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University of Baghdad
College of Science
Department of Biology

Advanced insect physiology

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الدراسات العليا / الدكتوراه / الفصل الثاني

أستاذ المادة

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Integument of insects.

Body wall or Integument of insect The outer most layer of insect covering of the whole insect body. It forms a composite structure which forms the skeleton of the insect body and ectodermal in origin. It provides area for muscle attachment; protection from desiccation, physical /mechanical injuries and shape, strength to the body and its appendages.

function of integument

Protection of internal organs and tissues

Protective barrier against entry of pathogens, parasites, and predators. Now also pesticides.

•determine the habit of the insect body (form, surface markings..)

•protects against harmful external effects (mechanical, physical, chemical and biological)

•keeps water, ion and thermal balance

•external skeleton (*exoskeleton*) providing places for muscle attachments within the body (*endoskeleton*)

•Other functions of the integument is forming of walls of fore- and hindgut , external genitalia ,trachea system ,sensory organs

Structure of the integument

1-basement membrane 2-epidermis (hypodermis) 3-procuticle 4-epicuticle.

1-Basement membrane

Basement membrane or basement envelope Mucopolysaccharide layer that is secreted by the epidermal cells, is penetrated by nerves and tracheae going to hypodermis, and is a selective barrier between hemolymph and epidermal cells. Hormones and other nutrients can pass through this selectively permeable layer to reach the hypodermal cells, main components: proteins, glycoproteins, collagen produced by the epidermal cells.

2- Epidermis

Structure of the epidermis:

It is an unicellular layer formed from polygonal cells which modifies in to cuboidal or columnar during the process of moulting. These cells consists of well developed nucleus and other cytoplasmic contents. Adjacent epidermal cells are held together by means of certain cytoplasmic processes. All the epidermal cells are glandular and secrete cuticle and the enzymes involved in production and digestion of old cuticle during moulting. The epidermal cells get differentiated in to following types based on the function they perform and may modify in to a) Dermal glands producing cement layer b) Trichogen cell producing hair like seta or trichome. c) Moulting glands secreting moulting fluid which digests the old cuticle d) Peristigmatic glands around the spiracles in case of Dipteran larvae

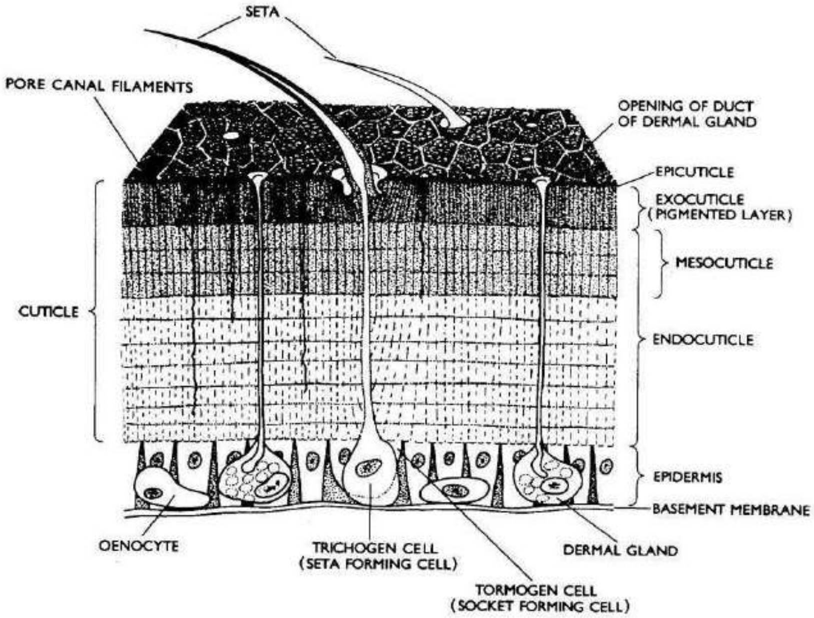


Figure 1**.** Diagrammatic cross-section of mature integument.

3- The cuticle

The cuticle, which is mainly produced by the epidermal cells, usually includes three primary layers, the inner procuticle,middle epicuticle, and outer cuticulin envelope (Figure 11.2). In older accounts of the integument the cuticulin envelope is treated as part of the epicuticle. However, Locke (1998, 2001) has argued that, because of its distinct origin, structure and functions, the cuticulin envelope should be considered separate from the epicuticle. All three primary layers are present over most of the body surface and in the cuticle that lines major invaginations such as the foregut, hindgut, and tracheae. However, the procuticle is very thin or absent, and certain components of the epicuticle may be missing, where flexibility or sensitivity is needed, for example, over sensory structures and the lining of tracheoles. Only the cuticulin envelope is universally present, except for the pores over chemosensilla.

The procuticle

The procuticle differentiated into two zones, endocuticle and exocuticle, which differ markedly in their physical properties but only slightly in their chemical composition**.**

Structure of the procuticule

A- **Exocuticle**: mostly rigid, hardened, dark coloured, pigmented upper layer Properties: produced before moulting; sclerotized and tanned after moulting; former exocuticle is removed during moulting, The exocuticle is the region of procuticle adjacent to the epicuticle that is so stabilized that it is not attacked by the molting fluid and is left behind with the exuvium at molting.

B- **Endocuticule:** generally soft, flexible, bright, pigment free lower layer Properties: produced after moulting; during next moulting will be dissolved and its ingredients will be utilized

In the exocuticle, adjacent proteinmolecules are linked together by a quinone molecule, and the cuticle is said to be tanned .The tanned (sclerotized) protein, which is known as "**sclerotin**," comprises several different molecules. Resilin is a rubberlike material found in cuticular structures that undergo springlike movements, for example, wing hinges, the proboscis of Lepidoptera, the hind legs of fleas , and the wing-hinge ligament that stretches between the pleural process and second axillary sclerite . Like rubber, resilin, when stretched, is able to store the energy involved.

Chemical composition of the procuticule:

Procuticle is composed almost entirely of protein and chitin. The latter is a nitrogenous polysaccharide consisting primarily of *N*-acetyl-*D*-glucosamine residues together with a small amount of glucosamine linked in a β1,4 configuration. In other words, chitin is very similar to cellulose, another polysaccharide of great structural significance, except that the hydroxyl group of carbon atom 2 of each residue is replaced by an acetamide group. Because of this configuration, extensive hydrogen bonding is possible between adjacent chitinmolecules which link together (like cellulose) to form microfibers.

Chitin:

Chitin is the major polysaccharide present in insects and many other invertebrates as well as in several microbes, including fungi. Structurally, it is the simplest of the glycos- aminoglycans, being a β(1→4) linked linear homopolymer of N-acetylglucosamine (GlcNAc, [C8H13O5N]n, where n >> 1). It serves as the skeletal polysaccharide of several animal phyla, such as the Arthropoda, Annelida, mollusks.

Chitin In insects, it is found in the body wall or cuticle, gut lining or peritrophic matrix (PM), salivary gland, trachea, eggshells, and muscle attachment points. In the course of evolution, insects have made excellent use of the rigidity and chemical stability of the polymeric chitin to assemble both hard and soft extra-cellular structures such as the cuticle (exoskeleton) and PM respectively, both of which enable insects to be protected from the environment while allowing for growth, mobility, respiration, and communication. All of these structures are primarily composites of chitin fibers and proteins with varying degrees of hydration and trace materials distributed along the structures.(Fig.2)

Proteins:

•40-80% of the dry weight of the procuticle

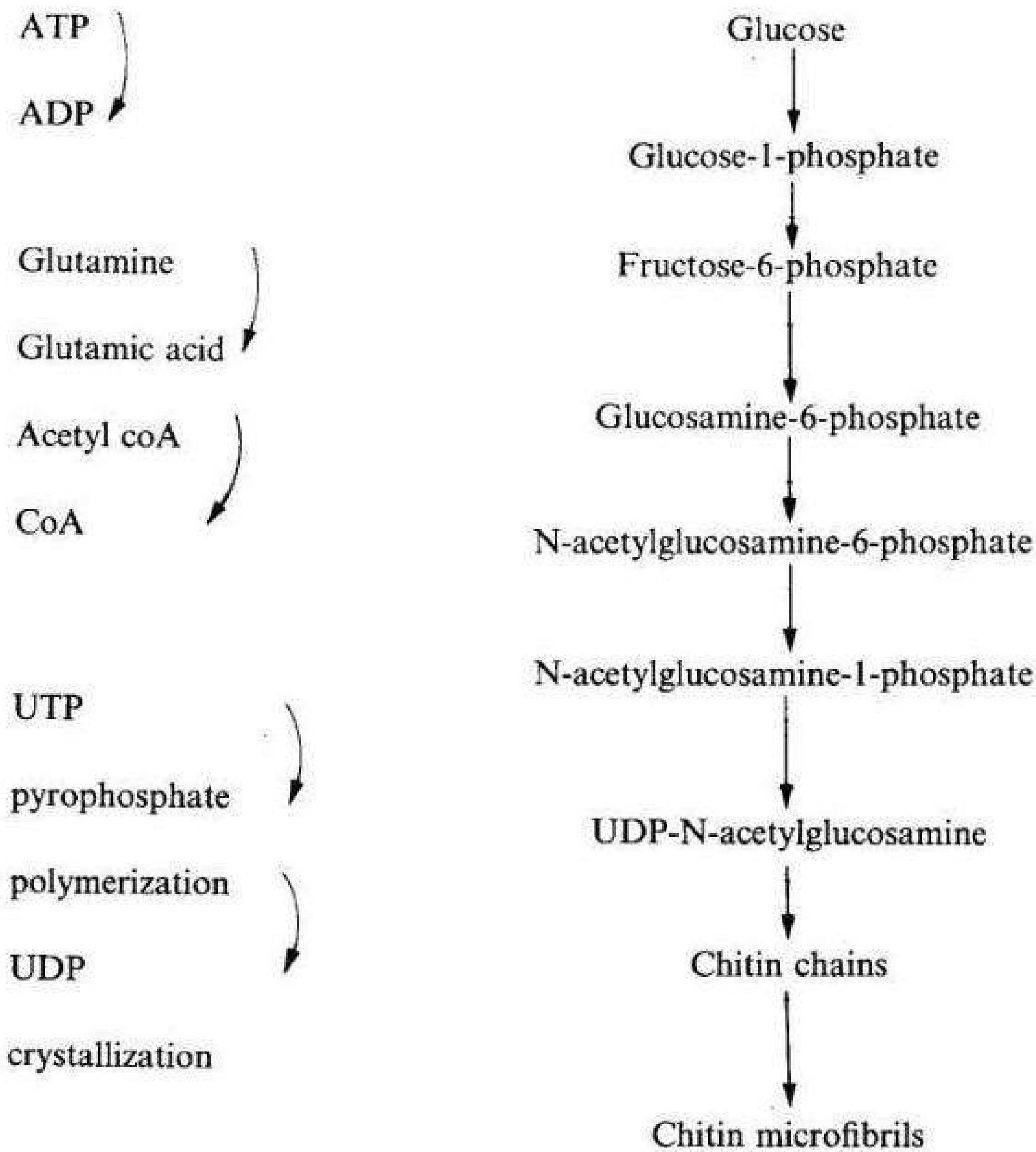
•lots of different proteins, more than 100; in a soft type of cuticle they are more hygrophilous

•many of them are soluble in water („*arthropodin*")

•they are able to make linkages with chitin chains („*chitino-proteins*"); linkage can be loose (H-bonding) or close (*quinone*)

•special protein: „*resilin*", which is rich in glycine amino acid and elastic like rubber, colourless and transparent.

Insect cuticle is composed of proteins and chitin. The cuticular proteins seem to be specific to the type of cuticle (flexible or stiff) that occur at stages of the insect development. The proteins found in the flexible cuticle of larva and pupa of different insects , also found in the soft endocuticle of adults insects as well as in other cuticular proteins including in arachnids. In addition, Many insect cuticle proteins also include a 35-36 amino acid .



Tanning of the exocuticle

•results in hard, rigid, resistant and generally dark exocuticle

Two main types of sclerotization

•quinone tanning - widespread way of tanning - it is generally accompanied by the darkening of the cuticle (melanisation) - proteins are linked to the rings

•β-sclerotization - cuticle remains bright - proteins are linked to the side-chains

Mineralization of the exocuticle

•infiltration and deposition of Ca-salts ($CaCO_3$, Ca-oxalate) heavy metals (Zn, Mn, Fe .

Summary of the quinone tanning process.

Epicuticle:

It is a thin outermost layer varying in thickness from 1-4µ. Chitin is absent in epicuticle. epicuticle consists of the following 4 layers.

Structure of the epicuticle:

1. Cement layer : It is secreted by dermal glands and is composed of lipoprotein. It protects the body from external damage. It is give the size and shape of insect body.
2. Wax layer: It is prominent layer , 0.25µ in thickness, consisting of long chain hydrocarbons, esters of fatty acids and alcohols. It is water proof layer preventing water loss from the body
3. Polyphenol layer: It is a non-static layer containing various types of phenols which are mainly used in the formation of the proteins. It is resistant to acids and organic solvents.
4. Cuticulin layer: It is an amber coloured thin layer over the surface of the epidermis which is strengthened by outer polyphenol layer.

Properties of certain layers:

1. **inner epicuticle** = inner homogeneous layer loose, thick (0.5-2 µm)
consist of tanned lipoproteins
2. **outer epicuticle** = cuticulin layer extends over the entire body surface
thinner (5-20 nm), compact, darker consist of lipoproteins and lipids
3. **wax layer**
• product of oenocytes
• consist of saturated aliphatic carbohydrates
• mostly aliphatic alcohols (12-50 C atoms)
• esters and free fatty acids (12-34 C atoms)

4. **cement layer**
• hard, protective layer, only in case of certain species
• generally thin and uncontinuous layer
• consists of mucopolysaccharids and lipids
• excretion of special epidermal gland cells (all other layers are the product of the epidermis!)

