channels have no lines. Axillary sclerites are omitted (after Clare and Tauber, 1942).

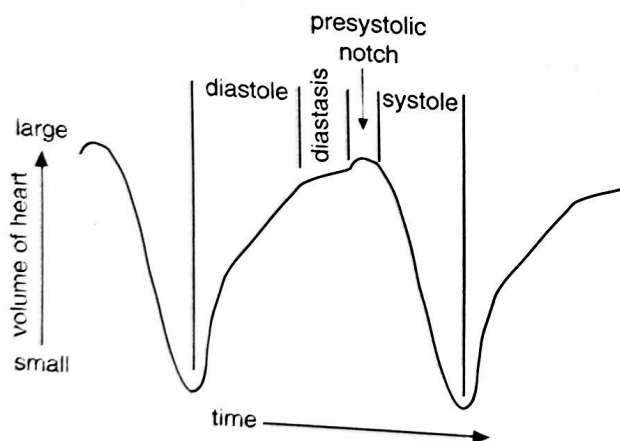
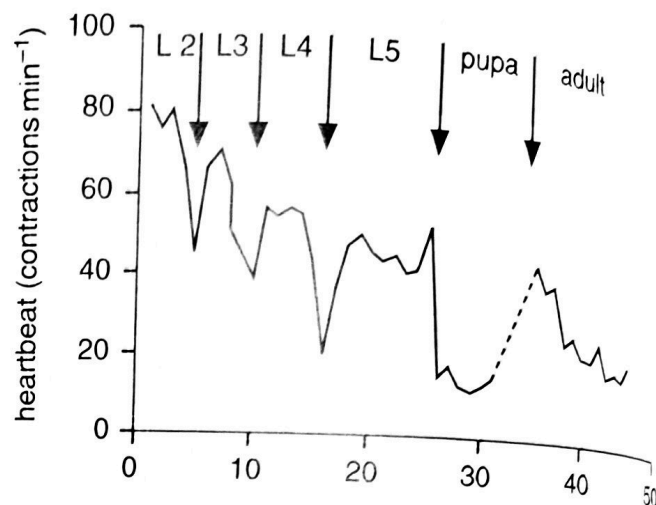


Figure 5.7 Changes in the volume of a section of the heart during beating. The presystolic notch results from an increase in hydrostatic pressure, producing a slight increase in volume within this part of the heart due to the start of systole in the more posterior segments.

rate occurs at eclosion, falling to a sustained but moderate rate when eclosion is complete.

In addition to these intrinsic changes, the rate of heartbeat is affected by environmental factors.

(a) *Bombyx*



(b) *Locusta*

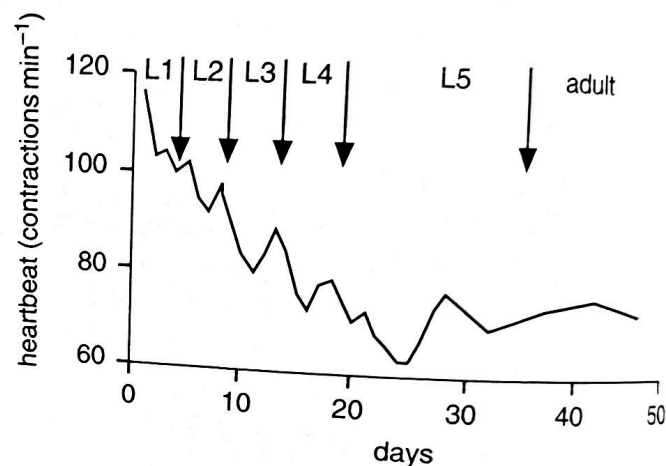


Figure 5.8 The frequency of heartbeat at different stages of development. L1–L5 indicate larval stages; arrows indicate eclosion. (a) *Bombyx* (after Masera, 1933); (b) *Locusta* (after Roussel, 1972).

Heartbeat increases with temperature within the thermal limits for the insect. In *Locusta* the rate of heartbeat is also higher in the light than in the dark. It is common for the heart to stop beating, sometimes for a few seconds, but sometimes for 30 seconds or more. The heart of the young pupae of *Anopheles* sometimes stops beating altogether, and in old pupae no activity of the heart is observed.

It is also common for the heartbeat to undergo periodic reversals, with waves of contraction starting at the anterior end. When this occurs, hemolymph is forced out of the "incurrent" ostia; in the mole cricket, *Gryllotalpa*, powerful currents pass out of the subterminal incurrent ostia. In female *Anopheles*, 31% of heartbeats start at the anterior end of the

heart. Reversal of heartbeat is rare in holometabolous larvae, but begin in the pupal stage or even at the larva-pupa ecdysis. Usually the rate of heartbeat is lower when the heart is pumping backwards. In the adult blowfly, *Calliphora*, the rates are about $175 \text{ beats min}^{-1}$ backwards compared with about $375 \text{ beats min}^{-1}$ forwards. In pharate adult Lepidoptera, periods of fast, forward beating lasting a few minutes alternate with periods when the heart reverses and beats more slowly, but during the period of wing expansion following eclosion no reversals occur (Fig. 5.9). Subsequently, periodic reversals are an essential feature of hemolymph circulation, at least in the Lepidoptera (see above). The distribution of hemolymph pressures is an important determinant of the direction of the heartbeat (if pressure at the front of the heart is so high that back pressure is set up, the heartbeat is reversed), but other factors, including oxygen supply, may also contribute to the incidence of beat reversal.

The activity of the pulsatile organs may be different from that of the heart. The antennal ampullae of *Periplaneta* pulse at about $28 \text{ beats min}^{-1}$, considerably slower than the heart rate. The wing pulsatile organs of Lepidoptera are only active during heart reversal (Fig. 5.5). At eclosion

the pulsatile organs pulsate more rapidly and without interruption.

The activity of the heart is basically myogenic, although the myogenic pattern may be modulated neurally or hormonally. As the segmental nerves leading to the heart contain the ramifying terminals of neurosecretory axons, it might be expected that their secretions exert modulatory effects. In addition, hormones released into the hemolymph at points remote from the heart are known to affect it. For example, the increase in the rate of beating at the time of eclosion in Lepidoptera is at least partly due to peptides released in the hemolymph at this time. These cardioacceleratory peptides are produced in neurosecretory cells in the ganglia of the ventral nerve cord and released into the hemolymph at the perivisceral neurohemal organs. The same peptides also increase the heartbeat during flight. Their effects are synergized by very low levels of octopamine, which is also present in the hemolymph during wing inflation and flight. A number of other neurohormones affect the heart rate in vitro, but they are not known to be involved in regulation of heartbeat in the intact insect.

5.3 Hemolymph

The hemolymph fills the hemocoel and circulates around the body, bathing the tissues directly. It consists of fluid plasma in which cells, known as hemocytes, are suspended. The properties and composition of the plasma, together with its function in transport, storage and immunity, are considered in this section. The diversity and functions of hemocytes are addressed in Section 5.4.

