(Insect Physiology)

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(الجزء النظري)

الفصل الدراسي الأول

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رؤية كلية العلوم

التميز في تعليم العلوم الاساسية والبحث العلمي للمساهمة في التنمية المستدامة

رسالة كلية العلوم

تقديم تعليم مميز في مجالات العلوم الاساسية وانتاج بحوث تعليمية تطبيقية للمساهمة في التنمية المستدامة من خلال اعداد خريجين متميزين طبقاً للمعاير الاكاديمية القومية وتطوير مهارات وقدرات الموارد البشرية وتوفير خدمات مجتمعية وبيئية تلبي طموحات مجتمع جنوب الوادي وبناء الشراكات المجتمعية الفاعلة .

رؤية القسم

خريجون وباحثون متميزون علمياً وبحثياً في دراسة ضرر ونفع الكائنات الحيوانية خدمة للمجتمع وتنمية للبيئة

رسالة القسم

يسعى قسم علم الحيوان والحشرات بكلية العلوم جامعة جنوب الوادي من خلال ما يقدمة من برامج تعليمية باستخدام الوسائل العلمية والتعليمية المتطورة والتي تكشف عن المزيد من ضرر ونفع الكائنات الحية وباحثين وخريجون متميزين علمياً وبحثياً ينتفع بهم المجتمع وترتقي بهم الامة .

INSECT PHYSIOLOGY

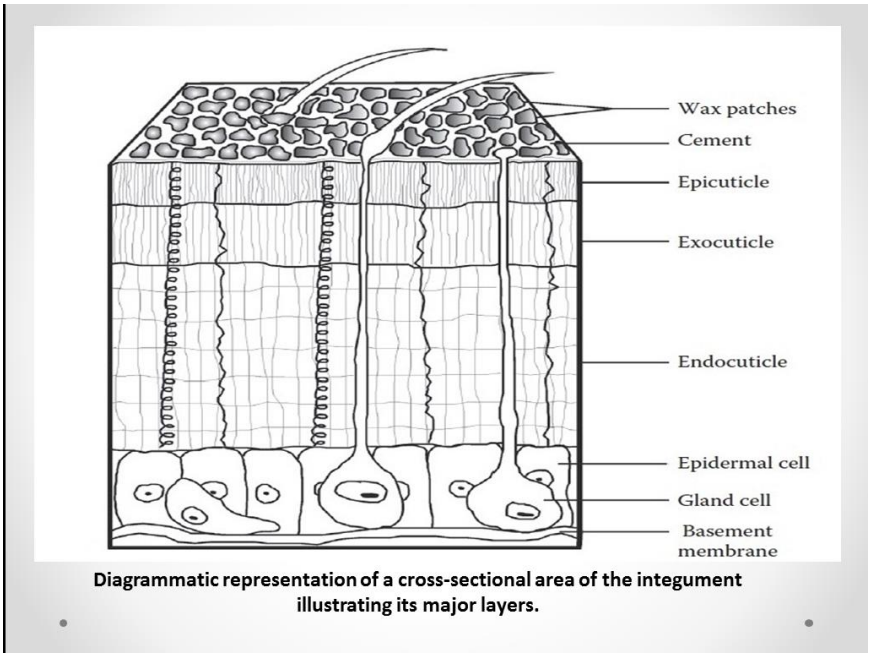
Insect Integument

- Insect body wall is called an exoskeleton or **integument**.
- It is the external covering of the body which is ectodermal in origin.
- It is a rigid, flexible, lighter, stronger and variously modified in different body parts to suit different modes of life.
- It serves as a protective covering over the body.

Integument Functions

- Gives shape to insect.
- Protects internal organs.
- Provides surfaces for muscles attachment.
- Conserve moisture and prevent desiccation.
- Prevents entering of pathogens and insecticides.
- Contains pigments to make insects attractive.
- Represents the sensory windows of the insect to the outside world.(forms sense organs).

Integument Structure



Diagrammatic representation of a cross-sectional area of the integument illustrating its major layers.

- The integument includes the **cuticle** on the surface of the body and the single layer of **epidermal cells** beneath the cuticle.
- the cuticle composed of three primary layers: 1- the **cuticulin envelope**, 2- the **epicuticle**, and 3- the **procuticle**.
- The epidermal cells secrete new cuticle at each molt.
- There is always a cuticulin or envelope layer in all insects and always an epicuticle layer.
- Beneath the epicuticle is the procuticle, and its chemical composition and degree of sclerotization varies greatly among different groups of insects and even in different stages of the same insect.
- When the outermost part of the procuticle is heavily sclerotized (cross-linked hard cuticle), it is called **exocuticle**.
- Not all insects have a hard exocuticle.

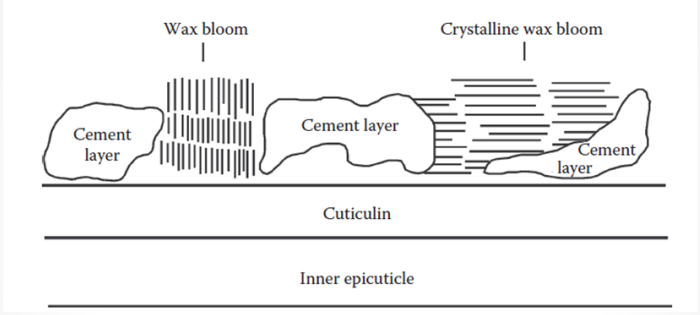
- For example, most soft bodied larvae and other soft parts of insects contain little or no exocuticle in parts of the body that are soft.
- The envelope, epicuticle, and exocuticle (if any) are not digested prior to a molt, and it is these parts of the cuticle that are shed at the molt.
- The part of the procuticle that is only lightly cross-linked is the **endocuticle**.
- Endocuticle may be greatly reduced or absent in particular parts of the cuticle of the same or different insects, as, for example, in the hard outer wing covers, the elytra, of scarab beetles.
- The epidermal cells secrete the cuticle, the lipids (waxes), cement, and often many additional chemical components that occur on or in the cuticle layers.
- When a new cuticle is secreted at molting, the envelope is secreted first, and the epicuticle is secreted on its inner surface.
- Procuticle is soft at first, but varying degrees of sclerotization occur in different insects soon after the cuticle is secreted.

1- Cuticulin Envelope

- The cuticulin envelope is from 10 to 30 nm thick and is formed at the external surface of the epidermal cell plasma membrane.
- It separates from the plasma membrane and is pushed upward as new epicuticle, and then procuticle is secreted beneath it.
- Because it is so thin, its chemical composition is poorly understood, although sclerotized or cross-linked protein is probably one of the main components.
- Neither the cuticulin envelope nor the epicuticle layer contains **chitin**.

2- Epicuticle

- The epicuticle layer typically is from 1 to 4 µm in thickness and, like the envelope, its detailed chemical structure has been difficult to discern.
- It is known to contain sclerotized proteins impregnated with lipoproteins, lipids, waxes, cement, and minor amounts of various minerals and other chemical components.
- It does not contain chitin, a major structural carbohydrate in the procuticle.



Diagrammatic illustration of the mosaic of wax (lipids) and cement on the surface of the epicuticle of some insects.

- The proteins and some of the lipids appear to be covalently linked, and the proteins are tanned or sclerotized by phenolic compounds and their oxidized products, quinones.
- **Sclerotization**, the cross-linking of molecules, gives the epicuticle strength, hardness, and low water permeability.
- Lipids and the cement layer on the surface also provide reduced permeability to water.

3- Procuticle

- The procuticle, containing both chitin and protein, lies just beneath the epicuticle.
- Parts of the procuticle (typically the outer part nearest the epicuticle) may be highly sclerotized and, therefore, is hard and rigid and called the exocuticle.
- Lamellae or layers within this exocuticle may refract light in such a way to produce structural colors in some insects. Many of the iridescent greens and blues of insects are structural colors due to refracted light rather than to pigments.
- The thickness of the exocuticle is variable and species specific.
- Adult insects generally have a thicker and more sclerotized exocuticle than larval insects.
- In particular, the thorax in flying insects has heavily sclerotized exocuticle to support the strong flight muscles.
- Many larvae have a soft flexible cuticle with little or no exocuticle.
- As in so many cases with insects, exceptions exist. There are larval insects with hard sclerotized exocuticle and soft-bodied adults with little or none.
- The harder the cuticle, the greater the degree of sclerotization.

- The content of chitin does not control hardness of the cuticle, but sclerotization does.
- Because of the sclerotization, little or none of the exocuticle is digested by molting fluid, and it is shed, along with the epicuticle, at molting.
- In some insects, the highly sclerotized exocuticle grades into less sclerotized cuticle, called **mesocuticle**, or there may be a rapid change to soft, little sclerotized cuticle called the **endocuticle**.
- The endocuticle is soft, flexible cuticle containing both chitin and proteins.
- It has little sclerotization, which is why it is soft and flexible.

4- Pore Canals and Wax Channels

- **Pore canals** are passageways from 0.1 to 0.15 μm in diameter, extending from the epidermal cells through the procuticle, but terminating at the interface between procuticle and epicuticle.
- Larger canals are often flattened, ribbon-like, and may be twisted or straight.
- Pore canals transport lipids and cement, and, sometimes, additional chemical components.
- Although pore canals do not penetrate the epicuticle, there are smaller passageways through the epicuticle called **wax channels** (about 0.006–0.013 μm in diameter).
- The wax channels are 10–20 times smaller than pore canals.

5- Epidermal Cells

- The cells underlying the cuticle are arranged in a single layer.
- They usually are simply called the **epidermal cells**, but sometimes referred to as the **cuticular epithelium**, the **epidermis**, and the **hypodermis**.

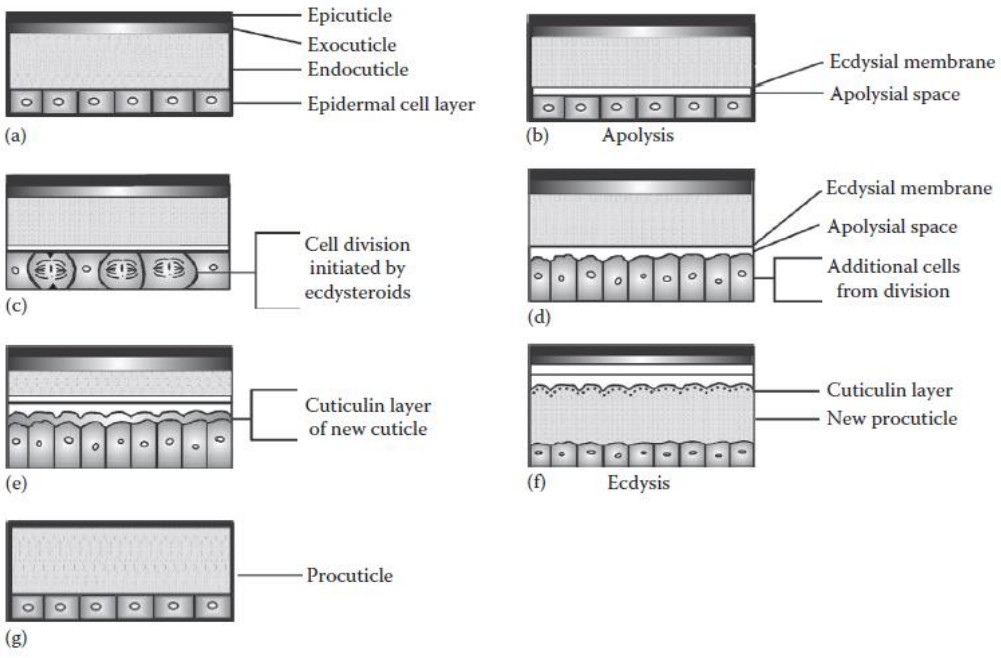
- All epidermal cells probably secrete **chitin** and **proteins**, and some may secrete **lipids**.
- Specially modified glandular epidermal cells may secrete cement. There frequently are specialized glandular cells in the cell layer, either in small groups or as scattered, isolated glandular cells that secrete special products.
- For example, sex pheromones in most female lepidoptera are secreted by small patches of tall, columnar epidermal cells located beneath the cuticle of the ventral intersegmental membrane between the eighth to ninth segments of the abdomen.

6- Basement Membrane

- Epidermal cells are separated from the circulating hemolymph by a **basement membrane**.
- A layer of poorly defined chemical composition, but with pores large enough to permit passage of larger hemolymph proteins and other molecules from hemolymph into the epidermal cells.
- The origin of the basement membrane is not defined for most insects; some evidence supports secretion by hemocytes, but some evidence suggests that the epidermal cells themselves secrete it.
- Tracheae, tracheoles, and nerves pass through the basement membrane to reach the epidermal cells.
- Hemidesmosomes hold the basement membrane to the epidermal cells.

MOLTING AND FORMATION OF NEW CUTICLE

- The external skeleton gradually becomes too small for the growing body tissues of an immature insect and it must molt its cuticle.
- Preparation for molting is under endocrine and nervous control.
- Molting is a vulnerable time for insects; they are easy prey for predators and subject to environmental hazards, particularly desiccation.
- The muscles that move the body must be detached from the old cuticle, but they are detached only immediately before the ecdysis and new muscle attachments are made quickly to the new epicuticle.
- The new cuticle must harden sufficiently to resist the pull of the musculature or muscle action can cause skeletal deformation and result in permanent restriction of movement, and especially failure of flight ability.



- The previous figure is a diagrammatic illustration of the process of apolysis, secretion of new cuticle, and ecdysis of the old cuticle.

❑ (a) Old cuticle just before molting begins.

- (b) Formation of the ecdysial membrane and apolysial space.
- (c) Initiation of cell division in epidermal layer in response to molting hormone.
- (d) New epidermal cells, usually developing an irregular apical surface.
- (e) New cuticle secretion begins with the secretion of cuticulin layer. Digestion of the old endocuticle continues.
- (f) New unsclerotized procuticle is formed.
- (g) The old cuticle shell has been ecdysed and the new cuticle will be covered with a wax and cement layer, and some of the procuticle may be sclerotized into exocuticle, depending upon the insect and location on the body.

Growth and Development

- **Insects:** during their postembryonic growth period pass through a series of stages (instars) until they become adult, the time interval (stadium) occupied by each instar being terminated by a molt.
- **Apterygotes:** continue to grow and molt as adults, periods of growth alternating with periods of reproductive activity. In these insects structural differences between juvenile and adult instars are slight, and their method of development is thus described as ametabolous.
- Among the Pterygota, which with rare exceptions do not molt in the adult stage, two forms of development can be distinguished.
- **Exopterygotes:** the later juvenile instars broadly resemble the adult, except for their lack of wings and incompletely formed genitalia. Such insects, in which there is some degree of change in the molt from juvenile to adult, are said to undergo partial (incomplete) metamorphosis, and their development is described as hemimetabolous.
- **Endopterygotes:** have larvae whose form and habits are very different from those of the adults. As a result, they undergo striking changes (complete metamorphosis) in the formation of the adult (holometabolous development). The final juvenile instar has become specialized to facilitate these changes and is known as the pupa
- **In insect evolution:** increasing functional separation has occurred between the **larval stage**, which is concerned with growth and accumulation of reserves, and the **adult stage**, whose functions are reproduction and dispersal. Associated with this trend is a tendency for an insect to spend a greater part of its life as a juvenile, which contrasts with the situation in many other animals.

Growth

Physical Aspects

- **Growth in insects and other arthropods** differs from that of **mammals** in various respects. In insects growth is almost entirely restricted to the larval instars, though in some species there is a short period of somatic growth in newly enclosed adults when additional cuticle may be deposited, and growth of flight muscles and the alimentary canal may occur.
- As a consequence, the length of the juvenile stage is considerably longer than that of the adult.
- An extreme example of this is seen in some **mayfly** species whose aquatic juvenile stage may require 2 or 3 years for completion, yet give rise to an adult that lives for only a few hours or days.

Mayfly

- **Growth laws:** for many insects grown under standard conditions the amount of growth that occurs is predictable from one instar to the next; that is, it obeys certain laws.

- **Dyar's law:** based on measurements of the change in width of the head capsule which occurs at each molt, states that growth follows a geometric progression; that is, the proportionate increase in size for a given structure is constant from one instar to the next.
- **Mathematically:** the law states x/y = constant (value usually 1.2–1.4), where x = size in a given instar and y = size in previous instar.
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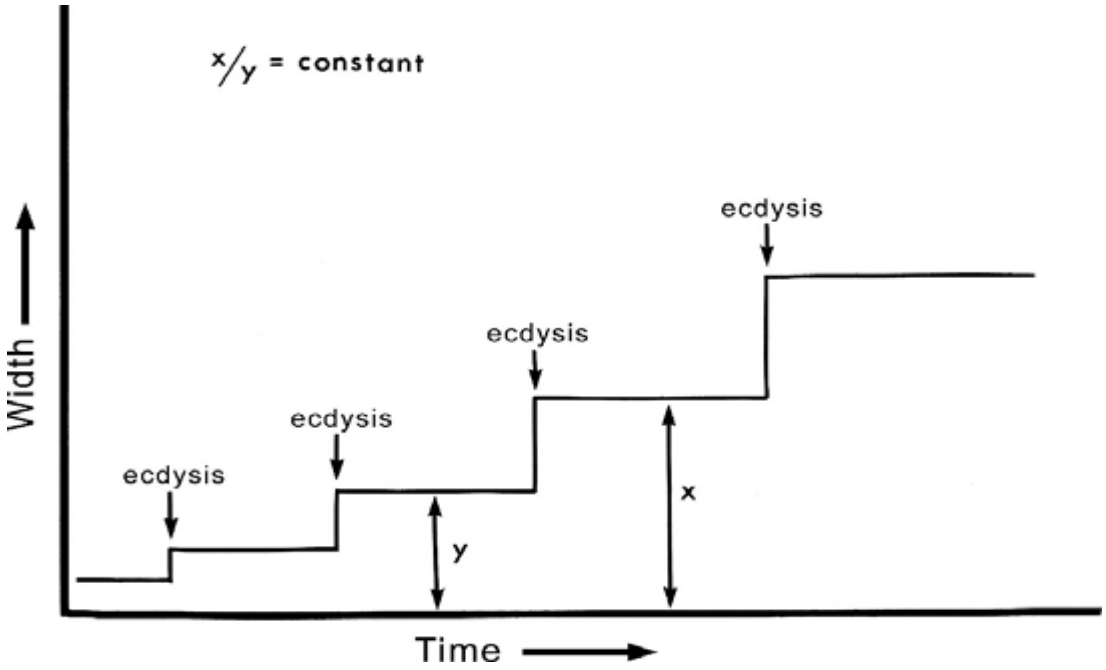

Change in head width with time to illustrate Dyar's law.

- However, so **many factors** affect growth rates and the frequency of ecdysis that the law is frequently inapplicable. In any event, the law requires that the interval between molts remains constant, but this is rarely the case.
- As winged insects grow, each part has its own growth rate. This disproportionate growth, which is not unique to insects, is described as "**allometric**" (heterogonic, disharmonic).

Biochemical Changes during Growth

- Like the physical changes, **biochemical changes** that occur during postembryonic development may also be described as **allometric**. That is, the relative proportions of the various biochemical components change as growth takes place.
- These changes are especially noticeable in endopterygotes during the final larval and pupal stages.
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- Though fat is the typical reserve substance in most insects, members of some species store **glycogen**.
- Again, this usually occurs in small amounts in newly hatched insects, but its proportion increases steadily through larval development, and at

pupation glycogen may be a significant component of the dry weight (one-third in the honey bee).

- Like fat, glycogen is stored in the **fat body**.
- In contrast, in some cases, during metamorphosis, the proportions of fat and/or glycogen decline as these molecules are utilized in energy production.
- In *Calliphora,* the fat content decreases from 7% to 3% of the dry weight through the pupal period.
- In honey bee, which mainly uses glycogen as an energy source, the glycogen content drops to less than 10% of its initial value as metamorphosis proceeds.
- The proportions of **water**, **protein**, and **nucleic acids** generally decline during larval development.
- However, this is often not the situation in larvae that require large amounts of protein for specific purposes, for example, spinning a cocoon.
- In *Bomybyx mori*, for example, the hemolymph protein concentration increases six fold in late larval development, and about 50% of the total protein content of a mature larva is used in cocoon formation.

Bomybyx mori

Based on the overall biochemical changes from hatching to adulthood, these changes occur in each stage in relation to the cyclic nature of growth and molting.

- Measurement of **oxygen consumption** shows that it follows a **U-shaped curve** through each stadium with maximum values being obtained at the

time of molting. The maxima are correlated with the great increase in metabolic activity at this time, associated especially with the synthesis of new cuticle and formation of new tissues.

Development

Forms of Development

In insects, three basic forms of postembryonic development can be recognized, described as **ametabolous**, **hemimetabolous**, and **holometabolous**, according to the extent of metamorphosis from juvenile to adult.

Ametabolous Development

- The degree of change from juvenile to adult form is slight and is manifest primarily in increased body size and development of functional genitalia as in Thysanura (and other primitive hexapods), which as adults remain wingless.
- Juvenile and adult apterygotes inhabit the same ecological niche, and the insects continue to grow and molt after reaching sexual maturity.
- The number of molts through which an insect passes is very high and variable.

Hemimetabolous Development (Incomplete Metamorphosis)

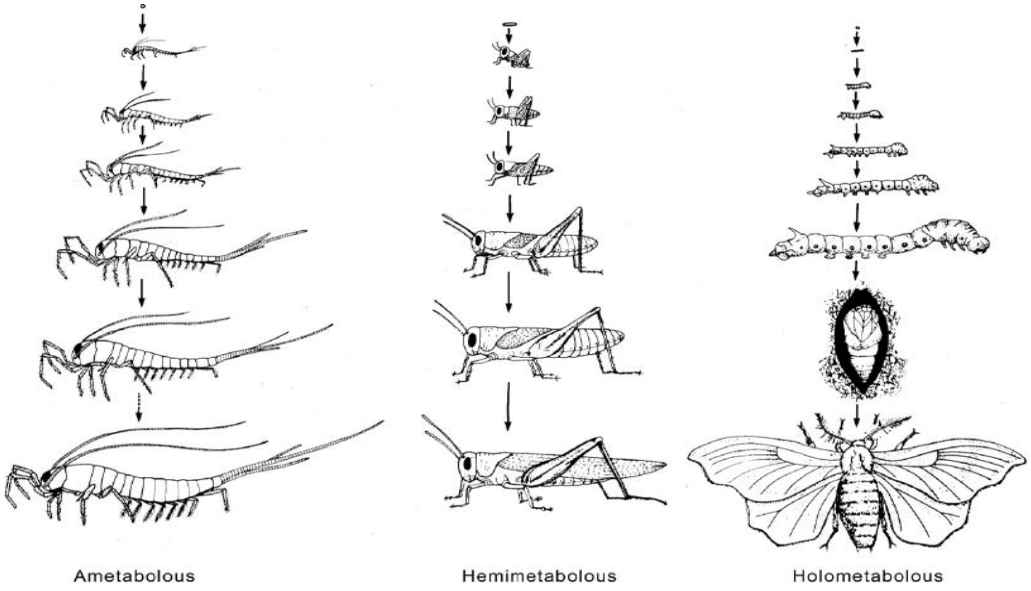
- The later juvenile instars broadly resemble the adult, except that their wings and external genitalia are not fully developed as in almost all exopterygotes.
- Other, less obvious, changes that occur during the growth of exopterygotes include the addition of neurons, Malpighian tubules, ommatidia, and tarsal segments, plus the differentiation of additional sensilla in the integument.
- Exopterygotes usually molt a fixed number of times. The number of molts is typically 4 or 5.

Holometabolous Development (Complete Metamorphosis)

- In which there is a marked change of form from larva to adult, occurs in endopterygotes and a few exopterygotes, for example, whiteflies (Aleurodidae: Hemiptera), thrips (Thysanoptera).

- Perhaps the most obvious structural difference between the larval and adult stages of endopterygotes is the absence of any external sign of wing development in the larval stages.
- The evolution of a pupal stage in the life history has made holometabolous development possible. The pupa is probably a highly modified final juvenile instar, which, through evolution, became less concerned with feeding and building up reserves (this function being left to earlier instars) and more specialized for the breakdown of larval structures and construction of adult features.
- In primitive endopterygotes most organs grow progressively during larval life, and metamorphosis consists mainly of the development of the flight mechanism. In most endopterygotes considerable differentiation of adult tissues occurs during metamorphosis, often from **imaginal discs**, groups of cells that remain embryonic through larval life, probably because of the hormonal milieu in juvenile instars.

Forms of Development



Basic types of development in insects

Eclosion

- For **exopterygotes**: adult emergence (eclosion) consists solely of escape from the cuticle of the previous instar.

- Many **endopterygotes**: force their way out of the cocoon or cell in which pupation occurred.
- For adults of many species, emergence is triggered by environmental factors, especially temperature and photoperiod.
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Control of Development

- Despite the apparently wide differences in the pattern of development seen in Insecta, the physiological system that regulates growth, molting, and metamorphosis is common to all members of the class, namely, the **endocrine system**.
- Variations in the relative levels of different hormones in an insect's body determine the nature and extent of tissue differentiation that is expressed at the next molt.
- In other words, it is the hormone balance that determines, in a holometabolous insect, for example, whether the next molt is larval-larval, larval-pupal, or pupal-adult.
- Hormones also coordinate the sequence of events in the growth and molting cycle and in some species ensure that an adult emerges when environmental conditions are suitable.
- The hormones act by regulating genetic activity.
- Many environmental factors can modify developmental patterns. Some of these factors, for example, temperature, may act directly to affect development; most factors, however, exert their effect indirectly via the endocrine system.

Endocrine Regulation of Development

- Postembryonic development is controlled by three endocrine centers:

1. Corpora cardiaca
2. Corpora allata
3. Molt glands
4. A molt cycle is initiated when, as a result of appropriate signals, the median neurosecretory cells of the brain release **ecdysiotropin** [prothoracicotropic hormone (**PTTH**)], which stimulates molting hormone (**ecdysone**)(**MH**) production by the molt glands.
5. In all insects studied there is a major peak of MH in the hemolymph during the second half of each molt cycle.
6. MH is then converted to the biologically active form, 20-hydroxyecdysone (**20-HE**),
7. The **corpora allata** produce juvenile hormone (**JH**).
8. JH can exert an influence on development only in the presence of MH, that is, after a molting cycle has begun.
9. It is the concentration of circulating JH during one or more critical periods of the stadium that determines the nature of the succeeding molt.
10. If the concentration of JH is above a threshold value during the critical period, the next molt will be larval-larval (for this reason, JH has been described as the "**status quo**" hormone).
11. 20-HE and JH are lipophilic and thus able to move through the cell membrane to the nuclear membrane where they bind to specific receptors.

Polymorphism

- **Polymorphism**: is the existence of several distinct forms of the same stage of a species. It may have a genetic basis (as in transient and balanced polymorphism) or be induced by changing external conditions (**polyphenism**) as seasonal polymorphism in aphids, whose effects are manifest via the endocrine system, specifically the concentration of JH at critical periods.

Digestion

- Insects feed on a wide range of organic materials.
- About 75% of all species are **phytophagous**.
- Others are **carnivorous**, **omnivorous**, or **parasitic** on other animals.
- In accord with the diversity of feeding habits, the means by which insects locate their food, the structure and physiology of their digestive system, and their metabolism are highly varied.
- The feeding habits of insects take on special significance for humans, on the one hand, because of the enormous damage that feeding insects do to our food, clothing, and health, and, on the other, because of the massive benefits that insects provide as plant pollinators during their search for food.

The Alimentary System

- The gut and its associated glands (Figure 1) triturate, lubricate, store, digest, and absorb food material and expel the undigested remains.
- Structural differences throughout the system reflect regional specialization for performance of these functions and are correlated also with **feeding habits** and the **nature of normal food material**.
- The structure of the system may vary at different stages of the life history because of the different feeding habits of the larva and adult of a species.
- The gut normally occurs as a continuous tube between the mouth and anus, and its length is broadly correlated with feeding habits, being short in carnivorous forms where digestion and absorption occur relatively rapidly, and longer (often convoluted) in phytophagous forms.
- In a few species that feed on fluids, such as larvae of Neuroptera and Hymenoptera-Apocrita, and some adult Heteroptera there is little or no solid waste in the food, and the junction between the midgut and hindgut is occluded.
- As Figure 1 indicates, food first enters the buccal cavity, which is enclosed by the mouthparts and is not strictly part of the gut.

- It is into the buccal cavity that the **salivary glands** release their products.
- The gut proper comprises three main regions: the **foregut**, in which the food may be stored, filtered, and partially digested; the **midgut**, which is the primary site for digestion and absorption of food; and the **hindgut**, where some absorption and feces formation occur.

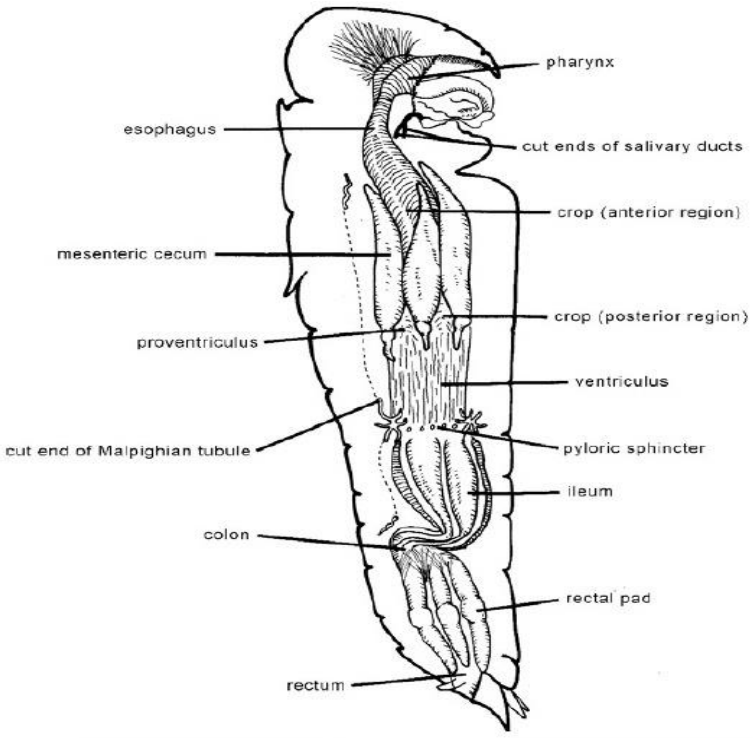

Figure 1: Alimentary canal and associated structures of a locust.

Salivary Glands

- Salivary glands are present in most insects, though their form and function are extremely varied, and they may or may not be innervated.
- Frequently they are known by other names according to either **the site at which their duct enters** the buccal cavity for example, labial glands and mandibular glands, or **their function**, for example, silk glands and venom glands.
- Typically, **saliva** is a watery, enzyme-containing fluid that serves to lubricate the food and initiate its digestion.
- Like that of humans, the saliva generally contains only carbohydrate digesting enzymes (amylase and invertase), though there are exceptions to this statement. For example, the saliva of some carnivorous species

contains protein- and/or fat-digesting enzymes only; that of bloodsucking species has no enzymes.

- In termite saliva there are cellulose-digesting enzymes.
- In the **innervated glands** of cockroaches and locusts, release of saliva is induced when food stimulates mechano- and chemosensilla on the mouthparts and antennae.
- The information travels to the **subesophageal ganglion** and then along aminergic or peptidergic neurons to the **glands** where it induces relaxation of the muscles that normally close off the opening of the salivary gland duct.
- In contrast, the **non-innervated glands** of *Calliphora erythrocephala* are stimulated to release saliva by a hemolymph factor, possibly serotonin.
- Other substances that may occur in saliva, though having no direct role in digestion, are important in food acquisition.
- A spectrum of compounds that assist feeding is present in the saliva of bloodsucking species. These include anticoagulants, inhibitors of platelet disintegration, pyrase (an enzyme prevent platelet aggregation), and vasodilators such as nitric oxide.
- In some species the glands have taken on functions quite unrelated to feeding, as, production of cocoon silk by caterpillars labial glands, and pheromone production by the mandibular glands of the queen honeybee.

Foregut

- The foregut, formed during embryogenesis by **invagination of the integument**, is lined with cuticle (**the intima**) that is shed at each molt.
- Surrounding the intima, which may be folded to enable the gut to stretch when filled, is a thin epidermis, small bundles of longitudinal muscle, a thick layer of circular muscle, and a layer of connective tissue through which run nerves and tracheae (Figure 2).
- The foregut is generally differentiated into pharynx, esophagus, crop, and proventriculus.

- Attached to the pharyngeal intima are dilator muscles. These are especially well developed in sucking insects and form the pharyngeal pump.
- The **esophagus** is usually narrow but posteriorly may be dilated to form the crop where food is stored.
- In Diptera and Lepidoptera, however, the crop is actually a diverticulum off the esophagus.
- During storage the food may undergo **some digestion** in insects whose saliva contains enzymes or that regurgitate digestive fluid from the midgut.
- In some species the intima of the crop forms **spines** or **ridges** that probably aid in breaking up solid food into smaller particles and mixing in the digestive fluid (Figure 2A).
- The hindmost region of the foregut is the proventriculus, which may serve as a **valve** regulating the rate at which food enters the midgut, as a **filter** separating liquid and solid components, or as a **grinder** to further break up solid material.
- Its structure is, accordingly, quite varied.
- In species where it acts as a **valve** the intima of the proventriculus may form longitudinal folds and the circular muscle layer is thickened to form a sphincter.
- When a **filter**, the proventriculus contains spines that hold back the solid material, permitting only liquids to move posteriorly.
- Where the proventriculus acts as a **gizzard**, grinding up food, the intima is formed into strong, radially arranged teeth, and a thick layer of circular muscle covers the entire structure (Figure 2B).