Pore canals (wax canals)

•tend upwards helically through the procuticle
• branch out within the epicuticle
•their cross-section can be round or flat, with1 µm in diameter

•there are 30-200 canals above one epidermal cell, which means 15000 canals/mm2

•they contain plasmafibers, wax and protein filaments

Process of cuticle formation, moulting (ecdysis) Phases

1) pre-ecdysis
2) ecdysis
3) post-ecdysis

1. Pre-ecdysis

•changes in the epidermis: active cell division (mitosis), growing of epidermal cells, cell density increases, intercellular spaces occur

•detachment of the old cuticle (apolysis): beneath the old cuticle ecdysial space or membrane occurs

•separation of the new inner epicuticle

•enzymatic dissolution of the old endocuticle (proteinases and chitinases for cuticle digestion)

•ecdysial liquid occurs, compounds gained from the old cuticle are recycled and utilized with an efficacy of 90%

•ecdysial liquid will be absorbed finally

2. Ecdysis

•special preparative behaviour: searching for safe places, handhold, or hand-climb, higher inner pressure by increase the volume of haemolymph, swelling air or water, muscle contractions

•The local increase in pressure in the anterior part of the body causes the old cuticle to split along a weak preformed ecdysial line where the exocuticle is thin or absent.

•Continuous swallow of air or water after moulting in order to stretch the new cuticle prior to tanning

•Leaving the old cuticle (exuvium)

3. Post-ecdysis

•expansion of the new cuticle, small grooves and folds will be flatten out

•scelrotization, tanning of the procuticle

•production of new endocuticle

•production of new wax and cement layer

•further expansion of soft cuticle parts (plasticization).

Moulting

The insect cuticle is hard and forms unstretchable exoskeleton and it must be shed from time to time to permit the insects to increase their size during growth period. Before the old cuticle is shed new one has to be formed underneath it ,this process is known as moulting

Moulting is a complex process which involve 3 process

1) Apolysis 2. Ecdysis 3. Sclerotization

1) Apolysis : [Apo = formation ; Lysis = dissolution] The dissolution of old cuticle and formation of new one is known as apolysis.
2) Ecdysis : The stage where the insect has both newly formed epi and procuticle and old exo and epicuticle is known as pharate instar. The ecdysial membrane starts splitting along the line of weakness due to muscular activity of the inner developing insect and also because of swallowing of air & water resulting in the distention of the gut. The breaking at the ecdysial membrane is also due to the pumping of blood from abdomen to thorax through muscular activity. After the breakage of old cuticles which is known as exuviae, the new instar comes out bringing its head followed by thorax, abdomen and appendages.
3) Sclerotization : After shedding of old cuticle the new cuticle which is soft, milky white coloured becomes dark and hard through the process known as tanning (or) sclerotization. The process of hardening involves the development of cross links between protein chains which is also known as sclerotization. This tanning involves the differentiation of procuticle in to outer hard exocuticle and inner soft endocuticle. Three types of hormones involved in the process of moulting which are as follows

JH : Juvenile Hormone :Produced from corpora allata of brain that helps the insects to be in immature stage.

MH : Moulting hormone: Produced from prothoracic glands of brain that induces the process of moulting

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Summary of Molting

Step1: Apolysis -- separation of old exoskeleton from epidermis

Step2: Secretion of inactive molting fluid by epidermis

Step3: Production of cuticulin layer for new exoskeleton

Step4: Activation of molting fluid

Step5: Digestion and absorption of old endocuticle

Step6: Epidermis secretes new procuticle
Step7: Ecdysis -- shedding the old exo- and epicuticle
Step8: Expansion of new integument
Step9: Tanning – sclerotization of new exocuticle

•produced by special epidermal cells
•bigger true hairs (macrotrichia, setae)
•produced by seta forming (trichogen) cells
•around them often socket constructing (tormogen) cells
•scales
•produced by special scale constructing cells
•other cuticle formations constructing sensory organs and other special organs.

Glands of the insect integument, dermal glands

Classification of glands according to their structure

• unicellular glands

• complex glands: a group of adjacent unicellular glands both can occur within the epidermis (intraepithelial) or under the epidermis (subepithelial)

•polycellular glands always occur subepithelial their structure is nodular or tubular.

Classification of glands according to their function

•wax glands, enamel glands, cement glands, lubricant producing glands, adhesion helping glands, pheromone producing glands, odour glands, stink glands, venom producing glands.

Colour of the integument

Pigmentary colours

(chemical or objective colours) Pigments are:
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•carotenoids (yellow, orange, red, violet) of plant origin

•flavonoids (yellow, yellowish white) of plant origin

•chromoproteins, pigment and protein complexes e.g.: insecto-verdin (green = blue bile+yellow charotenoid or pteridin pigments).

Physical colours

Physical colors are produced by scattering, interference, or diffraction of light though the latter is extremely rare. Most white, blue, and iridescent colors are produced using the first two methods. White results from the scattering of light by an uneven surface or by granules that occur below the surface. When the irregularities are large relative to the wavelength of light, all colors are reflected equally, and white light results.

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change and exchange of insects

•physiological (temporary): reversible process caused by movement of pigments particles

•morphological (permanent): irreversible process caused by ultimate deposition of pigment particles.

the inhibitors of chitin.

Chitin is an amino sugar biopolymer which together with a variety of proteins form elaborate structures such as insect cuticles and peritrophic membranes. This polymer serves mainly as a supporting element and resembles in this respect cellulose and collagen in plants and vertebrates, respectively. It was shown using chitin synthesis inhibitors that disruption of the polysaccharide formation resulted in malformed cuticles, lacking their normal exoskeletal properties and unable to withstand increased internal pressure during molting[1]. Since the cuticle is obviously of critical importance to survival of insects, chitin as a major structural element has been considered as a desired target site for selective pesticides.

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Lec. 3

Insect proteins

Structure protein

1- Contractile protein

They are found in muscle tissue. Including essential proteins such as actin and myosin, there are other proteins contribute in muscle contraction. These muscle proteins are similar to those in vertebrates that need calcium ions when they are active for example, the content of amino acids in actin protein in *phormia* insect similar to the content found in actin in the rabbit muscles actin. except for a slight increase in amino acid (glutamic acid).

Myosin, which is isolated from honeybees, is similar to that found in rabbits in the qualities and contents of amino acids.

The amino acid Myosin is forms 55% of muscle fiber proteins In one type of the insects. the other protein content in muscles is paramyosin its forms About 6.3-9.5 of muscular fibrosis. As well as have been diagnosed protein tropomyosin . The function of these two types of proteins is may be necessary to activate the enzyme ATPase It is important to release the energy needed to activate the muscles, The presence of these proteins is one of the differences between the chemical composition of the muscles of insects and vertebrates. It was also found that muscle proteins in insects need a lower level of calcium ion which is necessary for the activity of ATPase compared to vertebrates.

2- **Fibroin proteins**

ترجمهthese proteins secreted by special glands in different species of arthropods, they stored in these glands as a liquid and when it secret from gland convert to soled fiber, like fiber silk in *Bombyx mori*. Silk in its raw state consists of two proteins, **sericin** and **fibroin**, with a glue-like layer of sericin coating two singular filaments of fibroin called brins.

The fibroin protein consists of high rate of glycine and, lesser extent of alanine, their rate was 94% , That is, this protein is the polymerization of these two amino acids. While the rate of serine is 40% of the silk fiber

The cocconase enzyme secret from special gland in mouth part use to digest or open the cocoon to emerge the adult insect.

This enzyme digest the **fibroin** and **sericin** that with presence of potassium bicarbonate which regulates the pH to 8.3 (when the enzyme is active). The scientists found that the MH, and JH hormone has related in production and release this enzyme.

3- Collagens protein

It is a non-soluble fiber in the connective tissues such as muscle tendons, These fibers are not stretchable (non-expandable) and are inactive It, also the enzymes that dissolve the protein does not digest the collagens.

This protein transform into jelly-like when heated with water or with the alkaline compounds for a long time.

This protein is found in other animals. Its contents of amino acids are abnormal , about one-third of the content is the amino acid glycine, the remaining third consists of amino acid proline or hydroxyproline.

This protein is present in the elytra of insects and in the corpora cardiac gland , corpora allatum , PTG, FB , and muscles.

haemolymph proteins

The proteins found in insect blood are well studied by many researchers for several purposes including classification, and to find the function and activity in metabolic processes related to the differentiation.

Most of these proteins are created during the larval stage to be used later in the pupal stage to form adult tissues, so the scientists observed increasing in protein concentration at the middle of the last larval stage , the main proteins, which number of its reach 22% in some insects, are:

1- Carrier proteins

A- JH Binding protein

JH hormone is released from Ca gland to the heamolymph , In order to reach the target tissue it is found in order to reach the target tissues it was found to be associated with proteins or fatty proteins present in the heamolymph and when the hormone release the concentration of protein in the blood reach up to contact with 96% of the hormones molecules . The remaining four were found on the surface of other tissues without knowing the reasons.

These proteins protect the hormone from attacking of analytic enzymes.

Some lipoproteins were also diagnosed that is conducted with JH have a lower association, and a lower proportion of proteins alone

The source of these proteins which associated with JH is fat bodies.

B-Lipid- pending protein

This type of protein is associated with diglycerides which is release from Fat bodies to heamolymph , Where it was diagnosed in the blood of one type of insects genes *Hyalophora* Two types of these proteins its:

1- Diglyceride- carrying lipoprotein I

2- Diglyceride- carrying lipoprotein II

Where the first is associated with Diglyceride raised from FB, While the latter is associated with Diglyceride raised from another tissues , the rate of lipids in the first is estimated of 44% of its weight, and the rate of lipids in the latter is estimated 10% of its weight.

The second has been associated with polysaccharide It produces lipoglycoprotein where it is found at a high percentage in the female of *Hyalophora* ,compared with the male of this insect.

And at the pupa in the maturation stage, Its concentration decreases, it's found when ovaries are removed the concentration was not decreasing , This indicates its importance in the transfer of both proteins, carbohydrates and fats from heamolymph to ovareols in the ovaries, for the purpose of maturation of ovareols.

C- Xenobiotic –(binding proteins with foreign molecules)

There are lipoproteins associate with some foreign things which are present in the haemolymph of some insects like cockroaches, where it found that insecticide DDT and Alderin has been associated with two type of protein one of it is more associate with DDT more than alderin, and the other one has the same associate with both of pesticides These proteins may be important in the elimination of pesticide poisons.

D- Hemoglobins

Hemochlobin has been diagnosed in a few number of insects including Diptera like *Chironomus* and *Gastrophilus* ,and three insects of Himeptera . This protein is present in the haemolymph and is characterized by hemochlobin in the vertebrates throw apoprotein , the lengths of protein chains and sequencing of amino acids in insects apoprotein differ from those in vertebrates , while the Hemegroup is similar in all of them. This protein forms about 40% of insect blood proteins. The function of the hemoglobin in *Chironomus* is to store the oxygen and use it when needed. and may be acts as buffers. This protein is created in fat bodies and is released into the haemolymph to combine with oxygen.

2- Storage proteins

At the end of the last larval stage in insect *Calliphora erythrocephale* about 60% of total dissolved proteins is specific protein of this insect called calliphorin protein, it is a storage protein the insect use it to grate the special adult protein. This protein is created in fat bodies and is released into the haemolymph ,when the activity period of FB is end in the synthesis of proteins, an important part of the proteins in the haemolymph are re-absorbed by the FB cells to be clear Crystalline protein balls in cytoplasm of FB cells, This is one of the characteristics of last larval stage before becoming a pupa. At the stage of pupa Fat bodies begin to analyzed to release storage protein. Then convert to amino acids , the insect use it in create the special protein of the adult. The characters of this protein is has large amount of amino acid tyrosine and phenylalanine , so this protein is considered important in hardening the cell wall.

the other storage proteins is lipovitellin found in blood of many insect female pupa and It is present at a very low concentration in blood of male pupa . This protein is important in the process of vitellogensis and found that its synthesis in FB was under control of hormone JH. This protein after released to the blood enter to the ovaries by process called pinocytosis. the non-deposition of this protein inside the ovaries is not considered mature Even it has been fertilized Do not hatch eggs because the embryo non- develop.

3- Peptide hormones

There are hormones of a protein nature create created from epithelial cells in to the blood at certain stages, these hormones are Brain hor., Bursicon hor., diuretic hor., Hyperglyeamic hor., Hypoglyeamic hor., Adipokinetic hor.

4- Enzymes of haemolymph

There are many enzymes in haemolymph of insects , for example, oxidation ,reduction and analytical enzymes but most of these enzymes are related to insects are:

A- Trehalase enzyme

This enzyme analyzes the trehalose into two molecules of glucose , this enzyme is present in the haemolymph of insects through presence of trehalose, two type of these enzymes have been diagnosed:

a- Isozyme A

Occur in the midgut and haemolymph , optimal pH for its activity is 4.5 , Its molecular weight is 117.000- 115.000 , It is freely available in cytoplasm of the cells.

b- Isozyme B

Occur in the head , muscles , and rectal buds , optimal pH for its activity 5-5.5 Its molecular weight is 78.000- 90.000, it is present associate to the outer surface of the mitochondria .

B- JH esterase enzymes

There is JH esterase enzymes in the blood of insects, Specialized in analytic of hormone JH . it's found in haemolymph of *Mandusa sexta* (last larval stage). There are two types of these enzymes.

1- General esterases

This enzyme disappears in the haemolymph when freely and bond molecules of JH are dissolved . It is present at all stages of the larva , It also attacks another esters compounds.

2- JH specific esterses

This enzyme disappears in the haemolymph when freely and bond molecules of JH with protein are dissolved . it is present in high concentration on the fourth day of last larval stage of *Mandusa sexta*. These enzymes regulate concentration of JH in the haemolymph before transformation to pupa. Whereas the reduce of JH concentration is necessary at this stage to metamorphosis.

Lec. 4

Structure protein

4- Contractile protein

They are found in muscle tissue. Including essential proteins such as actin and myosin Other protein contents contribute to muscle contraction. These muscle proteins are similar to those in vertebrates that need calcium ions when they are activeFor example, the content of amino acids in actin protein in phormia insect similar to the content found in actin in the rabbit muscles actin. except for a slight increase in amino acid glutamic acid.