5.3.1 Hemolymph volume

The hemolymph volume, expressed as a percentage of the total body weight of the insect, varies widely among insects. In the heavily sclerotized tenebrionid beetle *Onymachus*, hemolymph constitutes about

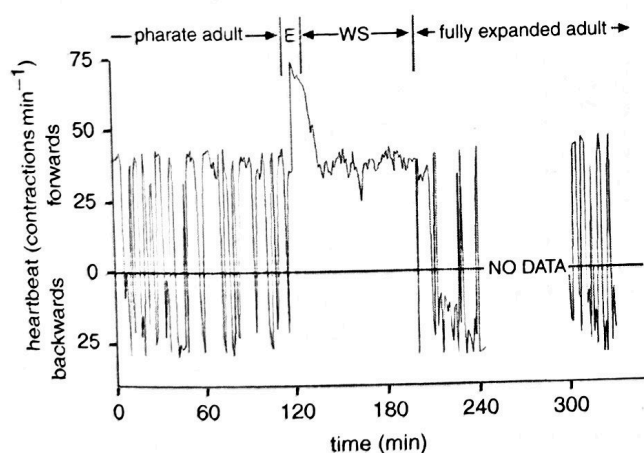


Figure 5.9 Changes in the heartbeat of an individual *Manduca* at the time of eclosion. E = eclosion, WS = wing spreading (after Tublitz and Truman, 1985).

11% of the beetle's total mass; in mid-stadium larvae of *Locusta*, the figure is about 18%, while in mature adults it is about 12%; in cockroaches it is about 17%; and in caterpillars 35–40%.

In most insects hemolymph water accounts for 20–25% of the total body water (the remainder being principally within cells). Generally, the water content and hydrostatic pressure of hemolymph increase just prior to ecdysis. For example, the hemolymph volume in *Schistocerca* locusts almost doubles in advance of each molt, and is then reduced again after the molt (Fig. 5.10). In *Periplaneta* the pre-molt increase in volume is associated with an increase in activity of an antidiuretic hormone and the post-ecdysial fall in volume is produced by a transient rise in the hemolymph titer of diuretic hormone (Fig. 5.11). Similarly, diuresis reduces the hemolymph volume of the cabbage butterfly *Pieris* by about 70% in the hours following eclosion. In caterpillars, however, the hemolymph water content is high, accounting for up to 50% of the total water content, and the associated high pressure provides for a hydrostatic skeleton that maintains body shape and contributes to locomotion. Caterpillar locomotion is considered further in Chapter 8.

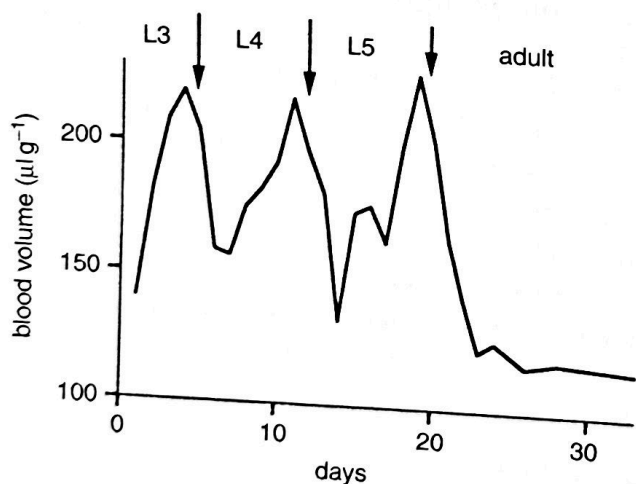


Figure 5.10 Changes in the blood volume (expressed as volume per unit weight) during the development of *Schistocerca*. L3, L4 and L5 refer to successive larval stages. Arrows indicate the time of ecdysis (after Lee, 1961).

An important function of the hemolymph is to provide a reservoir of water to sustain the levels of water in the tissues. This is illustrated vividly by experiments conducted on the desert tenebrionid *Onymachus*. When maintained in desiccating conditions, hemolymph volume is reduced by about 60%, but tissue water content does not change (Fig. 5.12). Some of the tissue water is drawn from the hemolymph; the remainder comes from fat metabolism. Similar changes can occur over shorter periods in some insects. For example, it is almost

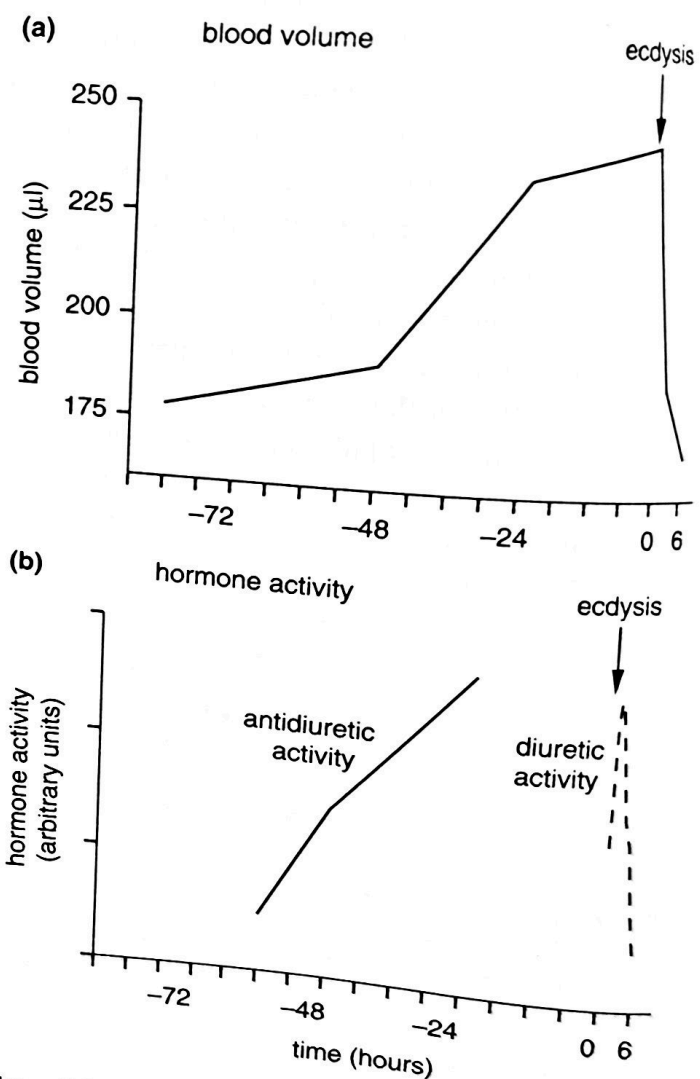


Figure 5.11 Regulation of blood volume in relation to ecdysis in *Periplaneta* (after Mills and Whitehead, 1970). (a) Changes in blood volume; (b) antidiuretic and diuretic activity of the hemolymph, suggesting that changes in hormonal activity regulate the changes in volume.