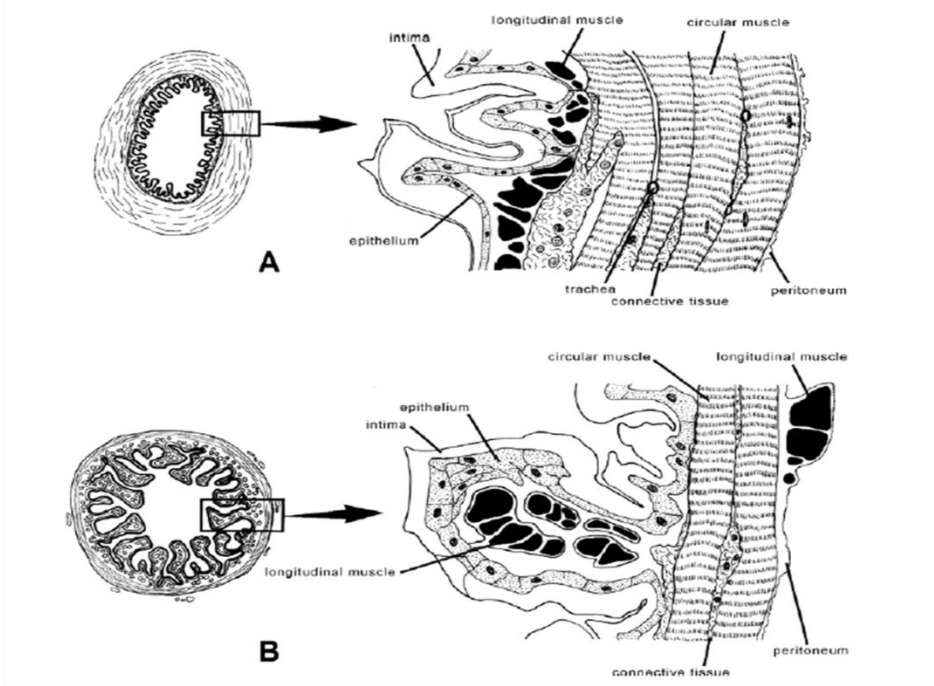

Figure 2: Transverse sections through (A) crop and (B) proventriculus of a locust.

- Posteriorly the foregut is invaginated slightly into the midgut to form the **esophageal** (= stomodeal) **invagination** (Figure 3).
- Its function is to ensure that food enters the midgut within the **peritrophic matrix**.

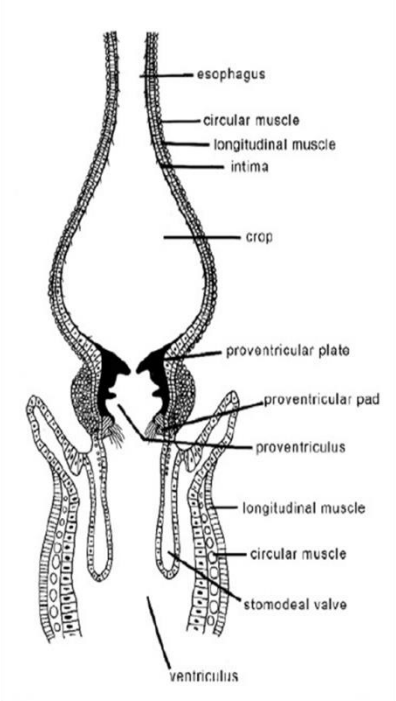

Figure 3: Longitudinal section through crop, proventriculus, and anterior midgut of a cockroach.

Midgut

- The midgut(= **ventriculus** = **mesenteron**) is of **endodermal** origin and, therefore, has no cuticular lining.
- In most insects, however, it is lined by a thin **peritrophic matrix** (PM) composed of proteins bound to a meshwork of chitin fibrils (Figure 4).
- Some PM proteins, the peritrophins, are heavily glycosylated like mucus in the intestine of vertebrates.

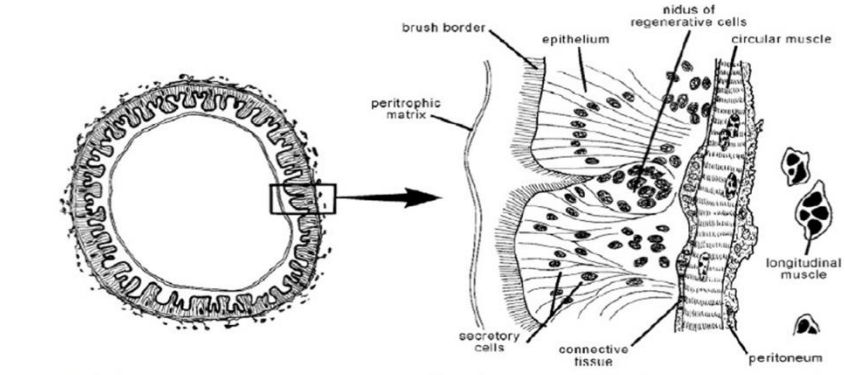

Figure 4: Transverse section through midgut of a locust.

- The **functions of the PM** are to prevent mechanical damage to the midgut epithelium, to prevent entry of microorganisms into the body cavity, to bind potential toxins and other damaging chemicals, and to compartmentalize the midgut lumen, that is, to divide it into an **endoperitrophic** space (within the matrix) and an **ectoperitrophic** space (adjacent to the midgut epithelium).
- This separation of the epithelium from the food improves digestive efficiency by segregating enzymes between the spaces and enabling some enzymes to be recycled.
- The **PM** is generally absent in fluid-feeding insects, for example, Hemiptera, adult Lepidoptera, and bloodsucking Diptera.
- However, some insects produce the PM only at certain times (e.g., female mosquitoes after a blood meal).

- The midgut is usually not differentiated into structurally distinct regions apart from the development, at the anterior end, of a varied number of blindly ending **ceca**, which serve to increase the surface area available for enzyme secretion and absorption of digested material.

- In many Heteroptera, however, the midgut is divided into three or four easily visible regions. In the chinch bug (*Blissus leucopterus*) four such regions occur (Figure 5). The anterior region is large and saclike, and serves as a storage region (no crop is present). The second region serves as a valve to regulate the flow of material into the third region where digestion probably occurs. Ten fingerlike ceca filled with bacteria are attached to the fourth region, which may be absorptive in function. The role of the bacteria is not known.

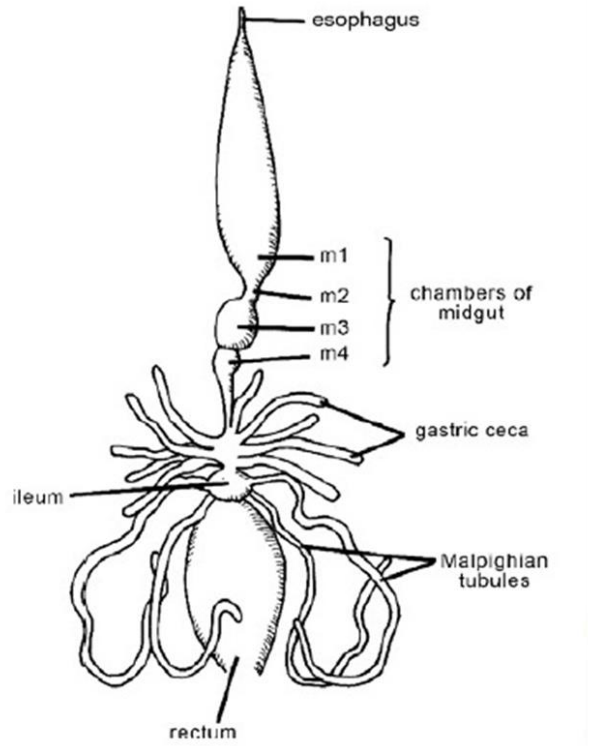

Figure 5: Alimentary canal of chinch bug (*Blissus leucopterus*) showing regional differentiation of midgut.

- In many homopterans, which feed on plant sap, the midgut is modified both morphologically and anatomically so that excess water present in the

food can be removed, thus preventing dilution of the hemolymph. Though details vary among different groups of homopterans, the anterior end of the midgut (or, in some species, the posterior part of the esophagus) is brought into close contact with the posterior region of the midgut (or anterior hindgut), and the region of contact becomes enclosed within a sac called the "**filter chamber**" (Figure 6).

- Such an arrangement facilitates rapid movement of water by osmosis from the lumen of the anterior midgut across the wall of the posterior midgut and possibly also the Malpighian tubules. Thus, relatively little of the original water in the food actually passes along the full length of the midgut.

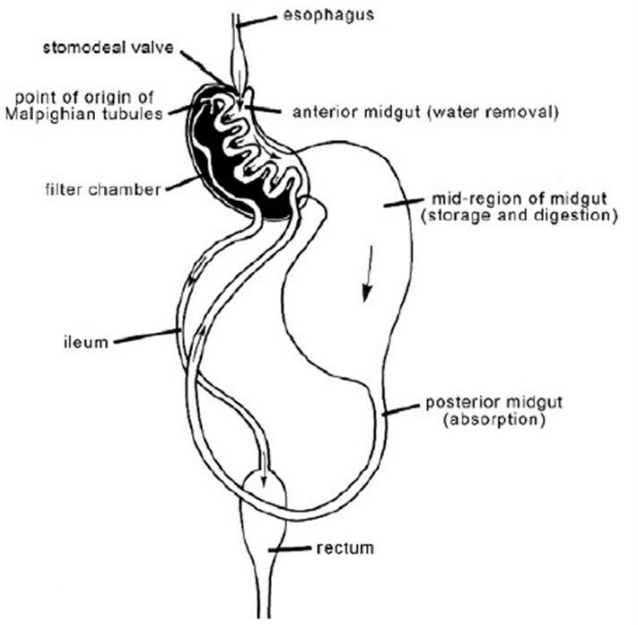

Figure 6: Alimentary canal of cercopid (Cercopoidea) showing filter chamber arrangement.

- Differentiation into digestive and absorptive regions occurs in some species.
- In tsetse flies the cells of the anterior midgut are small and are concerned with absorption of water from the ingested blood.
- They produce no enzymes and digestion does not begin until food reaches the middle region where the cells are large, rich in ribonucleic acid, and produce enzymes.

- In the posterior midgut the cells are smaller, closely packed, and probably concerned with absorption of digested food.
- In some species different regions of the midgut are apparently adapted to the absorption of particular food materials.
- In *Aedes* larvae the anterior midgut is concerned with fat absorption and storage, whereas the posterior portion absorbs carbohydrates and stores them as glycogen.
- In larval Lepidoptera goblet cells, with a large flask-shaped central cavity, are scattered among the regular epithelial cells. They are though to play a role in the regulation of the potassium level within the hemolymph.

Hindgut

- The hindgut is an **ectodermal** derivative and, as such, is lined with cuticle, though this is thinner than that of the foregut, a feature related to the absorptive function of this region.
- The epithelial cells that surround the cuticle are flattened except in the rectal pads where they become highly columnar and filled with mitochondria.
- Muscles are only weakly developed and, usually, the longitudinal strands lie outside the sheet of circular muscle.
- The hindgut usually has the following regions: **pylorus**, **ileum**, and **rectum**.
- The **pylorus** may have a well-developed circular muscle layer (pyloric sphincter) and regulate the movement of material from midgut to hindgut.
- Also, the **Malpighian tubules** characteristically enter the gut in this region.
- **The ileum** (Figure 7A) is generally a narrow tube that serves to conduct undigested food to the rectum for final processing.
- In some insects, however, some absorption of ions and/or water may occur in this region.

- In a few species production and excretion of nitrogenous wastes occur in the ileum.
- In many wood-eating insects, for example, species of termites and beetles, the ileum is dilated to form a fermentation pouch housing bacteria or protozoa that digest wood particles.
- The products of digestion, when liberated by the microorganisms, are absorbed across the wall of the ileum.

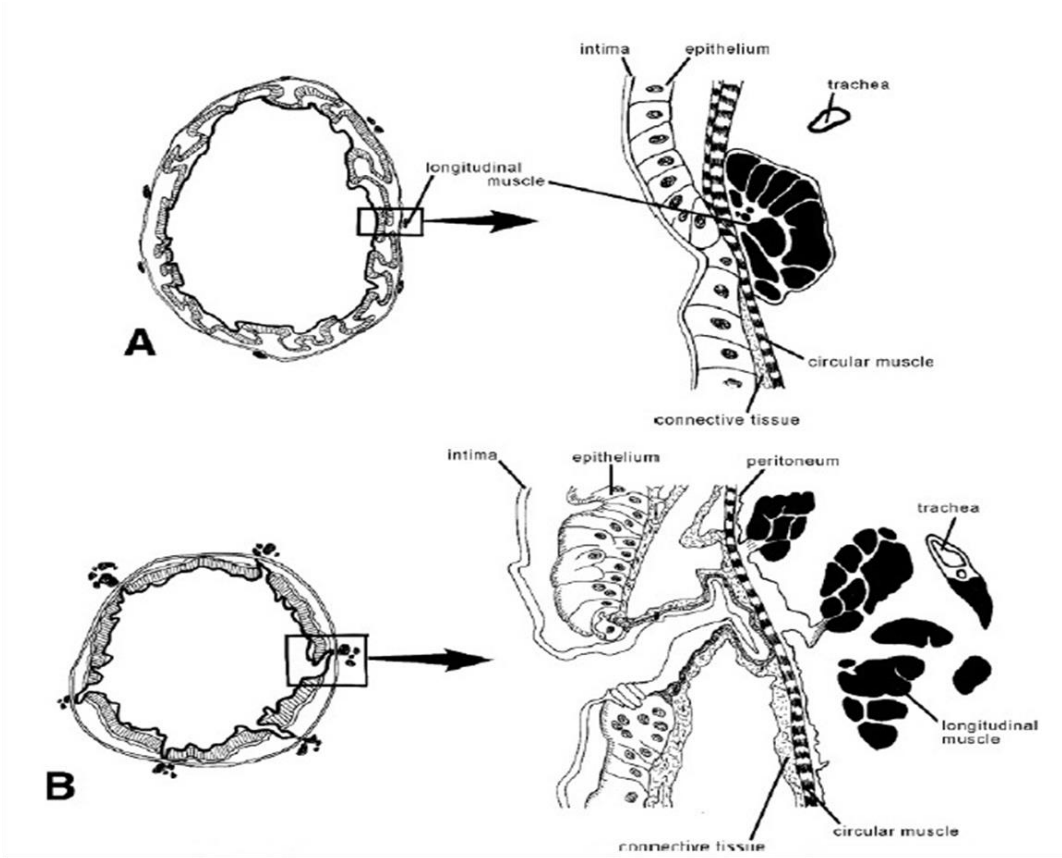

Figure 7: Transverse sections through (A) ileum and (B) rectum of a locust.

Gut Physiology

- The primary functions of the alimentary canal are **digestion** and **absorption**.
- For these processes to occur efficiently, food is moved along the canal.
- In some species, enzyme secretions are moved anteriorly so that digestion can begin some time before food reaches the region of absorption.

Gut Movements

- Though the alimentary canal is innervated, neural control is principally associated with the opening/closing of valves that occur within the canal.
- The **rhythmic peristaltic muscle contractions** that move food posteriorly through the gut are myogenic; that is, they originate within the muscles themselves rather than occurring as a result of nervous stimuli.
- **Antiperistaltic movements** also occur in some species and serve to move digestive fluid forward from the midgut into the crop.
- The rate at which food moves through the gut is not uniform.
- It varies according to the physiological state of an insect; for example, it is greater when an insect has been starved previously or is active.
- The rate may also differ between sexes and with age.
- Another important variable is the nature of the food. Some insects are able to move some components of the diet rapidly through the gut while retaining others for considerable periods.
- Within the gut, food moves at variable rates in different regions.
- The proventricular and pyloric valves are important regulators of food movement, though little is known about how their opening and closing are controlled.
- In *Periplaneta* opening of the proventriculus was shown to depend on the osmotic pressure of ingested fluid. As the concentration is increased, the proventriculus opens less often and less widely, and vice versa.
- In blood-feeding species, the abdomen, is known to cause release of neurosecretion from the corpora cardiaca which enhances gut peristalsis and, hence, the rate of food passage.
- Localized enhancement of peristalsis may be induced by release of peptide hormones from cells in the wall of the midgut.

Digestion

- As noted above, digestion may be initiated by enzymes present in the saliva either mixed with the food as it enters the buccal cavity or secreted onto the food prior to ingestion.
- Most digestion is dependent, however, on enzymes secreted by the midgut epithelium.
- Digestion mostly occurs in the lumen of the midgut, though regurgitation of digestive fluid into the crop is important in some species.
- In wood-eating forms, much of the digestion is carried out by microorganisms in the hindgut

Digestive Enzymes

- A wide variety and large number of digestive enzymes have been reported for insects.
- The produced enzymes reflect both qualitatively and quantitatively the normal constituents of the diet.
- The enzymes may have low specificity, enabling an insect to digest a variety of molecules of a given type, or may be highly specific, for example, when a species feeds solely on a particular food. Gut fluid is buffered within a narrow pH range to facilitate digestion and absorption.
- Omnivorous species produce enzymes for digesting proteins, fats, and carbohydrates.
- Carnivorous species produce mainly lipases and proteases; in some species these may be highly specific in action.
- The nature of the enzymes produced may change at different stages of the life history as the diet of an insect changes.
- For example, caterpillars feeding on plant tissue secrete a spectrum of enzymes, whereas nectar-feeding adult Lepidoptera produce only invertase.

- Insects can digest a wide range of **carbohydrates**, even though only a few distinct **enzymes** may be produced.
- α-glucosidase will hydrolyze all α-glucosides. Likewise, ß-glucosidase facilitates splitting of cellobiose, gentiobiose, and phenylglucosides; ß-galactosidase hydrolyzes ß-galactosides such as lactose.
- The normal polysaccharide-digesting enzyme produced is amylase for hydrolysis of starch, though particular species may produce enzymes for digestion of other polysaccharides.
- For example, silverfish (Zygentoma) have endogenous cellulases, though in most insects production of this enzyme is restricted to microorganisms present in the hindgut.
- As in other organisms, the **protein-digesting enzymes** produced by the midgut are divisible into two types: **endopeptidases**, which effect the initial splitting of proteins into polypeptides, **exopeptidases**, which bring about degradation of polypeptides by the sequential splitting off of individual amino acids from each end of a molecule.
- **Exopeptidases** can be further categorized into: **carboxypeptidases**, which remove amino acids from the carboxylic end of a polypeptide, **aminopeptidases**, which cause hydrolysis at the amino end of a molecule.
- A **dipeptidase** also is frequently present.
- In some species only endopeptidases occur in the midgut lumen (specifically **within** the endoperitrophic space), the exopeptidases being found **outside** the PM or even attached to the apical plasma membrane of the epithelial cells.
- Some insects produce specific enzymes for the digestion of particularly resistant **structural proteins**, such as **Keratin**, the primary constituent of wool, hair, and feathers.
- A **keratinase** has been identified in clothes moth larvae (*Tineola*) and may also occur in other keratin-digesting species, such as Mallophaga.
- The keratinase is active only under anaerobic (reducing) conditions and, in this context, it is interesting to note that the midgut of *Tineola* is poorly tracheated.

- Dietary **fats** of either animal or plant origin are almost always **triglycerides**, that is, glycerol in combination with three fatty acid molecules. The latter may range from **unsaturated** to **fully saturated**.
- **Lipases**, which hydrolyze fats to the constituent fatty acids and glycerol, have low specificity. Therefore, the presence of one such enzyme will normally satisfy an insect's needs.
- Fat digestion is generally somewhat slow as insects lack anything comparable to the bile salts of vertebrates that would emulsify and stabilize lipid droplets.

Absorption

- Absorption of digestion products occurs mostly in the anterior midgut and mesenteric ceca.
- It is generally a passive process, though carrier molecules may be used to facilitate the process.
- The rate at which **sugars are absorbed** is linked to the rate at which they are converted to trehalose and, hence, glycogen.
- **Amino acid absorption** may be preceded by absorption of water across the midgut wall to produce a favorable gradient for diffusion.
- **Absorption of lipids** is a passive process and is relatively slow compared to sugars and amino acids.

Respiration

- In all organisms gas exchange, the supply of oxygen to and removal of carbon dioxide from cells, depends ultimately on the rate at which these gases diffuse in the dissolved state.
- **The diffusion rate** is proportional to (1) the surface area over which diffusion is occurring and (2) the diffusion gradient (concentration

difference of the diffusing material between the two points under consideration divided by the distance between the two points).

- Diffusion alone, therefore, as a means of obtaining oxygen or excreting carbon dioxide can be employed only by small organisms where all cells are relatively close to the surface of the body) and organisms whose metabolic rate is low.
- Organisms that are larger and/or have a high metabolic rate must increase the rate at which gases move between the environment and the body tissues by improving (1) and/or (2) above.
- In other words, specialized respiratory structures with large surface areas and/or transport systems that bring large quantities of the gas closer to the site of use or disposal (thereby improving the diffusion gradient) have been developed.
- In insects the tracheal system, a series of gas-filled tubes derived from the integument, has evolved to cope with gas exchange.
- Terminally the tubes are much branched, forming **tracheoles** that provide an enormous surface area over which diffusion can occur.
- Furthermore, tracheoles are so numerous that gaseous oxygen readily reaches most parts of the body, and, equally, carbon dioxide easily diffuses out of the tissues.
- Thus, in most insects, in contrast to many other animals, the circulatory system is unimportant in gas transport.
- Because they are in the gaseous state within the tracheal system, oxygen and carbon dioxide diffuse rapidly between the tissues and site of uptake or release, respectively, on the body surface.
- Oxygen, for example, diffuses 3 million times faster in air than in water.
- Again, because the system is gas-filled, much larger quantities of oxygen can reach the tissues in a given time. (Air has about 25 times more oxygen per unit volume than water.)
- The eminent suitability of the tracheal system for gas exchange is illustrated by the fact that, for most **small insects** and many large insects at

rest, simple diffusion of gases in/out of the tracheal system entirely satisfies their requirements. In **large, active insects** the gradient over which diffusion occurs is increased by means of ventilation;

- that is, air is actively pumped through the tracheal system.

Organization and Structure of the Tracheal System

- A tracheal system is present in all Insecta and in other hexapods with the exception of the Protura and many Collembola.
- It arises during embryogenesis as a series of segmental invaginations of the integument.
- Up to **12 (3 thoracic and 9 abdominal) pairs of spiracles** may be seen in embryos, though this number is always reduced prior to hatching, and further reduction may occur in endopterygotes during metamorphosis.
- Various terms are used to describe the number of pairs of functional spiracles, for example, **holopneustic** (10 pairs, located on the mesothorax and metathorax and 8 abdominal segments), **amphipneustic** (2 pairs, on the mesothorax and at the tip of the abdomen), and **apneustic** (no functional spiracles).
- The last condition is common in aquatic larvae, which are said, therefore, to have a closed tracheal system.
- The proportion of the body filled by the tracheal system varies widely, both among species and within the same individual throughout a stadium.
- In active insects whose tracheal system includes air sacs the tracheal system occupies a greater fraction of the body than in less active species.
- The tracheal system volume may decrease dramatically during a stadium (e.g., in Locusta from 48% to 3%) as the air sacs become occluded by the increased hemolymph pressure that results from tissue growth.
- After ecdysis, when body volume has increased the tracheal system expands because of the lowered hemolymph pressure.

Tracheae and Tracheoles

- In **apterygotes** other than lepismatid Zygentoma, the tracheae that run from each spiracle do not anastomose either with those from adjacent segments or with those derived from the spiracle on the opposite side.
- In the **Lepismatidae** and **Pterygota** both longitudinal and transverse anastomoses occur, and, though minor variations can be seen, the resultant pattern of the tracheal system is often characteristic for a particular order or family.
- Generally, a pair of large-diameter, longitudinal tracheae (**the lateral trunks**) run along the length of an insect just internal to the spiracles.
- Other **longitudinal trunks** are associated with the heart, gut, and ventral nerve cord.
- Interconnecting the longitudinal tracheae are **transverse commissures**, usually one dorsal and another ventral, in each segment (Figure 1A, B).

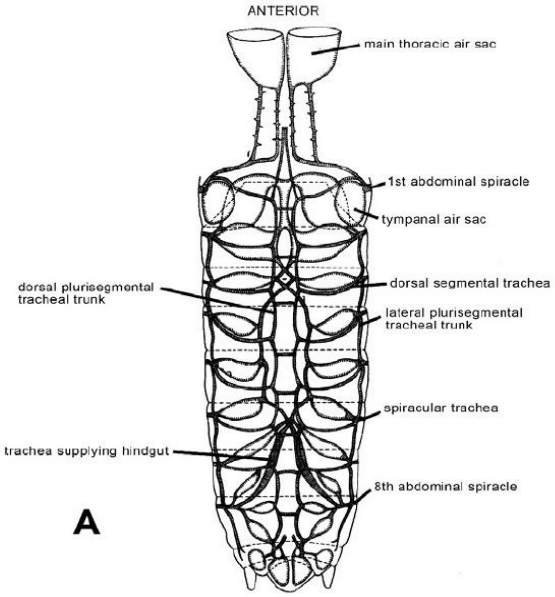

Figure 1A: Dorsal tracheal system of abdomen of locust

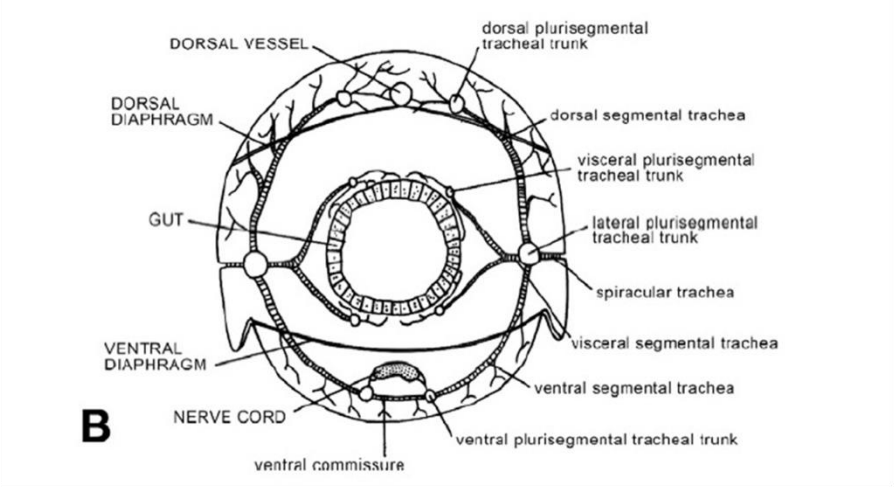

Figure 1B:diagrammatic transverse section through abdomen of a hypothetical insect to illustrate main tracheal branches

- Parts of the tracheal system, for example, that of the pterothorax, may be effectively isolated from the rest of the system by reduction of the diameter or occlusion of certain longitudinal trunks. This arrangement is associated with the use of **autoventilation** as a means of improving the supply of oxygen to wing muscles during flight.
- Also, tracheae are often dilated to form large thin-walled air sacs that have an important role in **ventilation** and other functions.
- Numerous **smaller tracheae** branch off the main tracts and undergo progressive subdivision until at a diameter of about 2–5 μm they form a number of fine branches each 1 μm or less across known as **tracheoles**.
- **Tracheoles** are intracellular, being enclosed within a very thin layer of cytoplasm from the **tracheoblast** (tracheal end cell) (Figure 2C), and ramify throughout most tissues of the body.
- **Tracheoles** are especially abundant in metabolically active tissues. Thus, in flight muscles, fat body, and testes, for example, tracheoles indent individual cells, so that gaseous oxygen is brought into extremely close proximity with the energy-producing mitochondria.

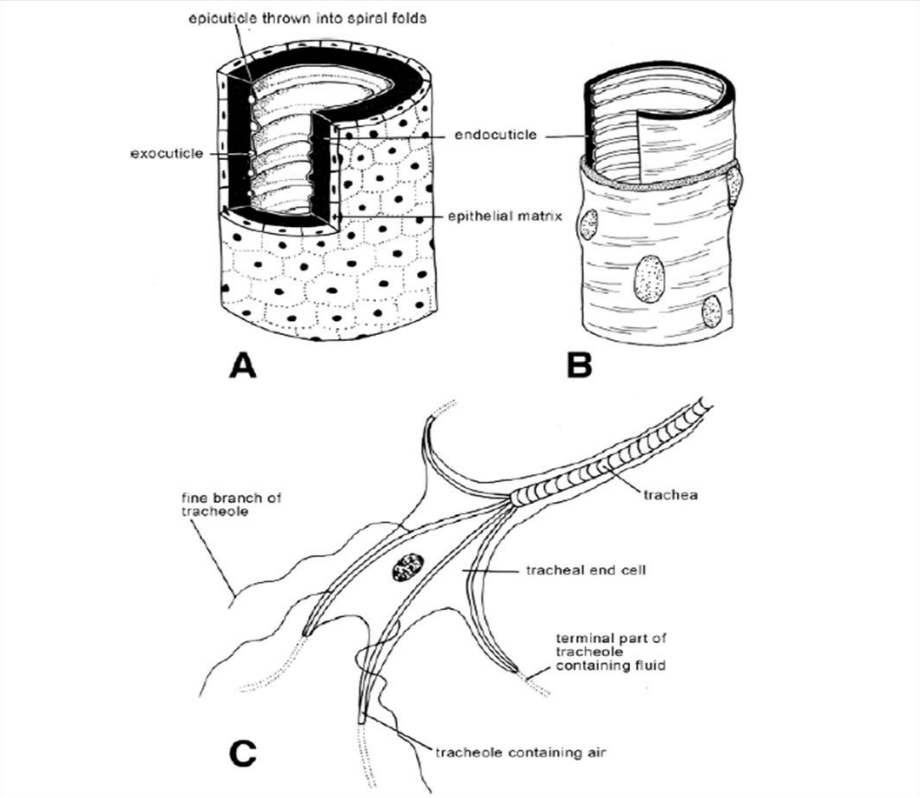

Figure2: Structure of (A) large; and (B) small tracheae. (C) Origin of tracheole.

- As derivatives of the integument, tracheae comprise cuticular components, epidermis, and basal lamina (Figure 2).
- Adjacent to the spiracle, the tracheal cuticle includes, the cuticulin envelope, epicuticle and procuticle; in smaller tracheae and most tracheoles only the cuticulin envelope and epicuticle are present.
- Providing the system with strength yet flexibility, tracheal cuticle has internal ridges that may be either separate (annuli) or form a continuous helical fold (**taenidium**).
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- Providing the system with strength yet flexibility, tracheal cuticle has internal ridges that may be either separate (annuli) or form a continuous helical fold (**taenidium**).

Spiracles

- Only in some apterygotes do tracheae originate at the body surface. Normally, they arise slightly below the body surface from which they are separated by a small cavity, the atrium (Figure 3A).
- Except for those of a few insects that live in humid microclimates, spiracles may be covered, for example, by the elytra or wings in Hemiptera and Coleoptera, or are equipped with various **valves** for prevention of water loss.
- The valves may take the form of one or more cuticular plates that
- can be pulled over a spiracle by means of a closer muscle (Figure 3B–D).
- Opening of the valve(s) is effected either by the natural elasticity of the surrounding cuticle or by an opener muscle.
- Alternatively, the valve may be a cuticular lever which by muscle action constricts the trachea adjacent to the atrium (Figure 3E,F).

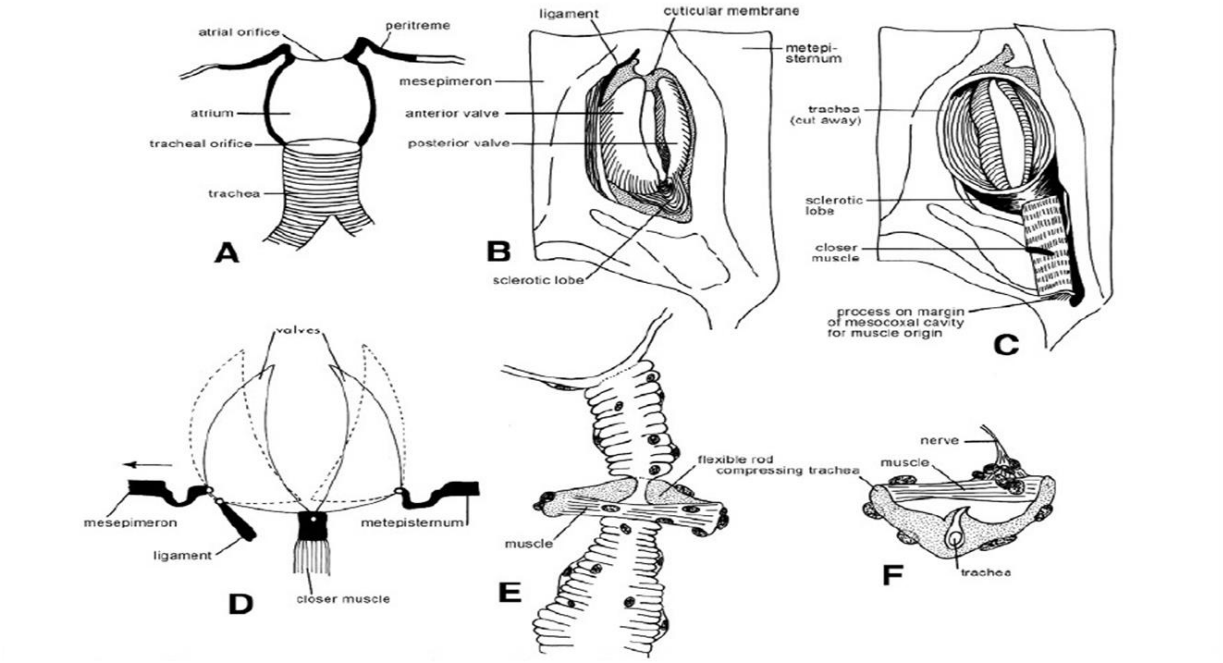


Figure 3: Spiracular structure. (A) Section through spiracle to show general arrangement; (B, C) outer and inner views of second thoracic spiracle of grasshopper; (D) diagrammatic section through spiracle to show mechanism of closure. The valve is opened by movement of the mesepimeron, closed by contraction of the muscle; (E) closing mechanism on flea trachea; and (F) section through flea trachea at level of closing mechanism.