Myosin, which is isolated from honeybees, is similar to that found in rabbits in the qualities and content of amino acids.

The amino acid Myosin is forms 55% Of muscle fiber proteins In one type of the insects. the other protein content in muscles is paramyosin its forms About 6.3-9.5 Of muscular fibrosis. As well as have been diagnosed protein tropomyosin . The function of these two types of proteins is The function of these two types of proteins may be necessary to activate the enzyme ATPase It is important to release the energy needed to active the muscles, The presence of these proteins is one of the differences between the chemical composition of the muscles of insects and vertebrates. It was also found that muscle proteins in insects need a lower level of calcium ion which is necessary for the activity ATPase compared to vertebrates.

5- **Fibroin proteins**

Fibroin is an insoluble protein present in silk created by spiders, the larvae of *Bombyx mori*, other moth genera as *Antheraea*, *Cricula*, *Samia* and *Gonometa*, and numerous other insects. Silk in its raw state consists of two proteins, **sericin** and **fibroin**, with a glue-like layer of sericin coating two singular filaments of fibroin called brins.[1][2]

The fibroin protein consists of layers of antiparallel beta sheets. Its primary structure mainly consists of the recurrent amino acidsequence (Gly-Ser-Gly-Ala-Gly-Ala)$_n$. The high glycine (and, to a lesser extent, alanine) content allows for tight packing of the sheets, which contributes to silk's rigid structure and tensile strength. A combination of stiffness and toughness make it a material with applications in several areas, including biomedicine and textile manufacture.

Fibroin is known to arrange itself in three structures, called silk I, II, and III. Silk I is the natural form of fibroin, as emitted from the *Bombyx mori* silk glands. Silk II refers to the arrangement of fibroin molecules in spun silk, which has greater strength and is often used in various commercial applications. Silk III is a newly discovered structure of fibroin.[3] Silk III is formed principally in solutions of fibroin at an interface (i.e. air-water interface, water-oil interface, etc.).

6- Collagens protein

It is a non-soluble fiber in the connective tissues such as muscle tendons, These fibers are not stretchable These fibers are non-expandable and are inactive It also does not affect the enzymes that dissolve the protein, This protein transform into jelly-like when heated with water or with the base for a long time This protein is found in other animals. Its contents different of amino acids like About one-third of the content is the amino acid glycine The other third consists of amino acid proline or hydroxyproline This protein is present in the elytra of insects and in the

Corpora cardiac gland , corpora allatum , PTG, FB , and muscles.

haemolymph proteins

The proteins found in insect blood are well studied by many researchers for purposes including classification, knowledge, the function and activity in metabolic processes related to the differentiation , Most of these proteins are created during the larval stage to be used later in the pupal stage to form adult tissues, so we observe the increased concentration of proteins at the middle of the last larval stage , the main proteins, which number of it in some insects was about 22% of protein , this protein is:

5- Carrier proteins

E- JH Binding protein

A JH hormone is released from Ca gland to the heamolymph , In order to reach the target tissue it is found In order to reach the target tissues it was found to be associated with proteins or fatty proteins Present in the heamolymph , And when it starts the concentration of protein in the blood up to enough to contact 96% of the molecules of the hormone. The remaining four were found on the surface of other tissues without knowing the reasons.

These proteins are protecting the hormone attacking it's from analytic enzymes.

Some lipoproteins were also diagnosed that is conducted with JH have a lower association, and a lower proportion of proteins alone

The source of these proteins that are associated with JH is fatty bodies.

F- Lipid pending protein

This type of protein is associated with diglycerides which is release from Fat bodies to heamolymph , Where it was diagnosed in the blood of one type of insects genes *Hyalophora* Two types of these proteins its:

3- Diglyceride- carrying lipoprotein I

4- Diglyceride- carrying lipoprotein II

Where the first is associated with Diglyceride raised from FB, While the latter is associated with Diglyceride raised from another tissues , the proportion of fat in the first is estimated of 44% of its weight, And the proportion of fat in the latter is estimated 10% of its weight.

The second has been associated with polysaccharide It produces lipoglycoprotein Where it is found at a high percentage in the female of *Hyalophora* ,compared with the of this insect.

And at the pupa In the maturation stage(vetelogenesis) Its concentration decreases, it's found when ovaries are removed concentration was not decreasing , haemoThis indicates its importance in the transfer of both proteins, carbohydrates and fats from heamolymph to ovareols in the ovaries, for the purpose of maturation of ovareols.

G- Xenobiotic –binding proteins

There are lipoproteins associate with some foreign things which are present in the blood of some insects like cockroaches, where it found that insecticide DDT and alderin has been associated with two type of protein one of it is more associate with both pesticides(DDT and alderin) and the other one has the same associate with both of pesticides These pesticides may be important in the elimination of pesticide poisons.

H- Hemoglobins

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occur in the head , muscles , and rectal buds , optimal pH for its activity 5-5.5 Its molecular weight is 78.000- 90.000, it is associate to the outer surface of the mitochondria .

D- JH esterase enzymes

There is JH esterase enzymes in the blood of insects, Specialized in analytic of hormone JH . it's found in haemolymph of *Mandusa sexta* (last larval stage). There are two types of these enzymes.

3- General esterases

This enzyme disappears when molecules of JH Which is found freely in the haemolymph of insects are dissolved . It is present at all stages of the larva , It also attacks another esters compounds.

4- JH specific esterses

This enzyme disappears when molecules of JH Which is found freely and connected with protein in the haemolymph of insects are dissolved, It is in high concentration on the fourth day of last larval stage of *Mandusa sexta*. These enzymes regulate concentration of JH in the haemolymph before transformation to pupa. Whereas the reduce of concentration JH is necessary at this stage to metamorphosis.

Lec. 5

Insect Carbohydrates

In general, the metabolism of carbohydrates in insects is similar to that found in vertebrates, However, the differentiation of metabolites in insects can be distinguished from vertebrates in the following points:

1- The presence of chitin in the outer structure of the insect body wall.

2- The presence of trehalose in high concentration in the insect.

3- presence of glycerol- 3- p cycle in insects.

4- The carbohydrates play an important role in the ability of insects to tolerate extreme cold

Role of carbohydrates in reproductive of insect.

Carbohydrates are essential for female and male reproductive systems As well as embryo growth in the egg.

In male reproductive system in *Apis mellifera* the most of carbohydrates are present in the testis. the rate of glucose and fructose were 83% in the testis.

Carbohydrates, trehalose and amino acids contribute to supply the energy to the sperm in the vesicles, and spermatheca in the females.

in female reproductive system the carbohydrates is essential In the maturation process of oocytes which called **vitellogenesis**, also in formation of glycoaminoglycans which contribute in constructing vitilline membrane and chorion.

In vitellogenesis process the carbohydrates, lipids and proteins where accumulate in oocytes which is important in the essential requirements of nutrition in development of embryo. The glycogen is the largest part of carbohydrates in the yolk of the eggs and usually synthesis from glucose and trehalose which is source in the fat bodies and haemolymph, Where they begin to acculmate in pharate adult stage in the eggs eight times more than fat body.

The polysaccharides are associated with the yolk protein vitellogennin in the eggs, Thus forming Complex compounds which is called clycoprotein

Which are considered to be storage molecules to Carbohydrates and proteins in the insects eggs and in some insect Make up 77% of the dry weight of the egg.

During embryonic development, the main source of energy production in the eggs are carbohydrates and lipids, the carbohydrates are used first and then lipids. the glycogen are convert to glucose and trihalose. the percent of trehalose was 90% found in the early stages of embryonic development, While the glucose ,mannose ,fructose , mannitol and glycerol was the lowest percentage of the total free sugars,(thats In one type of the locusts).

As well as the role of glycogen in energy saving during embryonic development ,it has other functions:

1- Provide glucose for the synthesis of chitin.
2- Synthesis the glycerol and sorbitol especially in species that enter the embryonic diapauses.

The role of carbohydrates in diapauses and the ability of the insect to tolerate the cold (cold hardiness).

diapauses is a genetically controlled condition in the life of insects , there are some behavioral adaptations(for example, choosing the appropriate place to spend diapause period), before entering the insect to diapauses status. Respiration rate decreases significantly (to less than 2% of Respiration before diapauses).

Most of insects in the diapause period depend on triglycerides, glycogen , trihalose and a little of proteins and amino acids to energy provided and conservation of various metabolic processes, where these compounds are stored in fat body's and haemolymph before beginning the diapauses period.

The glycogen and trehalose were used firstly to supply the energy and then synthesis the glycerol and sorbitol to contribute increased ability to resist the cold, these compounds distributed throughout the different side of body.

it's found in the pupa diapauses of cabbage butterfly *Pieris brassicae* the trehalose contributed as an anti-cold resistant compounds , where this compound was found in large amount (about 12% of dry weight) Compared with the rate of glycogen which was 4% . this is compared with the rate of 6% and 10% respectively in the un diapauses pupa and that's mean the diapauses pupa preserve adequate amount from trehalose Until the end of the diapauses period and its used it after diapauses.

The source of glycerol and sorbitol compound is glycogen and trehalose where glycerol is synthesis from the intermediate compound which is result of anaerobic oxidation , glycolysis of glucose .its dihydroxy acetone phosphate.

(reduction)

Dihydroxyacetone phosphate + NADH-------------→ Glycerol -3-p+NAD

↑

(Glycerol -3-p Dihydroxyacetonase)

(reduction) Glycerol -3- p + H^2O-----------------→ Glycerol + p

↑

(phosphatase)

The second most common compound is sorbitol:

(reduction)Glucose -6- p + NADPH --------→ sorbitol --6- p + NADP

↑

(dehydrogenase)

sorbitol --6- p + H^2O --------------→ sorbitol + P

↑

(phosphatase)

These compounds reduce the point of freezing in insects, maybe the glycerol effect on the sensitive enzymes to cold, and make them active always.

the other compounds that protect the insects from extreme cold are threitol.

Trehalose in insects

The first scientist who explain that the trehalose is very important and the main source to glucose in insects are Kalf and Wyatt (1956) when they isolate the trehalose from the hemolymph of silk worm *Bombyx mori* , Then it was identify in other types of insects.

Trehalose is common sugar in all types of insects , It is found in low concentrations in other types of organisms like bacteria, lichens, Fungi and nematodes , but it is not found in vertebrates.

The regulation of trehalose metabolism in insects

Trehalose is a non-reducing disaccharide comprising two glucose molecules. It is present as the main sugar in high concentration in haemolymph (blood) of in insects. The synthesis of trehalose in the fat body (an organ analogous the function of liver in vertebrates) is stimulated by neuropeptides (hormones), released from the corpora cardiaca, a neurohaemal organ associated with the brain. The peptides cause a decrease in the content of fructose 2,6-bisphosphate in fat body cells. Fructose 2,6-bisphosphate, acting synergistically with AMP, is a potent activator of the glycolytic enzyme1,6-phosphofructokinase and a strong inhibitor of the gluconeogenic enzyme fructose 1,6-bisphosphatase. This indicates that fructose 2,6-bisphosphate is a key metabolic signal in the regulation of trehalose synthesis in insects. Trehalose is hydrolysed by trehalase enzyme. The activity of this enzyme is regulated in flight muscle, but the mechanism by which this is achieved is unknown. Trehalose in locust, flight muscle is a glycoprotein bound to membranes of the microsomal fraction. The enzyme can be activated by detergents in vitro and by short flight intervals in vivo, which indicates that changes in the membrane environment modulate trehalase activity under physiological conditions.

Trehalose hydrolysis

Trehalose hydrolysis by trehalase enzyme to tow molecules of glucose .

This enzyme present in different parts of the body like fat bodies, muscles, salivary gland in larvae and adult of insects.

It has two forms

1- Freely in the cytoplasm of cell as in the mid gut.

2- Associated with membrane of cells in muscles.

Glycogen in insects

Glycogen is main store of sugars in insects , biosynthesis of glycogen in insects is the same in vertebrates, in insects store in fat bodies , flying muscles , gut, and another organs like nerve cells.

The consentration of glycogen In thes tissues is differs according to the stage , activities, and the type of insect , example. The flight muscles in grasshopper content high concentrations of glycogen , but in tse tse fly the concentration of glycogen is high in gut because the gut is the main source of glycogen in tse tse fly, and the flight muscles are oxidized the amino acid proline to obtain the energy.

Glycogen hydrolysis

Glycogen is convert to glucose by phosphorylation which results the G-1-p, This is due to the presence of glycogen phosphorylase enzyme.which was found in fat bodies and muscles, this enzyme help in break down the bonds between glucose molecules in glycogen to formed G-1-P

Glycogen + P ------------→ G-1-P + (1,4 &glucosyl)

↑

(Glycogen phosphorylase)

This interaction is controlled by hyperglycemic hormone. After that G-1-P convert to trehalose -6-P then to trehalose.

Hormonal regulation of carbohydrate metabolism

The rate of convert glycogen ,trehalos and glucose in insects was controlled by hyperglycemic hormone (insulin) or glucagon-like hormone, whereas the source of first hormone is Cc, and the source of second hormone is Ca.

Where the researcher Steele found in 1961 the first hormone leads to increases the concentration of trehalose in haemolymph with decrease of glycogen concentration in the fat bodies at the same time, this hormone release from Cc gland to haemolymph and effect on CAMP in fat bodies then activate glycogen phosphorylase enzyme and release trehalose , which is then released into the haemolymph.

While the second hormone released from Ca gland to haemolymph where it causes a decrease of trehalose in haemolymph, In addition, it helps to convert trehalose to glucose and increases the permeability of flight muscles cells to glucose then help in energy production in it. (Fig.1).

Lec. 6

Insect Diapause

Diapause is defined as a period of suspended development in insects and other invertebrates during unfavorable environmental conditions. And it's an important adaptation in many insect species enabling them to sustain in regions which would otherwise be unfavorable for permanent habitation, and to maintain high numbers in an environment which might support only a low population.

