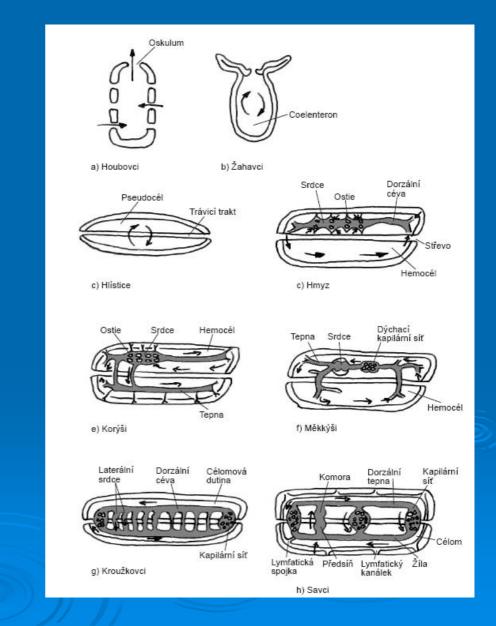
Oběhový systém a imunita



Otevřené a uzavřené sst.

1) Rozdíl mezi systolickým a diastolickým tlakem je malý. 2) Periferní odpor je malý, s čímž souvisí i malá intenzita srdeční činnosti (výkon srdce). 3) Krev neproudí plynule. 4) Podmínky výměny látek s tkáněmi jsou horší vzhledem k menší ploše styku hemolymfy s tkáněmi. 5) Transportní mechanizmus je sice méně energeticky náročný, je však také méně výkonný. 6) Omezená možnost regulace.

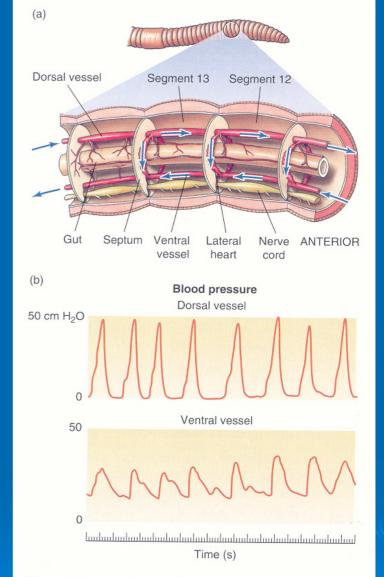


Figure 12-1 In the giant earthworm (Megascolides australis), peristaltic contractions of the dorsal vessel and pumping by the lateral hearts are both important in moving blood. (a) Blood flows from the dorsal vessel into the lateral hearts, present in the 13 anterior segments, and then is pumped into the ventral vessel. (b) Peak blood pressure in the dorsal vessel is about twice as high as in the ventral vessel owing to peristaltic contractions. [Adapted from Jones et al., 1994.]

Srdce Otevřených a Uzavřených sst.

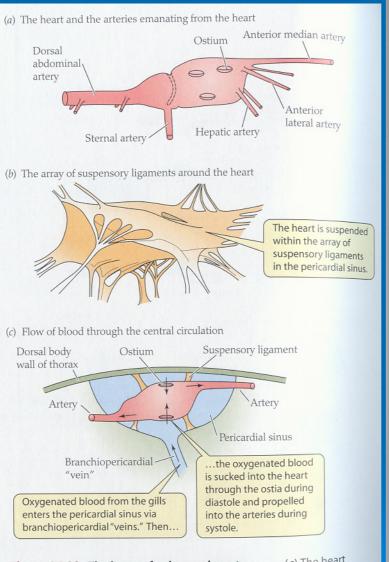
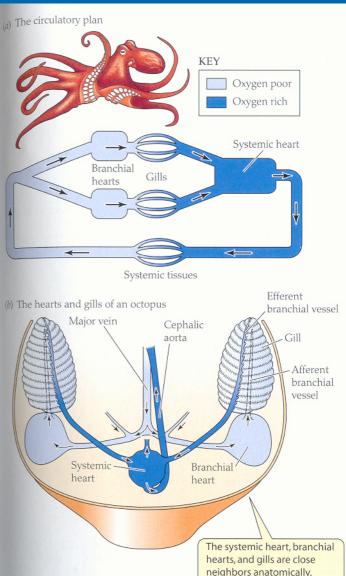