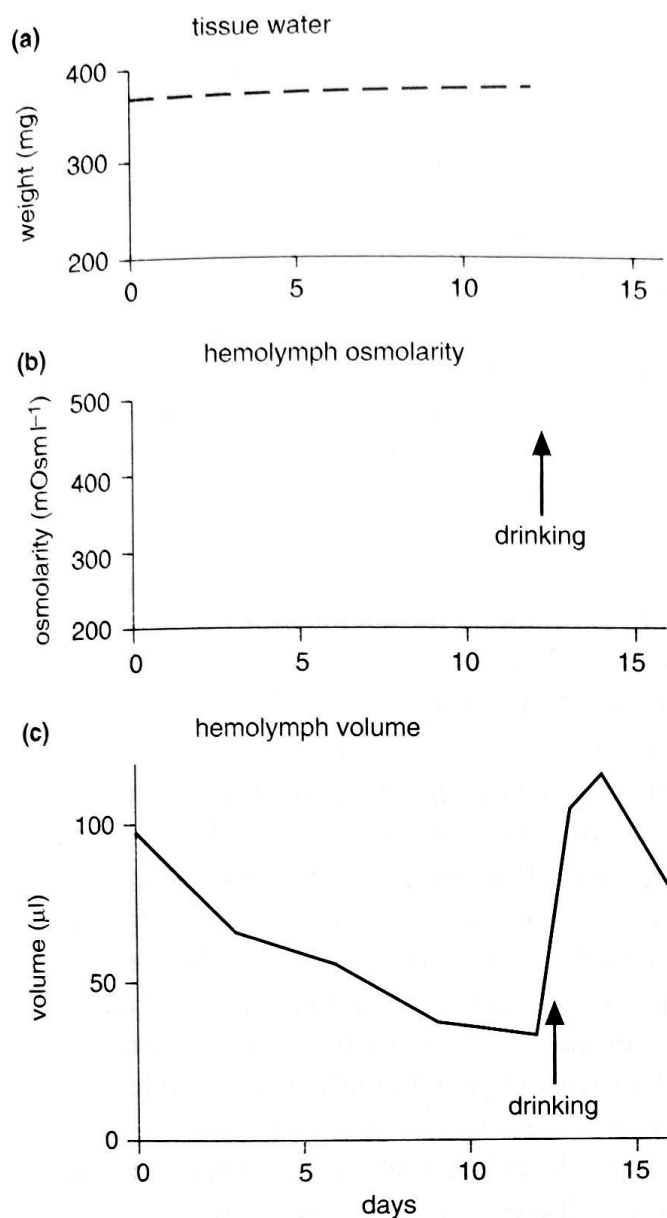


Figure 5.12 Regulation of tissue water and hemolymph osmotic pressure in *Onymacris*. The insects were without food or water at 26°C and 10–15% relative humidity for the first 12 days. On day 12 they were given distilled water to drink and then maintained at 50–60% relative humidity (after Nicolson, 1980). (a) Tissue water remained almost constant over the 12 days without food or water. (b) Hemolymph osmolarity rose very slightly during the first 12 days of concentration, despite the marked reduction in hemolymph volume (c).

impossible to obtain hemolymph samples from red desert locusts, *Nomadacris*, collected in the heat of the afternoon, but the insects have ample hemolymph in the morning and late evening.

5.3.2 pH of hemolymph

The pH of insect hemolymph is usually between 6.4 and 6.8, although values >7 pH units have been recorded in a dragonfly larva and in the larvae of the midge *Chironomus*. During activity the hemolymph tends to become more acidic due to the liberation of acid metabolites, including carbon dioxide. The buffering capacity of the hemolymph is mediated by various compounds, including bicarbonate and inorganic phosphate, the carboxylic and amino groups on various organic compounds, especially amino acids. The extrusion of protons from the hemolymph can also contribute to maintaining the hemolymph pH. In experiments on *Schistocerca*, the hemolymph recovered its normal pH within 24 hours after injection of acid (25 μl 0.4 M HCl); and this was accompanied by a significant reduction in pH of the gut contents, suggesting that the acid load in the hemolymph was relieved, at least partly, by protons extruded from the hemolymph.

5.3.3 Osmotic pressure and osmotically active solutes

The osmotic pressure of insect hemolymph is generally 0.7–1.2 MPa, with some variation with species, physiological condition and developmental stage of the insect. (In particular, overwintering insects in diapause sustain high hemolymph osmotic pressures, often greater than 1.2 MPa.) For a given insect, the osmotic pressure is regulated within narrowly defined limits.

The most important osmotic effectors are inorganic ions and small organic molecules, including sugars and amino acids. These compounds are also essential substrates for the maintenance and growth of insect tissues and organs. Consequently, the concentration of these solutes in hemolymph is controlled, despite variation in hemolymph volume (Section 5.3.1), by exchange with tissues (uptake by tissues when hemolymph volume is declining, and release from tissues into hemolymph when it is increasing).

Importantly, the osmotic pressure of hemolymph cannot be determined accurately from its solute content because some solutes are bound to macromolecules, reducing their osmotic effect. For example, in the larvae of the mosquito *Aedes*, the osmotic activity of sodium is lower than expected from its concentration, possibly because some is bound to large anionic molecules.

The relative importance of the different compounds varies among insect groups (Fig. 5.13). In many insects the most abundant inorganic ions in the hemolymph are Na^+ and Cl^- . Anions additional to Cl^- include phosphate and bicarbonate, usually at low concentrations; and the cations K^+ , Mg^{2+} and Ca^{2+} are also present. There is, however, considerable variation among insect groups, following both phylogenetic and dietary factors (Fig. 5.13).

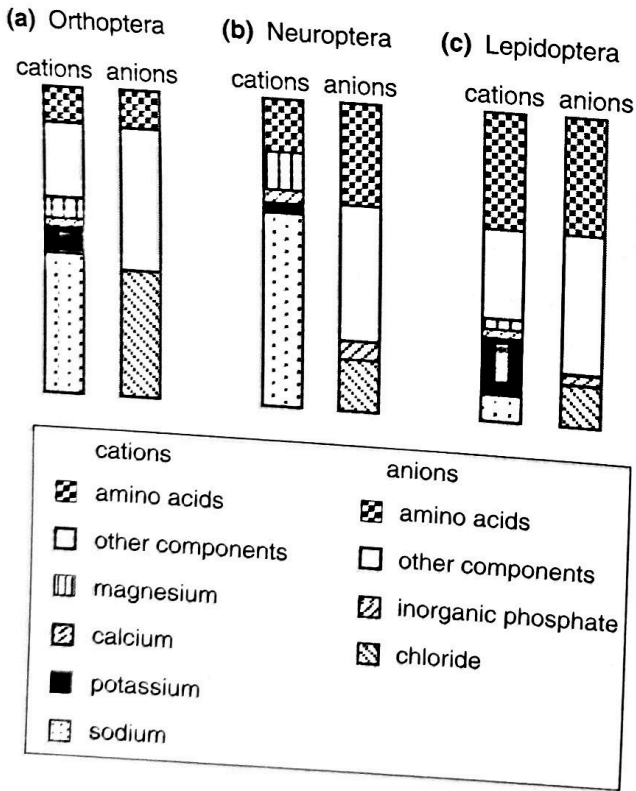


Figure 5.13 Osmotic components of the hemolymph in different groups of insects expressed as percentages of the total osmolar concentration. Each vertical column represents 50% of the total concentration (after Sutcliffe, 1963).

The Na^+ and Cl^- contents tend to be higher in hemimetabolous insects than holometabolous groups, in which the Na^+ is largely replaced by other inorganic cations (K^+ or Mg^{2+}) and Cl^- by organic anions. For example, the ratio of $\text{Na}^+:\text{K}^+$ is 10:1 in Orthoptera, but 1:5–10 in Lepidoptera. The high K^+ content of lepidopteran hemolymph has been linked to the high $\text{K}^+:\text{Na}^+$ ratio of plant tissues. Insect hemolymph contains low concentrations of transition metals, including zinc, manganese, copper and iron; the iron is not free in the hemolymph, but is bound to two proteins, ferritin and transferrin, which ensure that the iron is kept in a soluble and non-toxic form.

In most insects the dominant hemolymph sugar is the disaccharide trehalose, at concentrations of 10–50 mM. Glucose is generally present at much lower concentrations (<5 mM). It remains to be established whether the reports of high glucose concentrations in the hemolymph of some species, e.g., larval *Phormia* blowflies, are valid or the consequence of trehalose hydrolysis during sample preparation. Some insects additionally have appreciable hemolymph concentrations of polyols. For example, sorbitol accumulates in the hemolymph of the whitefly, *Bemisia tabaci*, reared at high temperatures.