Diapause can be categorized according to seasonal variations as aestivation (summer diapause) or hibernation (winter diapause); or according to life stages as an egg (embryonic), larval, nymphal or adult (imaginal, reproductive) diapause; or according to the influence of environmental factors as obligatory and facultative diapause. Depending on the species,

Obligate diapause is often universal, resulting in strictly univoltine life cycle with every individual in every generation experiencing diapause, irrespective of any possible environmental variations.

facultative diapause occurs due to environmental variations and results in a multivoltine life cycle. In this life cycle, one or more generations in which few individuals enter diapause alternate with a generation in which all or nearly all the individuals enter diapause. But in a particular environment (usually near the limit of geographic distribution of a species), facultative diapause may result in a virtual univoltine life cycle in which most individuals in every generation enter diapause.

Incidence of Diapause

Diapause may occur in any stage of the life cycle of insects, such as **eggs, larvae, pupae or adults.** For example, silkworm moth (*Bombyx mori*) overwinters in embryo stage, just before segmentation. The gypsy moth (*Lymantia dispar*) enters diapause as a fully formed larva with hatching occurring immediately after diapause ends.

The stage at which diapause occurs is highly characteristic for each species. Moreover, there is no case known in which diapause occurs in more than one stage in the same life cycle. At the **egg** stage, it may begin when the embryo is still very young (e.g., *Gryllulus*, *Austroicetes*); when embryo is half-grown (e.g., differential grasshopper, *Melanoplus diflerentialis*); or when embryo is fully grown and apparently almost ready to hatch (e.g., red-legged grasshopper, forest tent caterpillar).

In **nymphs and larvae,** diapause may occur more often in the last instar than other instars. The incidence of diapause may be quite variable, not only from species to species, but also between different populations of the same species.

Embryonic Diapauses

The best-understood hormonal mechanism regulating embryonic diapause is based on the silkworm. In this species diapause intercedes early during embryogenesis, just before segmentation. The developmental fate of the embryo is determined by the presence or absence of DH, a neuropeptide secreted by the femal's subesophageal ganglion. In the presence of DH, the ovariole produces eggs that enter diapause, and when the hormone is not present the eggs develop without interring to diapause. Whether the female releases DH is dependent upon the photoperiod it was exposed to as an embryo. Thus, the females photoperiodic history dictates whether it will release the DH needed to influence the diapause fate of its progeny.

Larval Diapause

Larval diapause is occurs at any larval stage but its common at the end of larval life, just before the onset of pupation and metamorphosis, In the absence of ecdysteroids from the Ptg, the larva fails to start the next molt. The failure of the brain to release ecdysteroids can usually be directly attributed to the brain's failure to release PTTH.

In a number of species, JH may also play a role. For example, in the southwestern corn borer, *D. grandiosella*, the JH level remains high throughout diapause, and the diapause can be terminated only when the JH level drops. In some other species such as the European corn borer, *O. nubilalis*, the JH level is high in early diapause but then declines and remains low throughout the remainder of diapause. No role for JH is apparent in several other insects: the larval diapause of both the parasitic wasp, *Nasonia vitripennis*, and the blow fly, *Calliphora vicina*, can be explained strictly as an ecdysteroid deficiency.

Pupal Diapause

Pupal diapause is the consequence of a shutdown in the brain-PG. Thus, in the absence of ecdysteroids from the PG the progression of adult differentiation is stoped. At the termination of diapause, ecdysteroids are again released, stimulate adult development. In *H. cecropia* a period of chilling is required before the brain can stimulate the Ptg to release ecdysteroids. Pupal diapauses can usually be quickly end with an injection of 20-hydroxyecdysone.

Usually the absence of ecdysteroids can be attributed directly to a failure of the brain to release the neuropeptide PTTH needed to stimulate the Ptg to synthesize ecdysteroids, but in some insects, for example, *Helicoverpa zea*, PTTH is released shortly after pupation, but pupa fail to develop until the Ptg has been chilled adequately.

Adult Diapause

A decrease in JH synthesis is a key feature in the regulation of adult diapause. The corpora allata, the endocrine glands that synthesize and release JH, are characteristically small during diapause.

Injection of JH into a diapausing individual usually stimulate the ending of diapause. Conversely, the cutoff corpora allata from a non diapausing adult causes the adult to enter a diapauses like state. Measurement of the JH level also supports the idea that adult diapause is the consequence of a shutdown of the corpora allata: the titer of JH typically drops as the insect enters diapause and increases again when diapause is terminated.

It is the brain that regulates the corpora allata, and both nervous and humoral pathways are involved in its regulation. In the Colorado potato beetle, the brain exerts its control over the corpora allata by a humoral mechanism, but in the linden bug, Pyrrhocoris apterus, nervous control is also involved. Ecdysteroids may also be involved in some species. The ecdysteroid titer is nearly twice as high in Colorado potato beetles destined for diapause than in those that are not destined to enter diapause, and an injection of ecdysteroids can terminate adult diapause in *Drosophila melanogaster*.

Intensity of Diapause

Diapause is also immensely variable in its intensity; duration of diapause can be taken as a measure of intensity. Diapause lasts for 9-10 months in the temperate zones, and may persist for a year or more in less common cases. During diapause, most insects do not feed at all or, in the case of some larvae and adults, feed very little. This indicates that the insect must sequester sufficient food reserves in the pre-diapause phase to meet its metabolic needs during diapause and still have sufficient reserves remaining at the end of diapause to complete development and resume activity.

Diapause lasting more than a year is known as **prolonged** or **extended diapause** , and has been documented in 64 insect species. For example, yucca moth (*Prodoxus y-inversus*) adults emerge after 19 years of diapause as prepupae . Another example, where larvae had been in soil up to three years followed by emergence of wheat-blossom midges (*Cantarinia tritici* Kirby), whereas larvae of wheat blossom midge (*Sitodiplosis mosellana*) overwintered for 12 years in the soil before adult emergence. In some sawflies, diapause stage lasts for 3-4 years.

Diapause and Quiescence

Dormancy is a generic term for any state of naturally occurring ecological or evolutionary adaptations of arrested development, and usually accompanied with metabolic suppression. Diapause and quiescence form two different types of dormancies in insects. In general, insects commonly confront two types of major environmental stresses. **The first category includes** unpredictable, irregular, temporary and localized stresses epitomized by short periods of seasonally high or low temperatures, food scarcity and drought. In this case, survival depends on appropriate and immediate response by insect. This type of response leads to quiescence or migration to favorable places. **The second category of stress** includes regularly occurring, seasonal fluctuations in temperature, humidity, food, natural enemies and other competitors over a wide geographic area. This stress is kind of predictable pressure occurring in some specific pattern and insects take advantage of this predictability by responding through physiological and behavioral alterations for the forthcoming changes. These types of changes constitute diapause.

Diapause is quite distinct from quiescence, but at times it may be difficult to distinguish the two phenomena. Quiescence is common seasonal (phenological), long duration adaptation in the life cycles of many insects. Unlike diapause, quiescence is directly induced and terminated by surrounding environmental conditions, for example, low temperatures induce and high temperatures terminate the quiescence stage of insects. Extrinsic factors are involved in the onset and termination of quiescence, and these factors act directly on metabolic rate and that eventually results in either slow down or complete arrest of development. In univoltine species, normally only one stage of the life cycle enter quiescence with the onset of winter. On the other hand, in multivoltine species (e.g., blowfly in New Zealand), each life stage has a capability to survive in quiescence. Unlike quiescence, diapause is defined as a dormancy state with the characteristics including:

(1) Diapause is a seasonally specific adaptation, which persists for a certain minimum period of time, regardless of environmental fluctuations;

(2) Day length (photoperiod) and temperature have been reported to be involved in the timing and induction process of diapause;

(3) It occurs in only one species-specific stage in the life cycle of insects.

Diapause is not induced in direct response to unfavorable environmental conditions. It is “anticipatory”, which means that diapause occurs before the onset of winter while conditions still permit growth and development. Above given postulates clearly demonstrate that in diapausing insect, an internally operating “clock” mechanism is involved, which can measure day length and hence the season can be discriminated. In addition to the above given facts, it can also be said that diapause is under complex hormonal control, whereas quiescence is a function of temperature acting on the metabolic rates. These points are helpful to some extent for distinguishing these two dormancy states

Theories of Diapause

Many theories are out there to explain the process of diapause. There are different hypotheses for theories of diapause including hormone theory of diapause, ecological consequences of hormone theory, the stimulus which activates the neuro-secretory cells in species without diapause, evidence from ecology for the “food mobilization” hypothesis and the food mobilization hypothesis as discussed below.

Hormone Theory of Diapause

Most research proves that diapauses is controlled by the hormones and This does not mean that hormones are the main reason of the diapausebut the environmental condition around the insect are the main reason of diapauses.

4. Factors Affecting Diapause

There are number of environmental factors that can play a crucial role in completion of diapauses, such as temperature, photoperiod, light and water or moisture. Most studies on seasonal ecology of insects are mainly focused on temperature and photoperiod as major regulatory cues. Insect species exhibit distinctive characteristics that help them to go through diapause stage during unfavorable conditions. Sensitive period, insect stages, physiological expression and intensity are all species specific diapause inducing characteristics that are eventually under the genetic control. Environmental factors affect expression of diapause which varies profoundly within each generation of an insect population. Based on this variation in expression, diapause is divided into two categories: facultative and obligatory (discussed earlier).

a- Photoperiod

In general, the days are long in summer and short in winter, The increase and decrease in the length of the day in spring and autumn effect on diapauses , When the length of the day is short in the autumn This is stimulation that winter entry. And this stimulat some insects to enter diapauses, example pupa of *Acronyeta* sp which was does not enter diapauses when the lengh of the day During the larval stage more than 16hours.but its enter the diapouse when the lengh of the day during the larval stage less than 16hours . there is a critical photoperiod in the most of insects, In insects that grow and develop without entering diapouse in the long photoperiod called **long –day insects** , and insects that grow and developed without entering diapouse in the short photoperiod called

short –day insects . There is an intermediate species like *Euproctis* sp the state of larval diapouse in this insect's accure when exposure either to the long photoperiod day or to short photoperiod day

FIGURE 1 Photoperiodic response curves for pupal diapause induction in two populations of the flesh fly, Sarcophaga bullata, from Illinois and Missouri. Fly cultures were maintained at 25°C under the range of daylengths indicated, and the incidence of pupal diapause was recorded. The critical daylength in this case is 13.5 h of light/day.

b- Temperature

After photoperiod, temperature is considered as an important factor which influences dormancy in a number of ways:

(1) temperature is a major diapauses inducing factor in some species;

(2) it can modify insects response to diapause inducing photoperiods to varying degrees;

(3) in some species, it is important for diapause maintenance;

(4) it can be an active stimulus in termination of diapause .It plays a major role in regulating the rate of post-diapause growth . Hibernating insects have evolved a number of physiological and behavioral adaptations that help them tolerate extremely low temperatures. These adaptations are associated with diapause that helps them protect from subzero temperatures. Two main physiological mechanisms which determine insect’s overall tolerance to cold are: supercooling (resistance to freezing by lowering temperature of body below the freezing point of body fluids) and freezing tolerance (survival despite freezing of body fluids). Some insects have high super cooling points (poplar sawfly, *Trichiocampus populi*) which help them survive freezing at temperatures below supercooling point, while others with low supercooling points may be susceptible to subzero temperatures.

c- Moisture

Insects undergo periods of water stress during both hibernation and aestivation, as both high and subzero temperatures cause available water scarcity to the insects. Two main physiological mechanisms come into play during drought: resistance to desiccation and tolerance to water loss. For example, during aestivation, eggs of grasshopper (*Austroicetes cruciate*) showed both mechanisms. Desert species are mostly drought hardy, but species inhabiting areas with seasonal droughts show adaptations to drought conditions mostly associated with diapause. Diapause in drought conditions needs resistance to desiccation, which comes from depressed metabolism, lowered water content, high fat accumulation during diapauses and increased secretion of waxy coverings. Ground pearl (*Margarodes vitium*is) is a significant example, where wax coated encysted nymph can survive for more than 10 years under dry conditions. Impact of moisture on diapause development does not appear that simple from many studies done in the past, rather it is a complex issue to understand. Water and moisture have both positive and negative effects on diapause development. Flooding along with high humidity can endanger the development of offsprings and delay oviposition to post-rain season. Females of Mexican bruchid beetle (*Acanthoscelides obtectus*) spend April to November (wet period) in rolled dead leaves hanging from plants as in reproductive diapause phase. Due to positive effects of moisture, these beetles postpone their oviposition till mid-November to late-December, the time when rain fall is below 50 mm/month. For details on examples of insects affected by moisture check.

Diapause development evidently initiates at dry conditions; however, wet conditions (rain, water or moisture) are must for restoration of development and pupation. The tropical borers are one of the most known examples of diapause termination by rains. Sorghum stem borer larvae diapause in dry stalks for 6-8 months, and pupate in spring after the onset of rain. It has been proven by series of experiments that previous exposure to drought is obligatory for the stimulatory effect of moisture.

Both wet and dry conditions play an important role in diapause process. There are many examples (e.g., *Diabrotica* spp.) where complete diapause is followed by quiescence due to low moisture. Water content should be replenished to or above a critical level to resume post-diapause morphogenesis. After the temperature regulated diapause, larvae of wheat blossom midges (*Contarinia tritici* and *Sitodiplosis mosellana*) need high moisture for pupation. In eggs of Australian plague locust (*Chortoicetes terminifera*), the development of “the second quiescent stage”, which is morphologically indistinguishable from diapause stage, can not start without replenishing with 10% moisture, which was lost in diapause stage .

d- Food

Availability of food in nature can be an important seasonal cue for insects. Insects have evolved different ways to utilize alterations in food quality and quantity as factors governing dormancy . Food has been observed as primary diapause regulating factor for insects, mostly in aestivating insects. It can be a primary diapause regulating factor or can play role in interaction with temperature and photoperiod. In two Australian species, a collembola (*Sminthurus virdis*), and a mite (*Halotydeus destructor*), the aestival diapause is influenced by food quality where maturity of host plant induces diapause [50]. In addition to aestivation, hibernating insects may also be influenced by food. Hibernal diapause is terminated in glassworm (*Chaoborus americanus*) , in response to prey and long day length, and in Indian meal moth (*Plodia interpunctella*) in response to rice bran extract.