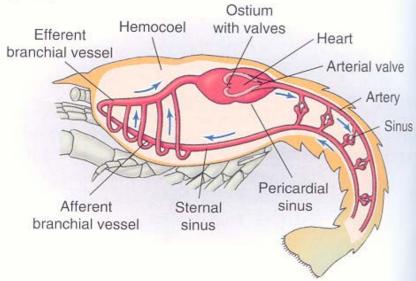
Figure 24.22 The heart of a decapod crustacean (a) The heart and the arteries leaving it. All the vessels connected to the heart in a crustacean are arteries. (b) The heart is surrounded by an array of elastic suspensory ligaments that run between the heart wall and the wall of the surrounding pericardial sinus, suspending the heart in the pericardial sinus. (c) The position of the heart in the pericardial sinus and the pattern of blood flow through the central circulation. (a after Wilkens 1999: b after Plateau 1880.)



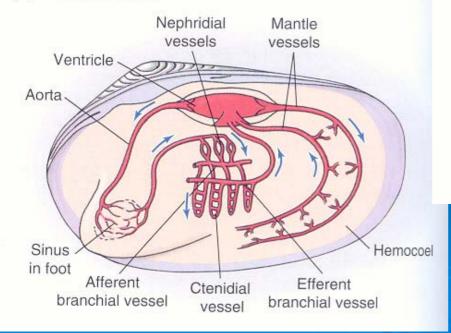
igure 24.20 The circulatory plan of squids and octopuses

a) The arrangement of the hearts, gills, and systemic tissues relative to one another. Hemocyanin, the respiratory pigment of squids and octopuses, turns blue when well oxygenated, but is clear or nearly clear when deoxygenated. (b) A more realistic drawing of the central circulatory system of an octopus. (b after Johansen and Lenfant 1966.)

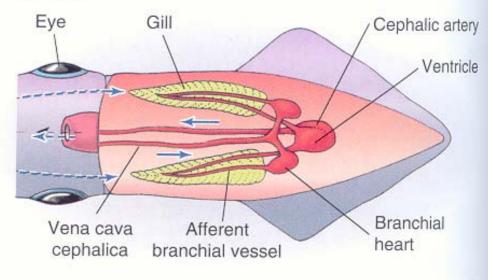
(a) Crayfish



(b) Bivalve mollusk



(c) Cephalopod

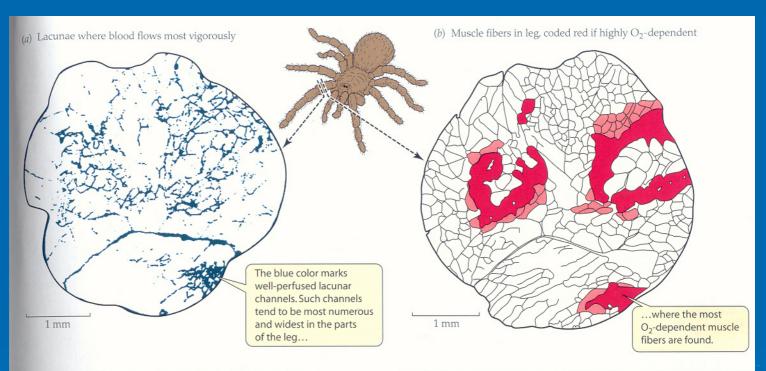


Otevřené a uzavřené oběhové soustavy – srovnání.

TABLE 24.2 Systemic circulatory function in two decapod crustaceans and two fish of similar body sizes

Characteristics of circulatory function	Spiny lobster (Panulirus interruptus)	Rock crab ^a (Cancer productus)	Starry flounder (<i>Platichthys</i> stellatus)	Rainbow trout trout (Oncorynchus mykiss)	
Principal features of circulatory function					
Rate of O ₂ delivery to tissues (mL O ₂ /kg•min) ^b	0.80	0.60	0.46	0.65	
Rate of blood flow through systemic circuit (mL blood/kg•min) ^c	128-148	125	39	18	
Pressure change to perfuse systemic circuit (mm Hg) ^d	14	3	16	22	
Systemic resistance (pressure change divided by flow rate) ^e	0.1	0.03	0.4	1.2	
Secondary information					
Heart rate (beats/min)	65	101	35	63	
Stroke volume (mL/kg•stroke)	2.1	1.2	1.2	0.3	
Blood pressure in major systemic arteries (mm Hg) ^d	35	10	18	26	
Blood pressure in major systemic veins or venous sinuses $(mm Hg)^d$	21	7	2	4	
Blood oxygen-carrying capacity (vol %)	2.0	1.3	5.7	7.8	
Temperature during studies (°C)	16	12-16	8–11	9–15	
Body weight (g)	515	~370	684	~210	