Insect hemolymph plasma is characterized by very high levels of free amino acids, ranging from 25 to 75 mM, and functioning as buffers in osmoregulation and as substrates for protein synthesis. Most or all of the 20 amino acids contributing to insect protein are detectable, although there is considerable variation in the relative abundance of the various amino acids among insect groups and with developmental stage. Of particular interest is tyrosine, which is poorly soluble and occurs predominantly as the more soluble glucoside in hemolymph. However, just before the insect molts, free tyrosine is released and accumulates and subsequently decreases sharply as it is used in tanning and melanization of the new cuticle (see Fig. 16.18).

Organic acids are present in some quantity in the plasma of insect hemolymph. The major components are acids associated with the citric acid cycle, including citrate, α -ketoglutarate, succinate and malate.

5.3.4 Hemolymph proteins

The plasma of insect hemolymph is protein-rich. The protein content varies among insect groups and with developmental age over an order of magnitude between 20 and 200 mg ml⁻¹, and proteome analyses reveal up to several hundred different proteins. Protein composition varies with developmental age and physiological condition. Many of the most abundant proteins play key roles in the transport and storage of nutrients between organs and in systemic immunity. The principal source of hemolymph proteins is the fat body, with some contribution from hemocytes (especially for immune-related proteins) and other organs.

One very important group of storage proteins are the hexamerins, which are very large proteins comprising (as the name suggests) six subunits (each 80 kDa). The hexamerins with a very wide phylogenetic distribution are the arylphorins, in which the aromatic amino acids phenylalanine and tyrosine comprise 18–26% of the total, but which contain little methionine. Lepidoptera also have methionine-rich hexamerins that contain 4–8% methionine. The hexamerins are synthesized in the fat body and released into the hemolymph, where they can accumulate to 50% of the total hemolymph protein. They are subsequently used as a source of amino acids, such as during pupation of holometabolous insects. Hexamerins are related to hemocyanins (respiratory pigments of many Crustacea and some insects, e.g., Plecoptera), and are believed to have evolved by the loss of the Cu²⁺-prosthetic group and oxygen-binding site of the hemocyanin molecule, and the shift in amino acid composition to favor aromatic amino acids or methionine.

Because lipids are not soluble in water, their transport in hemolymph involves combination with specific proteins of the large lipid transfer protein (LLTP) superfamily to form lipoproteins. Insects have a single class of lipoprotein called high-density lipophorin (HDLp), which transports diacylglycerol (DAG), together with small amounts of phospholipids and sterols. The HDLp particle is a re-usable shuttle: It circulates in the hemolymph between different tissues, alternately taking up lipids (e.g., from gut epithelium, fat body) and delivering lipids (e.g., to ovaries, flight muscle) without being internalized or degraded. The protein core of the HDLp particle is one copy each of apolipophorin I and apolipophorin II, derived by a single cleavage of the precursor protein apolipophorin II/I. Under conditions of high lipid demand (e.g., during sustained flight), the HDLp particle is transformed to a low-density lipophorin (LDLp) by association with several copies of a third apolipoprotein called apolipophorin III, together with substantially increased loading of DAG. The diameter of the LDLp is more than double that of the HDLp (Fig. 5.14).

The hemolymph of reproductive female insects contains appreciable amounts of a further protein of the LLTP superfamily: the glycolipophosphoprotein vitellogenin, which is usually transported as a homodimer of 200–660 kDa. Vitellogenin is a nutrient reserve for embryos. It is synthesized in the fat body and transported via the hemolymph to the ovaries, where it is taken up by developing oocytes (at a discrete developmental stage known as vitellogenesis). The vitellogenins of the honey bee, *Apis mellifera*, have been studied extensively, revealing that the hemolymph of adult workers (which are reproductively sterile) also contains appreciable amounts of vitellogenin. Overall, vitellogenin accounts for 70% of the protein in the hemolymph of adult queens and 50% of the hemolymph protein in young workers that reside in the nest ("nurse bees"). When workers are about two weeks old they switch to foraging, and this is accompanied by a rapid decline in hemolymph

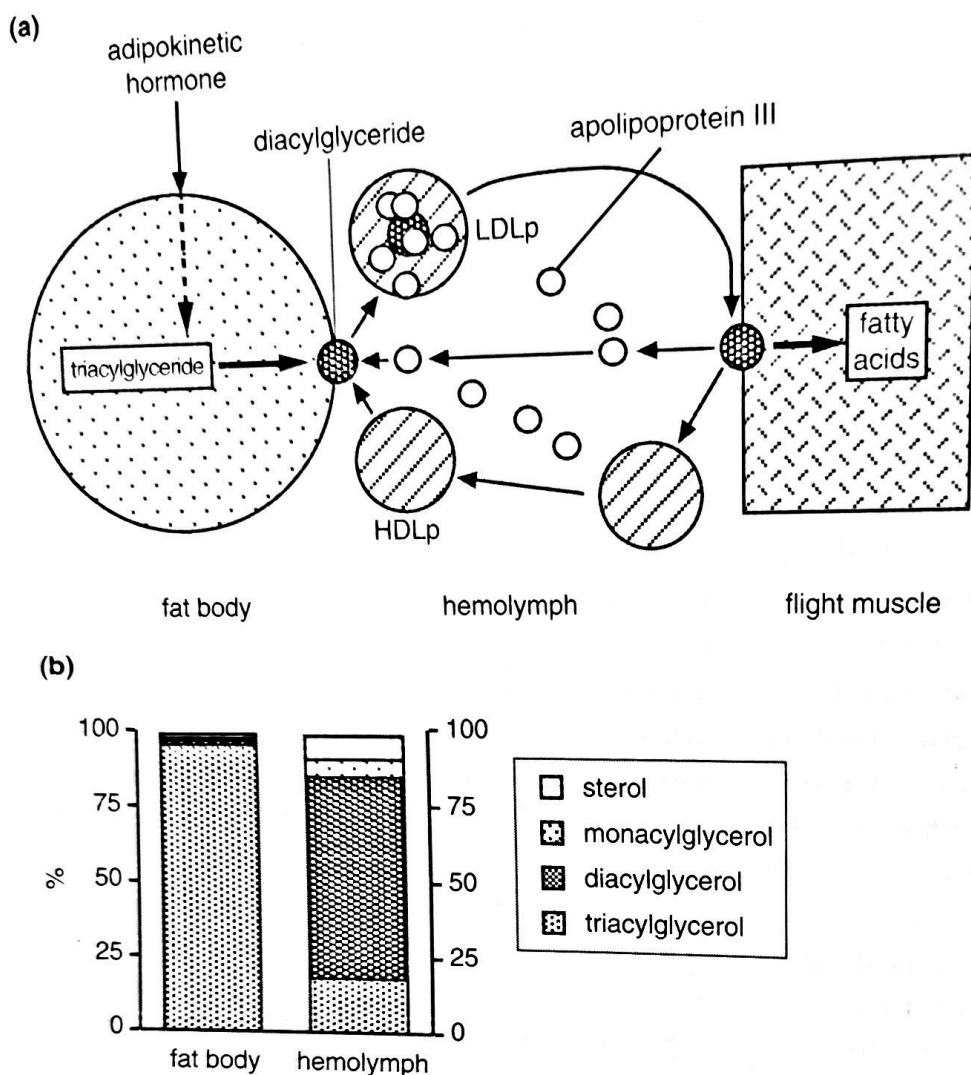


Figure 5.14 Lipid in the hemolymph. (a) Diagrammatic representation of the movement of lipids from the fat body to flight muscles in *Manduca* by the formation of low-density lipophorin (LDLp) (based on Shapiro *et al.*, 1988). (b) Proportions of major lipid components in the fat body and hemolymph of the pupa of *Hyalophora*. Most lipid is stored as triacylglycerol, but it is transported as diacylglycerol (data from Gilbert, 1967).

vitellogenin titer, to less than 20% of the total protein. Studies using RNAi-mediated knockdown of vitellogenin gene expression demonstrate that declining vitellogenin level is the signal responsible for the transition from nurse bee to foraging (Fig. 5.15).