Ways to Prevent Diapause

Diapause can be controlled by many ways including hormones, chemicals, wounding, alteration in chromosomes and modifications of environment factors, including moisture, temperature, photoperiod and oxygen.

a- Hormones

diapause hormone (DH) is known for regulating diapause in moths. Among members of the *Helicoverpa* complex of agricultural pests, DH prompts the termination of pupal diapause. Based on the structure of DH, several agonists were designed much more active than DH in breaking of diapause. One such agonist also been designed that when administered to larvae that are environmentally programmed for diapause prevents their entry into pupal diapause. In addition, the unique antagonist development strategy been designed by incorporating a dihydroimidazole trans Proline mimetic motif into one of the DH agonists, thereby converting the agonist into a DH antagonist that blocks the termination of diapause. These results suggest potential for using such agents or next-generation derivatives to dismay the success of overwintering in pest species.

b- Chemicals

In silkworm, hydrogen chloride (HCl) has been the most effective and primary method for the prevention of entry into embryonic diapauses, although another study in Japan showed dimethyl sulfoxide (DMSO) to do the same. The effect of diapause prevention was 78% with 100% DMSO concentration treatment, and the effect was comparable to that of the HCl treatment. DMSO analogs, such as dimethyl formamide (DMF) and dimethyl sulfide (DMS) did little preventive effect against the diapause . A study was carried out at Harvard University, and it was found that the diapauses can be reversed in the flesh fly (*Sarcophaga crassipalpis*) by treating the third instar larvae with tropical application of juvenile hormone analogues (10 g). Also, a 1 g dose of cholera toxin, a stimulant of adenylate cyclase can prevent diapause in flesh fly, if the dose is injected into larvae 24 h prior to pupation .

c- Oxygen Level

In Japan, the effect of in oxygen level on diapause development in leaf beetle (*Atrachya menetriesi*) was investigated to explain the role of oxygen in regulation of egg diapause. The effect of anoxia was temperature dependent; although anoxia alone had no effect on diapause termination, it decreased diapause intensity before chilling. Such an effect reached a maximum level when anoxia lasted for about 10 d. Diapause intensity was also reduced when anoxia was applied in pre-diapause stage. On the other hand, anoxia terminated diapause when the diapause intensity had been lowered by sufficient duration of chilling (50 d at 7.5 □C) .

d- Chromosomes Number

In UK, a study carried out in populations of the grasshopper (*Myrmeleotettix maculatus*) that were polymorphic (two or more clearly different phenotypes exist in the same population of a species called polymorphism) for the presence of B chromosomes. It was found that grasshoppers with two or more B chromosomes took longer time to develop from egg diapause to adult than those with one or no B chromosome.

e- Wounding

In addition to the above given examples, diapauses can also be terminated by modifying factors, including temperature, photoperiod, day length and moisture . A number of examples were cited in literature mentioning termination of diapause by wounding.

This phenomenon is usually associated with relatively weak or indefinite diapause. The mobilization of the food reserves, which activates the neuro-secretory cells, is essentially a breaking down of the food laden tissues of the fat body and other tissues. Wounding, which involves the destruction of a small amount of tissue, might be expected to produce in small quantities similar substances to those which are associated in larger quantities with the larger destruction of tissues which precedes metamorphosis. It is significant that wounding is rarely effective with those species in which diapause would be classed as firm .

Lec. 7

Endocrine system of insects

Hormones:

Hormones are biologically active compounds which are produced to control certain life processes and development. Hormones attain to the place of their action through the medium of hemolymph , insect hormones are generated to regulate physiological, developmental and behavioral events

Properties:

-hormones work along with the nervous system

-their effect is slower and more pervasive

-most of them are not specific to certain species, but there are some which can effect only on several species

-their effect is realized by specific receptors or secondary messenger molecules

-their biosynthesis, concentration, effect and removal is subtly regulated

-their production is under complex control

-they often have more places of action (polytropic effect)

-Their marking is based on the English abbreviations: (e.g. MH = moulting hormone, JH = juvenile hormone..), in case of species specificity (e.g. Lom-AKH = *Locusta migratoria* adipokinetic hormone).

Classification of insect hormones according to the place of production:

1. aglandular or tissue hormones: produced by special cells (neurosecretory cells (e.g. neurohormones)
2. glandular or endocrine hormones: produced by (corpora allata)**Ca**, (corpus cardiacun) **Cc** and (prothoracic Ganglion) **Ptg** e.g. moulting hormones, juvenile hormones.

Classification of insect hormones according to their chemical structure:

1. peptides – proteins with small or big molecule weight e.g. neurohormones
2. steroides – compound based on sterol tructure e.g. moulting hormones
3. terpenoids – certain compounds with sesquiterpene structure e.g. juvenile hormones.

Stages of hormone synthesis

1. **Biosynthesis and secretion** of the hormone from the neuroscrotory cellor or PTG,CA,and CC glands
2. **Transport** of the hormone to the target cells or tissues.
3. **Recognition** of the hormone by an associated cell membrane or intracellular receptor protein
4. **Relay and amplification** of the received hormonal signal via a signal transduction process: This then leads to a cellular response. The reaction of the target cells may then be recognized by the original hormone-producing cells, leading to reducing in hormone production.
5. **Breakdown** of the hormone. its include inactivation and metabolism of hormone .

These stages are the factors which affecting on concentration of the hormone in the haemolimph of insects. There are cells that create and release the hormone directly to haemolimph, It associated with special types of proteins (carrier protein), and there is another cells keep the hormone and convert it another compounds then release it to haemolimph again , the hormone may be associated with target cells which interact with hormone to occur specific response.and there is another cells remove the hormone. (Fig,1).

Prothoracicotropic Hormone (PTTH)(Brian hormone)

PTTH is consist of two polypeptides of 109 amino acids, as its name suggests, acts on the **prothoracic glands**.

it was the first insect hormone to be discovered. It was originally described simply as "brain hormone". Molting and pupation require the hormone, PTTH, secreted by a two pairs of cells in the brain of the larva. If these cells are cut out of the brain of a full-grown larva, pupation does not occur.

Later it was established that the insect brain produces a number of hormones, but the hormone which was the cause of the observations made by Kopeć and Wigglesworth was prothoracicotropic hormone.

PTTH is secreted by a neurohemal organ, the corpus cardiacum (in some insects the corpus allatum secretes PTTH). PTTH is released in response to environmental stimuli and as its name implies PTTH acts on the prothoracic glands, which respond by releasing molting hormone (an ecdysteroid) into the haemolymph. Molting hormone stimulates the molting process.

Function of prothoracicotropic hormone

-activates prothoracic glands (PG) to produce MH

-its secretion always precedes the MH secretion

Mode of action of prothoracicotropic hormone

It was found that stimulating the **prothoracic glands** in *manduca sexta* in vitro by (CAMP) Adenosine mono phosphate With a presence of (Aminophylline)(Which acts as an inhibitor to enzyme phosphordiesterase) Which analyzes the CAMP .

It was observed that the hormone was produced and released by **prothoracic glands ,** It was also observed that there was a significant increase in the activities of CAMP at the time of release and excretion PTTH hormone, so it seems the PTTH hormone stimulate the **prothoracic glands** to synthesis and excretion ecdyson hormone , Where it was found that this hormone activates synthesis RNA in the in vitro and in vivo . and the increase in RNA has a relation in the production of ecdyson hormone . (Fig,2)

Juvenile hormones (JHs)

are a group of acyclic sesquiterpenoids that regulate many aspects of insect physiology. The first discovery of a JH was by Vincent Wigglesworth.

Juvenile hormones are secreted by a pair of endocrine glands behind the brain called the **corpora allata.**

JH was isolated in 1965 by Williams and Slama and the first molecular structure of a final six was solved in 1967.

Physiological effects of JH-s:

1.regulate almost every aspect of the insect’s life including growth, development, immune response and reproduction

2. Inhibition of metamorphosis at the developmental stages

3.may determine the length of development, the growth of larvae and increase of their body weight

4.controls certain important processes of reproduction, important for the production of eggs in female insect,infiltration and deposition of yolks into the mating eggs, function of genital accessory glands

5.triggers and maintains certain types of diapause

6.controls the colour change.

Most insect species contain only juvenile growth hormone (JH) III To date JH 0, JH I, and JH II have been identified only in the Lepidoptera (butterflies and moths). The form JHB_3(JH III bisepoxide) appears to be the most important JH in the Diptera, or flies. Certain species of crustaceans have been shown to produce and secrete methyl farnesoate, which is juvenile hormone III lacking the epoxide group. Methyl farnesoate is believed to play a role similar to that of JH in crustaceans.

Being a sesquiterpenoid, JH chemical structure differs significantly from the structure of other animal hormones. Some JH analogs have been found in conifers.

biosynthesis of hormone JH

The biosynthesis of the hormone was came through the enables researchers to culture **corpora allata** gland in vitro and their control of continuous synthesis of this hormone , Therefore, most information was available about most of the biosynthesis steps of JH hormone especially JH1,JH2,JH3.
It appear that the source of carbonaceous structure of the JH3 is acetate composition ,while the source of JH1,JH2 was from propionate composition In addition to acetate composition.

It was found in 1992 by a researcher Brindle that amino acid isoleucine could be a the source to acetate and propionic acid ,These compositions consider the source to biosynthesis the hormone JH.

As for the final stages in hormone JH biosynthesis that has been clarified by culturing the **corpora allata** gland in vitro too, Studies have shown convert Farensyl pyrophosphate to JH hormone And that's after separation of the pyrophosphate and convert Farensyl pyrophosphate to Farnesenic acid which was identify in both of gradual metamorphosis insects and complete metamorphosis insects, After that epoxidation and methyltion process was occurs on end biosynthesis in complete metamorphosis insects , but in gradual metamorphosis insects the methyltion process happened then the epoxidation processas. it obtain in locust and Cockroaches, The opposite of that happens in complete metamorphosis insects like *M. sexta*, and perhaps the two processes will happen in some insects (Fig.4).

There are seven types of hormone JH

all known juvenile hormones are methyl esters of epoxy-farnesoic acid

1- JH 0 (C-19 JH)

isolated from the eggs of *Manduca sexta* .

2- JH I and JH II (C-18 JH and C-17 JH)

first time isolated from the abdomen of male adults of *Hyalophora cecropia* ,occurs mostly in Lepidopterans

3- JH III (C-16 JH)

fist time isolated from adults of *Manduca sexta*

most important , takes part in the control of reproduction in insects.

4. JH III bisepoxide (JH B III)

isolated from the ring gland of many Dipterans and in ticks

5. JH acids

natural degradation products of JH metabolism, are also produced by the CA of Manduca larvae and may serve as a hormone.

6- 4 methyl JH

The above JH forms is sesquiterpene compounds consist of epoxide group with ethyl croup. The JH forms is epoxy fatty acid methyl ester.

Physiological effects of JH-s:

1.regulate almost every aspect of the insect's life including growth, development, immune response and reproduction

2.controls the morphogenesis, the complex process of metamorphosis

3.may determine the length of development, the growth of larvae and increase of their body weight

4.controls certain important processes of reproduction: infiltration and deposition of yolks into the mating eggs, function of genital accessory glands

5.triggers and maintains certain types of diapauses.

6.controls the colour change

Mode of action of JH-s:

It was found after the release of the JH hormone From Ca to hemolymph associated with specific proteins and then transformed to target tissues which was effect on it .

And found one of the most common problems in insect physiology is the mode of action of this hormone and that's for the following reasons

1- Because this hormone differs in its composition from another hormones (peptides)
2- This hormone is unstable in different conditions.
3- not soluble in liquid media.

JH degradation

It is found in the haemolymph of many insects proteins(enzymes) having the ability to analyze the hormone , It is found in many orders of insects the hormone was degradation through ester hydrolysis and epoxide hydration That is some steps of decomposition accrue in haemolymph and the another happen in fat bodies.
Two types of Esterase enzymes were diagnosed, one of them is specialized(JH specific esters) and the other is (no specific general esterase). The first enzyme can attack the associated JH with protein . and the second associated with free JH. Studies indicated that the first enzyme appears in the last larval stage in the haemolymph before the release of the molting hormone.(fig .5).

Regulation of lipids

Adipokinetic hormone (AKH)

AKH was initially discovered in the locusts *Locusta migratoria* and *Schistocerca gregaria* and is generally associated with aiding flight. The source of this hormone is the glandular cells of corpora cardiac gland, It has been isolated and diagnosed by candy and mayer (1969)and It is the first hormone that has been diagnosed as its peptide , It consists of a number of linked amino acidsAs follows

Threonine ------proline-------asparagine-----------tryptophan -----------glycine-----------thereonine.

The release of the hormone AKH is Controlled by Instructions of Nervous cell in Corpora allata and the concentration is Its concentration varies according to the growth stage of the insect.

The function of this hormone is associated with activity of the flight muscles ,
This is done through regulating the level of fat body's in the hemolymph of the insects, Where it found that plays a role in the analysis of triacylglycerol, It also stimulates flight muscles to use the fat body's specially when a decrease in the level of necessary carbohydrates in flight muscles.

The following figure illustrates the increase in hormone concentration It leads to a decrease in fat body's concentration (triacylglycerol), and increase in level of triacylglycerol in lipids.

Where it was found that this hormone works on two sites, the first one is fat body's and the second is flight muscles In fat bodies it leads to decomposition triglyceride to Diglyceride And that through its effect on CAMP That stimulates the lipase enzyme in the fat body's, After that the Diglyceride is set out from fat bodies to hemolymph and transfer through the hemolymph like lipoprotein to flight muscles, As for the second impact site of hormone is flight muscles, Where it is believed its stimulates Diglyceride oxidation , As it affects on the permeability of the cell membrane to Diglyceride As the hormone does not enter into these cells It was also found that the high concentration of trehalose in the hemolymph Prevents hormone flow.