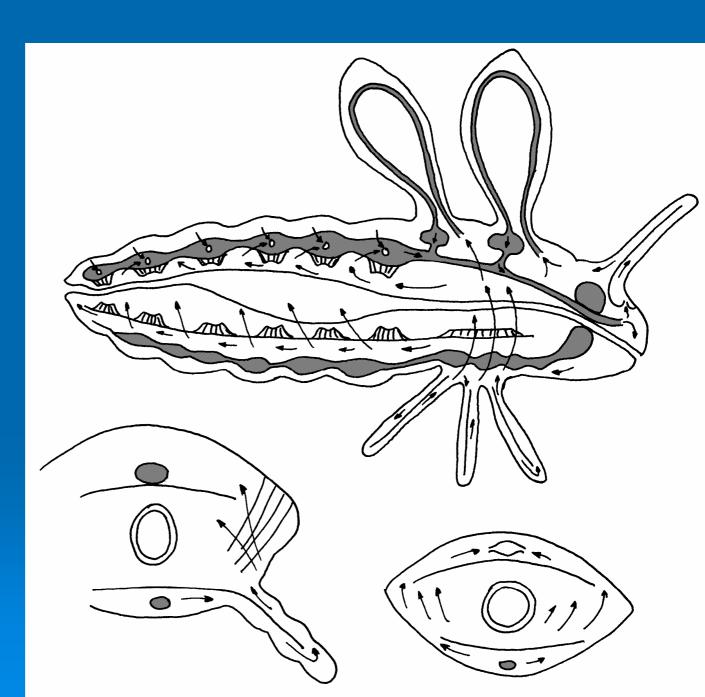
I v otevřených systémech regulace prokrvení lakun-obdoba kapilárních svěračů uzavřených sst.



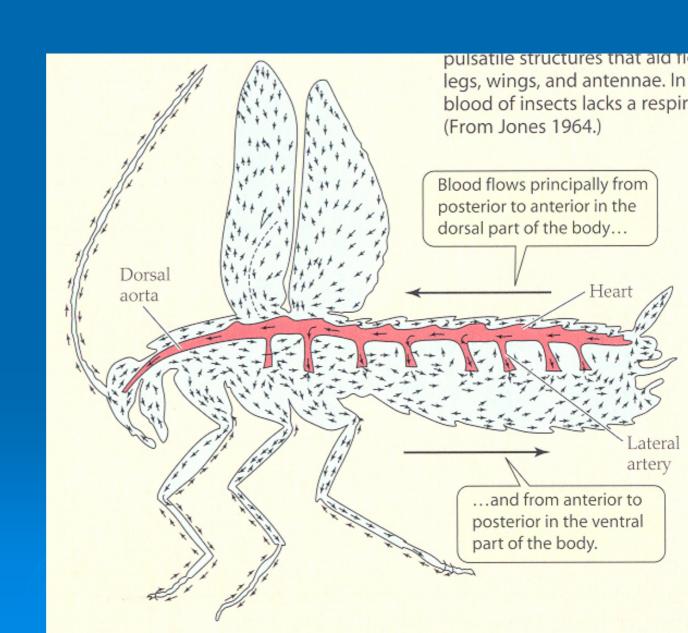
system: Blood flows most vigorously where it is needed most
Adjacent cross sections of a tarantula's leg are shown. The tissue seen
inside the cross sections is mostly muscle, that is, the tissue of the walking muscles. (a) Locations where blood flows particularly vigorously in
the lacunar networks of the muscles. In this cross section, hemocyanin
was stained immunohistochemically using monoclonal antibodies

(antibodies specific to hemocyanin). The stain was then visualized. (b) Locations of muscle fibers that are particularly O_2 -dependent. In this cross section, fibers were assessed using their profiles of catabolic enzymes (similar to Figure 7.10). The fibers colored dark red are the most poised to produce ATP aerobically and are the most O_2 -dependent. Those colored light red are also aerobically poised, but less so. (From Paul et al. 1994.)