The hemolymph additionally bears various immunity-related products that function in protecting the insect against systemic infection. These products are components of the humoral (i.e., non-cellular) part of the insect immune system. To a large extent, the humoral effectors are inducible, i.e., they are undetectable (or nearly so) in uninfected insects, and increase to high titers in response to mechanical wounding and infection by bacteria, fungal pathogens, nematodes, etc., or by the oviposition of parasitoid eggs. In this section,

two vitally important inducible humoral effectors are considered: the phenoloxidase system, which is induced very rapidly by a proteolytic cascade (see below) and anti-microbial peptides, whose production requires *de novo* gene expression and is somewhat slower.

Phenoloxidase is an enzyme that catalyzes the oxidation of phenols to quinones, and cytotoxic molecules (such as 5,6-dihydroxyindole) in reactions that also generate reactive oxygen and nitrogen intermediates. Subsequent reactions with phenoloxidase-derived quinones in the hemolymph lead to the synthesis of a brown-black insoluble pigment called melanin, which is also cytotoxic and contributes to the physical isolation of invading organisms. For example, various insects, including mosquitoes and aphids, enclose and inactivate

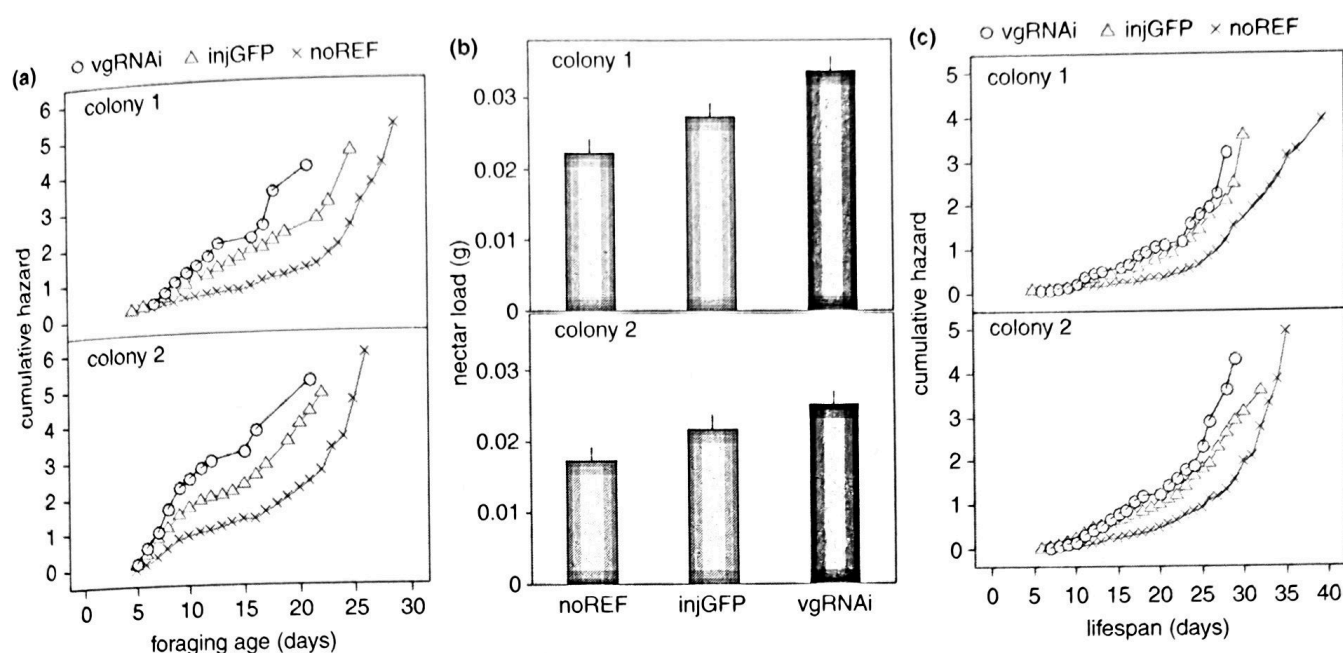


Figure 5.15 Impact of RNAi knockdown of expression of the vitellogenin gene in workers of the honey bee *Apis mellifera*. (a) Frequency of transition from nurse bee (in hive) to foraging bee activity; (b) nectar collection; (c) lifespan. vgRNAi: RNAi-mediated knockdown of vitellogenin gene expression; injGFP: injected negative control for RNAi against non-target gene (green fluorescent protein, GFP); noREF: uninjected control. Reproduced from Nelson *et al.* (2007), figures 2, 3 and 4.

bacterial clumps and parasitoid eggs in melanotic capsules, comprising non-cellular material dominated by melanin.

The phenoloxidase protein is stored as an inactive precursor, prophenoloxidase, in circulating hemocytes. In response to microbial products (e.g., peptidoglycan) or wounding, the prophenoloxidase is released from hemocytes and activated by limited proteolysis through the action of a specific serine protease. Prophenoloxidase activation and melanin formation also play a crucial role in the insect response to wounding by contributing to coagulation of blood and clot formation, thereby promoting bleeding control and wound healing. Studies with mutant *Drosophila* indicate that the primary clot requires the protein hemolectin (which has some sequence homology to the von Willebrand factor important in mammalian blood coagulation) but not phenoloxidase. In this insect the phenoloxidase-derived melanin is required for strengthening and stabilizing the initial clot, to form the mature, hardened clot that limits the risk

of infection after wounding. In some other insects, notably *Anopheles* mosquitoes, phenoloxidase is important for the formation of the initial clot.

Many of the humoral effectors in the hemolymph are derived from the fat body. They include anti-microbial peptides (AMPs), opsonins, which are essential components of the melanization clotting system (see above), catalase, which limits the reactive oxygen species produced by immune-related respiratory burst of immune cells, and transferrin, which reduces the availability of the free iron required for rapid microbial proliferation. By far the best characterized of these humoral immune effectors are the AMPs. Most AMPs are 15–50 amino acid residues in length and have an amphipathic structure, i.e., they include both charged and hydrophobic sequences, and this property enables them to partition into biological membranes. (The composition and net charge on microbial and insect membranes are different, and insect AMPs preferentially partition into bacterial membranes.) Some AMPs insert into the microbial membrane,

generating a pore that depolarizes the cell membrane; others penetrate through the microbial membrane to bind and disrupt an intracellular target. In response to infection, AMPs are synthesized in the fat body and released into the hemolymph. AMPs are gene products, and their production is regulated at the transcriptional level, specifically by transcription factors of the NF- κ B family. The transcription factor Relish is activated by the Imd signaling cascade in response to peptidoglycan fragments of Gram-negative bacteria that bind to receptors (peptidoglycan recognition proteins, PGRPs) on the surface of insect cells; and the transcription factor Dif is activated by the Toll signaling pathway, ultimately in response to molecular patterns of Gram-positive bacteria and fungi. Although there is cross-talk between the Toll and Imd pathways and other components of the intracellular signaling network, the AMPs regulated by the Imd pathway are primarily active against Gram-negative bacteria; and those regulated by the Toll pathway are active against Gram-positive bacteria and fungi.