Regulation of water and ion balance, osmotic regulation

A) Diuretic hormone (DH)

-increases the secretion of ions (Na^+, K^+ and Cl^-) and water from the distal Malpighian tubules

-oligopeptides with DH activity are produced by many regions of CNS.

This hormones regulate water balance through diuretic action. The insect excretory system, responsible for regulating water balance in the insect, comprises the Malpighian tubules and the hindgut (the ileum and rectum). Malpighian tubules secrete primary urine, most of which is passed into the hindgut where water, ions and essential metabolites are reabsorbed before the fluid is excreted. Excretion is under the control of diuretic and anti-diuretic factors, or hormones, .

B) Antidiuretic hormone (ADH)

This hormone are oligopeptides produced by *m*NSC, stored and secreted by CC-s
The function of this hormone is increases the reabsorption of ions (mostly Na+, K+ and rarely Cl-) and water by the hindgut (rectum) and proximal Malpighian tubules

lec. 8

Moulting hormone – (MH)

• Endocrine organs (PG) producing MH hormone which is control the processes of moulting,

They are generally paired of organs located on both side of brain consists of big, glands, their function is cyclic, starts shortly before moulting.

Most frequent MH producing organs:
1. prothoracic glands (PG)
2. lateral part of „ring glands" (Diptera-Cyclorrhapha)
3. pericardial glands (Phasmidae)
4. ventral glands (Odonata, Ephemeroptera)
5. oenocytes

First isolation of MH: Butenandt and Karlson (1954) purified 25 mg of the hormone starting with approximately 500 kg (a half ton!) of *Bombyx mori* pupae.

Types of natural MH-s:
1. ecdysone (α-ecdysone)

2. 20-hydroxy-ecdysone (β-ecdysone,)
-produced from ecdysone with the help of ecdysone-mono-oxigenase enzyme in the epidermis of integument, midgut and fat bodies
3. 26- hydroxy-ecdysone
4. 20-26 dihydroxy-ecdysone
The main type is the β-ecdysone

Biosynthesis of MH-s:
- from phytosterols (e.g. sitosterol, campesterol, stigmasterol);
-steroids derived from fungi and animals (e.g. ergosterol, cholesterol)

The mode of action of steroid hormones

The cell membranes are permeable to steroid hormones, so they pass through both the cell and the nuclear membranes. They bind to receptors that serve as transcription factors, so together they directly interact with DNA and regulate transcription of mRNA and the production of proteins.

Hormonal control of moulting

• The hormonal control mechanism is identical whether the moult is larval to larval, larval to pupal, or pupal to adult.

•The trigger for moulting generally correlates with some indicator of growth during the instar.

•In the few insects PTTH is secreted when a critical size is attained or when stretch receptors are triggered after a large meal is ingested.

•PTTH then stimulates the release of ecdysone from the PG-s, which is converted to 20HE

•PTTH release is governed by a photoperiodic inner clock, which opens a „*moulting gate*"

•PTTH release is short and shows a symmetric peak in titre

•secretion of MH is more extended, and remains until apolysis

•The 20HE circulates in the hemolymph and activates the epidermal cells beginning with apolysis until ecdysis starts with the cycle of epidermal cell division and the synthesis of the new cuticle.

•The presence of MH receptors and their particular varieties in the cells at various stages of their developmental programs also determines whether and how the cells will respond to the hormone.

Eclosion hormone

1- **eclosion hormone** A peptide hormone secreted into the hemolymph by the NSC, then stored and released by CC.
2- The release of EH from the brain is controlled by a circadian clock within the brain .
3- its leading to emergence of the adult from the pupa,. It is also involved, with other hormones (ecdysone), in moulting of the cuticle by immature stages.
4- wax synthesis and endocuticle deposition require its presence.
5- control the behavior of pharate adult,

Bursicon hormone(Tanning hormone)

Bursicon is an insect hormone which help in tanning the cuticle of adults .

function

Bursicon plays a very important role in insect wing expansion during the last step of metamorphosis: maturation of the wing. At this time, the newly emerged adult removes dead cells of larval tissues. In *Drosophila* and *Lucilia cuprina* fly, the epidermis of wing is detached by extensive cell death apoptosis, at the time of wing spreading.

The cells that undergo death are removed from the wing cuticle and are absorbed into the thoracic cavity through wing veins. Subsequent wing maturation is disrupted if the process of cell death is inhibited or delayed somehow.

Bursicon is released just after eclosion and induces epidermis cell death. At the same time it hastens the tanning reaction, and hardens the newly expanded cuticle of the wing.

Where the peptide is found

Bursicon is released directly after the ecdysis found in different insects and considered to be unspecific. It is produced by median neurosecretory cells in the brain, circulates in blood and stored in corpora cardiaca.

Mode of action of Bursicon Hor

According to the following scheme it appears that It increases the permeability of hemolymph cells to amino acid tyrocine and increases the permeability of the epidermis to Dopamine ,then the epidermis convert the Dopamine to N-acetyl dopamine which is very important in tanning process.

Scientists also noted that this hormone stimulates protein synthesis by fat bodies and then penetrates into the epidermis to be used in hardening of cuticle.

Lec. 9

Excretion

Excretion is eliminate the wastes that results of from metabolismswhich was be Harmful when accumulate in body of insects.

As well as regulate the balance between water content and salt concentration in the haemolymph to provide a suitable chemical medium for the effective of tissues inside the body.

The purpose of excretion are to provide optimal environmental conditions for the cells of different tissues to perform their activities, this conditions has very tiny rate which greatly affects the activity of cells.

Typical excretion organisms

1- **Malpighian tubule.**
2- **Rectum.**

Malpighian tubule.

The main excretory organ of the insect is the **Malpighian tubule**. Insects contain anything from 2 to 150 or more Malpighian tubules depending on the genus. Malpighian tubules are tubular outgrowths of the gut. They typically develop as pouches emerging from the junction between the midgut and the hindgut, though there actual final position varies.

Each Malpighian tubule is a blind-ending tube whose lumen is continuous with the lumen of the gut.

structure of malpighian tubule

the wall of malpighian tubule consist of single layer of epithelial cells, forming the tubule wall, enclosed by an elastic membrane (basement membrane). In most insects there is a thin layer of striated muscle around this membrane. Typically muscle cells spiral around **the distal end** (the end furthest from the gut) of the tubule, causing it movements with muscles contract. **The proximal end** (near the gut) may be coated in circular and longitudinal muscle fibers, the movement of muscles giving the tubes to empty the contents into the gut.

The typical malpighian tubule , the inner surface have sytoplasmic filament called microvillus, (brush border) the end of microvillus swollen when the excretion is active and the edge that is towards the haemolymph cavity have a lot of fold. 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 in folded areas, to supply the energy requirements for active transport of certain ions across the tubule wall.

The body cavity of the insect is filled with a fluid, usually colourless, called haemolymph. This fluid bathes the organs and tissues and is circulated around the insect body. The tubules are contact with fresh haemolymph (perhaps by circulating the heamolymph around the tubule). Metabolic wastes and other unwanted chemicals that entered the insect system pass into the haemolymph, or are excreted into the haemolymph by the cells. These include nitrogenous waste and plant toxins such as alkaloids. It is the job of the malpighian tubules to keep the haemolymph cleansed of these wastes — they remove wastes from the haemolymph and then excrete them into the gut lumen. Outside the muscle layer is a 'peritoneal covering' of cells with embedded tracheoles, which carry oxygen to the malpighian tubules which their mitochondria use to generate the needed ATP by aerobic respiration.

In some insects (e.g., Rhodnius), two distinct zones can be seen in the malpighian tubule (Figure 18.1C, D). In the distal (secretary) zone the cells possess large numbers of closely packed microvillus, but very few enfolding 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.

FIGURE 18.1. (A) Excretory system of *Rhodnius.* Only one Malpighian tubule is drawn in full; (B) junction of proximal and distal segments ofaMalpighian 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. [A, B, E, after V. B.Wigglesworth, 1965, *The Principles of Insect Physiology*, 6th ed., Methuen and Co. By permission of the author. C, D, from V. B. Wigglesworth and M. M. Saltpeter, 1962, Histology of the Malpighian tubules in *Rhodnius prolixus* Stal. (Hemiptera), *J. Insect Physiol.* **8**:299–307. By permission of Pergamon Press Ltd.]

General function of malpighian tubules

1- Pre-urine is formed in the **tubules**, when nitrogenous waste are transported through the tubule walls.
2- Wastes such as urea and amino acids are thought to diffuse through the walls, while ions such as sodium and potassium are transported by active pump mechanisms.
3- Water follows thereafter. The pre-urine, along with digested food, merge in the hindgut.

4- At this time, uric acid precipitates out, and sodium , potassium ions are actively absorbed by the rectum, along with water via osmosis.
5- Uric acid is left to mix with feces, which are then excreted.

Alternative function of Malpighian tubules

1- in Hemiptera reabsorption of water and essential ions directly to the hemolymph occurs in the proximal portion and the rectum.
2- in Coleoptera and Lepidoptera the distal end of the tubules are embedded in fat tissue surrounding the rectum. such an arrangement may serve to increase the reabsorption of water and essential ions in the Malpighian tubules.
3- Malpighian tubules have been modified in some insects to serve accessory functions. Larvae of *Arachnocampa luminosa* (Diptera:Mycetophilidae) use modified and swollen Malpighian tubules to produce a blue-green light attracting prey towards traps.
4- In insects which feed on plant material containing noxious allelochemicals, Malpighian tubules also serve to rapidly excrete such compounds from the hemolymph.

The Rectum

the rectum, is thin-walled, in some insects includes six to eight thick-walled rectal pads whose function is to absorb ions, water, and small organic molecules. As a result, the feces of terrestrial insects are expelled as a more or less dry pellet.

Malpighian tubules absorbed the beneficial and harmful substances from haemolymph ,specially low molecular weight(its absorb the amino acid instead of proteins), This means that the basement membrane in malpighian tubules allows the passage of low molecular weights. and don't permit to high molecular weights to pass in to malpighian tubules. The rectum reabsorbs the beneficial molecules and return it to haemolymph.

The malpighian tubules permit to the beneficial and harmful substances from haemolymph to malpighian tubules liquid which was pass to rectum , then the rectum return the beneficial molecules to haemolymph, if there is no rectum, the insects would died.

The importance of the rectum

1- It has a role in regulating osmotic pressure in haemolymph by controlling the amount of water absorbed from the rectal fluid and return it to haemolymph. This is due to the difference in osmotic pressure between the haemolymph and rectal fluid.

2- It has a role in absorbed inorganic ions by diffusion, if the concentration of chlorine, potassium, and sodium ions lower than its normal level in the haemolymph because of a defect, In this case the rectum absorbed these ions to preserve the concentration of its in haemolymph.

   Therefore, water and salts must remain stable in their levels in haemolymph. (the increase and decrease lead to defect).

3- Furthermore the rectum has a role in reabsorption of beneficial substances (low molecular weights) while harmful organic molecules cannot pass through the rectum, It remains in the rectal cavity to run out of gut.

Other Excretory Structures

1- excretion by alimentary canal

There are several cases that indicate that the alimentary canal contribute in excretion, and these cases are:

1- in cockroach, uric acid is found in the form of granules on hind gut, Therefore, it is believed that the hind gut has an excretory function.
2- In larvae of hymenoptera, uric acid is found on midgut.
3- In some insects, it's found the midgut consist ammonia then excrete it into haemolymph..
4- Aquatic insects excrete the ammonia directly from haemolymph to rectum.
5- In collembola where the malpighian tubules are absent, midgut store the uric acid in epithelial layer and get rid of it through molting.

2- Labial glands

labial glands are exist in Collembola and thysanura which do not have malpighian tubules , the function of labial glands isolation of pigments produced in haemolymph and throw it through an opening at the base of the labium.

In lepidoptera labial glands has excrete role, especially when adult emerge from pupa stage, it excrete cocconase enzyme and potassium bicarbonate from labial glands, the enzyme analyze the silk and the potassium bicarbonate provides optimum PH for enzymes.

Researchers consider this process ,excretory process because it eliminate the water and potassium.

3- Filter chamber

Filter chamber found in insects(or. Homoptera) that feeds heavily on plant juices, it content little amount of amino acid for that the insect feed large quantities of plant juice to obtain enough amount of amino acid.

So the filter chamber consider excretion organisms its function is prevent increasing the water rate in insects body.

4- Storage Excretion

An alternative strategy to the removal of wastes through the malpighian tubule-rectum system used by some insects is storage excretion.

In Dysdercus, for example, uric acid is deposited permanently in the epidermal cells of the abdomen, forming distinct, white transverse bands.

Adult Lepidoptera convert much of their waste nitrogen into pteridines that are stored in the integument, eyes, or wing scales, giving the insects their characteristic color patterns.

In the larval stages of many species uric acid crystallizes out in fat body cells and epidermis, even though the malpighian tubules are functional. It appears that this is caused by the metabolic activity of the cells themselves.

termites and cockroaches, retain large quantities of uric acid in special cells (urocytes) within the fat body.

Calcium salts (especially carbonate and oxalate) are found in the fat body of many plant-eating insect larvae. During metamorphosis they are released and dissolved, to be excreted via the malpighian tubules in the adult.

Dyes present in food are often accumulated in fat body cells where they appear to become associated with particular proteins. These proteins are then transferred to the egg during vitellogenesis and the dyes subsequently “excreted” during oviposition.

Nephrocytes (It is located on either side of the esophagus or on the salivary gland) accumulate a variety of substances, especially pigments, (of high molecular weight) that means are involved in the metabolism of hemolymph macromolecules.

Types of nitrogenous excretion

the major excretory product of nitrogenous is **ammonia** is highly toxic and very water-soluble. It contains a high proportion of hydrogen that can be used in production of water. It is generally found as the major excretory product, only in those insects that have available large amounts of water, for example, larvae and adults of freshwater species.

Most insects, excrete their waste nitrogen as **uric acid**. This is only slightly water-soluble, relatively non-toxic, and contains a smaller proportion of hydrogen compared with ammonia.