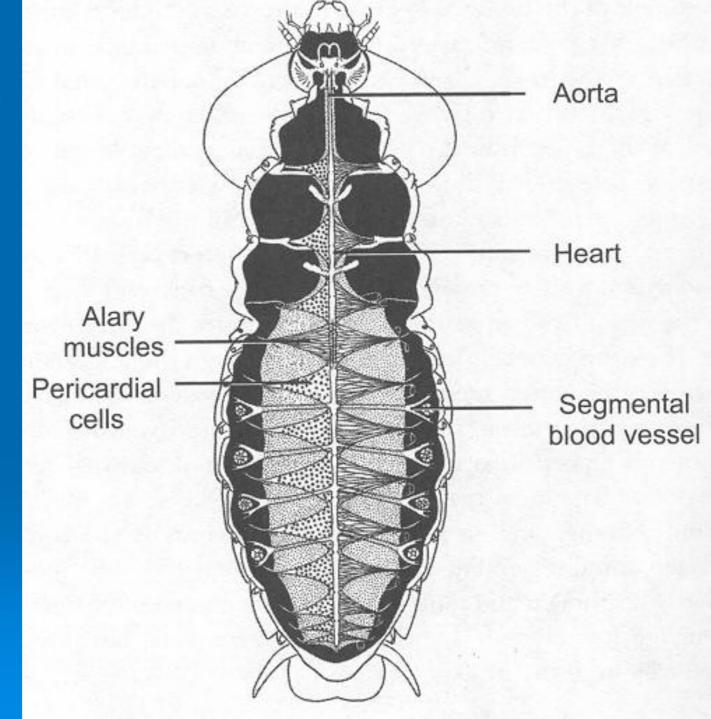
Oběh hmyzu: septa, siny, pomocná srdce.



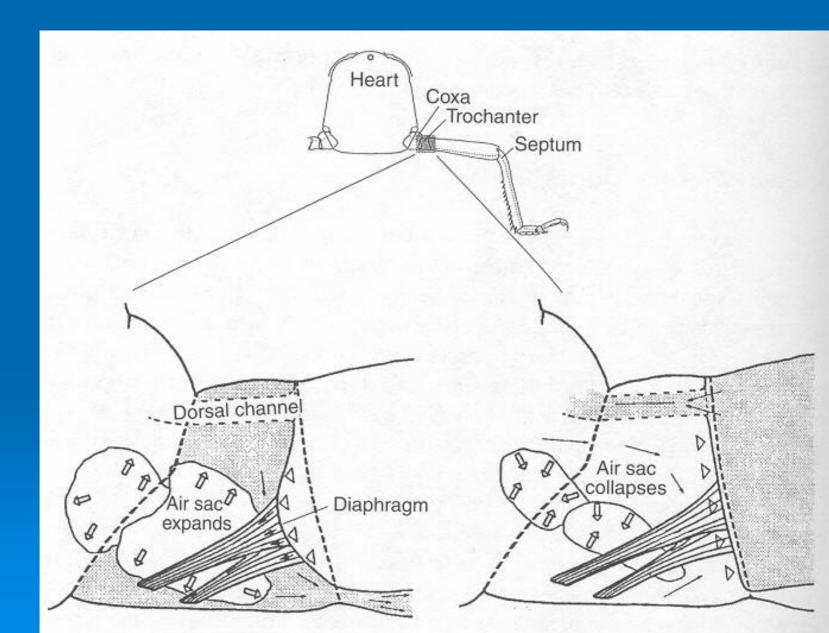
Oběh hmyzu: septa, siny, pomocná srdce.



Křídlaté svaly-Nezbytné pro plnění u otevřených soustav. Spolupráce na rytmu.



Pomocná srdce na bázi nohou a řízení směru průtoku.



Pomocná srdce na bázi nohou a řízení směru průtoku.

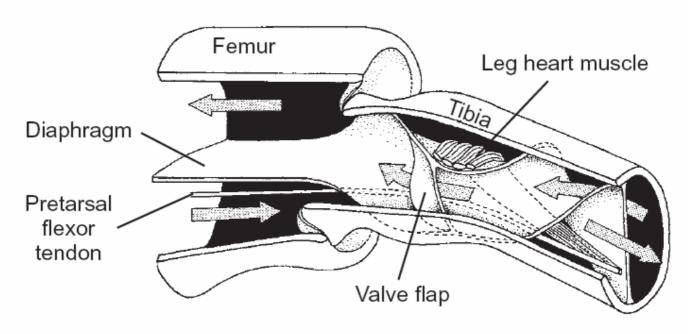


FIGURE 7.5. An accessory pulsatile organ in the insect leg. Arrows show the direction of hemolymph flow. From Hantschk (1991). Reprinted with permission.