The Imd and Toll signaling pathways are broadly conserved among the insects that have been studied (apart from the pea aphid, which lacks an intact Imd pathway), but the number and identity of AMPs regulated by these pathways vary widely. Some AMPs are taxonomically restricted. For example, only drosophilid flies are known to produce the anti-fungal AMPs drosomycin and metchnikowin, and gloverins are specifically lepidopteran AMPs. Other AMPs (e.g., cecropins, attacins) are known in various insects, and defensins occur widely across the animal kingdom.

The hemolymph of uninfected insects additionally contains lysozyme. This enzyme hydrolyzes β -1,4 linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan of bacterial cell walls. In the uninfected insect, the amount and activity of lysozyme in the hemolymph is low but generally detectable. It cleaves the peptidoglycan of invading bacteria, generating peptidoglycan fragments which act as elicitors of

the humoral response. Specifically, the fat body is triggered to synthesize and release high titers of lysozyme (an example of a positive feedback loop), phenoloxidase and AMPs.

5.4 Hemocytes

Hemocytes are the cells residing in hemolymph. They mediate the cellular arm of the insect immune response, especially phagocytosis and, in many insects, encapsulation. In this section the types of hemocytes, their origin and their function in immunity are addressed.

5.4.1 Types of hemocytes

The hemocytes have variously been classified by the criteria of morphology, function and molecular markers; and there is no universally recognized scheme for the classification of these cells (Fig. 5.16). Even so, the following types are recognized in many insects:

- (1) The prohemocyte: the stem cell, from which other hemocytes are derived. The prohemocyte is a small cell with very little cytoplasm. In most insects the prohemocytes are retained predominantly in hematopoietic organs (see below) and make a small contribution to the hemocyte population circulating in the hemolymph.
- (2) The granulocyte: a phagocytically active, adherent cell type that contains large numbers of intracellular granules. On activation, the granulocyte releases the granule contents. Granulocytes represent the most abundant hemocytes in many Lepidoptera. In mosquitoes, granulocytes inducibly express phenoloxidase activity following immune challenge.
- (3) The plasmatocyte: a large cell type that is strongly adherent and spreads across foreign surfaces (for example, to glass slides). Plasmatocytes have been described in various

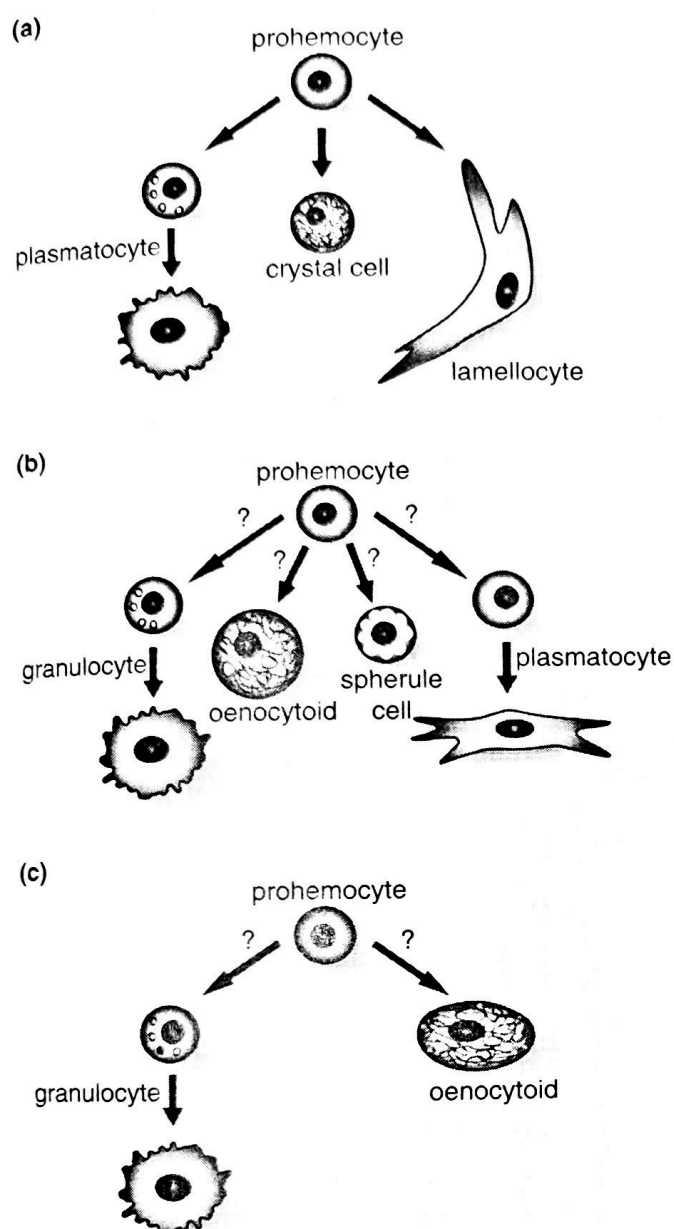


Figure 5.16 Types of hemocytes and their proposed lineage relationships in selected insects. (a) *Drosophila* larvae contain three differentiated hemocyte types in circulation: phagocytic plasmatocytes; phenoloxidase (PO)-containing crystal cells; and capsule-forming lamellocytes. Plasmatocytes are rounded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

Lepidoptera, but are not evident in some other insect groups, including *Anopheles* or *Aedes* mosquitoes or in aphids.

- (4) The oenocytoid: a non-adherent cell that is defined functionally as constitutively containing phenoloxidase activity.
- (5) The spherule: cells with large, refractile spherules in the cytoplasm and that contain cuticular components.

A different nomenclature is used to describe the hemocytes of *Drosophila* (Fig. 5.16a) in which three differentiated cell types are recognized: the phagocytically active plasmatocyte, which are broadly equivalent to the mammalian macrophages (and functionally very different from the plasmatocyte of other insect groups, see above) and account for >90% of circulating hemocytes of larval *Drosophila* under most conditions; the crystal cells (equivalent to oenocytoids of other insects), which contain prophenoloxidase and other components of the phenoloxidase cascade (see Section 5.3.4); and the lamellocytes, which are large, flat cells that mediate the cellular encapsulation of large foreign entities, such as parasitoid eggs.

5.4.2 Origin of hemocytes

Hemocytes are produced in structures known as hematopoietic organs. (Since, in insects, only the blood cells, not the plasma, are produced in these

oenocytoids. Granulocytes and plasmatocytes are rounded cells in circulation but both bind to foreign surfaces with granulocytes spreading symmetrically and plasmatocytes spreading asymmetrically. Hematopoietic organs contain putative progenitor prohemocytes and plasmatocytes, whereas proliferation of other hemocyte types occurs primarily in circulation. (c) Hemocytes of mosquitoes (*Aedes aegypti* and *Anopheles gambiae*) include three circulating types: prohemocytes; phagocytic granulocytes; and PO-containing oenocytoids. Prohemocytes are putative progenitor cells but lineage relationships have not been experimentally defined. Reproduced from Strand (2008).

structures, they should strictly be called hematocytopoietic organs, but the more general term is usually used.) The position of hematopoietic organs varies among insects. For example, in the cricket, *Gryllus*, and the mole cricket, *Gryllotalpa*, they are paired, segmental structures that open directly into the heart (Fig. 5.1), and in *Locusta*, *Periplaneta* and larvae of cyclorrhaphous flies and of the beetle *Melolontha* they consist of irregular accumulations of cells close to the heart, but not connected with it (Fig. 5.17a,b). By contrast, the lepidopteran hematopoietic organs comprise four structures, one in close proximity to each imaginal wing disc (Fig. 5.17c,d).

