ENT 502 INSECT ANATOMY, PHYSIOLOGY AND NUTRITION (2+1)

Theory

UNIT I Scope and importance of insect anatomy and physiology.

UNIT II Structure, modification and physiology of different systems- digestive, circulatory, respiratory, excretory, nervous, sensory, reproductive, musculature, endocrine and exocrine glands.

UNIT III Thermodynamics; physiology of integument, moulting; growth, metamorphosis and diapause.

UNIT IV Insect nutrition- role of vitamins, proteins, amino acids, carbohydrates, lipids, minerals and other food constituents; extra and intra-cellular microorganisms and their role in physiology; artificial diets.

Practical

- Dissection of different insects to study comparative anatomical details of different systems.
- Preparation of permanent mounts of internal systems
- Chromatographic analysis of free amino acids of haemolymph.
- Determination of chitin in insect cuticle.

- Examination of insect haemocytes.
- Determination of respiratory quotient.
- Preparation and evaluation of various diets.
- Consumption, utilization and digestion of natural and artificial diets.

Suggested Readings

- Chapman RF.1998. *Insects: Structure and Function*. ELBS Ed., London.
- Duntson PA. 2004. *The Insects: Structure, Function and Biodiversity*. Kalyani Publ., New Delhi.
- Kerkut GA & Gilbert LI. 1985. *Comprehensive Insect Physiology, Biochemistry and Pharmacology.* Vols. I-XIII. Pergamon Press, New York.
- Patnaik BD. 2002. *Physiology of Insects*. Dominant, New Delhi.
- Richards OW & Davies RG. 1977. *Imm's General Text Book of Entomology.* 10th Ed. Vol. 1. *Structure, Physiology and Development.* Chapman & Hall, New York.
- Saxena RC & Srivastava RC. 2007. *Entomology at a Glance*. Agrotech Publ. Academy, Jodhpur.
- Wigglesworth VB.1984. *Insect Physiology.* 8th Ed. Chapman & Hall, New York.

INSECT PHYSIOLOGY

Definition

Physiology (derived from two Greek words viz. Physis–Nature, function or processes and logos-study) is that branch of biological science which is concerned with the functions of living matter or living processes at different levels of cells, tissues and organism as a whole. It is closely associated with anatomy and morphology on one hand and biochemistry and biophysics on the other. A physiologist must have proper understanding of these fields of study for understanding various life processes.

History

The early history of insect physiology is indistinguishable from the history of biological sciences like anatomy which received impetus in the 17th century when microscope was invented. In 1668 Malpighi worked out silk worm anatomy. The progress of work on insect physiology remained very slow till the beginning of the 20th century.

The first book on insect physiology, "*Physiologie des Insects*" was published in 1911 by Paul Marchal. This was followed by a monograph, 'Insect physiology' in 1934 and a text book 'Principles of Insect Physiology' in 1939 by Wigglesworth, who can be regarded as a pioneer in the field of insect physiology.

The study of insect physiology gained more importance after World War II with the introduction of DDT and other new synthetic insecticides which posed such problems as resistance, specifically, phyto-and mammalian toxicity etc. To understand and solve these problems physiological approach was the only answer and thus insect physiology changed from a luxury science to a science of necessity.

Nature and Scope

The subject matter of insect physiology is more experimental and fundamental in contrast to anatomy and morphology. Its scope is wide enough to seek answer to such basic questions as how a particular phenomenon takes place and under what conditions? The scope of insect physiology has been under constant change, as more knowledge is gathered, from basic or fundamental science to applied science. With such problems arising as toxic hazards of insecticides to men and live stock and insect resistance to them, insect physiologists eagerly seek answer to such questions as how insecticides act and what makes insects resistant to them. Although, insect physiology does not directly furnish the means of controlling insect pests, yet the rational application of control measures like insecticides and artificial interferences with the insects' environment is often dependent upon knowledge of physiology of the insect in question. Anatomical studies support the physiological studies by providing detailed knowledge of internal organ systems.

Physiology of Insect Integument

Integument is the external covering tissue. Till 1940, very little was known about its physical and chemical properties. The subject was reviewed and updated in 1957 by Wigglesworth. Today a large volume of literature is available on special aspects of physiology of insect integument.

Integumentary Subdivisions Richards (1952) has given the following subdivisions of integument.

1. **Cuticle:** The cuticle consists of the outer non-chitinous epicuticle and the inner chitinous procuticle. The epicuticle, in most insects, has four sub layers, viz. (i) tectocuticle or cement layer (ii) wax layer (iii) polyphenol layer and (iv) cuticulin layer. The procuticle is differentiated in to (i) exocuticle, (ii) mesocuticle, and (iii) endocuticle. Cuticle is formed by the secretory products of the epidermis.

2. **Epidermis (or hypodermis):** It is composed of a single layer of epidermal cells which are differentiated in to (i) ordinary epidermal cells (ii) specialized epidermal cells forming the dermal glands and (iii) large occasional cells as well as the oenocytes and the dermal glands, that show cyclic activity correlated with moulting cycle.

3. **Basement membrane:** It is a thin inner limiting membrane on the under surface of the epidermis formed by its secretion or by certain dead blood cells.