However, **uric acid** is not the only form of nitrogenous waste. Usually traces of other materials (especially the related compounds **allantoin** and **allantoic acid**) can be detected, and in many species one of these has become the predominant excretory product .

Urea is rarely a major constituent of insect **urine**, usually representing less than 10% of the nitrogen excreted.

amino acids can be found in the excreta of many insects, but their presence should be regarded as accidental loss rather than deliberate excretion by an insect . for example, the clothes moth *Tineola* and the carpet beetle *Attagenus* excrete large amounts of the sulfur-containing amino acid cystine.

Although in tsetse flies uric acid is the primary excretory product, two amino acids, arginine and histidine, are important components of the urine.

The **amino acids** voided in honeydew by plant-sucking Hemiptera must be considered as largely fecal and not metabolic waste products. Because of the large amount of water taken in by aphids, it has been suggested that they might produce ammonia as their nitrogenous waste.

uric acid and the other nitrogenous waste products are derived from two sources, **nucleic acids** and **proteins**. Degradation of nucleic acids is of minor importance; most nitrogenous waste comes from protein breakdown followed by synthesis of hypoxanthine from amino acids. The biochemical reactions that lead to synthesis of this purine appear to be similar to those found in other uric acid excreting organisms.

In Rhodnius, whose tubules show structural differentiation along their length, the process of excretion is basically the same. However, in Rhodnius only the distal portion of the tubule is secretory and resorption of water and cations begins in the proximal part.

Hormonal Control

Both diuretic and antidiuretic hormones are known. The former stimulate malpighian tubule fluid production and may inhibit water resorption from the rectum; antidiuretic hormones mostly appear to act only by stimulating water resorption from the rectal lumen; however, in a few species the antidiuretic factor inhibits potassium ion transport (hence formation of the primary excretory fluid) in the malpighian tubule.

Lec. 10

Sensory Systems

1. Introduction

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) (Dethier, 1963). 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. Sensory structures are generally specialized so as to respond to only one energy form and are usually surrounded by accessory structures that modify the incident energy. As Dethier (1963) noted, the small size and exoskeleton of insects have had marked influence on their sensory and nervous systems. Smallness and, therefore, short neural pathways provide for a very rapid response to stimuli. However, it also means that there are relatively few axons and, therefore, a limited number of responses to a given stimulus. This has led to a situation in insects where stimulation of a single sense cell may trigger a series of responses. Further, 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. The cuticle provides protection and support by virtue of its rigid, inert nature, yet sense cells must beable to respond to very subtle (minute) energy changes in the environment. Thus, only where the cuticle is sufficiently “weakened” (thinner and more flexible) will the energy change be sufficient to stimulate the cell. An insect, therefore, must strike a balance between safety and sensitivity. In contrast to mammalian skin, which has millions of generally distributed sensory structures, the surface of an insect has only a few thousand such structures, and most of these are restricted to particular regions of the body.

Two broad morphological types of sense cells are recognizable (Dethier, 1963; French, 1988), those associated with cuticle (and therefore including invaginations of the body wall) (Type I neurons) and those that are never associated with cuticle and lie on the innerside of the integument, on the wall of the gut, or alongside muscles or connective tissue where they function as proprioceptors (Type II neurons) (Section 2.2). A Type I neuron and its associated cells are derived embryonically from the same epidermal cell. They and the associated cuticle form the sensillum (sense organ). All types of sensilla, with the possible exception of the ommatidia of the compound eye, are homologous and derived from cuticular hairs.

2. 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); they detect air movements, including sound waves; and they have gravitational sense, that is, through particular mechanosensilla they gain information about their body position in relation to gravity. This is especially important in flying or swimming insects which are in a homogeneous medium; they receive information about their body posture and the relationship of different body components to each other, and they obtain information on physical events occurring within the body, for example, the extension of muscles in movement, the filling of the gut by food, and the stretching of the oviduct when mature eggs are present. 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 (French, 1988).

2.1. Sensory Hairs

The simplest form of mechanosensillum is seen in sensory hairs (sensilla trichodea) (Figure 12.1), 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. Typically, they are single structures but on occasion they are found in large groups known as hair plates (Figure 12.2). In its simplest form a sensillum comprises a rigid, poreless hair set in a membranous socket and four associated cells; these are the inner sheath cell (also known as the trichogen or generative hair cell), outer sheath cell (tormogen or membrane-producing cell), neurilemma cell, which ensheathes the cell body and axon of the

sensory neuron, and the sensory neuron whose dendrite often is cuticularized and includes a terminal cuticular filament (scolopale) (McIver, 1985; Keil, 1997, 1998). In addition to their generative function, the outer sheath cells have an important physiological role in maintaining the appropriate ionic and molecular environment for stimulus transduction and conduction by the dendrites. Specifically, they pump K+ ions into the space that surrounds the tip of the sensory dendrite to facilitate generation of the receptor current (Section 2.3). A characteristic feature of the tip of sensory neurons are large numbers of microtubules. Because of their position and experiments with antimicrotubule drugs, it has been suggested that the microtubules may play a role in transduction. However, French's (1988) assessment of the evidence led him to conclude that their more likely roles are in the development and

structural maintenance of the sensilla.Within the above-generalized structure, hairs may differ widely in their detailed morphology, physiology, and function (Section 2.3). 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 thermosensory.

2.2. Proprioceptors

Proprioceptors are sense organs able to respond continuously to deformations (changes in length) and stresses (tensions and compressions) in the body. They provide an organism with information on posture and position. Five types of proprioceptors occur in insects: hair plates, campaniform sensilla, chordotonal organs, stretch receptors, and nerve nets. In
common, they respond tonically and adapt very slowly to a stimulus.

Acampaniform sensillum (Figure 12.3A) includes all of the components of a tactile hair with which it is homologous except for the hair shaft, which is replaced by a dome-shaped plate of thin cuticle. The plate may be slightly raised above the surrounding cuticle, flush with it, or recessed, but in all cases it is contacted at its center by the distal tip of the neuron and serves as a stretch or compression sensor. In many species the plate is elliptical and has a stiffening rod of cuticle running longitudinally on the ventral side, to which the neuron tip is attached (Figure 12.3B). The sensillum shows directional sensitivity, being stimulated by stress perpendicular to the longitudinal axis of the rod. Typically, the sensilla are arranged in groups. In *Periplaneta*, for example, the sensilla of the tibia occur in two groups, with their rod axes at right angles. During walking (Chapter 13, Section 2.3), contraction of the flexor and extensor muscles stimulates the proximal and distal groups of sensilla, respectively, which are thus important in the overall coordination of the process. In addition, when the insect is standing, the proximal sensilla whose axes are perpendicular to the axis of the tibia are continuously stimulated because of the stress in the cuticle. Information from sensilla passes to the central nervous system where it inhibits the so-called “righting reflex.” When the insect is turned on its back there are no longer stresses in the cuticle, the sensilla are not stimulated, the righting reflex is not inhibited, and the insect undertakes a series of kicking movements in order to regain the standing position. Chordotonal (scolophorous) sensilla (= scolopidia) (Figure 12.4A) are another widely distributed form of proprioceptor in insects. Unlike the sensilla discussed earlier, chordotonal sensilla lack a specialized exocuticular component, though, it should be emphasized, they are believed to be homologous with the sensillum trichodeum and other types of sensilla. A distinctive feature of scolopidia is the scolopale, an intracellular secretion of the sheath cells surrounding the dendrite of the sensory cell, that is assumed to be important in the transduction of the stimulus. They 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 12.4B). 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. Frequently, alteration of the positions of the points is brought about as a result of pressure changes, for example, in the air within the tracheal system, in the hemolymph within the body cavity, or in aquatic insects in the water in which they are swimming. In relatively few insects chordotonal sensilla are aggregated in large numbers and capable of being stimulated by changes in external air pressure, that is, sound waves (Section 3). Stretch receptors (Figure 12.5) comprise a multipolar neuron (Type II) whose dendrites terminate in a strand of connective tissue or a modified muscle cell, the ends of which are attached to the bodywall, intersegmental membranes, and/or muscles. As the points to which the ends are attached move with respect to each other, the receptor is stimulated. Stretch receptors are probably most important in providing information to the central nervous system on rhythmically occurring events within the insect, for example, breathing movements,,waves of peristalsis along the gut, and locomotion A peripheral system of multipolar sensory neurons is located beneath the body wall in many larvae whose cuticle is thin and flexible, or beneath the intersegmental and arthrodial membranes of insects with a rigid integument. The nerve endings are presumably stimulated by tension in the body wall or movements of joints. A similar arrangement is present in the wall of the bursa copulatrix of *Pieris rapae*, measuring the degree of stretching of this structure when a spermatophore is present (Chapter 19, Section3.1.3), and in the alimentary canal, though the sensory neurons in this case pass their information on to the visceral nervous system (Chapter 13, Section 2.2).

2.3. Signal Detection

Detection of stimuli by mechanoreceptors is a three-step process: coupling, transduction, and encoding (French, 1988). Coupling refers to the deformation of the sensory neuron's dendritic membrane caused by movement of the hair in its socket, the inward movement of the cuticular dome in campaniform sensilla, or the stretching of the chordotonal sensillum. Coupling in Type II proprioceptors (stretch receptors and peripheral nerve nets) presumably also results in distortion of the neuronal membrane, though these systems are much less studied. 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. Studies with

Drosophila mutants have elegantly demonstrated that the channels include specific proteins that serve as "gates" (Walker *et al.*, 2000).When the gates are opened as a result of membrane distortion, K+ions rush in from the extra-dendritic space, creating the receptor current. Encoding, the final step, is the transfer of information from the sensillum to the central nervous system. In common with typical neurons, this is seen as a train of action potentials induced by the receptor current. 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. Associated with this varied sensitivity are differences in the electrophysiology of the hairs. Delicate hairs typically adapt quickly, that is, rapidly lose their sensitivity to a continuously applied stimulus. More strongly built hairs, however, adapt only very slowly. Most hairs respond to a stimulus only while moving and are said to be "velocity-sensitive" and the response is "phasic." Such hairs are found on structures that "explore" the environment. The remainder responds continuously to a static deformation ("pressure-sensitive" forms with a "tonic" response). These are found usually as hair plates, at joints or on genitalia, which gather information on position with respect to gravity or posture. In these situations they are serving as proprioceptor

3. 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. Usually sound is thought of as an airborne phenomenon; however, it should be appreciated that sounds can pass also through liquids and solids. It will be apparent, therefore, that the distinction between sound reception and mechanoreception is not clear-cut. Indeed, many insects that lack specialized auditory organs can clearly "hear," in that they respond in a characteristic manner to particular sounds. For example, caterpillars stop all movements and contract their bodies in response to sound. If, however, their bodies are coated with water or powder, or the hairs removed, the response is abolished. Further, insects with specialized sound sensors may continue to respond to sounds of low frequency even after the specialized organs have been damaged or removed. The structures that respond to these low-frequency sound waves (see Figure 12.8) are the most delicate mechanosensilla, namely, the sensilla trichodea and, probably, chordotonal sensilla distributed over the body surface. In some species, hairs sensitive to sound may be restricted to particular areas, for example, antennae or cerci.Among Insecta, hearing has evolved independently in at least 12 groups (Michelsen and

Larsen, 1985). Though insect hearing organs include a number of common elements, their structural complexity reflects the interaction of three factors: the evolutionary history of the group, the size of the insect, and the acoustic features of the insect's environment. For example, the tympanal organs of most moths, which are sensitive only to the sounds emitted by bats preying on them, are relatively simple whereas the tympanal organs of crickets and grasshoppers tend to be complex because they need to distinguish the (equally complex) songs of conspecifics. Broadly speaking, insect-hearing structures can be divided into two categories: nearfield 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 (R¨omer and Tautz, 1992). 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 (Section 3.1). *Drosophila* males vibrate their wings at about 330 Hz during courtship. The sound produced is picked up by the aristae of a female, provided she is within about 2 mm. If she is unreceptive, she produces her own song (at about 300 Hz) that causes themale to turn away and stop courting (Bennet-Clark and Ewing, 1970). Caterpillars of the noctuid moth *Mamestra brassicae* have eight fine thoracic hairs that show maximal sensitivity to air-borne vibrations in the 100–600 Hz range. At these frequencies, crawling caterpillars stop moving, and may squirm and lose contact with the substrate. These have been interpreted as avoidance reactions, as the frequencies correspond with those made by wing beats of caterpillar parasitoids (tachinids and ichneumonids) and predators (wasps). Apparently, the caterpillars can hear the sounds at distances up to about 70 cm (Markl and Tautz, 1975).

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" (Section 3.2). Specialized auditory organs comprise groups of chordotonal sensilla and associated accessory structures that enhance the sensitivity of the organ. They include Johnston's organ, tympanal organs, and subgenual organs. The first two are sensitive to only airborne vibrations, the latter mainly to vibrations in solids, though in a few species airborne sounds are detected by these structures.

3.1. Johnston's Organ

Johnston's organ, that is, one or more groups of chordotonal sensilla located in the pedicel of the antenna, is present in all adult and many larval insects and generally serves a proprioceptive function, providing information on the position of the antenna with respect to the head, the direction and strength of air or water currents, or, in back swimmers (Notonectidae), the orientation of the insect in the water. However, in male mosquitoes and chironomids, as well as in worker honey bees, the structure has become specialized to perceive sounds, notably those produced by the wings of conspecifics.