- In lieu of, or in addition to, the valves, there may be hairs lining the atrium or a **sieve plate** (a cuticular pad penetrated by many fine pores) covering the atrial pore.
- It is commonly assumed that an important function of these hairs and sieve plates is to prevent dust entry.
- **Sieve plates** are not better developed on **inspiratory** than on **expiratory** spiracles and several other functions can be suggested:

✓ 1- They may prevent waterlogging of the tracheal system in terrestrial species during rain, in aquatic insects, and in species that live in moist soil, rotting vegetation.

✓ 2- They may prevent entry of parasites, especially mites, into the tracheal system.

✓ 3- They may reduce bulk flow of gases through the system caused by body movements, thereby reducing evaporative water loss.

- ✓ This would be disadvantageous in insects that ventilate the tracheal system, and it is of interest, therefore, that those spiracles important in ventilation commonly lack a sieve plate or have a plate that is divided down the middle so that it may be opened during ventilation.

Gas Exchange in Aquatic Insects

- ➢ Oxygen may enter the tracheal system in gaseous form, that is, via functional spiracles (**the "open" tracheal system**) or may pass, in solution, directly across the body wall to the tracheal system, in which arrangement the spiracles are sealed (non-functional), and the tracheal system is said to be "**closed**."
- ➢ Aquatic insects with open tracheal systems exchange the gas within the system by periodically visiting the water surface, by obtaining gas from gas-filled spaces in aquatic plants, or through the use of a "**gas gill**" (a bubble or film of air that covers the spiracles, in to or out of which oxygen and carbon dioxide, respectively, can diffuse from/to the surrounding water).
- ➢ A significant amount of gas exchange may occur by direct diffusion across the body surface (**cutaneous respiration**) in larvae with an open system whose integument is thin, for example, mosquito larvae.
- ➢ **Cutaneous respiration** may entirely satisfy the requirements of insects with closed tracheal systems.
- ➢ However, in many species supplementary respiratory surfaces, "**tracheal gills**," have evolved, though these often become important only under oxygen-deficient conditions.

Gas Exchange in Endoparasitic Insects

- ➢ It is probably not surprising that endoparasitic insects, as they too are surrounded by fluid, show many parallels with aquatic insects in the way that they obtain oxygen.

- Most endoparasites satisfy a proportion of their requirements by cutaneous diffusion.
- In some first-instar larvae of Hymenoptera and Diptera the tracheal system may be liquid-filled, but generally it is gas-filled with closed spiracles and includes a rich network of branches immediately beneath the integument.
- Endoparasites with greater oxygen requirements usually are in direct contact with atmospheric air either via the integument of the host or via the host's tracheal system.
- In larvae of many Chalcidoidea, for example, only the posterior spiracles are functional, and these open into an air cavity formed at the base of the egg pedicel that penetrates the host's integument (Figure 4A).
- Many larval Tachinidae (Diptera) become enclosed in a respiratory funnel produced by the host in an attempt to encapsulate the parasite (Figure 4B).
- The funnel is produced by inward growth of the host's integument or tracheal wall.
- Within it, the parasite attaches itself by means of mouth hooks while retaining contact with atmospheric air via the entrance of the funnel.

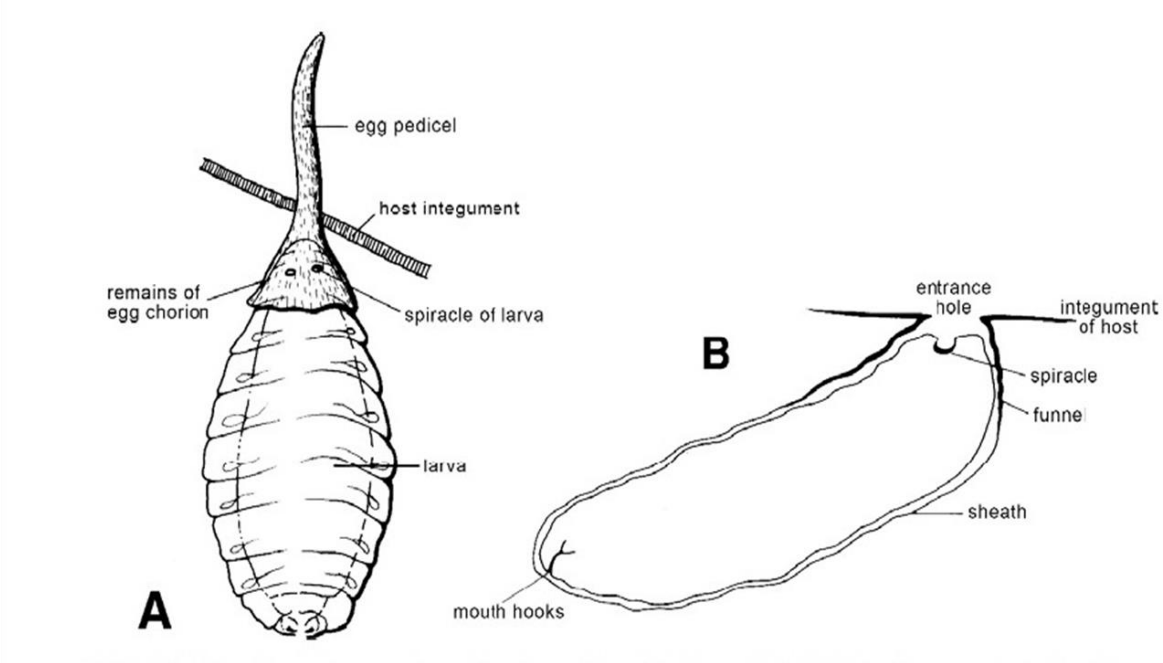


Figure 4: Respiratory systems of endoparasites. (A) Larva of *Blastothrix* (Hymenoptera) attached posteriorly to remains of egg, thereby maintaining contact with the atmosphere via the egg pedicel; and (B) larva of *Thrixion* (Diptera) surrounded by the respiratory funnel formed by ingrowth of the host's integument.

Excretion

- Insect excretory system is responsible for **removal** of unwanted materials and **retention** of those that are useful, to maintain as nearly as possible the best cellular environment.
- This regulation is of great importance in insects because they occupy such varied habitats and, therefore, have different regulatory requirements.
- Terrestrial insects lose water by evaporation through the integument and respiratory surfaces and in the process of nitrogenous waste removal.
- Brackish-water and saltwater forms also lose water as a result of osmosis across the integument; in addition, they gain salts from the external medium.
- Insects inhabiting fresh water gain water from and lose salts to the environment.
- The problem of osmoregulation is complicated by an insect's need to remove nitrogenous waste products of metabolism, which in some instances are very toxic.
- This removal uses both **salts** and **water**, one or both of which must be **recovered later** from the urine.

Excretory Systems

Malpighian Tubules—Rectum

- The **Malpighian tubules** and **rectum**, functioning as a unit, form the major excretory system in most insects.
- The blindly ending tubules, which usually lie freely in the hemocoel, open into the alimentary canal at the **junction** of the midgut and hindgut (Figure 1A).
- Typically they enter the gut individually but may fuse first to form a common sac or ureter that leads into the gut.

- Their number varies from two to several hundred.

- Malpighian tubules are absent in Collembola, some Diplura, and aphids; in other Diplura, Protura, and Strepsiptera there are papillae at the junction of the midgut and hindgut.

- With the tubules are associated **tracheoles** and, usually, **muscles** (Figure 1E). The latter take the form of a continuous sheath, helical strips, or circular bands and are situated outside the basal lamina. They enable the tubules to **writhe**, which ensures that different parts of the hemolymph are exposed to the tubules and assists in the flow of fluid along the tubules.

- A tubule is made up of a **single layer** of epithelial cells, situated on the inner side of a basal lamina (Figure 1B–D).

- The inner (apical) surface of the cells takes the form of a **brush border** (**microvilli**).

- The outer (basal) surface is also **extensively folded**.

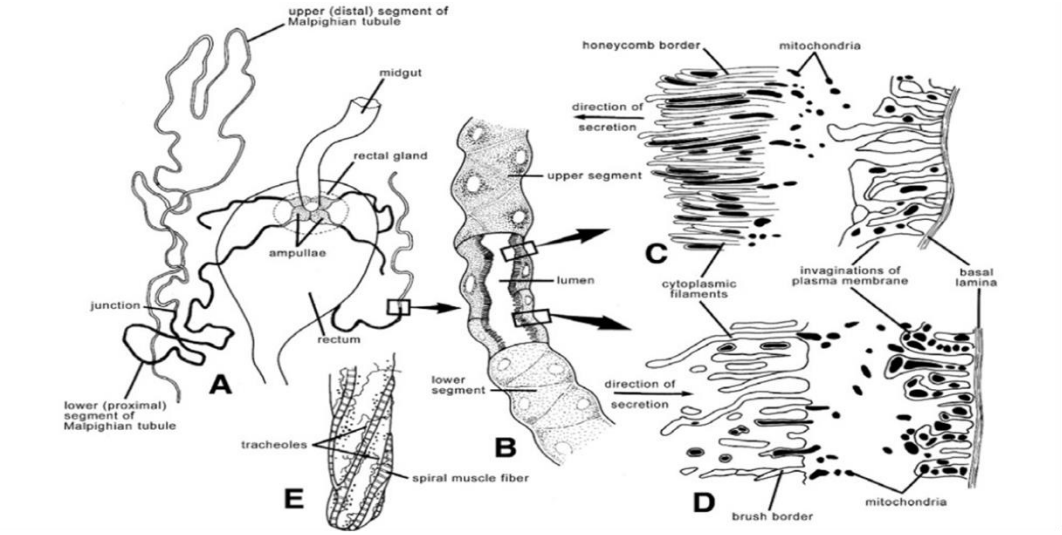


Figure 1: (A) Excretory system of *Rhodnius.* Only one Malpighian tubule is drawn in full; (B) junction of proximal and distal segments of a Malpighian tubule of *Rhodnius.* Part of the tubule has been cut away to show the cellular differentiation; (C, D) sections of the wall of the distal and proximal segments, respectively, of a tubule; and (E) tip of Malpighian tubule of *Apis* to show tracheoles and spiral muscles.

- Both of these features are typical of cells involved in the transport of materials and serve to increase enormously the surface area across which transport can occur.

- Numerous mitochondria occur, especially adjacent to or within the folded areas, to supply the energy requirements for active transport of certain ions across the tubule wall.
- In some insects (e.g., *Rhodnius*), **two distinct zones** can be seen in the Malpighian tubule (Figure 1C, D).
- In the **distal** (**secretory**) zone the cells possess large numbers of closely packed microvilli, but very few infoldings of the basal surface. Mitochondria are located near or within the microvilli.
- In the **proximal** (**absorptive**) part of the tubule the cells possess fewer microvilli, yet show more extensive invagination of the basal surface. The mitochondria are correspondingly more evenly distributed.

Other Excretory Structures

- Even in insects that use the rectum as the primary site of osmoregulation, the **ileum** may nonetheless be a site for water or ion resorption. In other species where the rectum is unimportant in osmoregulation, serving only to store urine and feces prior to expulsion, the ileum often takes on this role.
- The **midgut** of silkmoth larvae actively removes potassium from the hemolymph, thus protecting the tissues from the very high concentration of potassium ions present in the leaves eaten by these insects.
- In a few insects it appears that the Malpighian tubules, though present, play no part in nitrogenous excretion. In *Periplaneta americana*, for example, uric acid is not found in the tubules but does occur in small amounts in the **hindgut**, which may excrete it directly from the hemolymph.
- In *P. americana* much uric acid is stored in urate cells in the fat body, and the major form of excreted nitrogen in this species is ammonia.
- How this reaches the hindgut lumen in *P. americana* is unclear.

Physiology of Nitrogenous Excretion

- The **uric acid** is secreted into the lumen of the tubules as the sodium or potassium salt, along with other ions, water, and various low-molecular-weight organic molecules.

- In a typical insect, for example *Dixippus*, secretion occurs along **the entire length of the tubule**. No resorption of materials takes place across the tubule wall, and urate leaves the tubule in solution. In the rectum resorption of water and sodium and potassium ions occurs, and the pH of the fluid decreases from 6.8–7.5 to 3.5–4.5.
- The combined effect of water resorption and pH change is to cause massive precipitation of uric acid.
- Useful organic molecules such as amino acids and sugars are also resorbed through the rectal wall (Figure 2A).
- In *Rhodnius*, whose tubules show structural differentiation along their length, the process of excretion is basically the same as in *Dixippus*.
- However, in *Rhodnius* only the **distal portion** of the tubule is secretory and resorption of water and cations begins in the **proximal part**.
- Slight change in pH occurs (from 7.2 to 6.6) as the fluid passes along the tubule and this is sufficient to initiate uric acid precipitation.
- Further water and salt resorption occurs in the rectum (pH 6.0), causing precipitation of the remaining waste (Figure 4B).

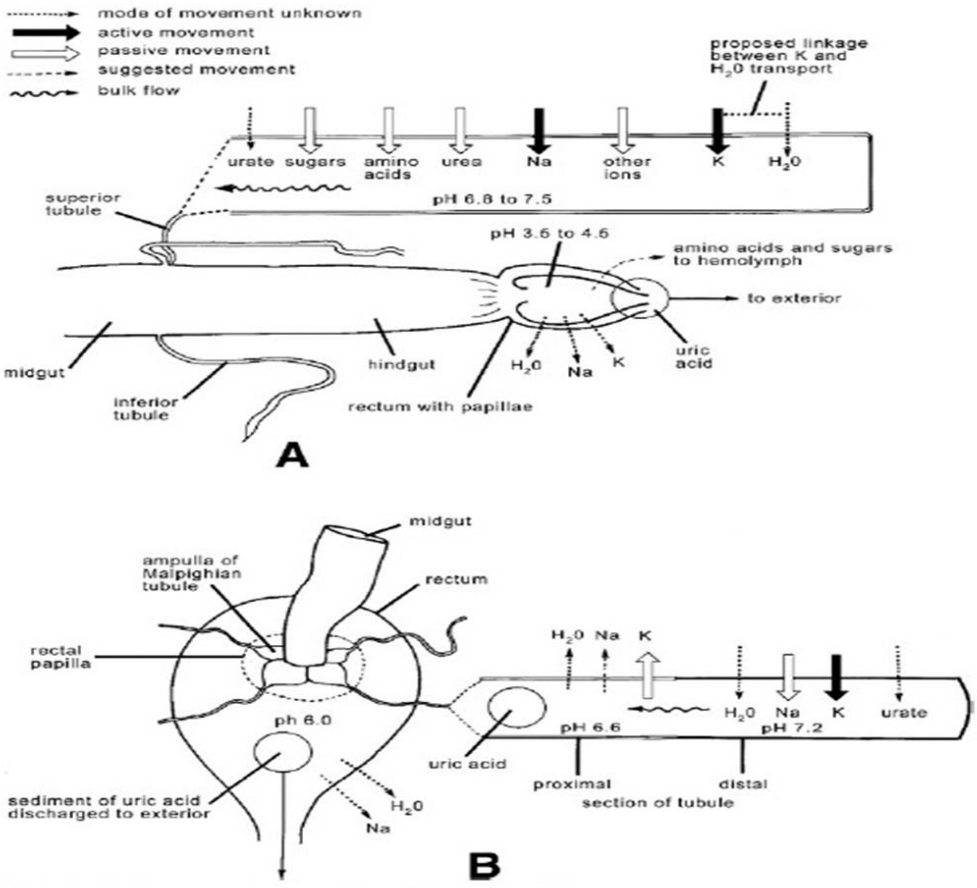


Figure 2: Movements of water, ions, and organic molecules in the excretory systems of (A) *Dixippus* and (B) *Rhodnius.*

Hormonal Control

- As in other systems with a homeostatic role, the activities of the excretory system, including both production of nitrogenous waste and osmoregulation, need to be regulated to suit the specific but changing requirements of the insect. This coordination is effected by hormones.
- In most species, neurosecretory cells in the brain produce **diuretic** and **antidiuretic** hormones that are then stored in the corpora cardiaca, though there are many reports of **diuresis-modifying factors** in extracts from other ganglia in the ventral nerve cord.
- Almost all identified osmoregulatory hormones are **peptides**, though in some insects (e.g., *Rhodnius*, locusts and crickets) **serotonin** also appears to be an important diuretic factor.

- Diuretic hormones appear to act primarily on the Malpighian tubules, **stimulating** them to **secrete potassium ions at a greater rate**, thereby creating an enhanced flow of water across the tubule wall.
- In some insects, for example *Calliphora* and *Schistocerca*, diuretic hormone has a dual action, causing **accelerated secretion** through the tubules and a **slowing down of water resorption** through the rectal wall.
- An ancillary effect of diuretic peptides and serotonin is to **stimulate contraction of the muscles** on the outside of the tubules, enhancing their writhing movements. This will reduce the thickness of the unstirred layer of hemolymph adjacent to the tubule, as well as improving fluid flow within the tubule.
- The nature and mode of action of antidiuretic factors are less well understood, and until recently it was thought that they act only at the level of the **rectum**, **enhancing fluid uptake**.

Circulatory System

- The circulatory system of insects, like that of all arthropods, is of the **"open" type**; that is, the fluid that circulates is not restricted to a network of conducting vessels as, for example, in vertebrates, but flows freely among the body organs.
- A consequence of the open system is that insects have only one extracellular fluid, **hemolymph**, in contrast to vertebrates, which have two such fluids, blood and lymph.
- Insects generally possess **pumping structures** and various **diaphragms** to ensure that hemolymph flows throughout the body, reaching the **extremities** of even the most delicate appendages.
- As the only extracellular fluid, it is perhaps not surprising that the hemolymph, in general, serves the **functions of both blood and lymph** of vertebrates.
- Thus, the fluid fraction (**plasma**) is important in providing the correct milieu for body cells, is the transport system for nutrients, hormones, and metabolic wastes, and contains elements of the immune system, while the cellular components (**hemocytes**) provide the defense mechanism against foreign organisms that enter the body and are important in wound repair and the metabolism of specific compounds.

Structure

- The primary pump for moving hemolymph around the body is a **middorsal vessel** that runs more or less the entire length of the body (Figure 1).
- The **posterior** portion of the vessel has **ostia (valves)** and is sometimes known as the heart, whereas the **cephalothoracic** portion, which is often a simple tube, may be termed the **aorta** (Figure 1A).
- In some insects the heart is the only part that contracts, but in many others the entire vessel is contractile.

- The vessel is held in position by connective tissue strands attached to the dorsal integument, tracheae, gut, and other organs and by a series of paired, usually fan-shaped, alary muscles.

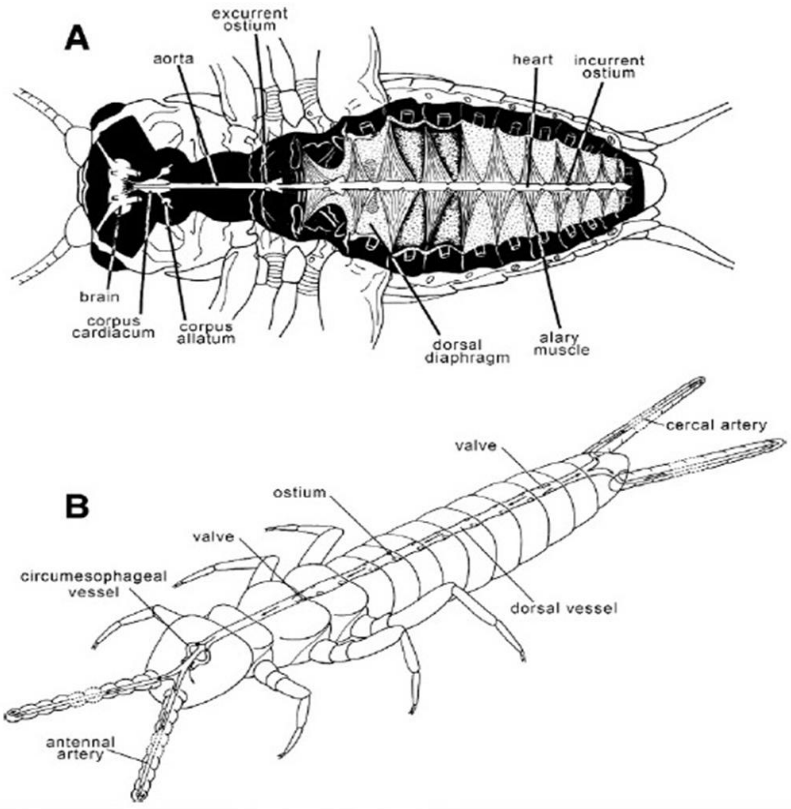


Figure 1: (A) Ventral dissection of the field cricket, *Acheta assimilis*, to show dorsal vessel and associated structures; and (B) circulatory system of *Campodea augens* (Diplura) showing anterior and posterior arteries running off the dorsal vessel.

- **Anteriorly** the aorta runs ventrally to pass between the corpora cardiaca and under the brain.
- Generally the dorsal vessel is closed **posteriorly**; however, in Diplura, Archaeognatha, Zygentoma, and some Ephemeroptera the dorsal vessel connects at its rear with arteries that run along the cerci and median caudal filament.
- In Diplura an artery also supplies each antenna (Figure 1B), and in Dictyoptera and some Orthoptera there are pairs of segmental arteries in the abdomen.

- However, except as noted, in pterygotes circulation to appendages is achieved by means of **accessory pulsatile organs** and **septa**.
- In most insects the dorsal vessel is well tracheated. The heart may not be innervated or may receive paired lateral nerves from the brain and/or segmental ventral ganglia.
- **Ostia** may be simple, slit like valves or deep, funnel-shaped structures in the wall of the heart, or internal flaps (Figure 2).
- Their position and number are equally varied. They may be lateral, dorsal, or ventral and may be as numerous as 12 pairs (in cockroaches) or as few as 1 pair (in some dragonflies).
- Ostia are usually incurrent, that is, they open to allow hemolymph to enter the heart but close to prevent backflow.
- In some orthopteroid insects, however, some ostia are excurrent.

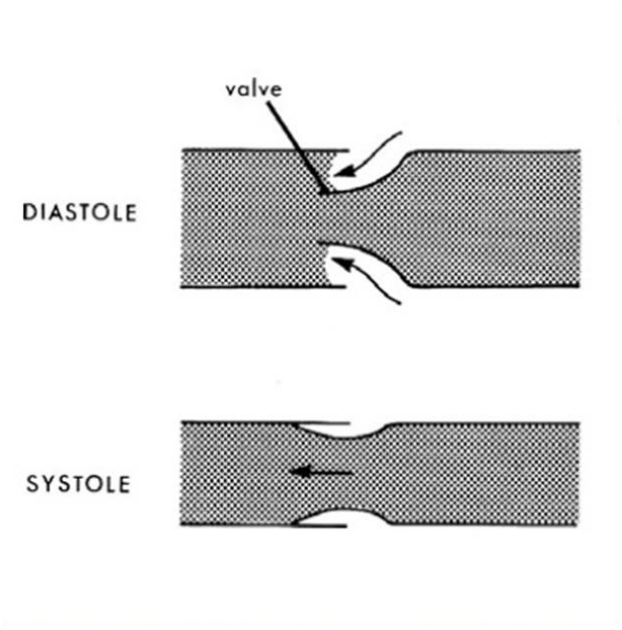


Figure 2: Incurrent ostia of *Bombyx* shown during diastole and systole. Arrows indicate direction of hemolymph flow.

- Histologically, the dorsal vessel in its simplest form comprises a single layer of circular muscle fibers, though more often longitudinal and oblique muscle layers also occur.

- Assisting in directing the flow of hemolymph, especially in post larval stages, are various **diaphragms** (**septa**) (Figure .3) that include both connective tissue and muscular elements.

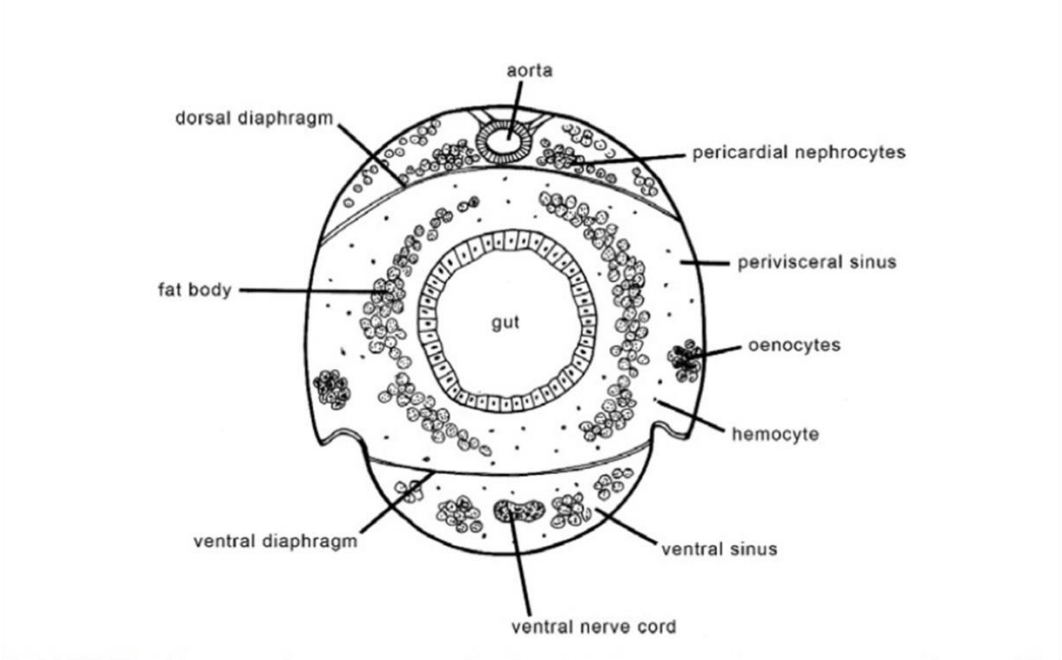


Figure 3: Diagrammatic transverse section through abdomen to show arrangement of septa.

- The spaces delimited by the diaphragms are known as **sinuses**.
- The **pericardial septum** (**dorsal diaphragm**) lies immediately beneath the dorsal vessel and spreads between the alary muscles.
- Laterally, it is attached at intervals to the terga and in most species has openings so that the **pericardial sinus** is in effect continuous with the **perivisceral sinus**.
- Ventrally, a **perineural septum** (**ventral diaphragm**) may occur, which cuts off the **perineural sinus** from the **perivisceral sinus**.
- Generally, the ventral diaphragm is restricted to the abdomen and occurs only in species whose ventral nerve cord extends into this region of the body. It is capable of performing posteriorly directed undulations and may have openings.

- Hemolymph circulation through the legs and palps of some insects is assisted by the presence of a **longitudinal septum** that partitions the appendage into **afferent** and **efferent** sinuses.
- To further facilitate hemolymph flow, especially through appendages, **accessory pulsatile organs** (**auxiliary hearts**) commonly occur.
- These have been identified in the head, antennae, thorax, legs, wings, and ovipositor.
- In many species they are saclike structures that have a posterior incurrent ostium and an anteriorly extended vessel.
- In antennal pulsatile organs the vessel may run the length of the appendage but is perforated at intervals to permit exit of hemolymph.
- The wall of the sac may be muscular, so that constriction of the sac is the active phase, and dilation results from elasticity of the wall, or the sac may have attached to it a discrete dilator muscle, and constriction is due to the sac's elasticity.
- In some situations, for example, the legs of Orthoptera and Hemiptera, the accessory pulsatile organ is simply one or two small muscles that attach to the longitudinal septum.
- Most accessory pulsatile organs are not innervated.
- **Hemopoietic organs** have been described for a number of insects. For example, in *Gryllus* there are pairs of such organs, in the second and third abdominal segments, directly connected with the dorsal vessel.
- Like those of vertebrates, the hemopoietic organs serve both as the site of production of at least some types of hemocytes and as centers for phagocytosis.
- At specific locations in the circulatory system are **sessile cells**, usually conspicuously pigmented, called **athrocytes**.
- They occur singly, in small groups, or form distinct lobes, and are always surrounded by a basal lamina, a feature that distinguishes them from hemocytes.

- In most species athrocytes are situated on the surface of the heart (occasionally also along the aorta), and these are referred to as pericardial cells.
- The cells are able to accumulate colloidal particles, for example, certain dyes, hemoglobin, and chlorophyll which led to an early suggestion that they segregated and stored waste products (hence their alternate name of **nephrocytes**).
- The usual view is that the cells accumulate and degrade large molecules such as proteins, peptides, and pigments, and the products are then used or excreted.

Physiology

Circulation

- Contractions of the dorsal vessel and accessory pulsatile organs, along with movements of other internal organs and abdominal ventilatory movements (coelopulses), serve to move hemolymph around the body.
- Generally hemolymph is pumped rapidly through the dorsal vessel but moves slowly and discontinuously through sinuses and appendages.
- The direction of hemolymph flow in most insects is indicated in Figure 4A–C.

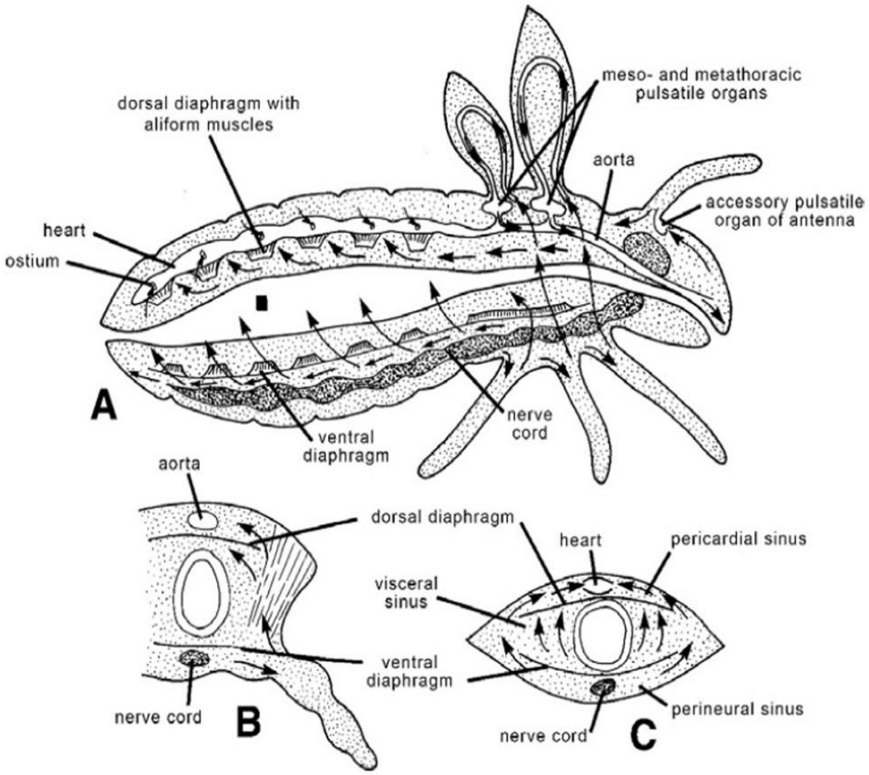


Figure 4: Diagrams showing direction of hemolymph flow. (A) Longitudinal section; (B) transverse section through thorax; and (C) transverse section through abdomen. Arrows indicate direction of flow.

- Hemolymph is pumped anteriorly through the dorsal vessel from which it exits via either excurrent ostia of the heart or mainly the anterior opening of the aorta in the head.
- The resultant pressure in the head region forces hemolymph posteriorly through the perivisceral and perineural sinuses.
- Undulations of the ventral diaphragm aid the backward flow of hemolymph.
- **Relaxation** of the heart muscle results in an increase in heart volume, and, by negative pressure, hemolymph is sucked in via incurrent ostia.
- **Circulation through appendages** is aided by accessory pulsatile organs. In most insects hemolymph enters the wings via the anterior veins and returns to the thorax via the anal veins. Though the structure of wing pulsatile organs is varied, they always operate by sucking hemolymph out of the posterior wing veins.