Pomocná srdce na bázi tykadel.

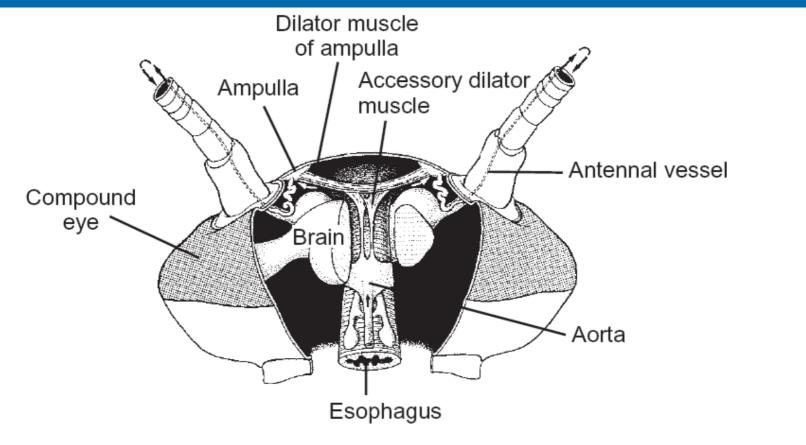


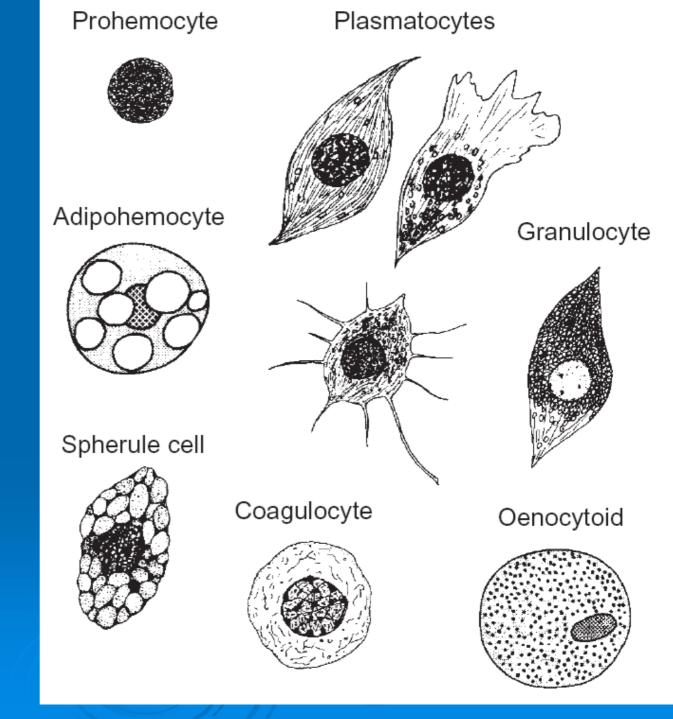
FIGURE 7.8. An ampulla and its dilator muscles that pump hemolymph into the antennae. From Pass (1985). Reprinted with permission.

Dýchací pigmenty – limit výkonu.





Hemolymfa, hemocyty.



Imunitní reakce.

TABLE 15-10

Summary of the development of immunity in animals. (Modified from Stites, Caldwell, and Pavia 1980.)

	Graft Rejection	Immunologic Specificity of Graft Rejection	Immuno- logic Memory	Phago- cytosis	Encapsu- lation	Non- specific Humoral Factors	Phagocytic Ameboid Coelomo- cytes	Leukocyte Differen- tiation	Anti- bodies
Protozoans	Yes	No	No	Yes	No	No	No	No	No
Poriferans	Yes	Yes	Yes	No	Yes	No	No	No	No
Cnidarians	Yes	Yes	Yes	No	Yes	No	No	No	No
Annelids	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Probable	No
Mollusks	Yes	?	?	Yes	Yes	Yes	Yes	No	No
Arthropods	Yes	?	?	Yes	Yes	Yes	Yes	No	No
Echinoderms	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Tunicates	Yes	Probable	Yes	Yes	Yes	Yes	Yes	Yes	No
Vertebrates	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes

THE IMMUNE SYSTEM OF INSECTS

- It was understood in the early 1930's that the immune response of insects is manifested both as cellular and humoral reactions.
- In cellular reactions the microorganisms or the apoptotic cells are phagocytosed, entrapped by nodule formation, or encapsulated by hemocytes.
- In humoral defence processes three immediate reactions are triggered: melanisation, clotting of the hemolymph and synthesis of antimicrobial peptides.