Male *Aedes aegypti* are attracted especially to sounds in the frequency range 500–550 Hz, which compare with the flight tone of females, 449–603 Hz. At these frequencies, males showacharacteristic mating response. Sounds of other frequencies (includingamale's flight tone, which is somewhat higher than that of a female) are also perceived by males, though these do not attract them. A male's antennae are extremely bushy, covered with long, fine hairs that vibrate in unison at certain frequencies, causing the flagellum to move in its socket, the pedicel (Figure 12.6). The bulbous pedicel accommodates a large number of sensilla that are arranged in two primary groups, the inner and outer rings. It is suggested that Johnston's organ resolves the sound into two components, a component running parallel to the flagellum, to which the inner sensilla are most sensitive, and a component perpendicular to the flagellum, which stimulates the outer sensilla. By "estimating" the relative strength of the stimulus from each component, a male is able to determine a female's position. The waggle dance has long been recognized as the means by which foraging worker honey bees communicate information on the distance to and direction of a food source on their return to the hive (see also Section 7.1.4). However, only recently was it realized that sounds produced during the dance provide the information on distance. Low frequency (up to 500 kHz) sounds produced by wing vibration are heard by the Johnston's organ, the duration of the auditory signal being a measure of the distance to the food source (Dreller and Kirchner, 1995).

3.2. Tympanal Organs

Tympanal organs are present in some species from at least seven orders of insects (Hoy and Robert, 1996; G¨opfert *et al.*, 2002): Orthoptera (fore tibiae of Tettigoniidae and Gryllidae, first abdominal segment of Acrididae), Lepidoptera (abdomen in Geometridae and Pyralidae, metathorax in Noctuidae and Notodontidae, fore or hind wing base in Nymphalidae and Hedylidae, mouthparts in Sphingidae), Hemiptera (abdomen in Cicadidae, thorax in Corixidae), Coleoptera (abdomen in Cicindelinae, cervical membrane in Scarabaeidae), Dictyoptera (metathorax in Mantodea, metathoracic leg in Blattodea), Neuroptera (wing base), and Diptera (ventral prosternum). Though their detailed structure varies, almost all tympanal organs have three common features: a cuticular membrane (the tympanum); a large tracheal air sac appressed to the membrane, the two structures forming a "drum"; and a group of chordotonal sensilla (Figure 12.7) (Yager, 1999). 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. Over this range the sensitivity of the organ varies greatly, with a maximum in the 2- to 15-kHz range (Figure 12.8). In contrast, the human ear is most sensitive to a frequency of 1–3 kHz. As pressure difference receivers, insect tympanal organs have directional sensitivity. Thus, insects with these organs can locate the source of a sound. The functional significance of tympanal organs varies (Spangler, 1988; Hoy and Robert, 1996). In Orthoptera and Hemiptera, the ability to hear is complemented by the abil to produce sounds (Chapter 3, Section 4.3.1), and in these orders the organs are important in species aggregation and/or mate location. Experimentally, it has been shown that the tympanal organs of nocturnal Lepidoptera, Dictyoptera, Neuroptera, Orthoptera and Coleoptera are sensitive to high-frequency sounds, and it is generally assumed that this enables these insects to detect the approach of predators, principally insectivorous bats. It must be noted, however, that for most groups this has not been observed under field conditions.

In Lepidoptera, for which good evidence is available, the tympanal organs are most sensitive to frequencies in the range of 15–60 kHz, which comes within the frequency range of the sounds uttered by the bats as they echolocate.

A moth's response varies according to the intensity of the sound.

At low intensity (i.e., when the bat is 30 m or more distant) a moth moves away from the sound. At high intensity, a moth takes more striking action, flying an erratic course, or dropping to the ground. In a few species of Lepidoptera ultrasound is used in sexual communication (Spangler, 1988). The phenomenon of acoustic parasitismwas first reported by Cade (1975) who observed that gravid females of the tachinid fly *Ormia ochracea* locate their host, the field cricket *Gryllus integer*, by homing in on the male cricket's mating call. Since then, other tachinids

and some sarcophagid flies that parasitize cicadas have been shown to use the same strategy for host location. On locating a cricket, the fly larviposits on or near it. The tachinid's ears are typical tympanal organs, located on the prosternum, and are most sensitive in the range of 4–6 kHz (the dominant frequency of the cricket's song is 4.8 kHz). Interestingly, in these nocturnally active flies males also have tympanal organs though these are insensitive to frequencies around 5 kHz. However, the ears of both male and female flies are sensitive to ultrasonic sounds (20–60 kHz). It is thought that this range of sensitivity is related flies' need to detect and avoid becoming prey for insectivorous bats

3.3. Subgenual Organs

These are chordotonal organs present in the tibiae of most insects, excluding Thysanura, Hemiptera, Coleoptera, and Diptera. The organ, which comprises between 10 and 40 sensilla, generally detects vibrations in the substrate though the mechanism by which the organ is stimulated is not known. In a few species, for example, the Madagascar hissing cockroach (*Gromphadorhina portentosa*), the subgenual organs respond to airborne vibrations. Their peak sensitivity is at 1.8 kHz, which coincides with the species' courtship song.

4. Chemoreception

Chemoreception, essentially taste (contact chemoreception) and smell (distance chemoreception), is an extremely significant process in the Insecta, as it initiates some of their most important behavior patterns, for example, feeding behavior, selection of an oviposition site, host or mate location, behavior integrating caste functions in social insects, and responses to commercial attractants and repellents.

Though taste and smell are distinguished traditionally, such a distinction has no firm morphological or physiological basis. The sensilla for the two senses are structurally very similar; indeed, in some species the same structure is used for both olfaction (smell) and gustation (taste). Further, stimulation of a sensillum by either tastes or odors probably entails comparable subcellular or molecular interactions. Any difference between smell and taste is, then, a matter of degree. Smell may be defined as chemostimulation by compounds in very low concentration but volatile at physiological temperatures, and taste as chemostimulation by higher concentrations of liquids that are not volatile at physiological temperatures.

In addition to taste and smell, insects have a third method of detecting chemical stimuli, the common chemical sense. This is the response of an insect (always an avoiding reaction) to high concentrations of noxious chemicals. It is not a response caused by stimulation of normal chemosensilla, because the response is not abolished after surgical removal of the structures bearing these sensilla. It would seem to be a non-specific response of other types of sensory neurons.

4.1. Location and Structure of Sensilla

Behavioral and electrophysiological experiments have been used to establish the location and nature of chemosensilla. Organs of taste are common on the mouthparts, especially the palps, though they have been identified also on the antennae (Hymenoptera), tarsi (many Lepidoptera, Diptera, and the honey bee), ovipositor (parasitic Hymenoptera and some Diptera), and on the general body surface. The antennae are the primary site of olfactory organs and often bear many thousands of these structures. The mouthparts also carry olfactory structures in many species. Earlier authors classified chemosensilla according to their morphology, but this system proved inadequate because structures that looked similar at the light microscope level were shown, using electron microscopy combined with electrophysiological studies, to have different structures and functions. Slifer (1970) grouped chemosensilla in two categories, “thick-walled” and “thin-walled,” which, though a seemingly simple structural criterion for separation, is valid in all except a few cases, and broadly correlates with their functions as organs of taste and smell, respectively.

Thick-walled (uniporous) chemosensilla (Figure 12.9A) take the form of hairs, pegs, or papillae (Mitchell *et al*., 1999; Ryan, 2002). Generally, they serve as taste sensilla, though some are also sensitive to strong odors. The chemosensitive hairs and pegs broadly resemble tactile hairs, though they can be distinguished with the electron microscope.

Whereas tactile hairs have a sharply pointed top and are innervated by a single neuron whose dendrite terminates at the base of the hair, thick-walled chemosensory hairs have a rounded tipwith a terminal pore, multineuronal innervation, and dendrites that extend along the length of the hair to terminate just beneath the pore. The dendrites are usually enclosed in a cuticular sheath. Occasionally, the thick-walled pegs may be set in pits, when they are known as sensilla coeloconica and perhaps have an olfactory function. Papillae having

the same general features as chemosensory hairs have been observed in the food canal of aphids, on the labellum of flies, and on the cockroach hypopharynx. Some thick-walled hairs are both mechano- and chemosensory.

4.2. Physiology of Chemoreception

Early studies, mainly behavioral, established broad parameters for the senses of smell and taste in insects and revealed some interesting comparisons between the senses in insects and those in humans. Like humans, insects appear to “recognize” the four basic taste qualities: sweet (acceptable), salty, acidic, and bitter (all non-acceptable). Further, their response to chemical stimuli varies according to their physiological state: age, sex, normal diet, and immediate history. The sensitivity of insects to taste stimuli is, broadly speaking, equal to or greater than that of humans. Sucrose, for example, can be detected by humans at a concentration of 2×10^{-2} M, by the honey bee at 6×10^{-2} M; hydrochloric acid stimulates at 1.25×10^{-3} M in humans, at 10^{-3} M (in 1 M sucrose) in the honey bee.

However, as in humans, sensitivity to a substance increases if that substance has not been experienced for some time. The red admiral butterfly, *Pyrameis atalanta*, for example, fed regularly on sucrose has a tarsal threshold sensitivity of 10^{-1}–10^{-2}M. If, however, the sugar is withheld for some time sensitivity increases so that a concentration of 8×10^{-5} M will elicit a response. Chemosensilla on different parts of the body have differing sensitivity to particular chemicals; for example, in the fly, *Calliphora vomitaria*, those on the tarsi are 16 times more sensitive to sucrose than those on the labellum. Disaccharides are more stimulating than monosaccharides.Trisaccharides are generally non-stimulating and polysaccharides never so. Of the inorganic ions, cations showincreasing stimulation in parallel with their partition coefficient and ionic mobility; that is, H+ is more stimulating than NH+ 4 > K+ > Ca2+ > Mg2+ > Na+. For anions, the situation appears more complex, and the relationship between ability to stimulate and physical properties of the ions is unclear. Organic acids stimulate in proportion to their degree of dissociation, indicating that the H+ ion is the principal factor in stimulation. The stimulating power of non-electrolytes is usually proportional to the oil:water partition coefficient, though contradictions to this generalization occur.

Some taste sensilla are capable of being stimulated by various substances. Electrophysiological work has shown that this is possible because sensory neurons in the sensillum respond differentially to the substances. For example, in the labellar hairs of *Protophormia* there are four chemoreceptor cells and one mechanoreceptor cell (which terminates at the base of the hair). Of the chemoreceptors, one is sugar-sensitive, one is salt-sensitive, one is water-sensitive, and the fourth responds to deterrent stimuli.

5. Humidity Perception

Many observations on their behavior indicate that insects are able tomonitor the amount of water vapor in the surrounding air. Insects actively seek out a “preferred” humidity in which to rest, or orient themselves toward a source of liquid water. The value of the preferred humidity varies with the physiological state of the insect, especially its state of desiccation. Normally, for example, the flour beetle, *Tribolium castaneum*, prefers dry conditions; however, after a few days without food and water, it develops a preference for more humid conditions. Ablation experiments have established that humidity detectors are typically located on the antennae, though they occur on the anterior sternites in *Drosophila* larvae, and surround the spiracles in*Glossina*, where they monitor the air leaving the tracheal system.

Hygroreceptors exist as thin-walled, aporous hairs or pegs that are typically also thermosensitive (Altner and Loftus, 1985). Each hygroreceptor contains the dendrites of two neurons: the dendrites that penetrate the full length of the hair are hygrosensitive, whereas those that terminate near the base of the hair are temperature sensitive (Figure 12.10). Because they are poreless, hygroreceptors clearly cannot operate in the same manner as chemoreceptors, that is, by water molecules binding to receptors in the dendritic membrane. In fact, it seems that they function as mechanoreceptors, behaving like a hair hygrometer. The sensillar cuticle or a substance associated with it is hygroscopic and undergoes deformations as humidity changes, leading to physical stimulation of the dendrites. It appears that there are two types of hygroreceptors: "moist" receptors respond to increasing humidity, while "dry" receptors are stimulated by a decrease in humidity. In both cases, the stimulus is encoded as an increase in the rate of generation of action potentials.

6. Temperature Perception

This is the least understood of insect senses. Insects clearly respond to temperature in a behavioral sense, by seeking out a "preferred" temperature. For example, outside its preferred range, the desert locust, *Schistocerca gregaria*, becomes active. This locomotor activity is random but may take the insect away from the unfavorable conditions. Within the preferred range, the insect remains relatively inactive. Under field conditions, the locustwill alter its orientation to the sun, raise or lower its body relative to the ground, or climb vegetation in order to keep its body temperature in the preferred range Parasitic insects such as *Rhodnius*, *Cimex*, and mosquitoes, which feed on mammalian blood, are able to orient to a heat source.

Though the ability to sense heat is present over the entire body surface, it appears that in bloodsucking species, the antennae and/or legs are especially sensitive and probably carry specialized sensilla in the form of thick-walled hairs

In the buprestid *Melanophila acuminata* each of the paired infrared organs, containing 50–100 sensilla, is located in a pit adjacent to the metathoracic coxae. The sensilla broadly resemble mechanosensilla; that is, they are innervated by a single neuron and are poreless. However, a unique feature is a thin cuticular lenslike structure thought to be where infrared radiation is focused. The dendrites of the neuron terminate immediately below this structure (Vondran *et al.*, 1995). In another buprestid, *Merimna atrata*, there are two pairs of ventral abdominal infrared organs. The dominant feature of the organ is a large multipolar sensory neuron whose dendrites reach two chordotonal organs in addition to the thermosensitive structure (.

7. Photoreception

Almost all insects are able to detect light energy by means of specialized photosensory structures: compound eyes, ocelli, or stemmata. In the few species that lack these structures, for example, some cave-dwelling forms, there is commonly sensitivity to light over the general body surface.

The use that insects make of light varies from a situation in which it serves as a general stimulant of activity, through one of simple orientation (positive or negative phototropism), to a state where it enables an insect to carry out complex navigation, and/or to perceive form, patterns, and colors. At all levels of complexity, however, the basic mechanism of stimulation is very likely the same; that is, the solar energy striking the photosensory cell is absorbed by pigment in the cell. The pigment undergoes a slight conformational change that causes a momentary increase in permeability of the receptor cell membrane and, thereby, the initiation of nerve impulses that travel to the optic lobes and central nervous system.

7.1. 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. In dragonflies, male tabanids, and horseflies, the eyes meet in the middorsal line, the holoptic condition. In some other species, the eye is divided into readily distinguishable dorsal and basal lamina.

The secondary pigment cells lie alongside the retinular cells, and the pigment within the former does not migrate longitudinally. Scotopic (superposition) 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.