- In apterygotes and mayflies hemolymph flow is bidirectional (Figure 1B). Anterior to a valve located in the heart at about the level of the eighth abdominal segment, hemolymph flows forward toward the head, while behind the valve the hemolymph is pushed backward along arteries that terminate at the tips of the cerci and median filament.
- Reversal of heartbeat may also occur and is characteristically seen in pupae and adults of Lepidoptera and Diptera.
- In some actively flying insects, for example, locusts, butterflies, saturniid moths, and possibly some Hymenoptera, as well as in diapausing lepidopteran pupae, hemolymph movements are closely coordinated with the ventilation movements for gas exchange

Heartbeat

- Contraction of the heart (**systole**) is followed, as in other animals, by a phase of relaxation (**diastole**) during which muscle cell membranes become repolarized.
- A third phase, **diastasis**, may follow diastole, when the diameter of the dorsal vessel suddenly enlarges because of the influx of hemolymph.
- In most pterygotes, where hemolymph flow is unidirectional, contraction of the dorsal vessel begins at the posterior end and passes forward as a peristaltic wave.
- Whether or not an insect heart is innervated, its beat is **myogenic**, that is, the beat originates in the heart muscle itself.
- The **rate** at which the heart beats varies widely both among species and even within an individual under different conditions. In the pupa of *Anagasta kuhniella*, for example, the heart beats 6–11 times per minute. In larval *Blattella germanica* rates of 180–310 beats/min have been recorded
- Many factors affect the rate of heartbeat.
- Generally, there is a decline in heartbeat rate in successive juvenile stages, and in the pupal stage the heart beats slowly or even ceases to beat for long periods.

- In adults the heart beats at about the rate observed in the final larval stage.
- Heartbeat rate **increases** with activity, during feeding, with increase in temperature or in the presence of carbon dioxide in low concentration, but is **depressed** in starved or asphyxiated insects.
- **Hormones**, too, may affect heartbeat rate. Authors have reported a wide range of cardioaccelerating and cardioinhibiting factors, including juvenile hormone, neurosecretory peptides, octopamine, and 5-hydroxytryptamine.

Hemolymph

- Hemolymph, like the blood of vertebrates, includes a cellular fraction, the **hemocytes**, and a liquid component, the **plasma**, whose functions are broadly comparable with those found in vertebrates.

Plasma

- **Composition**: plasma contains a large variety of components both organic (proteins, carbohydrates, lipids, amino and carboxylic acids) and inorganic (sodium, potassium, calcium, magnesium, chloride, phosphate and bicarbonate) whose relative proportions may differ greatly both among species and within an individual under different physiological conditions.
- **Function**:
- 1- Plasma serves as the medium in which nutrients, hormones, and waste materials can be transported to sites of use, action, and disposal, respectively.
- 2- It is an important site for the storage, usually temporary, of metabolites.
- 3- Plasma is the source of cell water, and during periods of desiccation its volume may decline at the expense of water entering the tissues.
- 4- By virtue of some components (proteins, amino acids, carboxylic acids, bicarbonate, and phosphate) it is a strong buffer and resists changes in pH that might occur as a result of metabolism.

- 5- As a liquid, plasma is also used to transmit pressure changes from one part of the body to another.
- 6- The plasma also has an important thermoregulatory function in many actively flying insects

Hemocytes

- **Forms of hemocytes**: several types of hemocytes have been recognized, which differ in size, stain ability, function, and cytology (including fine structure) (Figure 5). The three types common to most insects are prohemocytes, plasmatocytes, and granular hemocytes (granulocytes).

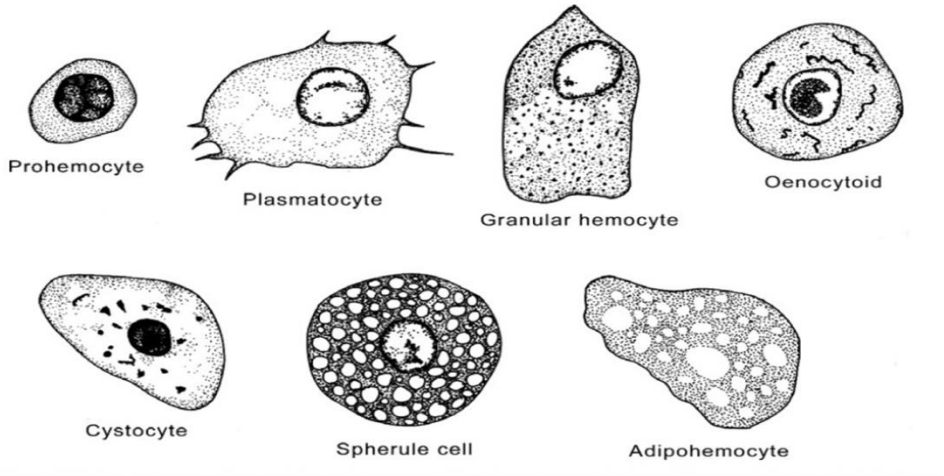


Figure 5: Different types of hemocytes.

- **Prohemocytes** (stem cells) are small (10 µm or less in diameter), spherical, or ellipsoidal cells whose nucleus fills almost the entire cytoplasm. They are frequently seen undergoing mitosis and are assumed to be the primary source of new hemocytes and the type from which other forms differentiate.
- **Plasmatocytes** (phagocytes) are cells of variable shape and size, with a centrally placed, spherical nucleus surrounded by well vacuolated cytoplasm. The cells are capable of amoeboid movement and are phagocytic.

- **Granulocytes** are usually round or disc-shaped, with a relatively small nucleus surrounded by cytoplasm filled with prominent granules. In some species they are amoeboid and phagocytic. More often, they are non-motile and appear to be involved in intermediary metabolism.
- **Adipohemocytes** are cells whose cytoplasm normally contains droplets of lipid. The cells, which occasionally are phagocytic, are considered by some authors to be a form of granulocyte.
- **Oenocytoids** are spherical or ovoid cells with one, occasionally two, relatively small, eccentric nuclei. They are almost never phagocytic. The oenocytoids are fragile cells that easily lyse.
- **Spherule cells** are readily identifiable cells whose central nucleus is often obscured by the mass of dense spherical inclusions occupying most of the cytoplasm. A variety of functions have been proposed for them, including phagocytosis, uptake and transport of materials, synthesis of some blood proteins, and a role in bacterial immunity.
- **Cystocytes** (**coagulocytes**) are spherical cells in whose small central nucleus the chromatin is so arranged as to give the nucleus a "cartwheel-like" appearance. The cytoplasm contains granules that, when liberated from these fragile cells, cause the surrounding plasma to precipitate. Thus, the cells, which are again a specialized kind of granulocyte, play a major role in hemolymph coagulation.

- **Functions**:
  - The major functions of hemocytes are **endocytosis**, **nodule formation**, **encapsulation**, and **coagulation**.
  - For the first three of these a key element is the ability to distinguish between foreign (including altered self) and self.
  - Hemocytes probably also have a variety of metabolic and homeostatic functions.

Nervous System

- Animals constantly monitor both their **internal** and their **external** environment and make the necessary **adjustments** in order to maintain themselves optimally and thus to develop and reproduce at the maximum rate.
- The **adjustments** they make may be immediate and obvious, for example, flight from predators, or longer-term, for example, entry into diapause to avoid impending adverse conditions.
- The nature of the **response** depends, obviously, on the nature of the **stimulus**.
- Almost always a **stimulus** is received by an appropriate **sensory structure** and taken to the **central nervous system**, which "determines" an appropriate response under the circumstances.
- When a response is **immediate**, that is, achieved in a matter of seconds or less, it is the nervous system that transfers the message to the effector system. Such responses are usually temporary in nature.
- **Delayed** responses are achieved through the use of chemical messages (viz., hormones) and are generally longer-lasting.
- The **nervous** and **endocrine systems** of an individual are, then, the systems that coordinate the response with the stimulus.

Neurons

- Like that of other animals, the nervous system of insects consists of nerve cells (**neurons**) and **glial cells**.
- Each neuron comprises a cell body (**perikaryon**) where a nucleus, many mitochondria, and other organelles are located, and a cytoplasmic extension, the **axon**, which is usually much branched, the branches being known as **neurites**.

- Axons may be long, as in sensory neurons, motor neurons, and principal interneurons, or very short, as in local interneurons.
- Often, insect neurons are **monopolar**, lacking the dendritic tree characteristic of vertebrate nerve cells, though **bipolar** and **multipolar** neurons do occur (Figure 1).
- **Motor** (efferent) neurons, which carry impulses from the central nervous system, are monopolar, and their perikarya are located within a ganglion.
- **Sensory** (afferent) neurons are usually bipolar but may be multipolar.

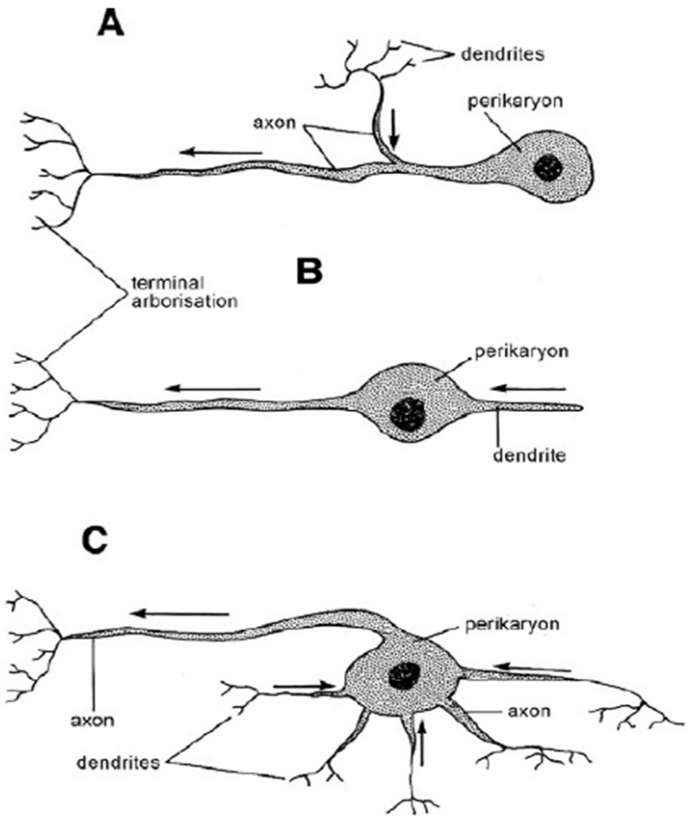


Figure 1: Neurons found in the insect nervous system. Arrows indicate direction of impulse conduction. (A) Monopolar; (B) bipolar; and (C) multipolar.

- **Interneurons** (also called **internuncial** or **association neurons**) transmit information from sensory to motor neurons or other interneurons; they may be mono- or bipolar and their cell bodies occur in a ganglion.
- Neurons are not directly connected to each other or to the effector organ but are separated by a minute space, the **synapse** or **neuromuscular junction**, respectively.

- Impulses may be transferred across the synapse either electrically or chemically.
- Neurons are aggregated into **nerves** and **ganglia**.
- Nerves include only the axonal component of neurons, whereas ganglia include axons, perikarya, and dendrites.

Central Nervous System

- Central nervous system in an adult insect comprises the **brain**, **subesophageal ganglion**, and a varied number of **ventral ganglia**.
- The brain (Figure 2) is probably derived from the ganglia of three segments and forms the major association center of the nervous system. It includes the **protocerebrum**, **deutocerebrum**, and **tritocerebrum**.

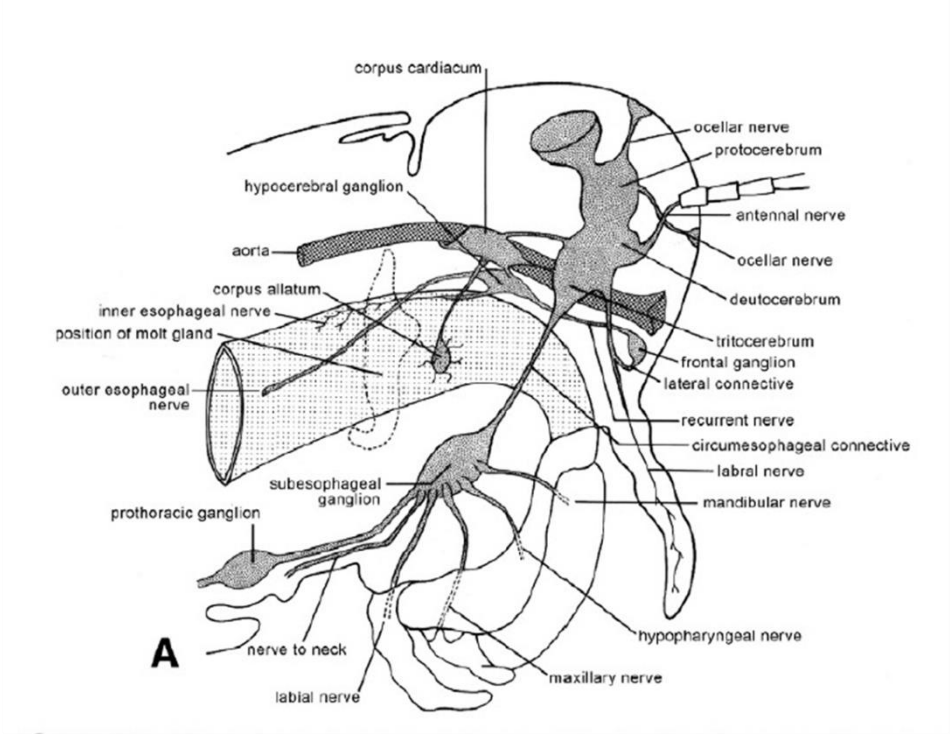


Figure 2: Lateral view of anterior central nervous system, stomatogastric nervous system, and endocrine glands of a typical acridid.

- The **protocerebrum**, the largest and most complex region of the brain, contains both neural and endocrine (neurosecretory) elements. Anteriorly it forms the proximal part of the **ocellar nerves** (the only occasion on which

the cell bodies of sensory neurons are located other than adjacent to the sense organ), and laterally is fused with the **optic lobes**.

- The **deutocerebrum** is largely composed of the paired antennal lobes.
- The **tritocerebrum** is a small region of the brain located beneath the deutocerebrum and comprises a pair of neuropiles that contain axons, both sensory and motor, leading to/from the **frontal ganglion** and labrum.
- The **subesophageal ganglion** is also composite.
- From this ganglion, nerves containing both sensory and motor axons run to the **mouthparts**, **salivary glands**, and **neck**. The ganglion also appears to be the center for maintaining (though not initiating) locomotor activity.
- In most insects the three segmental **thoracic ganglia** remain separate. Though details vary from species to species, each ganglion innervates the **leg** and **flight muscles** (direct and indirect), **spiracles**, and **sense organs** of the segment in which it is located.
- The maximum number of **abdominal ganglia** is eight.
- Varying degrees of **fusion** of the abdominal ganglia occur in different orders and sometimes there is fusion of the composite abdominal ganglion with the ganglia of the thorax to form a single **thoracoabdominal ganglion**.

Visceral (Sympathetic) Nervous System

- The visceral (sympathetic) nervous system includes three parts: the **stomatogastric system**, the **unpaired ventral nerves**, and the **caudal sympathetic system**.
- The stomatogastric system includes the **frontal ganglion**, **recurrent nerve** which lies mediodorsally above the gut, **hypocerebral ganglion**, a pair of **inner esophageal nerves**, a pair of **outer esophageal (gastric) nerves**, each of which normally terminates in an **ingluvial (ventricular) ganglion** situated alongside the posterior foregut, and various fine nerves from these ganglia that innervate the foregut and midgut, and, in some species, the heart.

- A **single** median ventral nerve arises from each thoracic and abdominal ganglion in some insects. The nerve **branches** and innervates the **spiracle** on each side. In species where this nerve is absent, **paired** lateral nerves from the segmental ganglia innervate the spiracles.
- The caudal sympathetic system, comprising nerves arising from the **composite terminal abdominal ganglion**, innervates the **hindgut** and **sexual organs**.

Physiology of Neural Integration

- As noted in the Introduction, an insect's nervous system is constantly receiving stimuli of different kinds both from the **external environment** and from **within its own body**.
- The subsequent **response** of the insect depends on the **net assessment** of these stimuli within the **central nervous system**.
- The processes of receiving, assessing, and responding to stimuli collectively constitute **neural integration**.
- Following the arrival of a stimulus of sufficient magnitude, an action potential is generated and the impulse travels along the axon as a wave of **depolarization** (Figure 3).

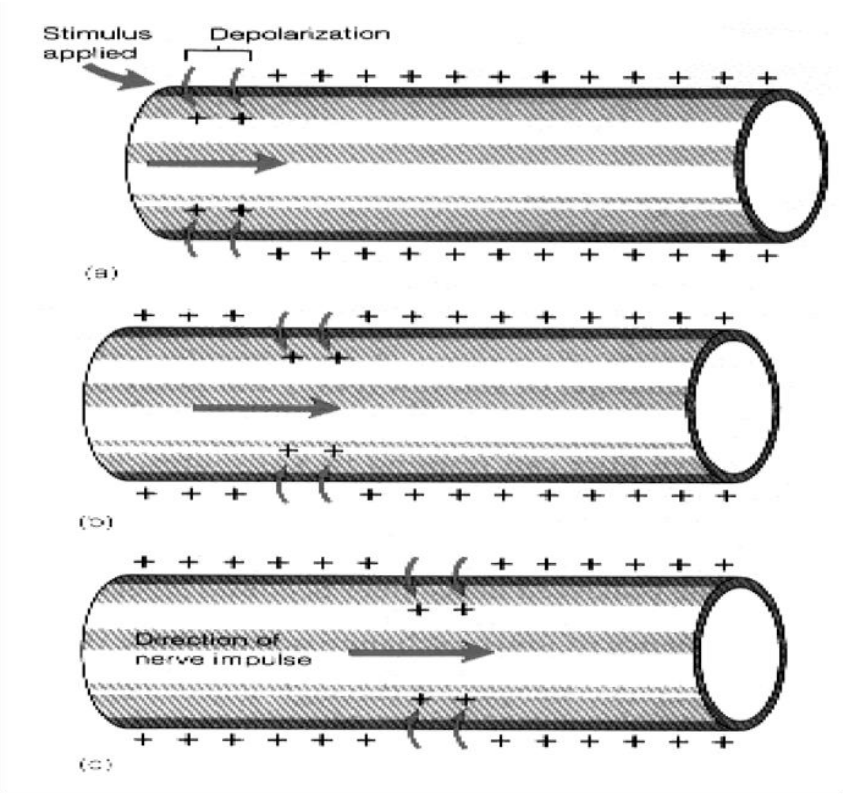


Figure 3: Depolarization wave through an axon

- Mostly, however, when an impulse reaches a **synapse**, it causes release of a chemical (a **neurotransmitter**) from membrane-bound vesicles (Figure 4). The chemical diffuses across the synapse and, in excitatory neurons, brings about depolarization of the postsynaptic membrane.
- **Acetylcholine** is the predominant neurotransmitter liberated at excitatory synapes, including those of interneurons and afferent neurons from mechanosensilla and taste sensilla

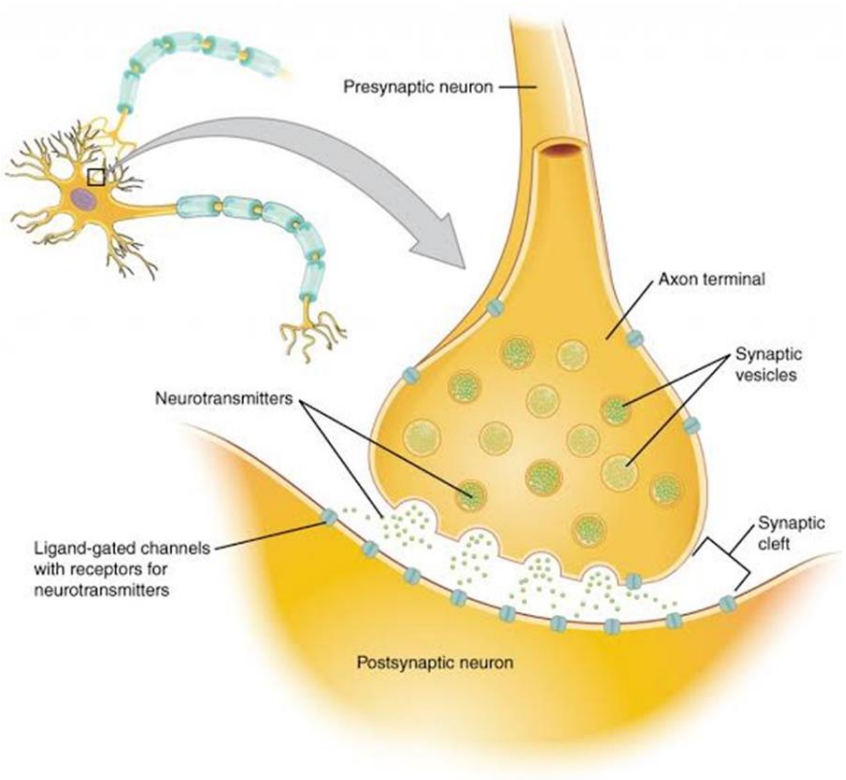


Figure 4: Synapse

- 5-Hydroxytryptamine (**serotonin**), **histamine**, **octopamine**, and **dopamine** function as central nervous system excitatory neurotransmitters in specific situations on occasion. These, and other amines, have an excitatory effect when applied in low concentrations to the heart, gut, reproductive tract, etc., and it may be that they also serve as neurotransmitters in the visceral nervous system.
- Eventually, an impulse reaches the effector organ, most commonly muscle.
- Between the tip of the motor axon and the muscle cell membrane is a fluid-filled space, comparable to a synapse, called a **neuromuscular junction**.
- Again, to achieve depolarization of the muscle cell membrane and, ultimately, muscle contraction, a chemical released from the tip of the axon diffuses across the neuromuscular junction.
- In insect skeletal muscle, this chemical is **L-glutamate**; in visceral muscles, **glutamate**, **serotonin**, and the **pentapeptide proctolin** have all been suggested as candidate neurotransmitters.

- In addition to stimulatory (excitatory) neurons, **inhibitory neurons** whose neurotransmitter causes hyperpolarization of the postsynaptic or effector cell membrane are also important in neural integration.
- When inhibition occurs at a synapse within the central nervous system, it is known as **central inhibition**. When inhibition of an effector organ occurs it is known as **peripheral inhibition**.
- At both synapses and neuromuscular junctions, the hyperpolarizing chemical is γ-aminobutyric acid.

Semiochemicals

- Insects interact with other members of their species, as well as with other organisms, by means of **semiochemicals**.
- When the interaction is intraspecific, the chemical messages are called **pheromones**.
- Chemicals involved in interspecific interaction fall into two major categories: **allomones** provoke a response that favors the emitter, whereas **kairomones** induce a response favorable to the receiver.
- A given semiochemical may fit into more than one of these groups; for example, pheromones emitted by prey insects may also function as kairomones by attracting predators and parasitoids.

1- Pheromones

- Pheromones are chemicals messages produced by one individual that **induce** a particular behavioral, physiological, or developmental response in other individuals of the **same** species.
- Like hormones, they are produced in small quantities; indeed, they are referred to in older literature as "**ectohormones**".
- Pheromones may be volatile and therefore capable of being detected as an odor over considerable distances, or they may be non-volatile, requiring actual physical contact among individuals for their dissemination.
- They may be **highly specific**, even to the extent that only a particular isomer of a substance induces the typical effect in a given species. As a result, closely related species often utilize different isomeric forms of a given chemical.
- Pheromones are released only under **appropriate conditions**, that is, in response to appropriate environmental stimuli.

- Thus, whereas the **neural and endocrine systems** coordinate the behavior, physiology, and/or development of **an individual**, **pheromones** regulate these processes **within populations**.
- Pheromones include the following categories:

✓ (1) **Sex pheromones**, which modify reproductive behavior or development.

✓ (2) **Caste-regulating pheromones**, which determine the proportion of different castes in colonies of social insects.

✓ (3) **Aggregation pheromones**, which attract insects to a common feeding and/or mating site.

✓ (4) **Alarm pheromones**, which warn of impending danger;

✓ (5) **Trail-marking pheromones**, which provide information on the location and quantity of food available to a colony of social insects.

✓ (6) **Spacing pheromones** that maintain optimum population density.

Sex Pheromones

- In the term "**sex pheromones**" are included chemicals that (1) excite and/or attract members of the opposite sex (sex attractants), (2) act as aphrodisiacs, (3) accelerate or retard sexual maturation (in either the opposite and/or same sex), (4) enhance fecundity and/or reduce receptivity in the female.
- Sex attractants are typically volatile chemicals produced by either **male** or **female** members of a species, whose release and detection by the partner are essential prerequisites to successful courtship and mating.
- Male-attracting substances are produced by **virgin females** of species representing many insect orders, but especially Lepidoptera and Coleoptera.
- Females release their pheromones only in response to a specific stimulus. Release may be influenced by age, reproductive status, time of day, presence of host plant, temperature, and wind speed.

- For example, many species of moths begin “calling” 1.5–2.0 hours before **dawn**.
- Other Lepidoptera are stimulated to release pheromone by the **scent** of the larval food plant.
- *Rhodnius prolixus* females release pheromone only after a **blood meal**.
- Pheromone production in *Periplaneta americana* is arrested when the **ootheca** is formed.
- It seems that in species such as R. prolixus, cockroaches, locusts, and beetles, which have repeated cycles of oocyte development over a period of time, pheromone production is under the control of the endocrine system, especially the **corpora allata**.
- In *Musca domestica* and perhaps other Diptera, pheromone synthesis is regulated by **ovarian ecdysteroids**.
- In many moths **PBAN** (Pheromone Biosynthesis Activating Neuropeptide) mediates production of sex attractant.
- Typically, the **pheromone-producing glands** of female Lepidoptera are located in the **intersegmental** membrane **behind the eighth abdominal segments**, in the homopteran *Schizaphis borealis* the glands are probably on the **hind tibiae**, in *Periplaneta* the pheromone seems to be produced in the **gut** and is released from **fecal pellets**, in the queen honey bee the **mandibular glands** are the source, in the house fly sex pheromone is secreted evenly over the **second through seventh abdominal segments**, in Coleoptera the glands are **abdominal**.
- The **components** of many male attractants have been identified, especially those of pestiferous Lepidoptera in which they appear generally to be **aliphatic straight-chain hydrocarbons**, **alcohols**, **acetates**, **aldehydes**, and **ketones** containing 10–21 carbon atoms.
- Usually the sex pheromone is a **blend** of two or more components, occurring in species-specific proportions. Interestingly, the proportions may vary among populations of the same species in different geographic locations.

- Further, only one isomeric form of a component is typically attractive in a given species. As a result, under natural conditions males respond only to the pheromone produced by females of their species.
- In many species, it is **males** that produce a sex pheromone.
- The **pheromone-producing glands** of males are much more varied in their location than those of females.
- For example, pheromone is secreted from **mandibular glands** in ants, from **glands in the thorax and abdomen** of some beetles, from **glands at the base of the fore wings** in some Lepidoptera, from **abdominal glands** in other Lepidoptera and cockroaches, and from **rectal glands** in certain Diptera.
- As in females, the attractants produced by males are usually **long-chain alcohols** or their **aldehydic derivatives**.

Caste-Regulating Pheromones

- in social insects very few members of a colony ever mature sexually, their reproductive development being **inhibited** by **pheromones** so that these individuals (forming the **worker caste**) can perform other activities for the benefit of the colony as a whole, in addition to the worker caste, there exists in termites and ants a **soldier caste**.
- The regulation of caste differentiation in social insects is a **morphogenetic phenomenon**, just as are the changes from larva to larva, larva to pupa, and pupa to adult. Such changes depend on the activity of the **corpora allata** (level of **juvenile hormone** in the hemolymph) for their manifestation.
- It is not surprising, therefore, that the pheromones regulating caste differentiation (including the development of reproductives) exert their effect, ultimately, via the corpora allata.

Aggregation Pheromones

- Aggregation pheromones are produced by either one or both sexes and serve to attract other individuals for **feeding**, **mating**, and/or **protection**.
- In Coleoptera the pheromones serve primarily to aggregate the beetles to a food source that may be isolated (e.g., stored grain), juvenile locusts produce an aggregation pheromone whose function is to keep the marching swarm intact. Aggregation pheromones are also common in a wide range of blood-feeding insects, serving to bring conspecifics together for mating, oviposition, and larviposition.
- Compared to sex pheromones, relatively little work has been done to establish the chemical nature of aggregation pheromones. Those studied mostly appear to be mixtures of compounds, often including **terpenoid compounds** and **cyclic alcohols** or **aldehydes**, that act synergistically.
- Like sex pheromones, aggregation pheromones are typically **highly specific**, particular isomers being attractive to a given species.

Alarm Pheromones

- As their name indicates, alarm pheromones **warn** members of a species of **impending danger**.
- They are produced by mites and insects that live in groups, including social forms, for example, cockroaches, treehoppers, aphids, bedbugs, termites, and social Hymenoptera.
- The pheromones may originate **internally**, being released as in treehoppers when the body wall is broken open, or in exocrine glands.
- Corpses (but not, living specimens) of *P. americana* release material that repels conspecifics and members of some other species. Thus, the repellent may be a cue that cockroaches use to avoid areas where others have died.
- Some Hymenoptera have more than one pheromone-producing gland; for example, in *Formica* species of ants there are **mandibular glands**, **Dufour's glands**, and **poison glands**.
- The chemical nature of alarm pheromones is highly varied but tends to be specific for each group.

- Mites, aphids, and termite soldiers produce **terpenoid compounds**, while honey bees produce a mixture of **acetates**, an **alcohol**, and a **ketone**.
- Typically, the alarm pheromones of non-social insects and mites stimulate dispersal (**escape behavior**).
- Such behavior is also seen in social species away from the nest. However, when the pheromone is released near the nest, the insects are **attracted toward the source** and may subsequently **attack** the intruder. In honey bees, for example, the pheromone is released from the sting shaft (embedded in the intruder!) causing more bees to attack.

Trail–Marking Pheromones

- Trail-marking pheromones are used by some insects to find mates, to 1) communicate information on the location and quantity of food (and thus to recruit nest mates for food collection), and to 2) ensure that a migrating group retains its integrity.
- The trails may be **terrestrial**, laid out on a solid substrate, or **aerial**, being released by a stationary insect and dependent on movement of the surrounding medium to generate a trail (in this sense, then, the sex and aggregation pheromones discussed earlier could equally be considered as trail marking pheromones). Terrestrial trail-marking pheromones, which are laid as a solid line or as a series of spots, are produced from a variety of glands.
- In ants trail-marking pheromones may be produced in the **hindgut**, **Dufour's gland**, **poison gland**, **ventral glands**, or on the **metathoracic legs**, and are released as the abdomen or limbs make contact with the substrate.
- Most trails are relatively **short-lived** and fade within a matter of minutes unless continuously reinforced. Some ants, however, make trails that last for several days. Furthermore, when a source of food is good, more returning workers secrete pheromone, thereby establishing a strong trail to which more workers will be attracted.

- Knowledge of the chemistry of trail-marking pheromones is almost entirely confine to those of social insects. In termites and many ants they appear to be mixtures of **long-chain fatty acids**, **alcohols**, **aldehydes**, **esters**, or **hydrocarbons**.

Spacing (Epideictic) Pheromones

- A relatively new discovery in pheromone research are those pheromones that stimulate insects to spread out so as to maintain an **optimal population density**.
- Spacing pheromones have been best studied in relation to oviposition.
- For example, after laying, the female apple maggot fly, *Rhagoletis pomonella*, releases a pheromone that deters oviposition on the fruit by other females. The pheromone appears to be released from the hindgut as the ovipositor is dragged over the fruit. It appears to be a water soluble peptide that remains biologically active for several days after deposition. Several Lepidoptera also produce oviposition-deterring pheromones that limit the number of eggs laid on a given plant, further, the presence of feeding larvae of *Pieris brassicae* inhibits egg laying, suggesting that the larvae also produce a pheromone.

2- Kairomones

- **Kairomones** are of special significance to many predators and parasitoids, which use **pheromones** released by their prey as cues enabling them to locate the host.
- **Examples**:
- The oviposition-deterring pheromone placed on fruit by the apple maggot fly allows the braconid parasitoid *Opius lectus* to locate its host's eggs.
- The spacing pheromone released by larvae of *Anagasta kuehniella* enables their predator, the ichneumonid *Nemeritis canescens*, to determine their position.

- Predators or egg parasitoids often “eavesdrop” on sex attractants released by female Lepidoptera: the predators locate and feed on the adult moths, while the parasitoids “hang around” until the moth lays its eggs soon after mating.
- The pheromones of social insects are exploited in various ways by predaceous and parasitic insects, as well as by other social species, for example, slave-making ants.

