Ventilation and Circulation

I. Ventilation
   A. Structure
   B. Function
   C. Ventilation in aquatic insects

II. Circulation
   A. Components of circulatory system
   B. Circulation
   C. Hemolymph
**Ventilation - Structure**

**Tracheae:** air filled tubes that branch and allow for gas exchange in all tissues of an insect’s body

**Tracheoles:** fluid-filled blind endings of the trachae that closely contact the respiring tissue; less than 1um in diameter

**Air sacs:** dilated or enlarged tracheae with reduced or missing taenidia

**Taenidia:** spiral ridges around tracheae that provides flexibility and strength
Fig. 3.11 Some basic variations in the open (a–c) and closed (d–f) tracheal systems of insects. (a) Simple tracheae with valved spiracles, as in cockroaches. (b) Tracheae with mechanically ventilated air sacs, as in honey bees. (c) Metapneustic system with only terminal spiracles functional, as in mosquito larvae. (d) Entirely closed tracheal system with cutaneous gas exchange, as in most endoparasitic larvae. (e) Closed tracheal system with abdominal tracheal gills, as in mayfly nymphs. (f) Closed tracheal system with rectal tracheal gills, as in dragonfly nymphs. (After Wigglesworth 1972; details in (a) after Richards & Davies 1977, (b) after Snodgrass 1956, (c) after Snodgrass 1935, (d) after Wigglesworth 1972.)
**Spiracles:** external tracheal openings

**Atrium:** chamber preceding spiracle with a closing mechanism
Fick's First Law of Diffusion:

\[ J_x = -D \frac{dC}{dx} \]

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Hemocytes (granulocyte) aggregate at tracheal tufts under O2 starvation

13 families of leps studied have these tracheal tufts
Discontinuous breathing (despite high metabolism many insects show discontinuous patterns of gas exchange)
I. Ventilation in aquatic insects
• Spiracular gills
• Cuticular gills
• Plastron
• Caudal siphon
Odonata nymphs

A. Aeshnidae; B. Calopterygidae; C, D. Coenagrionidae; E. modified labium of nymphs extended (left) and retracted (right) (pictured: Aeshnidae; all Odonata nymphs have this feature)
Surviving the flood: plastron respiration in the non-tracheate arthropod Phrynus marginemaculatus (Amblypygi : Arachnida)

Hebets EA, Chapman RF
Journal of Insect Physiology
CIRCULATION
Fig. 3.9 Schematic diagram of a well-developed circulatory system: (a) longitudinal section through body; (b) transverse section of the abdomen; (c) transverse section of the thorax. Arrows indicate directions of hemolymph flow. (After Wigglesworth 1972.)
Hemolymph

• Usually clear, or slightly green/yellow b/c of pigments, sometimes red if hemoglobin is present

• Hemocytes: cellular portion (nucleate)

• Plasma: fluid portion

• **COMPOSITION:** *Water:* 84 – 92%

  *Inorganics:* *Na, K, Ca, S, Mg, Cl, P, carbonate*

*Nitrogenous Wastes:* usually *Uric Acid*; other wastes include – *allantoin, allantoic acid, ammonia*
Composition of hemolymph (continued)

Organic Acids

Carbohydrates

Lipids: lipoproteins

Amino Acids

Proteins

Pigments

Gases

WHAT DOES IT DO??
Hemolymph Functions:

Lubricant

Hydraulic Medium

Transport and Storage

Heat Transfer

Protection
Hemocytes

**Coagulation**: rapid formation of fine granular ppt that can enmesh cells

**Wound Healing**: plasmocytes undergo mitosis in assoc w/ wounds

**Detoxification**: some have ability to detoxify metabolites

**Phagocytosis**: ability to ingest foreign particles

**Nodule Formation**: aggregates that entrap material contained w/in a coagulum

**Encapsulation**: flatten out and surround (encapsulate) object in several layers of hemocytes
Fig. 1. Haemocytes observed in light microscopy, using different techniques. Bars = 10 μm. (A) Haemocytes of S. littoralis, after fixation at blood collection. Each haemocyte shows the typical morphology of its type. GH, granular haemocyte: spherical and very refractive cells; Oe, oenocytoid: large cell with a low nuclear to cytoplasmic ratio and eccentric nucleus; PI, plasmatocyte: here it is spindle-shaped. Phase contrast microscopy. (B) Monolayer of haemocytes from third instar larva D. melanogaster parasitized by Leptopilina boulardi (Hymenoptera), after fixation at 30 s post-collection and toluidine blue staining. DPI, drosophila plasmatocyte; L, lamellocyte. Note the faint staining of lamellocytes compared to drosophila plasmatocytes, due to their flattened shape. (C)–(F) Shape modifications of plasmatocytes (PI) and granular haemocytes (GH) of S. littoralis in monolayers at different times after blood removal (C: 30 s, D: 3 min, E: 6 min, F: 10 min). Note the importance of plasmatocyte spreading, compared to granular haemocytes. Note also the small scattered chromatin masses in plasmatocyte nuclei. Phase contrast microscopy.
Fig. 7. Oenocytoid lysis in monolayers: comparative observation between lepidopteran and *Drosophila melanogaster*, in phase contrast microscopy. Bars = 10 μm. (A)–(C) Haemocytes were collected from *S. littoralis* larva, directly on a coverslip (A) and were observed in phase contrast microscopy. One oenocytoid (Oe) and two plasmocytes (Pl) are seen in the field of view. After 2 min incubation (B), the oenocytoid has lost its refringency and was lysed after 3 min (C), releasing its content in the surrounding medium, whereas the two Pl began to spread on glass. (D)–(F) Haemocytes were collected from third instar larvae of *D. melanogaster* and observed as above. One oenocytoid (= crystal cell) (Oe) and two drosophila plasmocytes (DPI) are seen in this field of view. D shows the cells at the time of collection. After 5 min incubation (E) the crystal-like inclusions begin to dissolve and after 8 min (F), the oenocytoid has released almost all its cytoplasmic content in the medium. Note that the two drosophila plasmocytes do not spread intensively.
Fig. 4. Phagocytosis by lepidopteran haemocytes. (A) and (B) Monolayer of *G. mellonella* haemocytes, after 30 min incubation with FITC-labelled heat-killed *Escherichia coli*. A: phase contrast microscopy; B: epifluorescence of the same picture. Note that bacteria are fixed in very large amounts on granular haemocytes (GH) and are very few (if any) on plasmatocytes (Pl). Bar = 10 μm. (C) Mode of engulfment (arrow) of yeast (asterisk) by granular haemocyte of *M. unipuncta*, 7 min after addition of the yeast cells to the previously formed monolayer. Arrowheads: short lamellipodia allowing the cell to adhere to the coverslip. SEM, bar = 10 μm.
Fig. 1 Stages in the melanotic encapsulation of the egg of *L. boulardi* by *D. melanogaster*. (A) Wasp egg (arrow) within the body cavity of a *Drosophila* seen through the larval cuticle shortly after oviposition. (B, C) Presence of melanin surrounding dead eggs approximately 12 h after parasitization. (D) Scanning electron micrograph showing hemocytes adhering on the periphery of a fully-formed capsule. The melanotic capsule is retained within the body of the pupa (E) and in the adult (F) host.
Figure 1. Enzyme activities in the haemolymph of last instar *T. ni* larvae originating from *N♀N♂, B♀B♂, B♀N♂* and *N♀B♂* crosses. (a) General antibacterial activity measured as the diameter of the lytic zone on agar plates with lyophilized *M. luteus* and transformed into lysozyme equivalents (μg μl⁻¹). Results represent mean values ± s.e. (b) proPO (open bars) and PO (filled bars) activities measured in the haemolymph samples. \( V_{\text{max}} \) is measured as the maximum change in optical density per minute ± s.e.

**Dietary-dependent trans-generational immune priming in an insect herbivore**

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Immune response

Is it effective against parasitoids?
Defenses Against Predators

- crypsis
- sequestration
- spiny
- regurgitant
- coloration
- gregarious
- shelter-building
- Parasitoids

hairy
Defenses Against Parasitoids

Gentry & Dyer, 2002
Barbosa & Caldas 2007
Effectiveness of immune response against parasitoids

- 16 species; 9 families
- Costa Rica parasitism dataset (Dyer & Gentry 1999, Gentry & Dyer 2002)
Injected with **beads** to simulate parasitism

Immune assay

24 hours

Dissection to retrieve beads

Photograph beads and compare melanization
Negative correlation between melanization and parasitism (Smilanich et al. 2009 *Ecology*)

Pearson Correlation Coefficient $r = -0.58$, $N = 15$, $P = 0.02$
Plate 1. A sample of species that have the lowest, intermediate, and highest levels of melanization. Inset pictures are representative beads from each species. A. *Desmia* sp. (braconid cocoons), B. *Cyclonia disparilis*, C. *Emesis lucinda* (1 braconid cocoon), D. *Opsiphanes tamarindi*, E. *Eois apyraria*, F. *Eois nympha*. Photos by authors.
Immune response is best predictor of parasitism

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Smilanich et al. 2009 *Ecology*
Encapsulation effective for different parasitoid taxa

![Graph showing parasitism % for different Lepidoptera species.](image)

- **Achlyodes busius**: High parasitism by wasps, low by flies, some by nematodes.
- **Antichloris viridis**: Moderate parasitism by wasps, negligible by flies and nematodes.
- **Caligo memnon**: High parasitism by wasps, negligible by flies and nematodes.
- **Chlosyne janais**: High parasitism by wasps, negligible by flies and nematodes.
- **Chlosyne gauderis**: Moderate parasitism by wasps, negligible by flies and nematodes.
- **Cyclomia disparis**: High parasitism by wasps, negligible by flies and nematodes.
- **Desmia sp.**: High parasitism by wasps, negligible by flies and nematodes.
- **Eois lucinda**: High parasitism by wasps, negligible by flies and nematodes.
- **Eois apyra**: High parasitism by wasps, negligible by flies and nematodes.
- **Eois nympha**: High parasitism by wasps, negligible by flies and nematodes.
- **Euptychia jessia**: High parasitism by wasps, negligible by flies and nematodes.
- **Opsiphantes tamarindii**: High parasitism by wasps, negligible by flies and nematodes.
- **Papilio thoas**: High parasitism by wasps, negligible by flies and nematodes.
- **Tarchon feldei**: High parasitism by wasps, negligible by flies and nematodes.
- **Xylophannes pluto**: High parasitism by wasps, negligible by flies and nematodes.