Buněčná i látková odpověď, nespecifická.

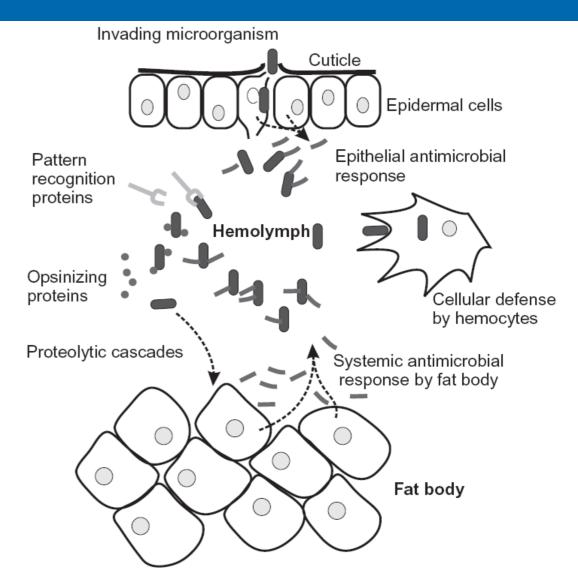
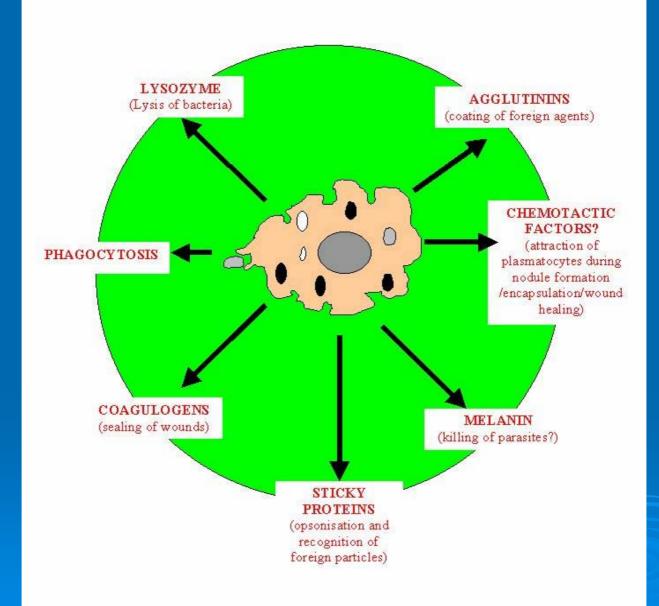


FIGURE 7.17. Possible mechanisms of response to invading parasites by epidermal cells, hemocytes, and fat body. Epidermal cells produce antimicrobial compounds, and pattern recognition and opsinizing proteins target invaders for attack by hemocytes. Fat body cells can also mount a systemic antimicrobial response.

Figure 5: Diagram of an insect granular cell emphasizing its multifunctional role.



Nodule formation

- During nodule formation insect hemocytes aggregate to entrap bacteria. Nodules can attach to tissues or may be encapsulated.
- An insect lectin scolexin was found to be involved in nodule formation in *Manduca sexta*. Scolexin is produced by epidermal and midgut cells upon wounding or bacterial infection.
- In the medfly (Ceratitis capitata), a protein with molecular mass of 47 kDa is secreted by hemocytes after LPS stimulation and aggregates E.coli cells by the presence of tyrosine and tyrosinase.



Figure 10: Nodule formation in fatbody and trachea of *B.mori*. Magnification 60X

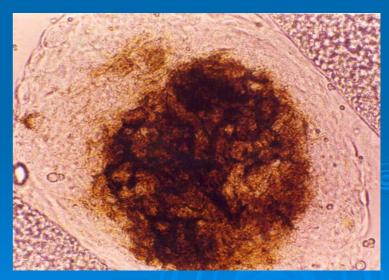


Figure 11: Mature dark melanized nodule of *B.mori* as observed under phase contrast microscope at 600X magnification