3- Allomones

- Insects release a wide array of volatile chemicals that affect the behavior of other animals, both vertebrate and invertebrate. The great majority of these secretions are used as defensive **allomones**.
- The chemical nature of allomones is extremely varied. However, it has long been recognized that some **allomones** are chemically very **similar**, even identical, to **alarm pheromones** and **sex attractants**, leading to the proposal that the original role for these compounds was defensive, with the pheromonal function arising secondarily.
- The allomones are typically produced in specific **exocrine glands**, though in a few species the allomone is **sequestered** within the **hemolymph**, to be released as a result of “**reflex bleeding**” that is, when hemolymph is exuded at joints and intersegmental membranes.
- The biosynthetic pathways are, for most allomones, not well understood, but it is evident that most insects produce these compounds **endogenously**. However, given the wide array of **plant** natural products, including many **feeding deterrents**, it is not surprising to discover that some specialist herbivores have evolved the ability to **sequester** these normally highly **toxic compounds** for their own use against would-be predators. As example, larvae of the sawfly *Neodiprion sertifer* sequester terpenoid compounds from their host, Scots pine (*Pinus silvestris*), and use them as defensive allomones.
- **Allomones** are occasionally used aggressively to **attract** prey or, in social Hymenoptera, to rob nests of other species.

Sensory Systems

- Organisms constantly monitor and respond to changes in their environment (both **external** and **internal**) so as to maintain themselves under the most favorable conditions for growth and reproduction.
- The structures that receive these environmental cues are **sense cells**, and the **cues** are always forms of **energy**, for example, light, heat, kinetic (as in mechanoreception and sound reception), and potential (as in chemoreception, the sense of smell and taste).
- The **sensory structures** use the energy to do work, namely, to generate a message that can be conducted to a decoding area, the **central nervous system**, so that an appropriate **response** can be initiated. The message is, of course, in the form of a **nerve impulse**.
- Almost all insect **sense cells** are **primary sense cells**, that is, they not only receive the stimulus but initiate and transmit information to the central nervous system; in other words, they are **true neurons**. In contrast, in vertebrates, almost all sensory systems include both a specialized secondary sense cell and a sensory neuron that transmits information to the central nervous system.
- **Two** broad morphological types of sense cells are recognizable.
- ✓ 1) Sense cells associated with cuticle (and therefore including invaginations of the body wall) (**Type I neurons**).
- ✓ 2) Sense cells never associated with cuticle and lie on the inner side of the integument, on the wall of the gut, or alongside muscles or connective tissue where they function as proprioceptors (**Type II neurons**)

Mechanoreception

- Insects **receive** and **respond** to a wide variety of **mechanical stimuli**. They are sensitive to physical contact with solid surfaces (**touching** and being **touched**); moreover, they detect air movements, including sound waves.

- Information on the above is gathered by a spectrum of **mechanosensilla** associated with which, in most cases, are accessory structures that transform the energy of the stimulus into usable form, namely, a mechanical deformation of the sense cell's plasma membrane.

Sensory Hairs

- The **simplest** form of mechanosensillum is seen in **sensory hairs** (sensilla trichodea) (Figure 1), which occur on all parts of the body but are in greatest concentration on those that frequently come into contact with the substrate, the **tarsal segments** of the **legs**, **antennae**, and **mouthparts**.

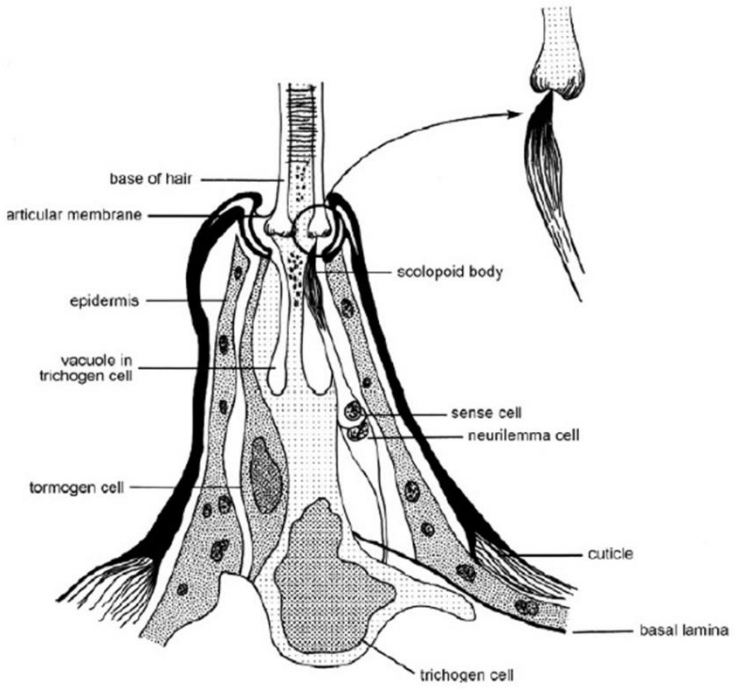


Figure 1: Section through the base of tactile hair.

- In its simplest form a sensillum comprises a rigid, poreless hair set in a membranous socket and **four** associated cells; these are 1) the inner sheath cell (also known as the **trichogen** or generative hair cell), 2) outer sheath cell (**tormogen** or membrane-producing cell), 3) **neurilemma** cell, which ensheathes the cell body and axon of the sensory neuron, and 4) the **sensory neuron** whose dendrite often is cuticularized and includes a terminal cuticular filament (scolopale).

- Within the above-generalized structure, hairs may differ widely in their detailed morphology, physiology, and function. Nevertheless, they are all designed such that the **slightest deformation** (a few nanometers) of the membrane of the sensory neuron will generate an **action potential**.
- Note also that some hairs include several neurons, but only one of these is **mechanosensory**, the remainder are **chemosensory** or **thermo-sensory**.

Proprioceptors

- **Proprioceptors** are sense organs able to respond continuously to deformations (changes in **length**) and stresses (**tensions** and **compressions**) in the body.
- **Chordotonal sensilla** are a widely distributed form of propriocepteros in insects.
- Chordotonal sensilla are associated with the body wall, internal skeletal structures, tracheae, and structures in which pressure changes occur. Though they are found singly, more commonly they occur in groups.
- Chordotonal organs exist as strands of tissue that stretch between two points.
- The **proximal end** of the sensory neuron is attached to **one point** by means of a **ligament** and the **distal end** is covered by a **cap cell**, which is attached to the **second point** (Figure 2).
- Chordotonal sensilla are **highly sensitive**. Thus, a change in the **relative position** of the **points** that causes the strand's length to be altered by as little as 1 nm will produce **bending** or **stretching** of the **dendritic membrane**, hence **stimulation** of the sense cell.

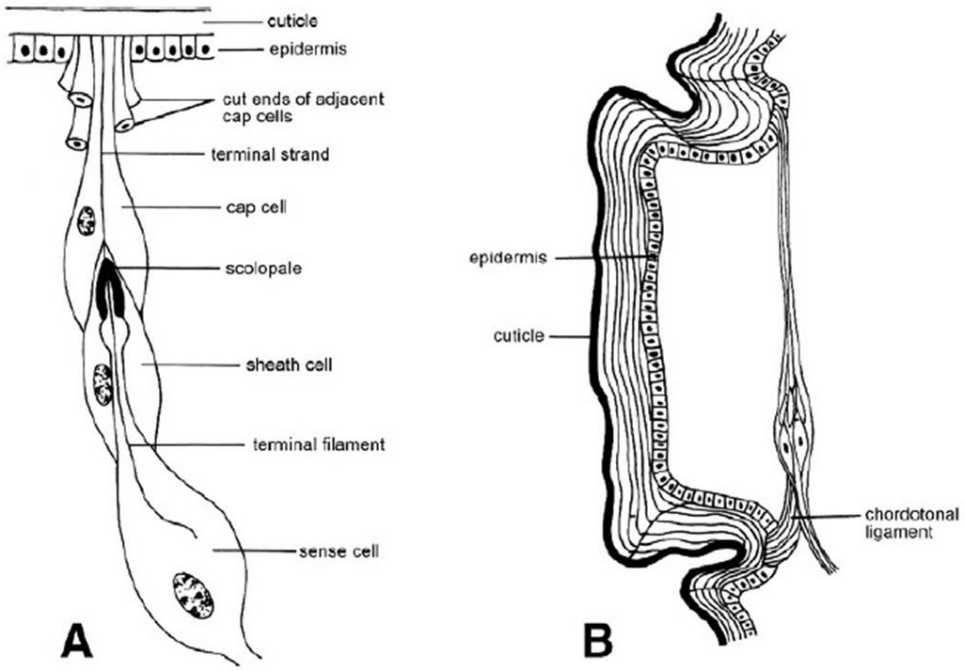


Figure 2: (A) Single chordotonal sensillum; and (B) chordotonal organ.

Signal Detection

- Detection of stimuli by mechanoreceptors is a three-step process: **coupling**, **transduction**, and **encoding**.
- **Coupling** refers to the deformation of the sensory neuron's dendritic membrane caused by movement of the hair in its socket.
- **Transduction** is the generation, followed by its flow through the dendritic membrane, of the receptor (generator) current. It results from the stretching of the membrane and the opening of transduction (stretch-activated) channels contained therein.
- **Encoding**, the final step, is the transfer of information from the sensillum to the central nervous system.
- **Hairs** differ in their **sensitivity**; **long**, **delicate** hairs respond to the slightest force, even air-pressure changes, whereas **shorter**, **thicker spines** (sometimes called sensilla chaetica) require considerable force for stimulation.

Sound Reception

- Sounds are **waves** of pressure detected by organs of hearing.
- A sound wave is produced when particles are made to **vibrate**, the vibration causing **displacement** of adjacent particles.
- The distinction between sound reception and mechanoreception is not clear-cut.
- Insect-hearing structures can be divided into two categories: **near field**
- **detectors** and **far-field detectors**.
- As their names indicate, the detectors are able to perceive sounds that originate a short distance (from a few millimeters up to about 1 m) or a long distance (tens of meters), respectively. However, there are several other features unique to each type of detector.
- **Near-field detectors** are **displacement** receivers (activated by vibrations of adjacent air particles), are sensitive to low-frequency sound (75–500 Hz), and usually have a relatively simple structure that does not include a tympanum.
- Examples of near-field detectors are the hairs on the cerci of cockroaches, on the aristae of Drosophila, and on the thorax of some noctuid caterpillars, as well as the specialized Johnston's organ
- In contrast, **far-field detectors** are **pressure difference** receivers (are stimulated by changes in air pressure created by sound waves), are sensitive to a wide range of high frequencies (2 to over 100 kHz), almost always have a tympanum, and hence are commonly called "**tympanal organs**"

Tympanal Organs

- Tympanal organs are present in some species from at least seven orders of insects.
- **Examples**: Orthoptera (fore tibiae or first abdominal segment), Lepidoptera (abdomen, metathorax, or fore or hind wing base), Hemiptera (abdomen, or thorax), Coleoptera (abdomen, or cervical membrane), Dictyoptera (metathorax, or metathoracic leg), Neuroptera(wing base), and Diptera (ventral prosternum).
- Almost all tympanal organs have **three** common features: 1) a cuticular membrane (the **tympanum**); 2) a large tracheal **air sac** appressed to the

membrane, the two structures forming a "**drum**"; and 3) a group of **chordotonal sensilla** (Figure 3).

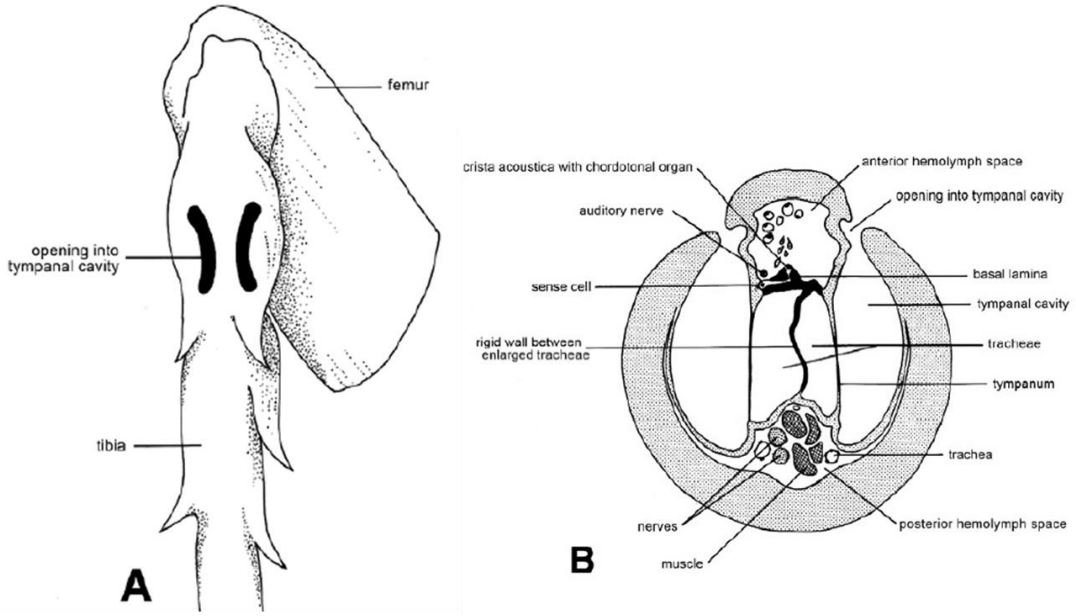


Figure 3: (A) Surface view of tibial tympanal organ of *Decticus* (Tettigoniidae); (B) transverse section through tympanal organ of *Decticus*.

- The tympanum is much **thinner** (1 µm in cicadas, 40–100 µm in some ensiferans) than the surrounding cuticle, providing the **sensitivity** required for sound reception.
- Sound waves that strike the **drum** cause it to **vibrate** and, therefore, the sensilla to be **stimulated**.
- The **range of frequency** of the waves that stimulate tympanal organs is **high**. For example, in Acrididae, it extends from less than 1 kHz to about 50 kHz.

Photoreception

- Almost all insects are able to detect light energy by means of specialized photosensory structures: **compound eyes**, **ocelli**, or **stemmata**.

Compound Eyes

- Paired compound eyes, the main photosensory system, are **well developed** in most adult insects and juvenile exopterygotes, but may be **reduced** or **absent** in parasitic or sedentary forms, such as lice, fleas, and female scale insects.
- Typically, the eyes **occupy** a relatively **large proportion** of head surface, from which they bulge out to provide a wide visual field.
- A compound eye comprises a varied number of photosensilla, the **ommatidia**.
- The **number** ranges from 1 in the ant *Ponera punctatissima* to as many as 28,000 in some dragonflies.
- Each **ommatidium** (Figure 4) includes 1) a light-gathering component (**corneal lens** plus **crystalline cone**); 2) the primary sense cells (**retinular cells**), which collect and transduce light energy; and 3) various enveloping **pigment cells**.

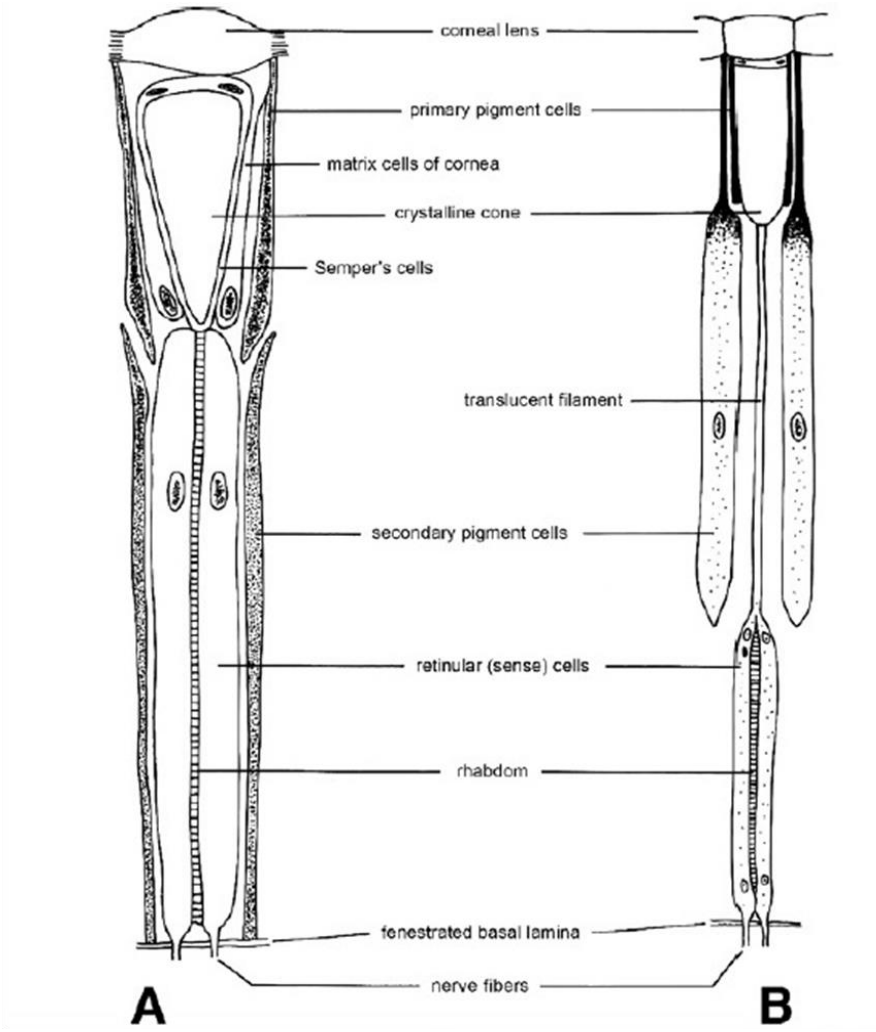


Figure 4: (A) Photopic ommatidium; (B) scotopic ommatidium.

- The **lens**, a region of **transparent cuticle**, is produced by the primary pigment cells. Its surface is usually smooth.
- The **crystalline cone** is a clear, hard material produced by four cells (Semper's cells). The material is typically intracellular, and the nuclei are situated around it (**eucone type**). In some species the material is extracellular (**pseudocone type**); in others there is no crystalline cone and the cells, which are transparent, occupy the area (**acone type**).
- Primitively, eight **retinular** cells occur beneath the crystalline cone, though one of these is usually degenerate or eccentrically located. The seven remaining cells are arranged around a central axis in most species; occasionally, they exist in two tiers of three and four cells, respectively.

- The mature sensory cells are **unipolar**, that is, lack dendrites. Instead, their inner surface is modified to form a receptive area, the **rhabdomere**. Collectively, the rhabdomeres form a **rhabdom**.
- The **rhabdom** contains **visual pigments** that resemble those of the vertebrate eye; that is, they are conjugated proteins called **rhodopsins**.
- Surrounding the photosensitive cells are the **secondary pigment cells** which, like the primary pigment cells, contain granules of red, yellow, and brown/black pigments (mainly ommochromes). These pigments, especially the browns and blacks are vitally important as they strongly absorb light that enters the eye at oblique angles.
- Proximally, the **retinular cells narrow** to form discrete **axons** that enter the **optic lobe**.
- **Two** ommatidial types can be distinguished according to the arrangement of retinular and pigment cells (Figure 4 A, B).
- 1) **Photopic ommatidia**: characteristic of diurnal insects, its retinular cells span the distance between the crystalline cone and basal lamina. The secondary pigment cells lie alongside the retinular cells, and the pigment within the former does not migrate longitudinally.
- 2) **Scotopic ommatidia**: found in nocturnal or crepuscular species, have short retinular cells whose rhabdom is often connected to the crystalline cone by a translucent filament that serves to conduct light to the rhabdom. The secondary pigment cells do not envelop the retinular cells and their pigment granules are capable of marked longitudinal migration, allowing light from adjacent ommatidia to reach each rhabdom, enhancing rhodopsin activation.

Reproduction

- An important factor in the success of the Insecta is their **high reproductive capacity**, the ability of a single female to give rise to many offspring, a relatively large proportion of which may reach sexual maturity under favorable conditions.
- As reproduction is almost always sexual in insects, there arise within insect populations **large numbers** of **genetic combinations**, as well as **mutations**, which can be tested out in the prevailing environmental conditions. As these conditions change with time, insects are able to adapt readily, through natural selection, to a new situation.
- Over the short term their high reproductive capacity enables insects to **exploit temporarily favorable conditions**, for example, availability of suitable food plants. The latter requires that both the timing of mating, egg production, and hatching, and the location of a suitable egg-laying site must be carefully "assessed" by an insect.
- Like other terrestrial animals insects have had to solve **two major problems** in connection with their reproductive biology, namely, the bringing together of sperm and egg in the absence of surrounding water and the provision of a suitable watery environment in which an embryo can develop.
- The **solution** to these problems has been the evolution of internal fertilization and an egg surrounded by a waterproof cover (chorion), respectively.
- The latter has itself created **two secondary problems**.
- **First**, because of the generally impermeable nature of the chorion, structural modifications have had to evolve to ensure that adequate gaseous exchange can occur during embryonic development.
- **Second**, the chorion is formed while an egg is still within the ovarian follicle, that is, prior to fertilization, which has necessitated the development of special pores (micropyles) to permit entry of sperm.

Structure and Function of the Reproductive System

Female

- Functions of the female reproductive system include **production** of eggs, including yolk and chorion formation, **reception** and **storage** of sperm, sometimes for a considerable period, and **coordination** of events that lead to fertilization and oviposition.
- Though details vary, the female system (Figure 1) essentially includes **a pair of ovaries** from each of which runs a lateral oviduct.
- The lateral **oviducts** fuse in the midline, and the common oviduct typically enters a saclike structure, the **vagina**.
- In some species the vaginal wall evaginates to form a pouchlike structure, the **bursa copulatrix**, in which spermatophores and/or seminal fluid is deposited during copulation.

- Also connected with the vagina are the **spermatheca** in which sperm are stored and various **accessory glands**.

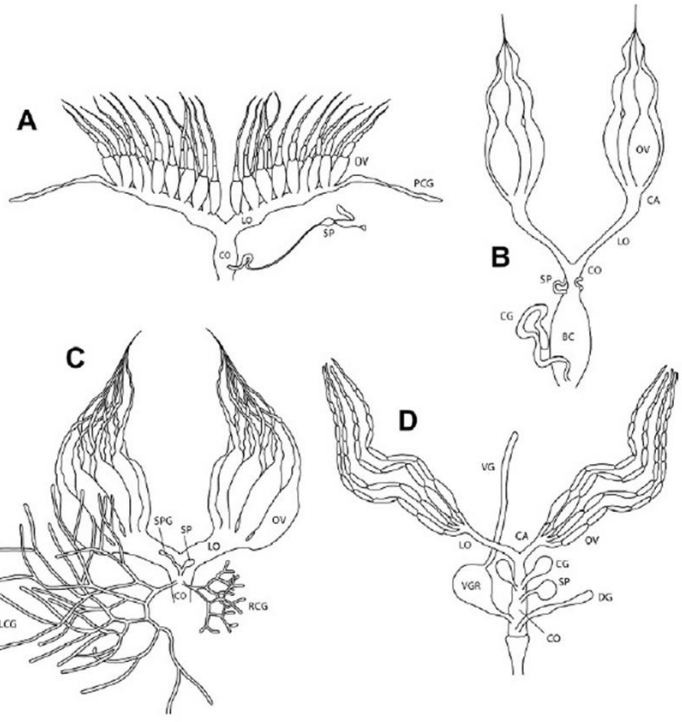


Figure 1: Representative female reproductive systems (not to scale). (A) *Melanoplus sanguinipes* (Orthoptera); (B) *Rhodnius prolixus* (Hemiptera); (C) *Periplaneta americana* (Dictyoptera); and (D) *Nasonia vitripennis* (Hymenoptera). Abbreviations: BC, bursa copulatrix; CA, calyx; CG, collateral (accessory) glands; CO, common oviduct; DG, Dufour's gland; LCG, left colleterial gland; LO, lateral oviduct; OV, ovariole; PCG, pseudocolleterial gland; RCG, right colleterial gland; SP, spermatheca; SPG, spermathecal gland; VG, venom gland; VGR, venom gland reservoir.

- In some species part of the spermatheca takes the form of a diverticulum, the **spermathecal gland**.
- It is noteworthy that the ovaries themselves lack innervation though the ductal components of the system receive nerves from the terminal abdominal ganglion.
- The ovaries are usually **dorsolateral** to the gut, and each comprises a number of tubular ovarioles ensheathed by a network of connective tissue in which numerous tracheoles and muscles are embedded.
- The **number** of **ovarioles** per ovary, though approximately constant within a species, varies widely among species. For example, in some viviparous aphids and in dung beetles there is one ovariole per ovary in contrast to the more than 2000 ovarioles per ovary in some higher termite queens.
- The **wall** of each **ovariole** includes an outer epithelial sheath and an inner acellular, elastic layer, the tunica propria.
- Each ovariole (Figure 2) consists of a **terminal filament**, **germarium**, **vitellarium**, and **pedicel** (ovariole stalk).
- The terminal filaments may fuse to form a sheet of tissue attached to the dorsal body wall or dorsal diaphragm by which an ovary is suspended within the abdominal cavity.

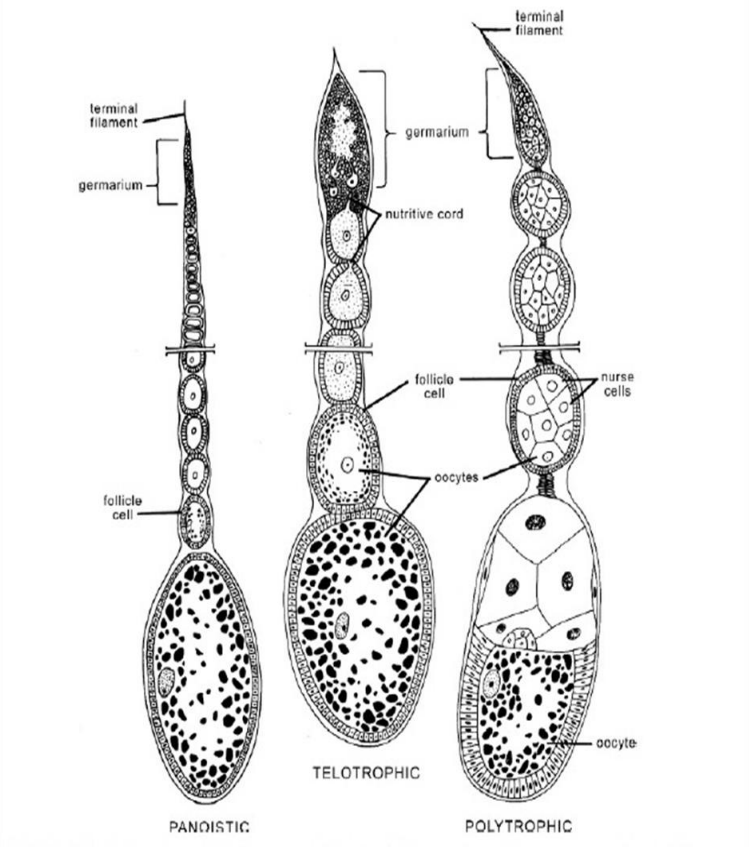


Figure 2: Types of ovarioles. The upper portion of each figure is enlarged to a greater extent than the lower in order to make details of germarial structure clear.

- Within the germarium, **oogonia**, derived from primary germ cells, give rise to **oocytes** and, in some types of ovarioles, also to nutritive cells.
- As oocytes mature and enter the vitellarium they tend in most insects to become arranged in a linear sequence along the ovariole.
- Each oocyte also becomes enclosed in a one-cell-thick layer of **follicular epithelium** derived from mesodermal prefollicular tissue located at the junction of the germarium and vitellarium.
- As its name indicates, the vitellarium is the region in which an oocyte accumulates yolk, a process known as **vitellogenesis**.
- Normally vitellogenesis occurs only in the terminal oocyte, that is, the oocyte closest to the lateral oviduct, and during the process the oocyte's volume may increase enormously, for example, 10^5 by as much as times in *Drosophila*.
- Each ovariole is connected to a lateral oviduct by a thin-walled tube, the **pedicel**, whose lumen is initially occluded by epithelial tissue.
- This plug of tissue is lost during ovulation (movement of a mature oocyte into a lateral oviduct) and replaced by the remains of the follicular epithelium that originally covered the oocyte.

- Ovarioles may **join** a **lateral oviduct linearly**, as in some apterygotes, Ephemeroptera and Orthoptera, or, more often, **open confluently into** the distal expanded portion of the oviduct, the **calyx**.
- **Three** types of ovarioles can be distinguished (Figure 2).
- The most primitive type, found in Thysanura, Paleoptera, most orthopteroid insects, Siphonaptera, and some Mecoptera, is the **panoistic** ovariole in which specialized nutritive cells (**trophocytes**) are absent.
- **Trophocytes** occur in the two remaining types, the **polytrophic** and **telotrophic** ovarioles, which are sometimes grouped together as **meroistic** ovarioles.
- In **polytrophic** ovarioles, several trophocytes (nurse cells) are enclosed in each follicle along with an oocyte.
- The trophocytes and oocyte originate from the same oogonium.
- Polytrophic ovarioles are found in most endopterygotes, and in Dermaptera, Psocoptera, and Phthiraptera.
- In Hemiptera and Coleoptera **telotrophic** (acrotrophic) ovarioles occur in which the trophocytes form a syncytium in the proximal part of the germarium and connect with each oocyte by means of a trophic cord.
- The **lateral oviducts** are thin-walled tubes that consist of an inner epithelial layer set on a basal lamina and an outer sheath of muscle.
- In many species they include both mesodermal and ectodermal components. In almost all insects they join the **common oviduct medially beneath** the **gut**, but in Ephemeroptera the lateral oviducts remain separate and open to the exterior independently.
- The common oviduct, which is lined with cuticle, is usually more muscular than the lateral oviducts.
- Posteriorly, the common oviduct is confluent with the **vagina** that, as noted above, may evaginate to form the **bursa copulatrix**.
- In nearly all Lepidoptera the bursa is physically distinct from the oviduct and opens to the outside via the vulva (Figure 3).
- A narrow **sperm duct** connects the bursa with the oviduct and forms the route along which the sperm migrate to the **spermatheca**.
- Usually a single spermatheca is present in which sperm are stored, though in some higher Diptera up to three such structures occur.

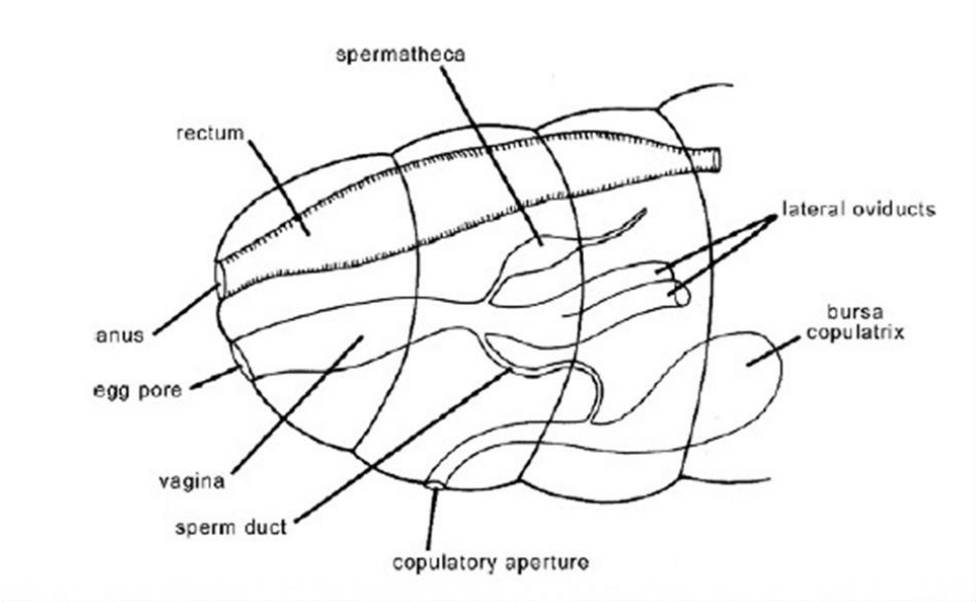


Figure 3: Reproductive system of female Lepidoptera-Ditrysia.

- The spermatheca and the duct with which it joins the bursa are lined with **cuticle**.
- The cuticle overlays a one-cell-thick layer of **epithelium** whose cells are **glandular** and assumed to secrete nutrients for use by the stored sperm.
- Typically, also, the cells have a much folded apical **plasmalemma**, adjacent to which are many **mitochondria**. These features may indicate that the sperm stored in the spermatheca require an ionic milieu different from that of the surrounding hemolymph.
- Various **accessory glands** (also called **collateral** or **colleterial** glands) maybe present and usually open into the bursa.
- However, in Acrididae (Orthoptera) the glands, known as **pseudocolleterial** glands, are anterior extensions of the lateral oviducts (Figure 1A).
- Normally, there is one pair of glands, which secrete materials that form a protective coating around the eggs or stick the eggs to the substrate during oviposition.
- Less commonly the glands produce **antibacterial** substances that coat the eggs, toxic egg **protectants**, and **oviposition** stimulating or oviposition-deterring **pheromones**.
- In some species the glands may be structurally distinct bi- or multipaired structures, each pair presumed to have discrete functions.

- In Hymenoptera, the glands are single, not paired, and produce the venom used in the sting, secrete trail- or oviposition site-marking pheromones, or lubricate the ovipositor valves (Figure 19.1D).

Male

- **Functions** of the **male reproductive system** include **production**, **storage**, and, finally **delivery** to the female of sperm.
- In some species, the system produces **substances** transferred during copulation that regulate female **receptivity** and **fecundity**.
- An additional function may be to supply the female with **nutrients** that can be incorporated into developing oocytes, thereby increasing the rate and number of eggs produced.
- The male system includes **paired testes** (in Lepidoptera these fuse to form a single median organ), **paired vasa deferentia and seminal vesicles**, a **median ejaculatory duct**, and various **accessory glands** (Figure 4).
- The testes, which lie either above or below the gut, comprise a varied number of **tubular follicles** bound together by a connective tissue sheath.
- The follicles may open into the vas deferens either confluently or in a linear sequence.