In all insects studied in detail, the hematopoietic organs are not the sole source of hemocytes. All the hemocytes of embryos and most in larvae are derived from progenitor cells of embryonic origin. For example, in *Drosophila*, embryo-derived hemocytes differentiate in the mesodermal tissue of the embryo head, migrate throughout the embryo and are subsequently allocated to the larval hemolymph. The hemocytes derived from the hematopoietic organ (which in *Drosophila* comprises the bilateral lymph gland along the anterior dorsal vessel) are released into the hemolymph in the final larval stadium. This is when the lymph glands degrade. Similarly, the

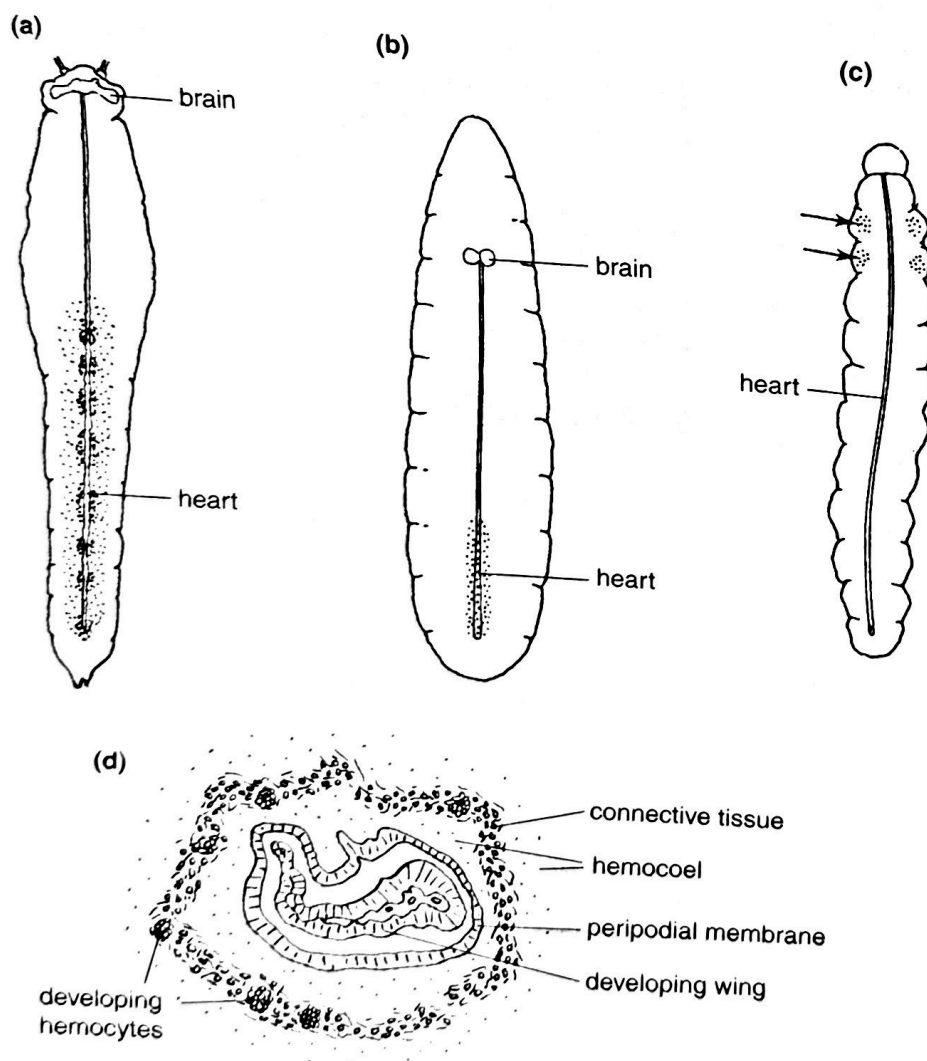


Figure 5.17 Hemopoietic organs in different insects. Stippling indicates hemopoietic tissue (see also Fig. 5.2). (a) *Locusta*; (b) *Calliphora* larva; (c), (d) caterpillar. (c) Showing the positions of the organs (arrows); (d) section through one wing disc (after Monpeysson and Beaulaton, 1978).

hemocytes from the hematopoietic organ of Lepidoptera contribute to the hemocyte pool late in larval development. The genes that specify hemocytes include homologs of the GATA (Serpent) and Runx (Lozenge) families of transcription factors, which are involved in vertebrate hematopoiesis.

Hemocytes are usually quantified as the number of cells per unit hemolymph volume, which is known as the "total hemocyte count." Values vary widely among insects, and with developmental age and physiological condition. Aphids are reported to have just 1800 hemocytes per microliter, and there are 15 000 hemocytes per microliter in the bark bug, *Halys dentate*, 80 000 cells per microliter in the cockroach, *Periplaneta americana*, and 300 000 cells per microliter in *Spodoptera* just prior to the onset of metamorphosis. In hemimetabolous insects, the numbers are generally similar in larval and adult insects, but in holometabolous species it is usual for larvae to have more cells per unit volume of hemolymph than adults. In general, adult females have a higher number of hemocytes than males.

These generalities about the variation in hemocyte counts notwithstanding, values of hemocyte counts can be misleading for two reasons. First, hemocyte counts are normalized to hemolymph volume, which can vary widely because of the role of the hemolymph in water balance (see Section 5.3.1). Second, some hemocytes are not in circulation, but attached to epithelial tissues, forming a distinct pool of sessile cells. The sessile hemocytes can be released into circulation following an immune challenge, a process that may be accompanied by one or both of proliferation and differentiation. For example, the encapsulation of parasitoid eggs in larval *Drosophila* is mediated principally by lamellocytes that are derived from sessile hemocytes associated with the epithelial cells and imaginal discs. It appears that the cells undergo the final stages of differentiation into lamellocytes as they are released into circulation.

5.4.3 Functions of hemocytes

Hemocytes play a crucial role in both development and immunity. Studies on *Drosophila* in which hemocytes are ablated have revealed that these cells are absolutely required for normal development. In the embryo, the hemocytes phagocytose cells that have undergone programmed cell death as a normal part of development, and secrete extracellular matrix and other proteins that promote organogenesis. In particular, hemocyte-derived material is required for normal development of the ventral nerve cord and Malpighian tubules. In holometabolous insects, hemocytes are also important in elimination of larval tissues that have undergone programmed cell death at metamorphosis.

The hemocytes play a central role in wound healing and protection against infection in both the insect embryo and throughout the life of the insect. This is partly through the secretion of compounds, such as phenoloxidase that contribute to humoral immunity (Section 5.3.4), and also through direct involvement, i.e., cellular immunity. Hemocytes remove a variety of small particles, including isolated bacterial cells and yeasts, as well as synthetic beads, by phagocytosis. After internalization, microbes that lack appropriate defenses are delivered to lysosomes and destroyed.

Where the hemolymph is infected by large numbers of bacteria or fungal spores, the hemocytes do not phagocytose the microbes, but produce nodules (Fig. 5.18). Within a few minutes of entry, bacteria are localized into aggregations by humoral compounds, sometimes accompanied by melanization (see Section 5.3.4). Subsequently, plasmatocytes become associated with the aggregation, forming a layer around the outside of the clump of melanized and dying cells. Eventually the clusters of coagulated microbes are enclosed within a hardened melanized nodule often containing hemocyte debris and necrotic cells, as well as dead microbes.