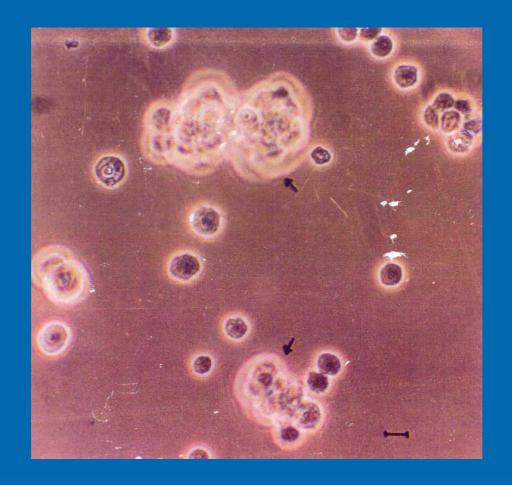


Figure 14: Encapsulation of bacteria as evidenced by clumped mass of bacteria and degenerated cells of B.mori. Bar = $10 \mu m$

The Humoral Reactions

Melanisation

- > The formation of the black pigment, melanin is catalysed by the enzyme phenoloxidase, which is converted to its active form by a serine protease cascade.
- The inactive proenzyme, prophenoloxidase is synthesized in the hemocytes and after releasing by cell rupture it is either actively transported to the cuticle or deposited around wounds and encapsulated parasites.
- Prophenoloxidase has been purified and subsequently characterized from the hemolymph of a range of insect species.
- > The insect prophenoloxidase enzyme contains a sequence with similarity to the thiol-ester region of the vertebrate complement component proteins C3 and C4.

Enkapsulace a nodulace. Fenoloxidázová kaskáda.

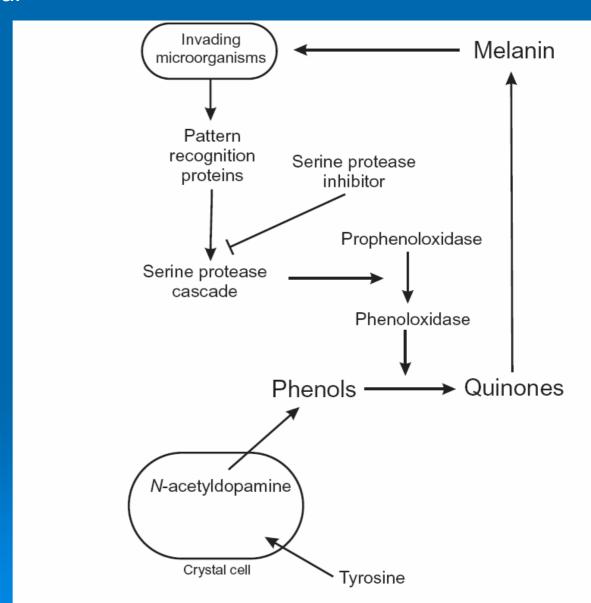


FIGURE 7.18. Mechanism of humoral encapsulation by the deposition of melanin on foreign invaders. The serine protease inhibitor restricts phenoloxidase activity to the site of the infection.

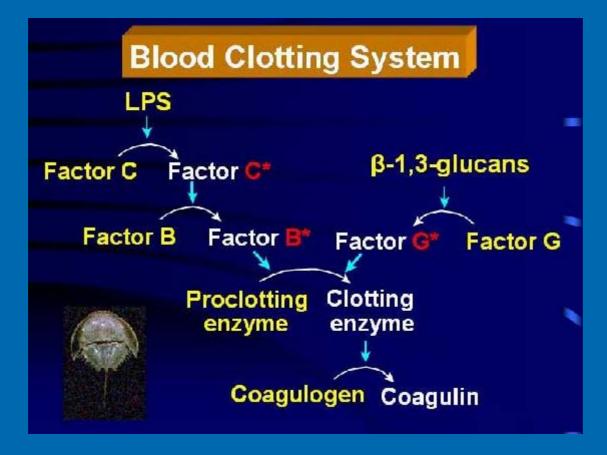


Figure 16: Blood clotting system (horseshoe crab)

Horseshoe crab blood clotting system can be activated by bacterial LPS or fugal beta-1,3-glucans. Binding of LPS to Factor C auto activates Factor C, which then triggers the serine proteinase cascade, leading to activation of proclotting enzyme. Clotting enzyme cleaves coagulogen to form gel-like materials coagulin. Another pathway of this system is activated by beta-1,3-glucans, in which binding of glucans to Factor G auto activates Factor G.