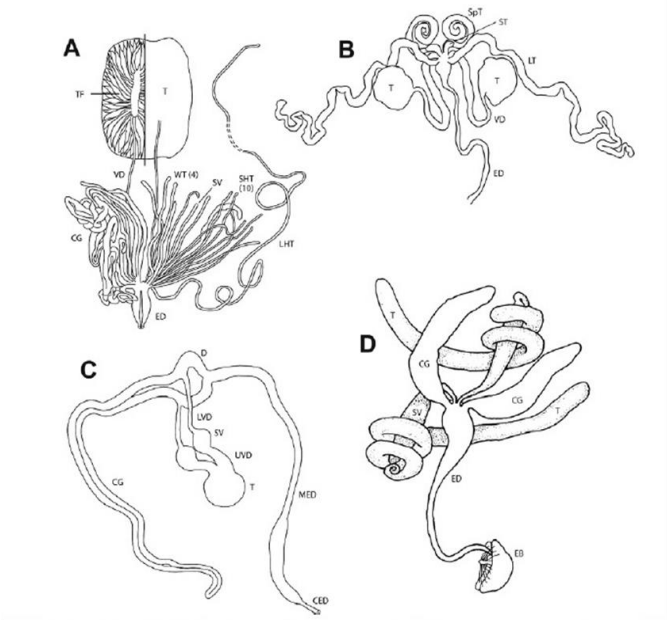


Figure 4: Examples of male reproductive systems (not to scale). (A) *Melanoplus sanguinipes* (Orthoptera); (B) *Lytta nuttalli* (Coleoptera); (C) *Anagasta ku¨hniella* (Lepidoptera); and (D)*Drosophila melanogaster* (Diptera). In *M. sanguinipes* 16 pairs of tubules make up each collateral gland (CG). There are 4 white tubules (WT), 10 short hyaline tubules (SHT), and a long hyaline tubule (LHT). The 16th tubule serves as a seminal vesicle (SV). In *L. nuttalli* there are three tubules in each collateral gland; a spiral tubule (SpT), short tubule (ST), and a long tubule (LT). *Other abbreviations*: CED, cuticular ejaculatory duct; D, duplex; EB, ejaculatory bulb; ED, ejaculatory duct; LVD, lower vas deferens; MED, mesodermal ejaculatory duct; T, testis; TF, testis follicle; UVD, upper vas deferens; VD, vas deferens.

- The **wall** of each **follicle** is a layer of **epithelium** set on a basal lamina.
- Within the follicles several zones of development can be readily distinguished (Figure 5).
- The distal zone is the **germarium** in which **spermatogonia** are produced from **germ cells**.
- In Orthoptera, Dictyoptera, Hemiptera, and Lepidoptera a prominent **apical cell** is also present whose presumed function is to supply nutrients to the spermatogonia.

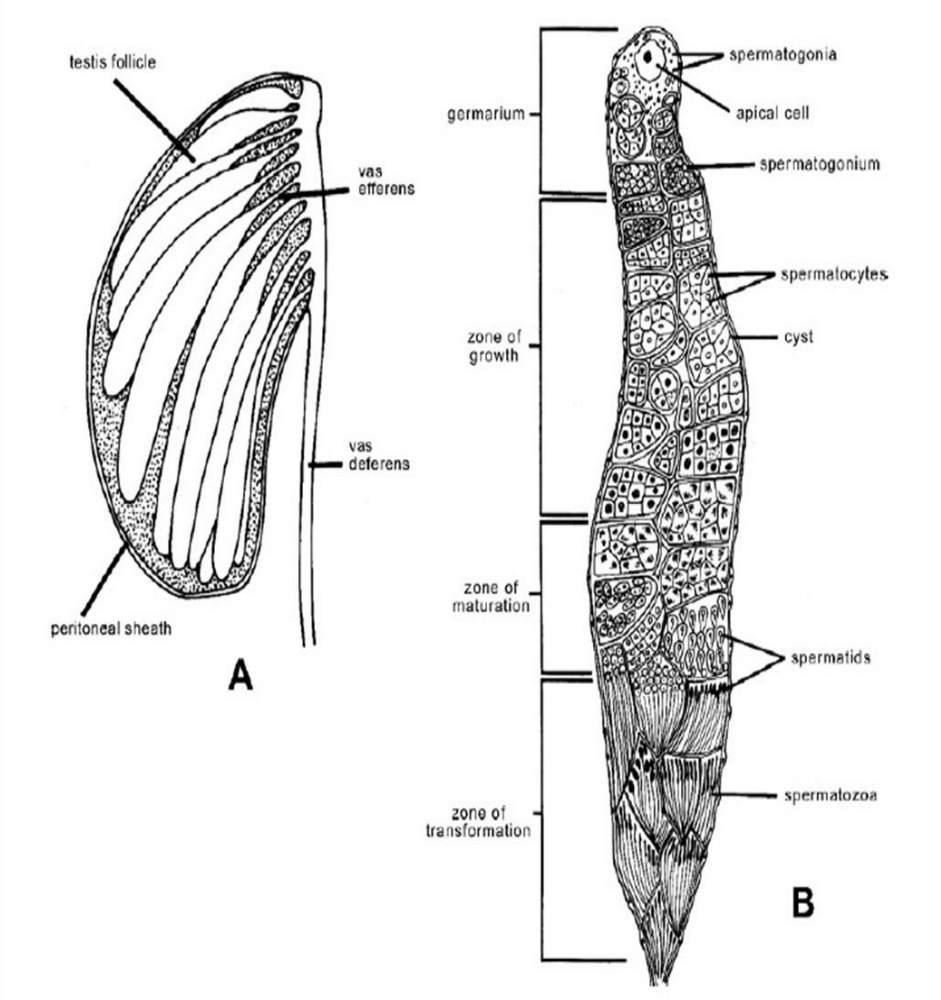


Figure 5: (A) Section through testis to show arrangement of follicles; and (B) zones of maturation in testis follicle.

- As each spermatogonium moves proximally into the zone of growth, it becomes enclosed within a layer of somatic cells, forming a "**cyst**".
- Within the cyst, the cell divides mitotically to form a varied number (usually 64–256) of **spermatocytes**.

- In the zone of maturation, the spermatocytes undergo two maturation divisions, so that from each spermatocyte four haploid **spermatids** are formed.
- In the proximal part of the follicle, the zone of transformation, spermatids differentiate into flagellated **spermatozoa**.
- At this time the cyst wall normally has ruptured, though often the **sperm** within a bundle (**spermatodesm**) remain held together by a gelatinous cap that covers their anterior end.
- This cap may be lost as the sperm enter the **vas deferens** or persist until the sperm have been transferred to the female.
- In **Lepidoptera two types** of **sperm** occur. **Pyrene** (nucleate) sperm are those that fertilize eggs, while **apyrene** (anucleate) sperm are speculated to have several functions, including assisting in the movement of pyrene sperm from the testes to the seminal vesicles, providing nourishment to the pyrene sperm, and destroying sperm from previous matings.
- In each species within the *Drosophila obscura* complex **two size classes** of **nucleated sperm** are produced, which differ in head and tail lengths. Only the long-sperm type fertilize eggs, though the function(s) of the short sperm remain unidentified.
- Sperm are moved from the testes to their site of storage (normally, the **seminal vesicles**) by peristaltic contractions of the vas deferens.
- The seminal vesicles are dilations of the vasa deferentia. Their walls are well tracheated and frequently glandular, which may indicate a possible nutritive function.
- In Acrididae (Orthoptera) sperm are stored in a pair of highly modified accessory gland tubules (Figure 4A).
- In many Lepidoptera the migration of sperm follows a **circadian rhythm**. Typically sperm are released from the testes into the upper vasa deferentia shortly before or just after dark, and then are moved into the seminal vesicles during the next light phase. However, they quickly leave

this site, being moved into the duplex region of the reproductive tract (Figure 4C), where they remain until the next copulation occurs.

- The timing of sperm movement is such that the sperm produced each day move into the duplex a few hours after the male's daily period of receptivity to female pheromone. This ensures that when the male next has an opportunity to mate, a substantial amount of new sperm will be available for insemination.
- The vasa deferentia enter the anterior tip of the **ejaculatory duct**, an ectodermally derived tube lined with cuticle whose walls normally are heavily muscularized.
- Posteriorly, the ejaculatory duct may run through an evagination of the body wall, which thus forms an intromittent organ.
- In insects that form a complex spermatophore, subdivision of the ejaculatory duct into specialized regions may occur.
- In Ephemeroptera no ejaculatory duct is present, and each vas deferens opens directly to the exterior.
- The **accessory glands** may be either mesodermal (mesadenia) or ectodermal (ectadenia) in origin and are connected with either the lower part of the vasa deferentia or the upper end of the ejaculatory duct. In some species considerable morphological and functional differentiation of the glands occurs. Essentially, however, their secretions may contribute to the seminal fluid and/or form the spermatophore. In some species the glands produce substances that, when transferred to the female during insemination, cause increased egg production and/or decreased receptivity (willingness to mate subsequently).

Immunity

- The **first defense** of insects against microbial organisms and fungi is the **physical barrier** presented by the tough sclerotized cuticle that covers the body, and thinner, more flexible cuticle that lines the tracheae, parts of the internal reproductive tract, foregut, and hindgut.
- Chemicals on the cuticular surface such as free **fatty acids** in some species can be effective against some bacteria and fungi.
- When organisms succeed in getting past the cuticular barrier, insects rapidly mobilize innate immune responses to invading foreign organisms.
- Insects combat invading microbial organisms by several innate mechanisms including: (1) **phagocytosis** of small objects and **encapsulation** of larger objects with layers of hemocytes, (2) localized **coagulation of hemolymph** at wound sites, (3) **melanization reactions** at wound sites and usually at encapsulated objects, it involves the action of phenoloxidase (PO) on phenolic compounds to produce quinones that autopolymerize and produce melanin, (4) synthesis of **antimicrobial peptides**.
- Insects lack the complement system of acquired immunity with memory that occurs in vertebrates, although some experiments suggest that there is increased sensitivity and response to repeated challenges if the challenges are temporally close together.
- Insect innate immune responses include both **cellular defenses** and **humoral defenses**.
- **Cellular events** are initiated by the cells that encounter the invading organisms, usually epithelial cells beneath the cuticle, hemocytes in the hemolymph, fat body cells, and epithelial cells lining the gut.
- These cells rapidly respond to the invasion by secreting **pattern recognition proteins** that have a variety of functions, including eliciting synthesis of antimicrobial peptides.
- Hemocyte proliferation occurs making increased numbers available to attack the invaders by phagocytosis, encapsulation, and nodule formation.

- Hemocytes release clotting agents in the hemolymph at wound sites, and **prophenoloxidase**, a zymogen circulating in the hemolymph, is activated to **phenoloxidase** (PO), which promotes melanization of encapsulated objects and wound sites (Figure 1).

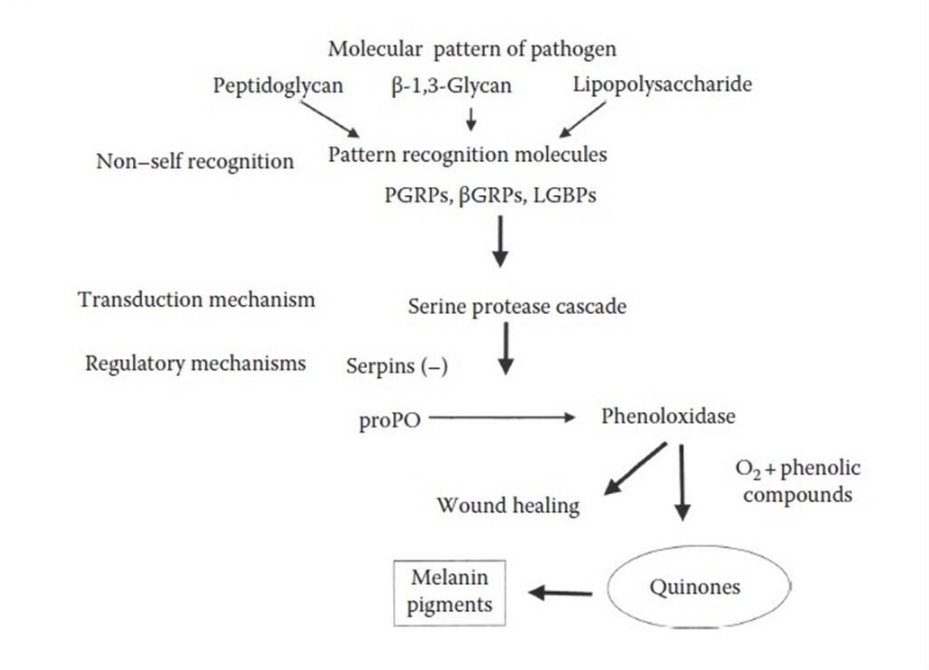


Figure 1: A conceptual scheme to illustrate non-self recognition by pattern recognition proteins and signal transduction through a serine protease cascade to activate prophenoloxidase to phenoloxidase and the production of melanin. PGRP, βGRP, and LGBPs are peptidoglycan recognition protein, betaglucan recognition protein, and liposaccharide recognition protein, respectively. Serpins are enzymatic proteins that have a negative effect by attacking the kinase cascade. proPO is prophenoloxidase.

- **Humoral responses** usually are considered to be the elaboration of response agents that circulate in the hemolymph, which will include pattern recognition proteins.
- The **pattern recognition proteins** elaborated from **epithelial cells**, **hemocytes**, and **fat body cells** set in motion a cascade of enzymatic reactions in the cytoplasm that lead to the activation of nuclear genes that encode enzymes for the synthesis of **antifungal** and **antibacterial peptides**.

Physical barriers to invasion

- The **external cuticle** of insects is a natural barrier to many microorganisms and fungi, although some organisms have chitinase and protein-digesting enzymes that aid penetration through the cuticle.
- Probably the thickness of the cuticle is the main physical barrier, but in many insects there are numerous thin areas of cuticle, particularly at intersegmental boundaries between segments in the abdomen and in the lining of the tracheal system.
- Insect **ingest** many **microorganisms** with their food and, for some pathogens, the oral route is the main entry point into the insect.
- The foregut and hindgut have an attached cuticular lining on the lumen surface of the gut epithelial cells that protects the epithelial cells, but the midgut is more vulnerable.
- The midgut may have an unattached protective layer, the **peritrophic matrix** composed of chitin and protein that protects the delicate brush border on midgut cells from harsh food particles as well as ingested microorganisms. Some insects, however, do not have a peritrophic matrix. The peritrophic matrix has holes or pores through which digestive enzymes secreted by the midgut cells pass into the food bolus and through which small digested molecules pass for absorption by midgut cells. Invading microorganism find these pores a potential site of entry to the midgut cells.

Cellular immune reactions

- Cellular reactions are initiated immediately upon the invasion of a foreign microorganism and involve direct attack of the foreign object by hemocytes in the circulating hemolymph.
- When stimulated by invading microorganisms, epithelial cells in various parts of the body secrete **pattern recognition proteins** (PRPs also called **pattern recognition receptors** [PRRs]).
- Some of the PRPs are released into the circulating hemolymph while others are attached to the surface of the cells that produce them.

- The PRPs recognize and bind to particular carbohydrate or carbohydrate-peptide linkages in the structure of invading fungi or bacteria by acting as receptors for characteristic bacterial or fungal wall components.
- The bacterial and fungal structures recognized by PRPs are called **pathogen-associated molecular patterns** or PAMPs.
- Identified PAMPs include **ß-1,3-glucans** as a part of the fungal cell wall, and **lipopolysaccharides** (LPS) and **peptidoglycans** (PGNs) as part of the cell surface of bacteria.
- Binding of the PRPs to invading microbial cells marks them for destruction.
- The open circulatory system of insects makes PRRs especially well suited to communicate rapidly the presence of nonself because the marked microorganisms are conveyed directly to hemocytes (for cellular responses as phagocytosis or encapsulation) and to fat body cells (for humoral responses as synthesizing of antimicrobial peptides).

Recognition of nonself

- Recognition of nonself by fat body cells, hemocytes, midgut epithelium, and cuticular epithelium is the first step in mounting a humoral defense.
- Insects may use the **basement membrane** that lies at the basal surface of all insect cells (except hemocytes) as an **indicator of self** against which they direct non-self reactivity.

الجزء العملي

Lab. 1 **Date / /**

The Nervous System

The nervous system coordinates the activities of the insect to conditions, both inside and outside the body. The basic elements in the nervous system are nerve cells which are produced into long processes or axons, along which nerve impulses are conducted. The bodies of the nerve cells are aggregated to form ganglia while bundles of axons form the nerves.

The nervous system composed of three parts: (1) the central nervous system, (2) the visceral nervous system, and (3) the peripheral nervous system.

(1) Central nervous system:

- The central nervous system composed of a double series of ganglia which are joined together by means of longitudinal connectives.
- Typically there is a pair of ganglia in each segment of the body, but these always show some degree of fusion that they appear as a single ganglion. A typical ganglion is differentiated into a peripheral (cortical) region containing nerve and glial cell bodies, and a medullary (neuropile) central region containing nerve fibers (axons) and their supporting glial elements. The whole ganglion is invested in a non-nervous sheath which is differentiated into a non-cellular neural lamella and a cellular perineurium.
- The ganglia are joined to each other longitudinally by connectives made up only of axons and supporting cells.
- The central nervous system is divisible into three main parts:
    a. The brain or cerebral ganglion.
    b. The suboesophageal ganglion.
    c. The ventral nerve cord.

Exp.1: Examine T.S. of a ganglion and an interganglionic connective of the locust, *Schistocerca gregaria* showing the general histology.

a. The brain:

The brain is the principle association center of the body receiving a sensory impute from the sense organs of the head and from the more posterior ganglia. It is located in the head above the oesophagus and is formed of a fusion of the first three pairs of ganglia, so three regions are recognized which are:

1- The protocerebrum forms the greater part of the brain and innervates the compound eyes and ocelli.
2- The deutocerebrum contains the antennal lobes which innervate the antennae.
3- The tritocerebrum which is divided into two small widely separated lobes that unite the brain with the oesophageal ganglion and innervate the labrum.

b. Suboesophageal ganglion:

This is a compound ganglion, lying ventrally in the head, arising from the fusion of the ganglia of the mandibular, maxillary and labial segments. It gives off paired nerves innervating the mandibles, maxillae and labium.

c. Ventral nerve cord:

The ventral nerve cord consists of a series of ganglia united into a longitudinal chain by means of a pair of connectives. The first three ganglia are situated in the thorax (thoracic ganglia) which control the locomotory organs. They innervate the muscles of segments and the muscles of legs. In meso and meta thorax, an additional pair of nerves is present and controls the movements of wings. The rest of the ganglia lie in the abdomen (abdominal ganglia) which are variable in numbers. Each gives off a pair of nerves to the muscles of its segment.

Exp.2: Examine the central nervous system of the locust, *S. gregaria* showing external morphology and histology.

(2) The Visceral Nervous System:

The visceral or sympathetic nervous system is divided into: (i) the esophageal sympathetic, (ii) the ventral sympathetic, and (iii) the caudal sympathetic nervous systems.

(i) The Oesophageal sympathetic (stomatogastric) nervous system:

It is directly connected with the brain and innervates the fore and mid gut, heart and certain other parts. Typically, it consists of a small triangular frontal ganglion lies above the oesophagus, a short distance in front of the brain. Anteriorly the frontal ganglion gives off a frontal nerve which passes to the clypeus, and a pair of lateral roots connects the frontal ganglion with the tritocerebrum. Posteriorly it emits a recurrent nerve which passes just beneath the brain, expands a short distance behind the latter into a hypocerebral ganglion. The recurrent nerve leaves the hypocerebral ganglion as a median oesophageal nerve and pass back to the hinder region of the fore-gut, where it terminates in a ventricular ganglion, which innervates the adjacent region of the fore and midgut. A pair of corpora cardiaca lies on the oesophagus just behind the brain, each joined with the hypocerebral ganglion, and also connected with the protocerebrum and includes both nervous and endocrine structure.

(ii) The ventral sympathetic nervous system:

It consists of a pair of transverse nerves associated with each ganglion of the ventral nerve-cord. These transverse nerves pass to the spiracles of their segment.

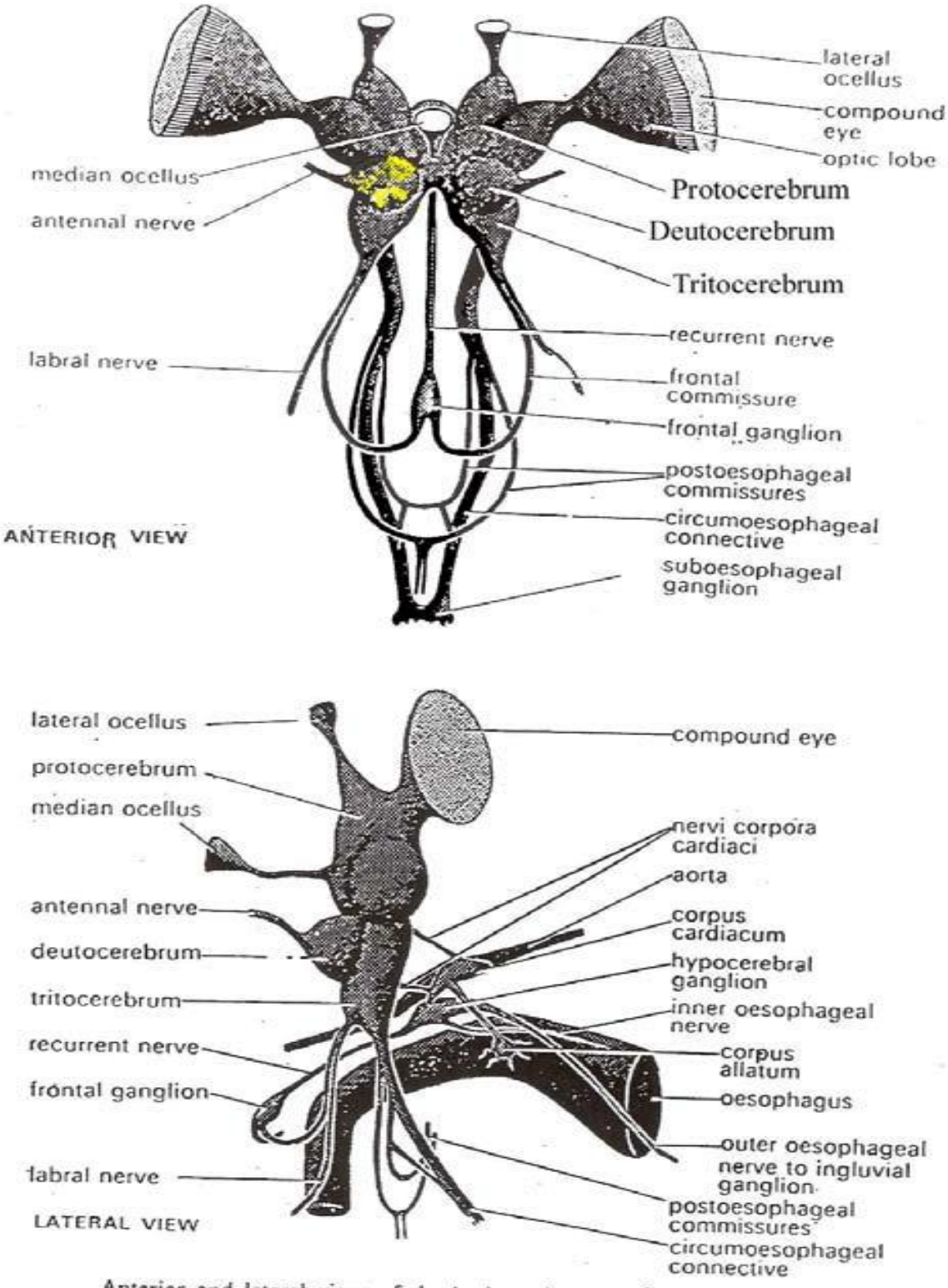
(iii) The caudal sympathetic nervous system:

This arises from the posterior compound ganglion of the ventral nerve-cord and supplies the reproductive system and posterior part of the gut.

(3) The Peripheral Nervous System:

This includes all the nerves radiating from the ganglia of the central and sympathetic nervous systems, and dealing only with the innervations of particular organs such as salivary glands, integument, various regions of the gut and reproductive system.

brain



Anterior and lateral views of the brain and stomatogastric nervous system of *Locusta* (Orthoptera) (after Albrecht, 1953).

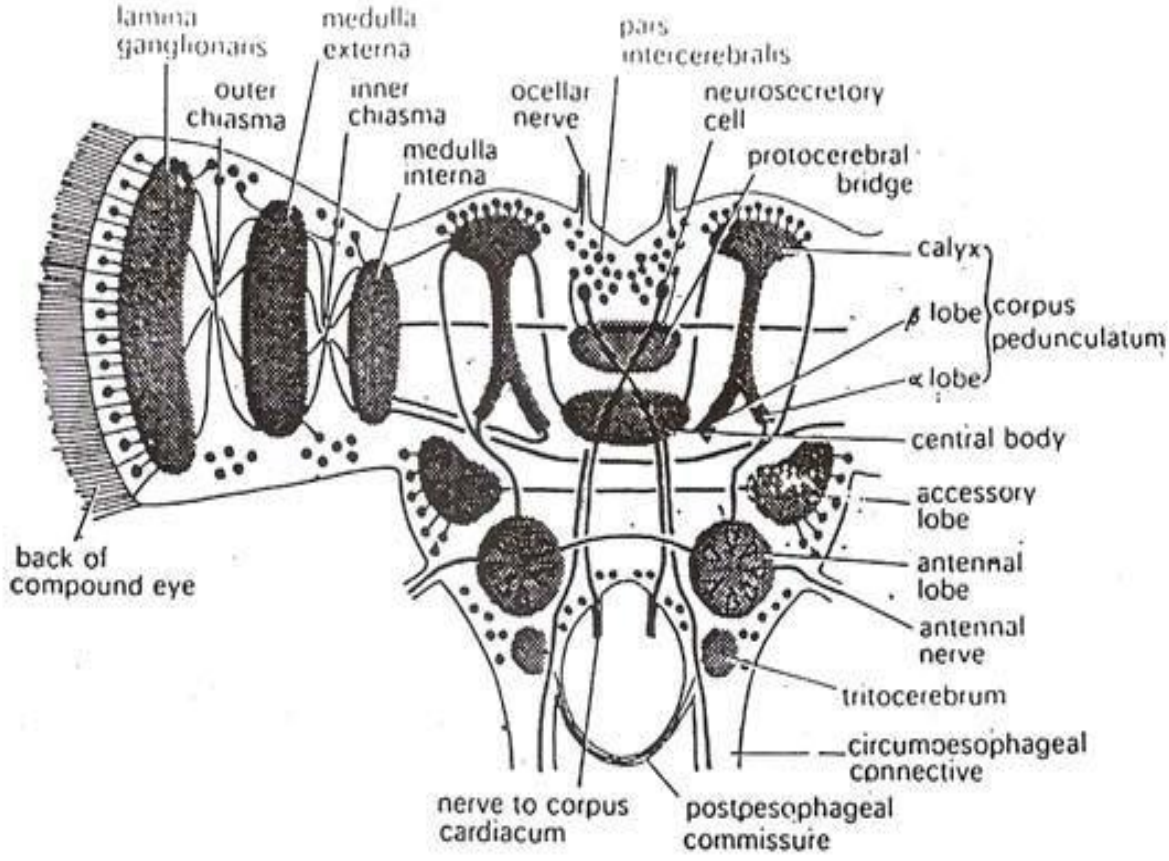
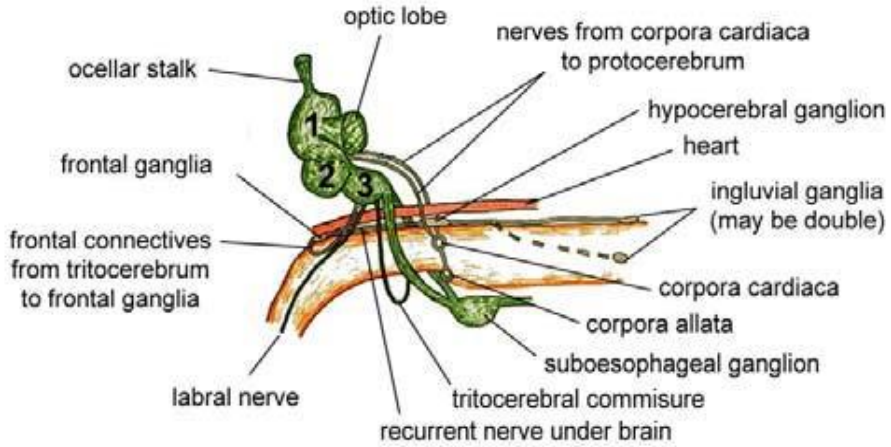


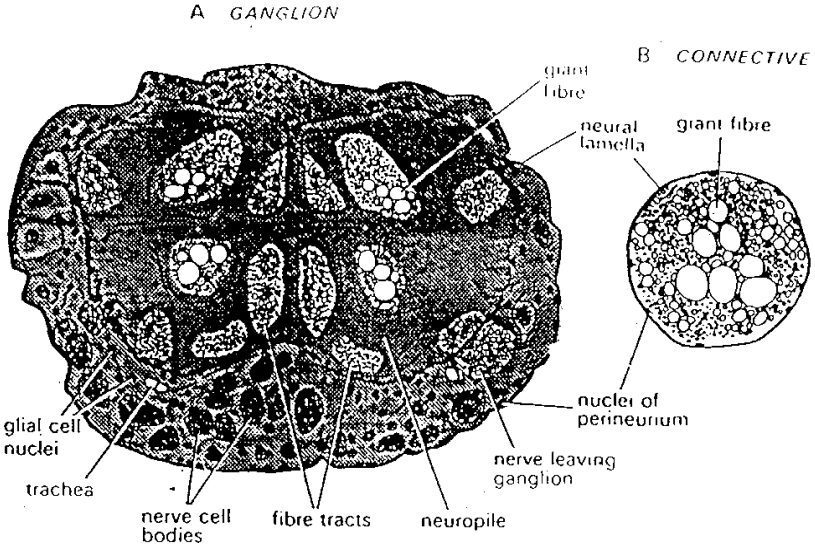
Diagram of the brain showing the more important areas of neuropile (hatched) and a few of the main connections between these areas. Black dots represent zones containing perikarya.

BRAIN - SIDE VIEW

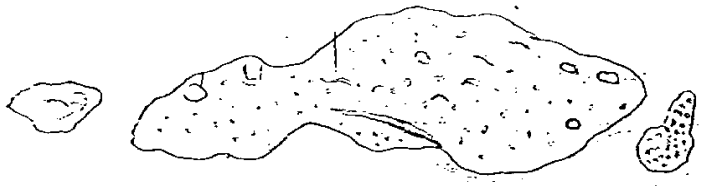


1 Protocerebrum **2** Deutocerebrum **3** Tritocerebrum

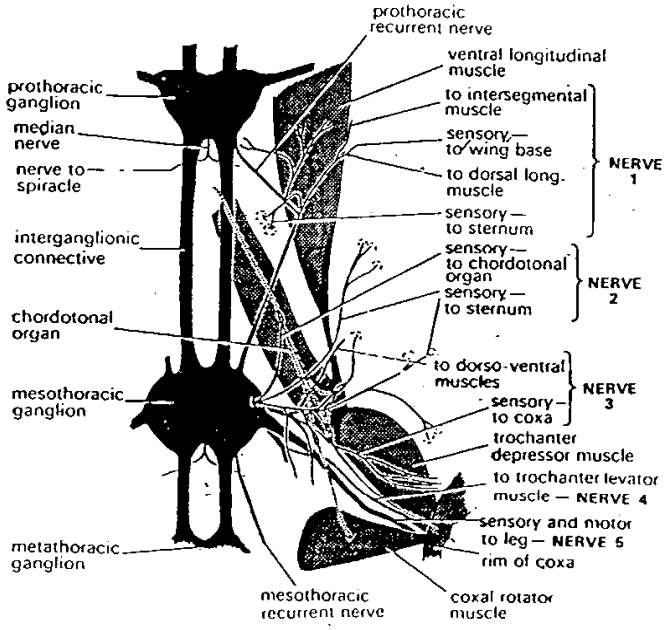
ganglia



Transverse sections of (A) an abdominal ganglion and (B) an abdominal interganglionic connective of *Periplaneta*. Not to same scale (after Roeder, 1953, 1963).



ventral nerve cord



Ventral view of part of the central nerve cord in the thorax of *Locusta* showing some of the nerves of the mesothoracic segment (after Campbell, 1961).

The Endocrine Organs

The endocrine organs of insects are of two types: (a) neurosecretory cells (NSCs) mainly within the central nervous system, and (b) specialized endocrine glands, such as the corpora cardiaca, corpora allata and prothoracic glands.