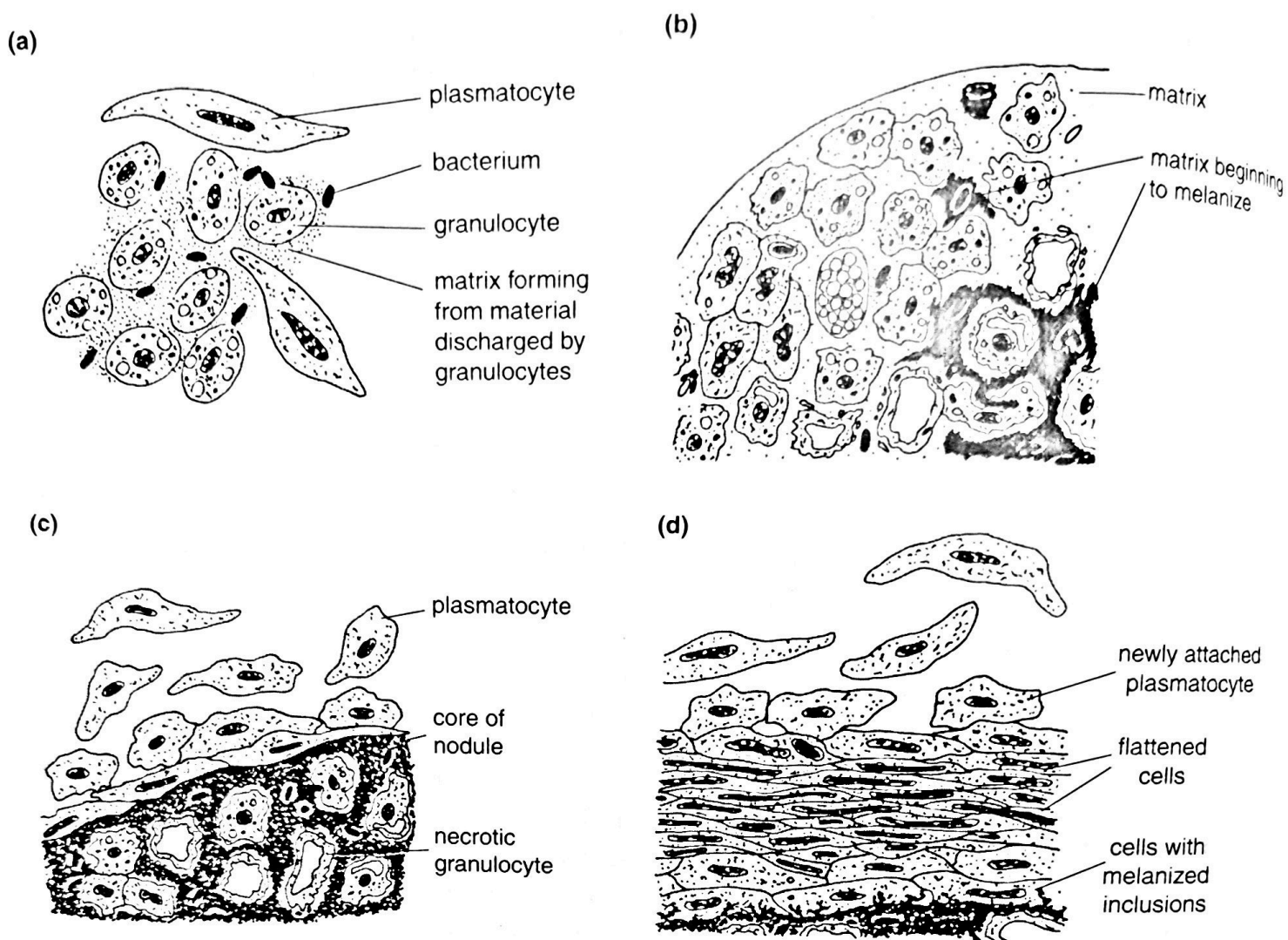


Figure 5.18 Nodule formation (after Ratcliffe and Gagen, 1977). (a) One minute after injection of bacteria. Granulocytes have degranulated and bacteria are trapped in the flocculent material produced by the cells. (b) 30 minutes after injection. The clumps of granulocytes and bacteria have compacted, and melanization of the matrix is beginning. (c) Plasmotocytes arrive at the nodule and melanization of the matrix is advanced. (d) 24 hours after injection. Nodulation is complete. Three regions are recognizable in the layers of plasmotocytes.

In many insects, large invaders, such as parasitoid eggs or larvae and nematodes, evoke a cellular response known as encapsulation, which is essentially the same as nodule formation but produces a larger melanized structure. Encapsulation involves large numbers of hemocytes, generally the plasmatocytes, together with some granulocytes, in *Lepidoptera* and lamellocytes in *Drosophila*. For example, in the lepidopteran *Pseudoplusia includens*, granulocytes first adhere to the foreign structure. The granulocytes are believed to produce factors that activate and recruit plasmatocytes, which generate a multilayered sheath that completely surrounds the foreign structure. The process is usually complete

within 24 hours. Melanin is often produced in this layer as a consequence of the activation of the prophenoloxidase system.

The microbes in nodules and parasites in capsules are almost certainly killed by a combination of being entombed in a hard capsule and thus isolated from the nutrients in the hemolymph, as well as the effects of the cytotoxins, including the products of the prophenoloxidase cascade. The nodules and capsules remain inside the insect's hemocoel until it dies.

If the epidermis is damaged, a clot forms beneath the wound. Formation of the clot involves components from both the hemocytes and the plasma. Granulocytes release material which forms a gel. This gel is stabilized

by plasma lipophorins; phenoloxidasases from the hemocytes may also be important.

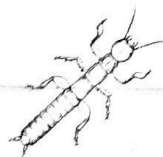
Some time after clotting has occurred, plasmatocytes migrate to the site. In *Rhodnius* the cells become linked to each other by zonulae adherens

within 24 hours of the wound occurring and subsequently tight junctions and septate desmosomes are formed. In this way, hemocytes become bound together to form a continuous tissue. The epidermal cells migrate over the clot to repair the wound.



Summary

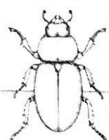
- The circulatory system of insects comprises the dorsal vessel, which mediates the circulation of the hemolymph (blood) around the insect body, and multiple accessory pulsatile organs that promote blood flow through appendages, including legs, wings and antennae. The circulatory system is open, meaning that hemolymph is in direct contact with the insect organs.
- Hemolymph is generally pumped forwards from the abdomen to head by peristalsis of the dorsal vessel. The heartbeat is myogenic in origin, with modulation by neuronal input in some insect groups. Blood flow is sometimes reversed by waves of contraction starting at the anterior end of the dorsal vessel.
- The hemolymph accounts for 10–40% of the volume of the insect. It contains inorganic ions, sugars (especially trehalose), amino acids and other organic compounds that collectively maintain the osmotic pressure of the hemolymph. Hemolymph proteins at 20–200 mg ml⁻¹ include proteins that function in storage (hexamerins, vitellogenin), transport (lipophorins that mediate lipid transport) and immunity, notably phenoloxidase and anti-microbial peptides that are induced by immunological challenge.
- The hemolymph contains hemocytes (blood cells) measured in cells per microliter. Some hemocytes are phagocytic and function to eliminate microbial pathogens and tissue debris generated during embryonic development, metamorphosis and by wounding. Other hemocytes protect against large foreign bodies (e.g., parasitoid eggs, nematodes) by encapsulation.



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