Immune proteins

- The third humoral reaction to infection is the rapid de novo synthesis of a battery of antimicrobial peptides (Boman, 1996; Hetru et al., 1998).
- > The principal site of synthesis is the fat body, but also the hemocytes, the cuticular epithelial cells, the gut, the salivary gland, and also the reproductive tract.
- During the research work conducted in the last decade nearly 60 peptide antibiotics have been described in insects. Though diverse in structure, they are basically amphipathic molecules acting at membranes and thereby killing the target cell eventually by lysis.
- > The insect antimicrobial proteins are grouped into families, based on structural and sequence similarities and their proposed target in the bacterial cell wall.
- The attacin-like bacteria inducible proteins have been identified in butterflies and in *Drosophila*. These proteins are active only against gram negative organisms, where they affect cell division mechanisms by inhibiting the synthesis of outer membrane proteins. The family of these factors includes glycine-rich peptides (20-28kDa) characterized by the presence of one or more copies of the G domain (Hultmark, 1993).

- Lysozyme hydrolyses β-(1,4)-glycosidic bonds in peptidoglycan of bacterial cell wall. Insect lysozymes are proteins(14 kDa) with sequence similarity to vertebrate lysozymes. The lysozyme gene or cDNAs have been cloned in several insect species (Kylsten et al., 1992; Sun et al., 1991; Lee & Brey, 1995). In *Drosophila*, the lysozymes are encoded by at least seven genes and expressed in different parts of the digestive tract and at different stages of development.
- > Cecropins have antibacterial activity against both gram-positive and gram negative bacteria since they interact with lipid membranes forming voltage dependent ion channels. The cecropins (4 kDa) are devoid of cystein, and exhibit a structure of two α-helices joined by a hinge region. Families of cecropin genes with some sequence differences have been found in butterfly species, in the flesh fly (Sarcophaga peregrina) and in Drosophila. A mammalian cecropin was identified in pig intestine (Lee et al., 1996) and bovine adrenal glands (Strub et al., 1995) which implies that cecropins may be widespread in the animal kingdom.
- Defensins attack mainly Gram-positive bacteria in contrast to attacin-like antibacterial peptides. They act on the cytoplasmic membrane and lyse cells by the formation of membrane channels. Insect defensins are cationic peptides (4 kDa) containing six conserved cystein residues engaged in three disulphide bridges. They possess three distinct domains: amino-terminal loop, an α-helix, and an antiparallel β sheet. About thirty defensins have been characterized in various insect species. Although numerous defensins have been isolated from mammalians and from plants.

- bacteria by increasing the membrane permeability. They are peptides with molecular mass of 2 4 kDa, lacking cysteine and containing at least 25% proline. The *O*-glycosylation at the threonin residues is essential for their biological activity. Apaedicins and abaecin from honey-bee, drosocin and metchnikowin from *Drosophila*, pyrrhocoricin, lebocin and metalnikowin belong to this family. Also, the pig intestine and bovine leukocytes have been shown to produce proline-rich antibacterial peptides, although these peptides do not share sequence homology to the proline-rich peptides of insects.
- Diptericins have so far been described only in dipteran species. They are 9-kDa peptides containing both an attacin-like G domain, a C-terminal glycine rich residue and a short N-terminal prolinerich region containing a consensus site for O-glycosylation. Diptericins are lytic for Gram-negative bacteria which may be due to a way of action similar to that of attacins.
- Other inducible antibacterial proteins have been isolated from insects not fitting into the groups described above. Coleptericin, holotricin-2, hemiptericin and gallysin-1 act on Gram negative bacteria, while moricin, thanatin (homologous to frog-skin antimicrobial peptides of the brevinine family), and hymenoptaecin can lyse both Gram-negative and Gram-positive bacteria.

Recently inducible peptide antibiotics against fungi have been discovered in insects. The peptide named AFP, tenecin-3 and holotricin-3 share similarities while drosomycin shows a significant homology with a family of plant antifungal peptides. Furthermore, the antibacterial peptides metchnikowin and thanatin have antifungal activity.

Parallels between humoral immune response of insects and mammalian acute phase response:

•The acute phase response of mammals is stimulated by tissue injury or bacterial challenge and characterized by the immediate synthesis of acute phase proteins mainly in the liver and in cells of the innate immune system. The synthesized proteins act as opsonins, blood clotting and wound healing factors. In insects, the rapid humoral immune reactions are also triggered by wound or microbial infection. They involve proteolytic cascades leading to hemolymph clotting and the rapid de novo synthesis of antimicrobial peptides by the fat body, the functional analogue of the mammalian liver, and the hemocytes.