The endocrine organs produce hormones which travel, usually in the blood, to various organs of the body, coordinating their longer term activities. The endocrine system is thus complementary to the nervous system. The hormones of insects are many and various in their effects and even hormones from a single organ may have a variety of effects. Among others, activities which are affected by hormones are growth, post-embryonic development, moulting, metamorphosis and diapause, as well as certain aspects of oocyte production, water balance and excretion, and such metabolic activities as the regulation of blood sugar and lipid levels. Various modes of behaviour, the hardening and darkening of cuticle, digestive enzymes secretion, and perhaps even the control of cast and morph differentiation in certain polymorphic species are also among those activities that are subject to hormonal influences.

(a) ***Neurosecretory cells:***

- Neurosecretory cells normally occur in the ganglia of the central nervous system.
- They resemble typical nerve cells with axons, but they are characterized by showing cytological evidence of secretion.
- The neurosecretory cells of the brain are found in two groups on each side. One group is in the pars intercerebralis. The axons from these cells pass backward through the brain and some or all of them cross over to the opposite side, emerging from the brain as a nerve which runs back to the

corpus cardiacum. Most of the fibers end here, but a few pass through the corpus cardiacum to the corpus allatum. The second group of cells is variable in position.

- The products of the NSCs of the brain pass along the axons usually to the corpora cardiaca or allata where they may be stored or released.
- The secretions of the NSCs in the pars intercerebralis promote the function of the prothoracic glands; stimulate protein synthesis and possible control water loss, oocyte development and activity.
- The NSCs are often large and lobulated nerve cells with granular cytoplasm, an inclusion stained deeply with paraldehyde fuchsin (PF) or with chromhematoxylin (CH) of permanganate oxidation.
- A second secretion which seems to be constant in the presence in their cytoplasm of numerous dense granules of 1000 to 3000 A^{o} in diameter.
- There are several types of the NSC, designated A, B, C and D cells and can be differentiated by their reaction to staining (Table 1).

Table (1): The characteristics of the different types of the neurosecretory cells (NSCs).

	Parameter	**A**	**B**	**C**	**D**
1-	**Size**	Very small		Small with large vacuole	Large
2-	**Diameter (in microns)**	10-13	10-14	20-90	20-90
3-	**Granules**	Big and clear	Big and clear	Free of granules with large vacuoles	Granulated with small vacuoles
4-	**Staining reactions**				
(a)	**CH after permanganate**	Blue black	Red	Red	Purple

	oxidation				
(b)	**PF**	Cytoplasm greenish with characteristic reddish pink granules	Pink	Reddish purple	Pink reddish purple inclusions

Exp.3: Examine T.S. in pars intercerebralis of the brain showing different types of NSCs stained with either PF or CH after permanganate oxidation.

(b) Endocrine glands

1- ***Corpora cardiaca***

- The corpora cardiaca are a pair of organs often closely associated with the aorta, and forming part of its wall.
- In higher groups such as Lepidoptera, Coleoptera and some Diptera, they become separated from the aorta.
- Each organ contains the endings of axons from cells in the brain and other axons passing through to the corpora allata.
- The corpora cardiaca store and release hormones from the NSCs of the brain.
- In addition, the intrinsic secretory cells produce hormones which are concerned with the regulation of the heartbeat and have other physiological effects.

2- ***Corpora allala***

- The corpora allata are glandular bodies, usually one on either side of the oesophagus. Each is connected with the corpus cardiacum of the same side by a nerve which carries fibers from the neurosecretory cells of the brain. In addition, a fine nerve connects each corpus allatum with the suboesophageal ganglion.
- Corpora allata produce juvenile hormones which maintain the larval characteristics and yolk deposition in the egg. Juvenile hormones also have a number of other effects: they break the adult reproductive diapause of several species, and they modify epigamic behavior or cocoon construction in other.

3- ***Prothoracic glands***

- The prothoracic, or thoracic, glands are a pair of glands at the back of the head or in the thorax but in Thysanura they are in the base of the labium.
- The prothoracic glands produce moulting hormones, ecdyson and in most cases, they break down soon after the final moult to adult.

4- ***Ring gland (Weismann's Ring):***

The larvae of Cyclorrhaphan Diptera do not display the usual arrangement of retrocerebral endocrine glands. Instead, there is present behind the brain and around the aorta a small ring-like structure supported by tracheae. In addition to the tracheal matrix cells and the hypocerebral ganglion, Weismann's ring contains three types of glandular cells which are respectively homologous with the corpora allata, corpora cardiaca and prothoracic glands, all fused together, although the component elements can still be identified.

Exp.4: Examine the stomatogastric nervous system of *Schistocerca* (L.V. of the brain) showing retro-cerebral endocrine glands.

Lab. 2

Date / /

THE INTEGUMENT

The integument is the outer layer of the insect, comprising the epidermis (hypodemis) and the cuticle. The cuticle is a characteristic feature of arthropods and is, to a large extent, responsible for the success of insects as terrestrial animals. It affords support and protection through its rigidity and hardness and is of primary importance in restricting water loss from the body surface. It is secreted by the epidermis and oenocytes and consists of a number of layers serving different functions.

Basic structure of cuticle:

The cuticle is a complex, non-cellular layer covers the whole of the outside of the body and its appendages but is invaginated locally to form endoskeletal

structures and also provides the lining of the tracheal system, some glands, parts of the alimentary canal and reproductive tract.

It is differentiated into two major regions: an inner region (procuticle) up to 200 μm thick, which contains chitin and forms the bulk of the cuticle, and the thin outer epicuticle which contains no chitin and is only 1-4 μm thick.

Chemistry of chitinous cuticle:

The two major components of insect cuticle are the carbohydrate chitin, which accounts for 25-60% of the dry weight of various cuticles, and a number of proteins and lipids. Chitin is a polysaccharide made up largely of N-acetylglucosamine, but also probably containing some glucosamine.

The sugar residues are linked by 1-4 β-linkages so that they form a chain in which all the residues are orientated in the same direction. Adjacent chitin chains are held together by hydrogen bonds to form microfibrils, and hydrogen bonds probably also link the oxygen atoms of adjacent acetylglucosamien residues. On hydrolysis, chitin yields acetic acid and glucosamine.

Chitin chains are apparently jointed to proteins by covalent linkages involving aspartic acid, aglutamic acid, histidine, lysine and tyrosine. The chitin-protein complex is in fact polydisperse glycoproteins in which rod-like microfibrils are embedded in a protein matrix. The main structural proteins of the insect cuticle are sometimes known collectively as athropodin.

The proteins differ in amino-acid composition from one species to another, but those of related species show some resemblances. Of special insects is the distinctive protein, resilin, being an isotropic three-dimensional network of polypeptide chains held together by stable covalent cross-links and showing remarkable rubber-like properties.

Chitin is insoluble in water, dilute acids and organic solvents, but dissolves with decomposition in concentrated mineral acids and sodium hypochlorite. It has a

specific gravity of about 1-4,a refractive index of about 1.55 and is best detected by the Van Wisselingh test.

Hardening of the cuticle:

Hardening of cuticle is primarily a consequence of cross-linking between the protein molecules so that they form a rigid matrix, sclerotin. The process of cross-linking is called tanning or sclerotisation and the cuticle is then said to be sclerotised. The process involves the production of N-acetyldopamine, which forms links between the protein chains. There are also various waxes ad other lipids secreted by the oenocytes and responsible for waterproofing of the cuticle. These form a layer of a complex mixture of hydrocarbons with smaller amounts of fatty acids, alkyl esters and other constituents.

Exp. (1): Qualitative chemical test for chitin in the cuticle (Van Wisselinsh's test)

Materials:

- Glycerin or paraffin oil bath,
- saturated solution of KOH,
- 90 and 80 % alcohol,
- 0.2% iodine solution in 1% H_2SO_4,
- 3% acetic acid,

- 10% H_2SO_4,
- test tubes, thermometer 200 °C, glass slides and
- cockroaches.

Preparation of 0.2 % iodine solution:

1.6 g I_2 crystals + 43.4g KI in one liter of 1 % H_2SO_4.

Procedure:

1- Place small fragments of the insect integument in a saturated solution of KOH in a test tube.
2- Immerse the tube in a glycerin or paraffin bath and heat up to 150 to 160 °C for 20 min (or longer if the material is not decolorized).
3- Transfer the fragments to a glass slide, wash with 90 % and 50 % alcohol and then with water.
4- Flood the slide with I_2 solution.

Control:

1- Repeat the same steps on other fragments of the integument, but without heating with KOH solution.
2- Compare the colour developed with that of the experiment.

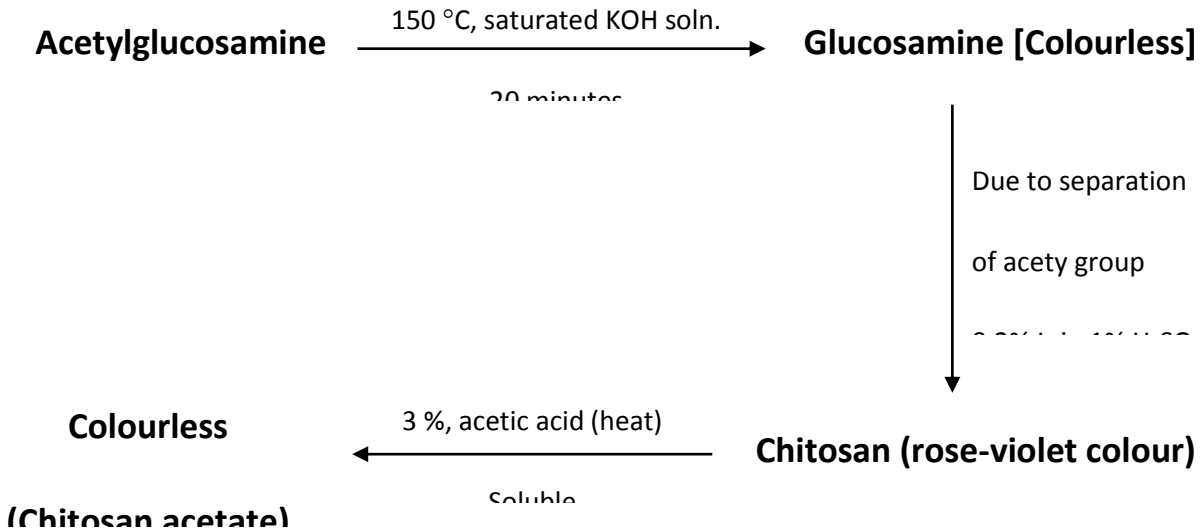
Observation:

Intense rose-violet colouration in the experiment is produced, which is disappeared by adding 3% acetic acid or 10 % H_2SO_4 on boiling.

Comment:

This is due to the separation of the acetyl group from chitin leading to the production of chitosan which gives a rose-violet colour with I_2 solution in 1%

H_2SO_4. Chitosan prepared in this way is soluble in 3% acetic acid on boiling forming chitosan acetate (colourless). It may be dissolved also by boiling in 10% H_2SO_4 and if left to stand at 70 °C, spherites of chitosan-sulphate separate out which are colourless.



Exp. (2): Qualitative chemical test for protein in the cuticle (Biurt test)

Materials:

- 10% NaOH,

- very diluted $CuSO_4$ solution,
- test tubes and cockroaches.

Procedures:

1- Place small fragments of the insect integument in 10% NaOH in a test tube and heat.
2- Leave the tube to cool in room temperature and then odd one drop of diluted $CuSO_4$ solution.

Control:

1- Repeat the same steps on other integumental fragments, but without heating with 10% NaOH.
2- Compare the developed colour with that of the experiment.

Observation:

The colour of the solution of the experiment becomes dark blue (purple).

Comment:

The principle of the experiment based on the change of colour due to copper salts in an alkaline medium link with protein chains forming a complex mixture of copper containing proteins with dark blue (purple) colour. The intensity of the produced colour is proportional to the protein concentration in the integument.

Exp. (3): Removal of the waxy elements from the cuticle by chemical treatment.

Materials:

- Paraffin oil,
- methyl alcohol,
- embryo dishes,
- stereoscopic microscopes and
- flies.

Procedure:

1- Submerge an insect in a mixture of paraffin oil and methyl alcohol (1:1) in an embryo dish.
2- Observe under a stereoscopic microscope.

Control:

Repeat the same experiment but immerse the fly in each of the used solutions separately.

Observation:

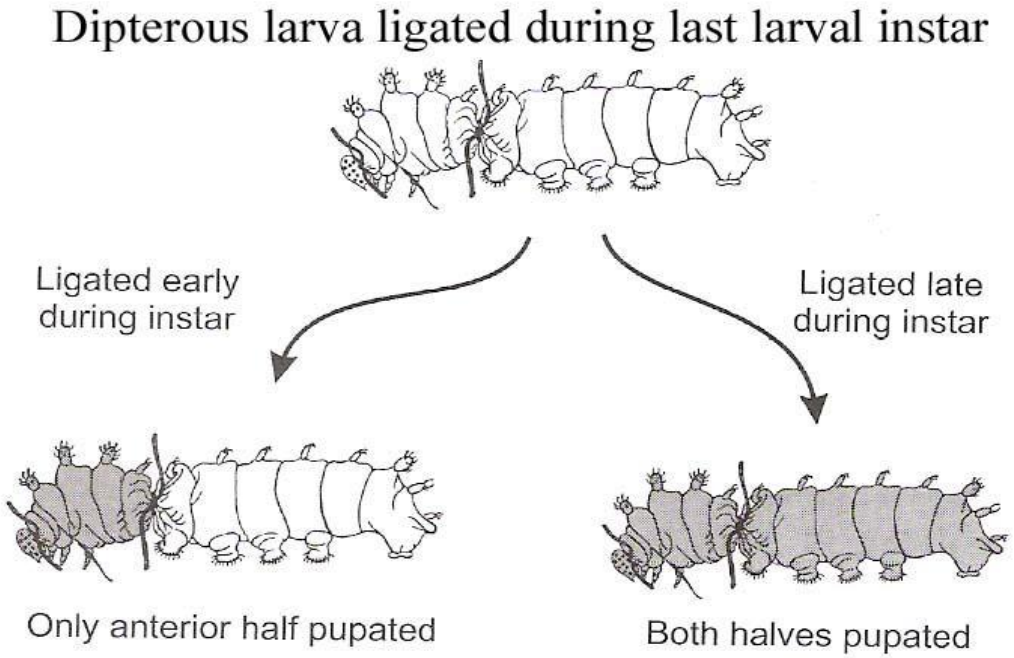
After a few seconds, hundreds of small bubbles can be seen coming out of the cuticle. This phenomenon lasts for a few minutes.

Comment:

The expanding bubbles are droplets of water which expand by the intake of the alcohol, after removal of the waxy layer from the cuticle by the action of paraffin. This phenomenon ends after few minutes, when all of the water is drown out of the insect body.

Exp. (4): Moulting (ligation experiment)

- Mature *Musca* or *Sarcophaga* larvae when their gut become empty are ligated by a cotton thread on the anterior third of the body. Examine such larvae 24 hr later.
- Ligation of the last larval instar at the middle portion (between the thoracic region and the abdomen) before the critical period, leads to pupation of the anterior half only.
- The critical period is the time at which the hormone was released into circulation from the anterior portion "i.e. PTTh and prothoracic gland".
- Ligation of the last larval instar after the critical period, leads to pupation of both halves.
- If no change in the larval body, this means that the larva was dead before the secretion of the hormone, due to miss handling.



Lab. 3 **Date / /**

Digestive System and Digestion

Insects feed on a very wide variety of animal, vegetable and dead organic materials. Food recognition involves sensilla on the mouthparts, which are distributed so as to monitor the food before and during feeding.

Once the insect has recognized its food as suitable it starts to feed. The process by which food is ingested varies considerably. Food is pushed back from the pharynx by the pharyngeal pump, aided by cibarial pump when this is present and subsequently passed along the gut by persistaltic movements. The movement of food from the crop to midgut is controlled by the proventriculus and its associated sphincter. In *Periplaneta* the rate of emptyring of the crop is inversely proportional to the concentration of food in it, so that food in high concentration is passed back to the midgut very slowly. In midgut the passage of food is aided by the peritrophic membrane which, as it moves down the gut, will carry the enclosed food with it. The movements of the hindgut are primarily

concerned with the elimination of undigested material. The time taken by food in its passage through the gut is very variable.

Digestion process begins once food swallowed by secretion of amylase and invertase enzymes to digest sugary materials found in food. Enzymes concerned with digestion are present in the saliva and in the secretions of the midgut cells.

An **extract** is a substance made by extracting a part of a raw material (such as chemical substance), often by using a solvent such as ethanol or water. **Homogenate** is a material that has been homogenized (especially tissue that has been ground and mixed).

Enzymes in the salivary glands:

The saliva serves to lubricate the mouth parts, more is produced if the food is dry, and is also contain enzymes which start digestion of the food. The presence of particular enzymes is related to diet, but an amylase, converting starch to sugar, and an invertase, converting sucrose to glucose and fructose are commonly present.

Exp. (1): Detection of the presence of amylase:

(A) Starch solution

Materials:

1- Cockroaches (*Periplaneta* and *Blattella*]
2- Diluted I_2 solution (5cc. of conc. 0.2 % I_2 + 95 cc H_2O 0.01% I_2).

* Conc 0.2% I_2 [1.6 gm I_2 crystals + 43.4 gm KI in liter H_2O].

3- 1 % NaCl [1gm of NaCl in 100 ml H_2O].
4- Water extract:
- 1: 10 salivary gland: 10 cc. H_2O or [10 heads of cockroaches: 50 cc. H_2O].
- or [1-10 Zymogen : 10 cc. H_2O].

5- 2 % starch solution (hot) [2 gm starch + 100 ml H_2O steer then heat].
6- 4 % Agar solution (hot) [4 gm. Agar + 100 ml of boiled H_2O then heat].
7- Glass slides covered with starch agar mixture [2% starch solution (hot) + 4% agar solution (hot) 1:1].
8- Petri-dishes, cotton, and test tubes.

Procedures:

1- Put 1 cc. of extract in a test tube.
2- Add 2 cc of 2% starch.
3- Add 2 drops of 1% NaCl (activator).
4- Leave in incubation (37 °C) for 1-2 hrs.
5- After the incubation period add 1cc of diluted I_2.

Control:

Repeat the same steps but by using boiled extract.

Observation:

The first mixture of the un-boiled extract will become colourless while the second tube becomes blue.

Comment:

The spot of the experiment drops becomes clear due to digestion of starch by amylase. While that of control remains blue due to the break-down of amylase through boiling.

Exp. (1): Detection of the presence of amylase:

(B) Starch-agar slide

Procedures:

- Prepare the water extract [1-10 head of cockroach: 50 ml water or 1-10 zymogen: 10 cc H_2O.
- Prepare the starch-agar slide [one drop of the agar-starch mixture is placed on the glass slide then smear it by using another glass slide].

1- Place a drop of the extract at one end of the starch-agar slide together with a drop of NaCl solution (1%) (activator).

2- Keep the slide in a Petri-dish in which a small piece of cotton, soaked with water, is placed (to keep a moist atmosphere).
3- Incubate at room conditions about one hour.
4- After the incubation period put one drop of diluted I_2 solution on the water extract.

Control:

- A control experiment is better carried out simultaneously, by repeating the same steps but after boiling the extract.
- A drop of this extract is placed at the other end of the starch agar slide, with a drop of 1% NaCl (activator).

Compare the spots left in place of both the experimental and control drops.

Observation and comment:

If amylase is present the spot of the experimental drop will be clear due to digestion of starch by amylase. The spot of the control drop remain blue due to the break-down of amylase through boiling so starch remain as it is and react with I_2 giving the blue colour.

Exp. (2): Determination of the achromatic point of amylase:

Procedures:

1- Put 5cc of 2% starch solution in a test tube.
2- Add 5cc. of distilled water and 2cc of a salivary extract (in which amylase has been proved to be positive).

3- Mix well and incubate at 37 °C.

4- At (5) minutes intervals transfer 0.5 cc of the digestive mixture to a tube containing 1 cc of 0.01 % I_2 solution with 2 drops of acetic acid.

Control:

- Repeat the same experiment but with boiled extract.

Observation:

Notice the gradual break-down of starch as indicated by the change in colour of the mixture of starch-iodine from dark blue to colourless.

- Determine the time at which the mixture becomes colourless.

Comment:

Starch $\overset{\text{Amylase}}{\rightleftharpoons}$ higher dextrine (dark blue)

H. dextrine $\overset{I_2}{\rightleftharpoons}$ erythro dextrine (blue)

e. dextrine $\rightleftharpoons$ acrodextrine (faint blue)

a. dextrine $\rightleftharpoons$ maltose (colourless)

Amylase is specific to digest starch and converts it to maltose passing by the previous steps.

Exp. (3): Detection of the presence of invertase (sucrase)

Procedures:

1- Add 2cc of 1% sucrose solution to 1 cc of the extract.
2- Then directly from this solution take 1 cc and add it to a test tube containing 1 cc of Fehling solution (0.5 cc Fehling A + 0.5 cc Fehling B) (control).
3- Heat gently till boiling and observe of any precipitate is formed.
4- Repeat the experiment with Fehling solution after incubation of the rest part of the digestive mixture at 37 °C for 1-2 hours (experiment).

Observation:

Red ppt. in the experiment, while the control remains blue.

Comment:

The formation of red ppt. indicates the presence of reducing sugars as a result of sucrose hydrolysis by invertase.

Invertase

Sucrose ⇌ Glucose + Fructose

Feh. A,B

Glucose ⇌ Red ppt..

Reduction

Observe that invertase is present in the salivary extract of *Blattella* only so, for the preparation of the extract, take 15 heads of *Blattella* in 50 cc dist. H_2O.

Lab. 4 **Date** / /

Enzymes in the alimentary canal

Materials:

1- Cockroaches

2- Test tubes, Petri-dishes, and cotton

3- Phosphate-buffer solution at:

a- pH (4.5) : 9.078 gm $K(H_2PO_2)_4$ in 1 liter dist H_2O.

b- pH (7) : 6 gm Na_2HPO_4+4gm KH_2PO_4 in 1 liter dist H_2O.

c- pH (8.5) : 11.1876 gm Na_2HPO_4 in 1 liter dist H_2O.

4- Porcelain, Spot-plate

5- Olive oil

6- Phenolphethaline (Ph Ph) 0.5 gm Ph.Ph. $\rightarrow$ 100 ml 50 % alcohol.

7- 0.1 N NaOH

Equivalent wt = 40 in 1000 cc H_2O $\rightarrow$ give 1 normal

$\therefore$ 4gm in 1000 cc H_2O $\rightarrow$ 0.1 N

8- 1 % maltose solution (10 gm maltose $\rightarrow$ liter dist H_2O)

9- **Barfoed's** reagent

13.3 gm crystalline Cu acetate in 200 cc. H_2O.

Filtrate, then add 1.8 cc glacial acetic acid.

10- Water extract

6 midguts : 10 cc dist H2O (add 2 drops of touluene) as a preservator.

1:10 zymogen: 10 cc dist H_2O.

11- Toluene.

12- Photographic plates.

Exp. (1): Test for the presence of amylase and invertase as in case of the salivary glands.

Exp. (2): Test for the presence of maltase and lactase

Procedures:

1- Add 2 cc of the extract to a test tube containing 2 cc of 1% maltose (or lactose) solution.

2- Directly boil 1 cc of the digestive mixture with 1 cc of **Barfoed's** reagent (control).

3- Incubate the rest of the mixture at 37 °C for 2 hours.

4- Test for the products of digestion by **Barfoed's** reagent (to 1 cc of **Barfoed's** solution add 1 cc of the solution to be tested drop by drop, heat vigorously for 10 minutes.

Observation:

Red ppt. in the experiment. while the control remains blue.

Comment:

The formation of red ppt. indicates the presence of reducing sugars as a result of maltose (or lactase) which is hydrolyzed by maltase (or lactase).

Maltase

Maltose ~~2 glucose~~

Lactase

Lactose ~~glucose +~~ galactose

Glucose reduces the copper ion from **Barfoed's** reagent and transfers it from blue to red.

Exp. (3): Test for the proteolytic activity in the extract:

Procedures:

1- In a spot plate, mix 1 cc of the buffer solution at different pH values, each with 1/2 cc of the extract.
2- Pipette a drop of each of the resultant mixtures on the gelatin side of a photographic plate.
3- Leave the plates in a Petridis and incubate (for about 2 hours at room temperature) with them a piece of cotton soaked with water to avoid evaporation.
4- After the incubation period wash under strong running tap water.
5- **Control:** repeat the same experiment on a separate photographic plate but after boiling the extract for a few minutes.
6- Compare the effects of the extract at different pH values and with those in the control group.
7- Record your results.

Observation:

Gelatin side appears as white spot in the experiment.

The maximum activity occurs at pH …followed by pH … and then pH …

Comment:

The proteinacious material (gelatin) on the photographic plate is completely digested by the actions of proteolytic enzyme (protease) at the pH 4.5, pH 7 and pH 8.5 in the experiment leaving a clear spot in place of experimental drops.

$$\textit{Protein} \xrightarrow{\textit{Protease}} \textit{peptones + polypeptides + free amino acids}$$

The control experiment gives no change in the gelatin side as a result of the destruction of the enzymes by boiling the extract.

Exp. (4): Test for lipolytic activity in the extract:

Procedure:

1- Put 1cc of the extract in a test tube.

2- Add 1/4 cc of the olive oil, few drops of Ph.Ph.th and carefully add 0.1 N NaOH drop by drop to make the mixture alkaline (light pink colouration appear).

3- Incubate at 37 °C, shake from time to time.

Control:

Repeat the same steps in another test tube but with boiled extract.

4- Observe the difference in colour between the two tubes.

Observation:

- Colourless in the experiment, no change of colour of the control (pink).

Comment:

- Lipids (or fats) (olive oil) are decomposed to fatty acids and glycerin in the presence of the lipase, so the pink colour disappear due to the formation of fatty acids which neutralize the NaOH.

$$\text{Lipid} \xrightleftharpoons{\text{Lipase}} \text{fatty acids + glycerin}$$

$$\text{Ph. Ph.} \xrightarrow[\text{NaOH}]{} \text{Pink colour}$$

$$\text{Ph. Ph} \xrightarrow[\text{Medium}]{\text{Neutral}} \text{colourless}$$

Exp. (5): Estimation of pH values of the different regions of the alimentary canal:

Materials:

Procedure:

1- Dissect the cockroach on cold ice.
2- Separate the alimentary canal then grind it well keeping all on the cold ice.
3- Measure the pH value of the alimentary canal using litmus paper and write your notes.
4- Record down your own observations and comments.

Observation:

Comment:

Lab. 5 **Date / /**

Estimation of amylase activity by Hagedorn-Jensen method:

The amount of enzyme present in any mixture is expressed in terms of its activity as compared with a freely chosen standard. Either the time required for a given amount of enzyme preparations to bring about a definite degree of conversion of the substrate or the amount of preparations needed to bring about a definite degree of conversion in a specified time may be made the basis of comparison in, as much as, enzyme action is greatly influenced by the pH of the solution, by the presence of inorganic salts and activators, and by temperature. The speed of action must naturally also vary with the concentration of the enzyme and the concentration of the substrate. The effects of co-enzymes and of a proper concentration of electrolyte are of importance in many cases, as well as, the negative effects of inhibitors or "poisons". As digestion proceeds, the products of the reaction may have an inhibiting effect. It is important that the conditions in the digestion mixtures should be made as nearly identical as possible and that the enzyme, if in inactive form, should be properly activated.

To establish these limits, an experiment must be conducted. This experiment aims at the estimation of amylase enzyme activity at different temperature points, or different hydrogen ion concentrations. This activity was measured through estimating the amount of maltose sugar produced by the action of amylase on starch (substrate) through an enzyme-substrate reaction (Lampitt *et al*., 1947).

The amount of maltose sugar produced will be measured through its capability in reducing potassium ferricyanide K_3 [Fe (CN) $_6$] into pot. ferrocyanide K_4[Fe $(CN)_6$], when it is present in a basic medium.

Reduction refers to the process in which the substance acquires an electron, while the oxidation refers to a process in which the substance loses an electron

Exp. (1): Estimation of amylase activity at different PHs:

Materials:

1. Potassium ferricyanide solution.
2. Iodide sulphate chloride solution.
3. 3% Acetic acid solution.
4. 2% Starch solution.
5. Starch-NaCl solution.
6. Sodium thiosulphate solution.
7. HCl solution (1 N).
8. KIO_3 solution.
9. 0.2 M phosphate buffer solution.
10. Water extract of the midgut of cockroach 1:20, or water extract of zymogen 1:30.

Method:

In this experiment the amount of maltose sugar produced by amylase-starch reaction is estimated as shown in the following steps:

1-Prepare three groups of test tubes; each group consists of two tubes (one tube as a control, and the other as an experiment mix tube).

2-Put in the experiment mix tube 0.5 cc extract, 1.1 cc dist. H_2O, 0.4 cc buffer and 2cc 2% Starch solution.

Note: In one of the three groups of test tubes the buffer solution is of PH 4.5, in the other it is of PH 7 and in the last group it is of PH 8.5

3- Repeat the previous step for the control but with adding 0.5 cc dist. H_2O instead of the extract.

4- Incubate both tubes in a water bath at 37ºC for 15 minutes.

5- After incubation take 0.1 cc of the mix to new test tubes and then

add to each 2cc of potassium ferricyanide solution (make two replicates of the experiment and two for the control to ensure accurate results).

6- Add 11.9 cc dist. H_2O to the four tubes, then put them in a boiling water bath for 15 seconds.

7- After that pour the test tubes' contents into conical flasks and leave them to cool at the room temperature in a dark place to prevent the reverse reaction.

8- After cooling, add to each conical: 3 cc iodide sulphate chloride, 2 cc acetic acid and 0.5 cc Starch-NaCl.

Observation:

- A blue color appears.

9- Titrate against 0.005 N sodium thiosulphate ($Na_2S_2O_3$) till the blue colour becomes colourless.

10- Determine the volumes V_{C1}, V_{C2}, V_{E1}, and V_{E2}.

11- From the table provided obtain the amount of glucose in mg equivalent to the volume of the sodium thiosulphate used.

12- Subtract from each value of the experiment that of the control titration. This value represents the increase in mg glucose.

13- Convert the amount of increase of the mg glucose obtained to mg maltose as follows: **mg maltose = mg glucose x 100/75**, since the reducing power of 100mg maltose is equivalent to 75 mg glucose.

The mg glucose obtained in the control reflects the whole volume of pot. ferricyanide which was not reduced at all, because no reaction was carried out in the control tubes "no starch digestion", in contrast with the experiment tubes where a certain amount of $K_3[Fe(CN)_6]$ was not reduced.

So when we estimate the amount of $K_3[Fe(CN)_6]$ in the two tubes we find that it is larger in the control one than that of the experiment. The difference between the two tubes gives the amount of $K_3[Fe(CN)_6]$ reduced by the sugar, which in turn represents the enzyme activity at this PH.

The mechanism of reaction:

1. $K_3[Fe(CN)_6] + \text{sugar} \rightleftharpoons K_4[Fe(CN)_6] + \text{acid}$

Pot. ferricyanide — Pot. ferrocyanide

This reaction is reversible, in which pot. ferrocyanide is easily oxidized by atmospheric O_2 into pot. ferricyanide, and to prevent reversion $ZnSO_4$ is added to reaction mix.

2. $2\,K_4[Fe(CN)_6] + ZnSO_4 \longrightarrow 2\,K_2Zn_3[Fe(CN)_6] + 2\,K_2SO_4$

Complex compound

The rest of potassium ferricyanide which was not reduced by the sugar is estimated by adding the mix of acetic acid and iodide sulphate chloride (zinc sulphate + pot. Iodide + NaCl) as shown in the following equations:

3. $K_3[Fe(CN)_6] + 3CH_3COOH \longrightarrow 3CH_3COOK + H_3[Fe(CN)_6]$

Hydroferricyanic acid

4. $3CH_3COOH + KI \longrightarrow CH_3COOK + HI$

Then, hydrogen iodide reduces hydroferricyanic acid to hydroferrocyanic acid, and I_2 is liberated.

5. $2\,H_3[Fe(CN)_6] + 2\,HI \longrightarrow 2\,H_4[Fe(CN)_6] + I_2 \uparrow$

Then titrate against 0.005N $Na_2S_2O_3$ till the blue color, which is produced due to liberation of I_2, becomes colorless and determine V_{C1}, V_{C2}, V_{E1}, and V_{E2}.

6. $2\,Na_2S_2O_3 + I_2 \longrightarrow 2\,NaI + Na_2S_4O_6$

Sod. iodide Sod. tetrathionate

The volume of $Na_2S_2O_3$ is proportional with I_2 and consequently with $K_3[Fe(CN)_6]$. So, the volume of $Na_2S_2O_3$ titrated in the experiment is less than that used in the control.

PH	Control	Mean	mg glucose (C)	Exp.	Mean	mg glucose (E)	E-C mg glucose
PH 4.5	V_{C1} V_{C2}	$(V_{C1}+ V_{C2}) / 2$	From the table	V_{E1} V_{E2}	$(V_{E1}+ V_{E2}) / 2$	From the table	
PH 7	V_{C1} V_{C2}			V_{E1} V_{E2}			
PH 8.5	V_{C1} V_{C2}			V_{E1} V_{E2}			

Use the following equation to obtain mg maltose from the value obtained of mg glucose:

mg maltose = (E-C) x 100/75

Since the reducing power of 100 mg maltose is equivalent to 75 mg glucose.

Ml.	0.00	0.01	0.02	0.03	0.04	0.05	0.06
0.0	0.385	0.382	0.379	0.376	0.373	0.370	0.367
0.1	0.355	0.352	0.350	0.348	0.345	0.343	0.341
0.2	0.331	0.329	0.327	0.325	0.323	0.324	0.318
0.3	0.310	0.308	0.306	0.301	0.302	0.300	0.298
0.4	0.290	0.288	0.286	0.284	0.282	0.280	0.278
0.5	0.270	0.268	0.266	0.264	0.262	0.260	0.259
0.6	0.251	0.249	0.217	0.245	0.243	0.241	0.240
0.7	0.232	0.230	0.228	0.226	0.224	0.222	0.221
0.8	0.213	0.211	0.209	0.208	0.206	0.204	0.202
0.9	0.195	0.193	0.191	0.190	0.188	0.186	0.184
1.0	0.177	0.175	0.173	0.172	0.170	0.168	0.166
1.1	0.159	0.157	0.155	0.154	0.152	0.150	0.148
1.2	0.141	0.139	0.138	0.136	0.134	0.132	0.131
1.3	0.124	0.122	0.120	0.119	0.117	0.115	0.113
1.4	0.105	0.101	0.102	0.104	0.099	0.097	0.095
1.5	0.088	0.086	0.081	0.083	0.081	0.079	0.077
1.6	0.070	0.068	0.066	0.065	0.063	0.061	0.059
1.7	0.052	0.050	0.048	0.047	0.045	0.043	0.044
1.8	0.034	0.032	0.034	0.029	0.027	0.025	0.024
1.9	0.017	0.015	0.014	0.012	0.010	0.008	0.007

Millimeters 0.005N Sod. thiosulphate used and Milligrams glucose present*

Monometric methods for reducing sugars (Van Slyke and Hawkins, 1929)

0.07	0.08	0.09
0.364	0.364	0.385
0.338	0.336	0.333
0.346	0.314	0.312
0.296	0.294	0.292
0.276	0.274	0.272
0.257	0.255	0.253
0.238	0.236	0.234
0.249	0.217	0.215
0.200	0.199	0.197
0.182	0.181	0.179
0.164	0.163	0.161
0.146	0.145	0.143
0.129	0.127	0.125
0.144	0.110	0.108
0.093	0.092	0.090
0.075	0.074	0.072
0.057	0.056	0.051
0.039	0.038	0.036
0.022	0.020	0.019
0.005	0.003	0.002

Lab. 6
Date / /

Estimation of amylase activity "Hagedorn-Jensen method" *conti.*

Exp. (2): Estimation of amylase activity at different temperatures:

Method:

In this experiment the amount of maltose sugar produced by amylase-starch reaction is estimated as shown in the following steps:

1. Prepare three groups of test tubes; each group consists of two tubes (one tube as a control, and the other as an experiment mix tube).

2. Put in the experiment mix tube 0.5 cc extract, 1.1 cc dist. H_2O, 0.4 cc buffer and 2cc 2% Starch solution.

3. Repeat the previous step for the control but with adding 0.5 cc dist. H_2O instead of the extract.

4. Incubate the three groups of tubes at three different temp. points; one group at 15 ºC, the other at 37 ºC and the

last one at 45 ºC

5. After incubation take 0.1 cc of the mix to new test tubes and then add to each 2cc of potassium ferricyanide solution (make two replicates of the experiment and two for the control to ensure accurate results).

6. Add 11.9 cc dist. H_2O to the four tubes, then put them in a boiling water bath for 15 seconds.

7. After that pour the test tubes' contents into conical flasks and leave them to cool at the room temperature in a dark place to prevent the reverse reaction.

8. After cooling, add to each conical: 3 cc iodide sulphate chloride, 2 cc acetic acid and 0.5 cc Starch-NaCl.

- **Observation:**

A blue color appears.

9. Titrate against 0.005 N sodium thiosulphate ($Na_2S_2O_3$) till the blue colour becomes colourless.

10. Determine the volumes V_{C1}, V_{C2}, V_{E1}, and V_{E2}.

11. From the table provided obtain the amount of glucose in mg equivalent to the volume of the sodium thiosulphate used.

12. Subtract from each value of the experiment that of the control titration. This value represents the increase in mg glucose.

13. Convert the amount of increase of the mg glucose obtained to mg maltose as follows: **mg maltose = mg glucose x 100/75**, since the reducing power of 100mg maltose is equivalent to 75 mg glucose.

The mg glucose obtained in the control reflects the whole volume of pot. ferricyanide which was not reduced at all, because no reaction was carried out in the control tubes "no starch digestion", in contrast with the experiment tubes where a certain amount of $K_3[Fe(CN)_6]$ was not reduced.

So when we estimate the amount of $K_3[Fe(CN)_6]$ in the two tubes we find that it is larger in the control one than that of the experiment. The difference between the two tubes gives the amount of $K_3[Fe(CN)_6]$ reduced by the sugar, which in turn represents the enzyme activity at this temperature.

The mechanism of reaction:

1. $K_3[Fe(CN)_6]$ + sugar $\rightleftharpoons$ $K_4[Fe(CN)_6]$ + acid

Pot. ferricyanide — Pot. ferrocyanide

This reaction is reversible, in which pot. ferrocyanide is easily oxidized by atmospheric O_2 into pot. ferricyanide, and to prevent reversion $ZnSO_4$ is added to reaction mix.

2. $2\,K_4[Fe(CN)_6] + ZnSO_4 \longrightarrow 2\,K_2Zn_3[Fe(CN)_6] + 2\,K_2SO_4$

Complex compound

The rest of potassium ferricyanide which was not reduced by the sugar is estimated by adding the mix of acetic acid and iodide sulphate chloride (zinc sulphate + pot. Iodide + NaCl) as shown in the following equations:

3. $K_3[Fe(CN)_6] + 3CH_3COOH \longrightarrow 3CH_3COOK + H_3[Fe(CN)_6]$

Hydroferricyanic acid

4. $3CH_3COOH + KI \longrightarrow CH_3COOK + HI$

Then, hydrogen iodide reduces hydroferricyanic acid to hydroferrocyanic acid, and I_2 is liberated.

5. $2\,H_3[Fe(CN)_6] + 2\,HI \longrightarrow 2\,H_4[Fe(CN)_6] + I_2 \uparrow$

Then titrate against 0.005N $Na_2S_2O_3$ till the blue color, which is produced due to

liberation of I_2, becomes colorless and determine V_{C1}, V_{C2}, V_{E1}, and V_{E2}.

6. $2\ Na_2S_2O_3 + I_2 \longrightarrow 2\ NaI + Na_2S_4O_6$

Sod. iodide Sod. tetrathionate

The volume of $Na_2S_2O_3$ is proportional with I_2 and consequently with $K_3[Fe(CN)_6]$. So, the volume of $Na_2S_2O_3$ titrated in the experiment is less than that used in the control.

Temp.	Control	Mean	mg glucose (C)	Exp.	Mean	mg glucose (E)	E-C mg glucose
15 ºC	V_{C1} V_{C2}	$(V_{C1}+ V_{C2})$ / 2	From the table	V_{E1} V_{E2}	$(V_{E1}+ V_{E2})$ / 2	From the table	
37 ºC	V_{C1} V_{C2}			V_{E1} V_{E2}			
45 ºC	V_{C1} V_{C2}			V_{E1} V_{E2}			

Use the following equation to obtain mg maltose from the value obtained of mg glucose:

mg maltose = (E-C) x 100/75

Since the reducing power of 100 mg maltose is equivalent to 75 mg glucose.

Millimeters 0.005N Sod. thiosulphate used and Milligrams glucose present*

Monometric methods for reducing sugars (Van Slyke and Hawkins, 1929)

MI.	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	0.385	0.382	0.379	0.376	0.373	0.370	0.367	0.364	0.364	0.385
0.1	0.355	0.352	0.350	0.348	0.345	0.343	0.341	0.338	0.336	0.333
0.2	0.331	0.329	0.327	0.325	0.323	0.324	0.318	0.346	0.314	0.312
0.3	0.310	0.308	0.306	0.301	0.302	0.300	0.298	0.296	0.294	0.292
0.4	0.290	0.288	0.286	0.284	0.282	0.280	0.278	0.276	0.274	0.272
0.5	0.270	0.268	0.266	0.264	0.262	0.260	0.259	0.257	0.255	0.253
0.6	0.251	0.249	0.217	0.245	0.243	0.241	0.240	0.238	0.236	0.234
0.7	0.232	0.230	0.228	0.226	0.224	0.222	0.221	0.249	0.217	0.215
0.8	0.213	0.211	0.209	0.208	0.206	0.204	0.202	0.200	0.199	0.197
0.9	0.195	0.193	0.191	0.190	0.188	0.186	0.184	0.182	0.181	0.179
1.0	0.177	0.175	0.173	0.172	0.170	0.168	0.166	0.164	0.163	0.161
1.1	0.159	0.157	0.155	0.154	0.152	0.150	0.148	0.146	0.145	0.143
1.2	0.141	0.139	0.138	0.136	0.134	0.132	0.131	0.129	0.127	0.125
1.3	0.124	0.122	0.120	0.119	0.117	0.115	0.113	0.144	0.110	0.108
1.4	0.105	0.101	0.102	0.104	0.099	0.097	0.095	0.093	0.092	0.090
1.5	0.088	0.086	0.081	0.083	0.081	0.079	0.077	0.075	0.074	0.072
1.6	0.070	0.068	0.066	0.065	0.063	0.061	0.059	0.057	0.056	0.051
1.7	0.052	0.050	0.048	0.047	0.045	0.043	0.044	0.039	0.038	0.036
1.8	0.034	0.032	0.034	0.029	0.027	0.025	0.024	0.022	0.020	0.019
1.9	0.017	0.015	0.014	0.012	0.010	0.008	0.007	0.005	0.003	0.002

Lab. 7 **Date / /**

Excretion

Exp. (1): Test for the presence of uric acid by the Murexide Reaction.

Materials:

- Conc. HNO_3 nitric acid
- Roaches
- Conc. NH_4OH solution [1: 100]
- Porcelain plate
- Pipettes

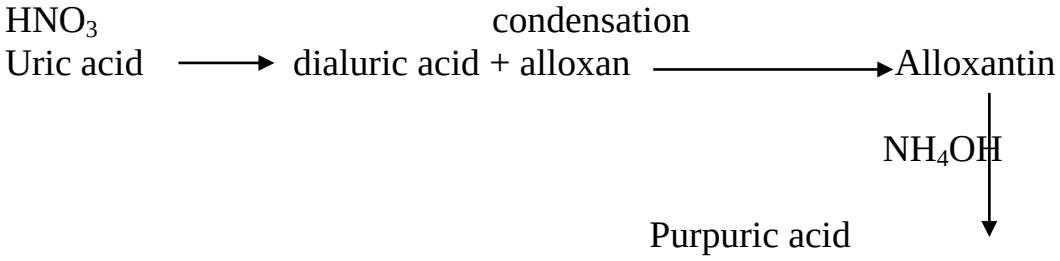
Method:

1- Dissect the roaches to get out the Malpighian tubules, Hind gut content and the fat body separately in the porcelain plate.
2- Add 2 drops of HNO_3.
3- Evaporate till dry in a water path (reddish or yellow residue will appear).
4- Cool.
5- Add one drop of NH_4OH.

Observation and comment:

Purple colour will appear

The purple colour resembles the Tyrian purple dye extracted from snail *Murex*, hence the name Murexide.



In this reaction, the uric acid is oxidized to dialuric acid and alloxan. These two substances condense to form alloxantin (red or yellow). Alloxantin, in turn,

reacts with NH_4OH to form purpuric acid. The purple colour is due to the formation of ammonium purpurate of murexide.

Lab. 8
Date / /

Circulatory system and circulation

Insects have an open blood system. The blood or haemolymph is contained in the general body cavity, and is the only extracellular fluid in the insect body makes up 15 – 75 percent of the volume of the insect. The amount and composition vary with species and its physiological conditions. It consists of fluid plasma and numerous blood cells or haemocytes.

The plasma, which contains about 85% water is usually slightly acidic and include inorganic ions, amino acids, proteins, fats, sugars, organic acids and other substances in variable amount. It serves primarily as a mean by which substances like food materials and hormones may be transported around the body, It may also provide a store of water, sugars and protein.

Several types of haemocytes occur and their number in circulation varies considerably from time to time. The number present in circulated blood of various insect species varies from about 1000 cells to about 100 times this number per microlitter haemolymph. Their functions include phagocytosis and encapsulation of foreign invading materials, wound healing and perhaps food storage since they contains inclusions of glycogen and fats.

Really this part of practical physiology course is divided into three main divisions:

Division 1: aims at studying of haemocytes in an alive insect like differential haemocyte count (DHC) and Total haemocyte count (THC).

Division 2: aims at estimation of some physical properties of the haemolymph like specific gravity (SG), blood volume (BV) and blood pH (BpH).

Division 3: aims at investigation of *in vitro* induction of insect immune reaction mechanisms.

Division I: Studying of haemocytes present in an alive insect

Exp. (1): Identification of different haemocyte types (Differential haemocytes counts):

General information:

- Price and Ratcliffe (1974) attempted to equate the terminology of haemocytes by different authors and recognized six main types of cells which have been designated in all insects studied, although there are many classification schemes of haemocytes are proposed.
- Classification of haemocytes based on cytological parameters (such as size of the cell, shape of the cell, size, shape and position of the nucleus and nuclear-cytoplasmic ratio) and chemical parameters like staining affinity.
- Haemocyte categories are:

1) **Prohaemocytes (PRs):**

Large round nucleus represent 75-95%, cytoplasm have no inclusion, small rounded or ovoidal cells. Cell cytoplasm is usually homogenous, or occasionally contains a few granule-like inclusions.stains deepely with Giemsa.

2) **Plasmatocytes (PLs):**

Variable in shape: polymorphic, spherical, ovoid or fusiform, often with pseudopodia.Nucleus is central round to ovoid and occupies about (52-69%) of

cell volume.Cytoplasm faintly stained and loaded with fine granules and numerous vacuoles.

3) **Spherule cells (SPs):**

Roundto ovoid in shape, or spindle-like. Nucleus round and small, which accounted for (26-37%) of the cell volume. Cytoplasm with round to ovoid spherular inclusions called "spherule" filling up the whole cell and masking the nucleus.

4) **Granular cells (GRs):**

Similar in appearance to prohaemocytes except that the nucleus is round to ovoid and represent about (42-51%) of the cell volume. Cytoplasm contains a largenumber of similar sized granules.

5) **Cystocytes (CYs):**

Small nucleus. Cytoplasm with black granules (Vacuoles).

6) **Oenocytoids(OEs):**

Relatively large cells, round, oval or spindle in shape. Nucleus small and represent about (21-40%) of the cell volume, and located in accenteric position. Cytoplasm
contain crystals or complex strand.

- The practical importance of study insect haemocytes is:
  (1) Focus attention on comparative immunology.
  (2) Understanding both innate and acquired resistance to make a wiser choice in the use of biological control agents.

(3) Understanding the immunological reaction mechanisms, e.g, immunorecognition,

Materials:

- Haemolymph
- Distilled water

- Clean slide
- Methanol
- Giemsa stain
- Canada Palsam
- Filter paper
- Porcelain plate

Procedures:

A) Haemocytes smear preparation:

1- Smear haemolymph on a clean glass slide, and then allow drying:

For preparing the smears, a drop of the larval blood had been obtained from the base of the 4th abdominal proleg of the larva by puncturing with a very fine insect pin. The haemolymph exuding from the puncture was touched with the surface of a clean glass microscopic slide then the drop was quickly smeared to a thin film on the slide by applying an edge of another slide across the 1st one at a 45 °C angle. The 1st slide was hold with the thumb and index fingers of the left hand and the narrow edge of the 2nd slide is placed in the drop and held there till the blood has spread across it. It then drawn slowly over the whole length of the first slide but without any pressure. After the blood is spread, it should be dried by being waved rapidly in the air to prevent undue shrinkage of the cell (Hunter and Bomford, 1959).

2- Fix haemocytes by methanol for 2 min:

The dry film was fixed in absolute methyl alcohol for 2 minutes.

3- Stain with Giemsa stain for 30 min.

Fixed cells were then stained with Giemsa's solution (diluted 1: 20 in distilled water) for 25-30 minutes

4- Wash with distilled water and then with tape water.

Wash several times with distilled and then with tap water (Gray, 1973).

5- Let the slide on filter paper to be dried.

6- Examine the slide (at least count 100 haemocytes) under microscope with oil immersed lens (100X).

7- Record your data in the following table, represent by a sketch drawing:

B) Haemocytes Count:

Type of haemocyte	**Size** (small, moderate, large)	**Shape** (Round, oval, spindle)	**Nucleus position** (Centric, accentric)	**Cell diameter**	**Nucleus diameter**	**No. of haemocytes**	**Nuclear cytoplasmic**	**Total haemocytes examined.**	**% haemocyte**

The smears were examined under oil immersion (X1000) and 100 cells from random fields were differentiated on each slide to determine the percentage of each type. Cell-shape, diameter, nuclear-cytoplasmic ratio and cytoplasmic inclusions were used for the classification of haemocytes using the classification scheme of Brehelin and Zachary (1986). The percentage of haemocyte types may be calculated by the formula:

$$\% = \frac{Number\ of\ each\ haemocyte\ type}{Total\ number\ of\ haemocytes\ examined} x100$$

PRs									
PLS									
SPs									
GRs									
OEs									
CYs									

Exp. (2) Total haemocyte s count (THC):

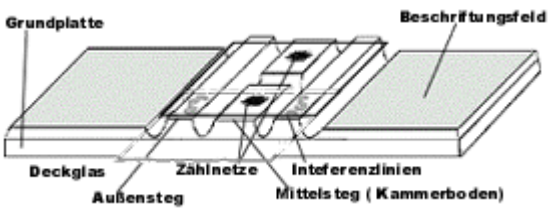
General information:

1- What is a counting chamber and what is it used for؟

A counting chamber is a precision measuring instrument made of special optical glass. It is used to count cells or other particles in suspensions under a microscope .Counting chambers are mainly used for blood analysis (counting leucocytes, erythrocytes, thrombocytes and insect haemocytes) and to count cells of liquor. Counting chambers are also used, however, to count bacteria and fungus spores.

2- Design principle

- All counting chambers have the same basic design principle (as seen in figure below)

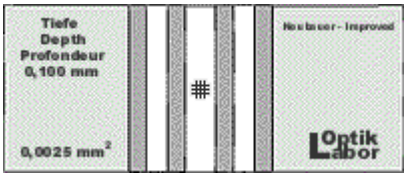


- There are four longitudinal grooves in the central third of a rectangular and thick base plate made of special optical glass. The grooves are parallel to the short sides of the base plate and the central third has the same size as the coverglass used with the counting chamber. The two larger external surfaces are unfinished and are used for marking purposes.
- The central support and the two external supports are ground smooth and polished. The surface of the central support is deeper than that of the two external supports. The counting nets are engraved in the central support (chamber base).
- If a cover glass is placed on the external supports, a capillary gap is produced between the underside of this cover glass and the central support of the counting chamber.

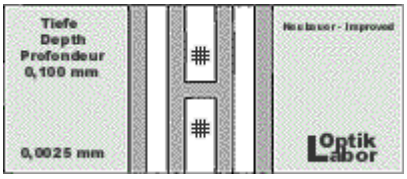
3- Design and identification of a counting chamber

You can differentiate between two types:

1- Single net ruling: middle support without division (one counting net)



2- Double net ruling: middle support with one division (two counting nets)



Furthermore there are two different designs of net:

- Standard: the counting net is directly engraved in the glass.
- Bright-lined: the chamber base is initially coated with rhodium and the counter net is then etched into the coating of rhodium. By shifting the

contrast, colour inversion under the microscope is possible so that the counter net can be viewed either in light or dark colouring.

4- Identification

The following details are printed on both un-worked surfaces of the counting chamber

- counting net system
- name and trademark of the manufacturer
- chamber depth in mm
- area of the smallest square in mm2

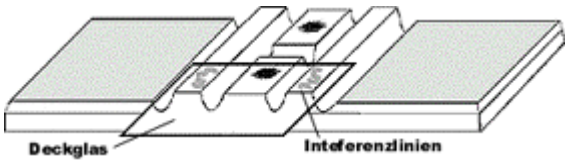
5- how to fill the counting chamber

5.1. Sliding on the cover glass

The external supports are to be moistened with distilled water and the cover glass then is gently pushed onto the counting chamber from the front.

Important: The cover glass is fragile!

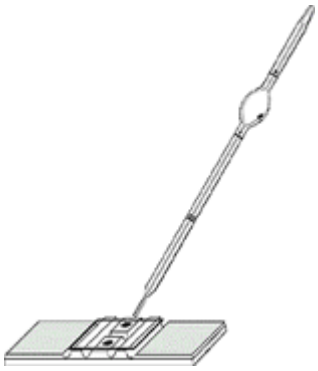
The formation of interference lines (Newton rings) between the external support and the cover glass shows that the cover glass is correctly positioned (as shown in fig. below)



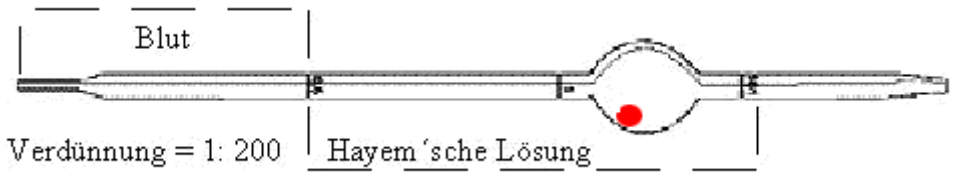
5.2. Feeding

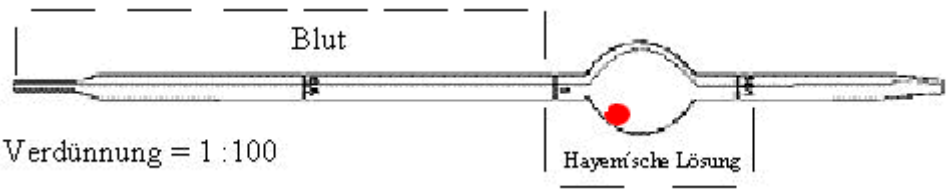
- Take a well mixed pipette from the shaker and dispose off the first few drops.
- Wipe the pipette dry on the outside and then hold it at an angle until a small drop has arisen at the tip of the pipette.

- This drop is then to be placed between the cover glass and the counting chamber.
- As a result of the capillary effect the gap between the cover glass and the chamber base fills up. Before the thinned blood solution can overflow at the edges of the chamber section, the tip of the pipette must be removed. If any air bubbles are visible or if the liquid has overflowed over the edges and into the grooves, the chamber must be cleaned and feeding must be started again



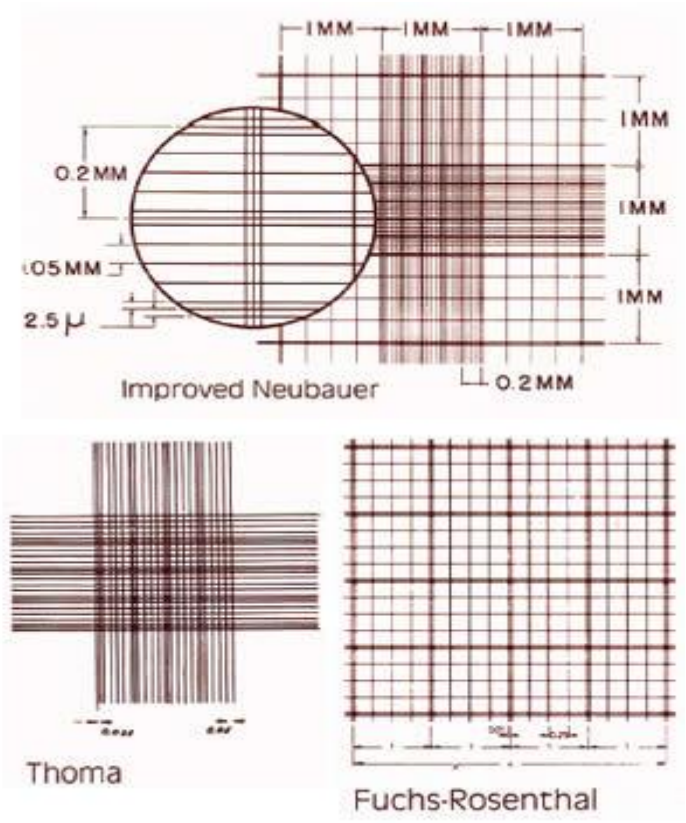
5.3 Haemocytometer:



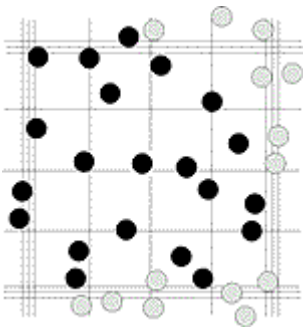


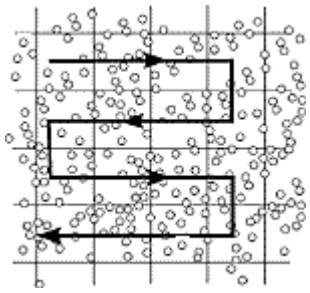
6- Counting the particles

6.1. Counting technique



- Counting assumes precise knowledge of the limit lines of the counting chambers used. These are shown in the illustration.
- To ensure that cells which are on or along the limit lines are not counted twice or are not missed during the count, certain rules have to be observed (eg. see illustration to the right).
- To ensure that cells which are on or along the limit lines are not counted twice or are not missed during the count, certain rules have to be observed (eg. see illustration to the right).





- The count should be started at the top left-hand corner and follow the direction shown by the arrow

6.2. Notes on counting

a) The trim of the capacitor on the microscope must be almost closed for all chamber counts.

b) The difference of the counter cells in the large squares and the group squares must not exceed 10 cells.

c) Double checks must be performed for all cell counts. After counting the two counting nets the bottom counting net is to be counted in the same way as a check. When doing this it is to be ensured that the chamber has not dried out. This can be prevented by filling the bottom chamber only shortly before the count and the counting after the sedimentation time.

d) The difference between the totals of the counts for the two counting nets must not exceed 10 cells. The average value of the counts is then used in the calculation formula or multiplied by the corresponding factor.

7- Calculation

Formula:

$$\textbf{Cells per mm}^3 = \textbf{No. of Cells/ Counted area (mm}^2\textbf{)} \times \textbf{Chamber depth(mm)} \times \textbf{dilution}$$

Example:

a) Haemocytes:

- 1. Counted cells 161 haemocytes
- 2. Counted area: four squares (= 4 x 1 mm^{2}) = 4 mm^{2}
- 3. Chamber depth 0.1 mm
- 4. Dilution 1:20

Haemocytes per 1 mm^{3}= 161/4 mm^{2}×0.1mm×1/20=161×20/4×0.1=8050 cells.

8- How to clean the counting chamber

Immediately after completing the count the cover glass is to be removed and the counting chamber has to be cleaned with water or (if necessary) with a mild cleaning solution. After- wards, the chamber is to be dried with a soft cloth or rinsed with acetone.

Materials:

- Thomas counting slide
- Haemocytometer
- Alcohol and ether
- Diluting fluid
- Gasterophilus larvae
- Porcelain plate.

Methods:

1- Clean H-shaped slide and cover with ether and alcohol (the correct position of cover is indicated by appearance of Newtons rings).
2- Press the cover gently on the slide.
3- Cut the coxa of larvae; allow haemolymph to flow out in porcelain plate.
4- Drow the haemolymph by haemocytometer till mark (l) then drow the diluting fluid to mark (ll).
5- Introduce some of haaemocytometer content on the slide, then examine number of cells.
6- Check the data you obtain and complete the data in the following page:

To determine the total haemocyte counts, 3 or 4 drops of haemolymph were allowed to flow on a clean glass slide as previously described in (DHCs). The haemolymph was quickly drawn up to the 0.5 mark of a Thoma White Blood Cell diluting pipette and immediately diluted to 11 mark with the diluting solution (NaCl-4.65g, KCl-0.15g, $CaCl_2$-0.11g, crystal violet-0.05 gm and acetic acid -1.25ml/ liter distilled water). The first 3 drops were discarded to avoid errors (Jones, 1967a and Shapiro, 1967). The mixture was dispersed to both chambers of the counting slide. After about 2 minutes, the total numbers of cells recognized in 64 small squares of the four corners were counted and cells recognized in 64 small squares of the four corners were counted and multiplied by a factor of 50 to get the number of cells per cubic millimeter. Duplicate counts were made with each sample to check counting error (Patton and Flint, 1959). If either agglutination or poor distribution (a difference of more than 20 cells between any two squares) of haemocytes occurred, the sample was discarded (Wheeler, 1963). The count was repeated ten times for each time interval.

7- Calculations:

Dilution of blood = 1 : 20 ml

Side of each small square = 1/4 mm

Depth of each small square = 1/10 mm

Volume of each small square $= \frac{1}{4} x \frac{1}{4} x \frac{1}{10} = \frac{1}{160} mm^3$

Number of WBC's in 64 small squares = x

Number of WBC's /mm^3 $= \frac{x}{64} x \frac{1}{volume\ of\ \ each\ small\ square} x \frac{1}{Dilution}$

$= \frac{x}{64} x160x20\ WBC's / mm^3$

Exp. (1): Determination of the speed of heart beat

Materials:

1- Lepidopterous larvae.
2- Binocular microscope.

Procedures:

1- Fix the larvae under the microscope on a cork piece by pines and determine the place of the dorsal blood vessel.
2- Count the heart beat within 3 min for 3 different times.
3- Repeat this with other larvae of the same age and determine the mean of heart beat/minute.
4- Repeat the experiment in older larvae (instars) and compare the no of its heart beats with that of the early instar.

5- Treat the larvae with sub-lethal dose of D.D.T. and after half an hour determine the mean heart beat.
6- Put the larvae in hot environment for one hour and detect the mean heart beat.
7- Tabulate your results in a table.

No. of heartbeat	3rd instar	5th instar
Normal larvae. D D T- treated larvae. larvae at high temperature.		

Exp. (4): Estimation of the specific gravity of the haemolymph by copper sulphate method

Materials:

- Botles, Lepidopterous larvae, test tubes and stack solution of $CuSO_4$.
- **Stock solution:** 159.63gm $CuSO_4 \rightarrow$ 1 liter dist. H_2O stear then filtrate.

Method:

1- From the stock solution of $CuSO_4$ dilute several concentrations of different specific gravities as follows:
   a- For the specific gravity 1.020: 19 ml $CuSO_4 \rightarrow$100 ml dist. H_2O.

b- For the specific gravity 1.025: 24ml $CuSO_4 \rightarrow$100 ml dist. H_2O.

c- For the specific gravity 1.030: 29ml $CuSO_4 \rightarrow$100 ml dist. H_2O.

d- For the specific gravity 1.035: 34ml $CuSO_4 \rightarrow$100 ml dist. H_2O.

2- Then put each solution in a tube labeled.

3- Put one drop of the haemolymph in these tubes and observe its movement.

At the point at which the drop doesn't fall down, make another serial of dilution with lower differences between it and that one behind and repeat the dropping.

4- The drop at which the blood is hunged for 15-20 sec. without any move refers to the blood specific gravity.

Comment:

We use $CuSO_4$ because it has known specific gravity and also because it does not affect the blood of the insect (has no effect on the cytoplasmic membrane of the cells).

Exp. (5): Estimation of the blood volume by the direct method (the absorption method)

Materials:

- Beetles and larvae.
- Filter paper.

Method:

1- Detect the weight of the insect body = X`

2- Detect the weight of the filter paper strip = y.

3- Bleed the larva and absorb its blood with the filter paper weighted before.

4- Determine the weight of the filter paper and the blood absorbed y`.

5- Determine the weight of the larva after bleeding = X.

$\therefore$ the blood weight $= y` - y$

$= x` - x$

6- Repeat the above with other larva and determine the body ratio in each by:

$$\text{H-Blood ratio} = \frac{\text{wt. of blood}}{\text{wt. of the body}} \times 100$$

$$\text{B-Blood volume} = \frac{\text{wt. of blood}}{\text{specific gravity}}$$

Estimation of some physical parameters of larval haemolymph

Exp. (6): Estimation of haemolymph density:

The densities of haemolymph was determined ar 27 ± 2°C for normal and treated larvae after the different times interval (6,12,24,48 hours). The method followed is essential that of **Carrel *et al.* (1990)**. The larvae were immobilized by cooling in the refrigerator at 4 °C for 30 minutes. The 4^{th} abdominal prolegs were cut and the oozed blood was withdrawn directly into microcapillary tubes (haematokreet) calibrated at 1 μl which were weighed before they filled with the haemolymph. The filled tubes were quickly re-weight and the haemolymph density was calculated as the difference between the two weights. The haemolymph density was expressed as mg /μl. Ten measurements (ten tubes) were used for each time interval.

Exp. (7): Estimation of haemolymph volume:

Firstly, the blood density was determined as described above at different time interval. The blood weight was determined as follows: a piece of filter paper was weighted to determine its net weight; all the haemolymph of one larva was squeezed on this weighted filter paper. The haemolymph weight calculated as the difference between the two weights; and the following equation was adopted to evaluate the blood volume:

$$\text{Blood volume} = \frac{Blood\ \ weight}{Blood\ \ denisty}$$

The blood volume is expressed as μl / larva