Cuticle It is secreted on the outer surface of the epidermal cells and solidifies there to form exoskeleton. It serves as a protective barrier between the outside environment and the internal organs systems. It also holds the parts of the body together and gives shape and strength to the body. It contains physical external structures of the sensory receptors (sense organs). It has remarkable properties of hardness and rigidity at some places for protection and fatigue free flexibility at others for ease the movement. It has also important permeability characteristics.

The thickness of cuticle ranges from a fraction of a micron to several microns in different species and in different areas. The epicuticle or non-chitinous cuticle is usually 1 micron thick but ranges from a small fraction of a micron, as in Culex larvae, to about 4 microns thick, as in *Periplaneta* and Sarcophaga larva. It was first considered a single layer. Richards and Anderson (1942) recognized two distinct sub layers in it. Wigglesworth and his colleagues (1947-48) have reported that in most insects like Tenebrio, ticks etc. the epicutcile is formed of four distinct sub-layers viz. (i) the inner cuticulin layer (ii) polyphenol layer (iii) wax layer and (iv) the outer tectocuticle or cement layer.

FORMATION OF DIFFERENT SUB-LAYERS OF EPICUTICLE

1. **The cuticulin layer** It is the innermost thin membrane over the epidermal cells, sometimes penetrated by pore canals and formed of conjugated protein (polymerized lipo-protein) produced by oenocytes. It is tanned by quinones which are secreted by epidermal cells. It is first sub layer of epicutcile to appear and is formed before moulting.

2. **The polyphenol layer** It is the second sublayer formed above the cuticulin layer. It is formed of protein rich in dihydroxyphenol. It is secreted by epidermal cells. Minute droplets rich in polyphenol penetrate the cuticulin layer, perhaps through porecanals, and fuse in to a continuous layer of semifluid material. It is also formed before moulting.

3. **The wax or lipid layer** It is deposited above the polyphenol layer a few hours before moulting in form of an emulsion. It is also secreted by the epidermal cells. The wax in the form of emulsion perhaps passes through the pore-canals. The emulsion contains a series of waxes which harden in layers on deposition. Its thickness ranges from 0.1 to 0.4 micron. The innermost layers of wax molecules are highly oriented, tightly packed and bound chemically to the underlying portion of the epicuticle (i.e. the polyphenol layer). It is the water proofing layer of the cuticle. A few spp. living in moist environments do not show the presence of a different wax layer.

4. **The tectocuticle or cement layer** It is the outermost layer of the epicuticle overlying the wax layer. It is extremely thin layer, not more than 0.1 micron thick, formed by the secretion of the dermal glands called Verson's Glands of the epidermal layer shortly after moulting. It is formed of a shellac like material and has protein and lipid components.

The Procuticle or chitinous cuticle

The procuticle is formed of linked up chains of chitin and protein (Richards, 1951). In cross-section, it is seen as a series of horizontal alternating light and dark laminae or bands which range from 0.2 to 10 microns in thickness. The procuticle is much thicker than the epicuticle, ranging from 40 microns in caterpillars to 240 in Sarcophaga puparium. The outer part of the procuticle is formed before moulting but much of the inner part of it is secreted and formed after moulting. The horizontal layers of the procuticle differ both in density and refractive index, resulting in the common form of physical (metallic or iridescent) colours of insects due to differential refraction of light rays.

Porecanals Extending through the outer part of the procuticle and sometimes in to the epicuticle are minute ducts or canals, called the pore canals, which are present in all except very thin cuticles. Their structure can be adequately determined only when they are exceptionally large e.g. in blow fly larvae, or when examined under electron microscope. They are formed around cytoplasmic filar (filamentous) projections of the epidermal cells and are helical. The pore canals, with their filaments, are probably thrown into helices as a result of subsequent contraction of the horizontal laminae of procuticle in thickness due to their partial dehydration and molecular packing (Dennell, 1946). In many insects the pore canals have very fine diameter and their length is almost twice the thickness of the cuticle.

Functions of pore-canals

1. Their cytoplasmic filaments secrete procuticle around them, except in thin cuticle and in inner part of the thick procuticle.
2. These transport polyphenols and wax for the formation of polyphenol and wax layers of the epicuticle (Dennell, 1946).
3. They transport the oxidizing enzymes, viz. the tyrosinase to the epicuticle for sclerotization of cuticle (Wigglesworth, 1939).
4. They transport the phenolic substrate viz., tyrosine, an amino-acid (monohydric phenol) and do its partial oxidation. These substances as well as the enzyme tyrosinase diffuse from the blood through the epidermal cells and procuticle.

Sclerotization and differentiation of procuticle into exocuticle, mesocuticle and endocuticle

In most insects the outer part of the soft procuticle becomes sclerotized (hardened) in certain predetermined areas in the body where the appropriate chemicals are added for the purpose after moulting. These hardened sclerotized areas become sclerites marked by the surroundings soft infolded lines, the sutures. The sclerites provide rigidity to the cuticle and the sutures provide flexibility to the body. The extent of sclerotization may differ much in different insects and in different parts of the same insect from very slight yellowish brown to very intense dark brown with great rigidity.

Analyzing the development of sclerotization by histo-chemical methods, Schatz (1952) has concluded that the procuticle is differentiated by sclerotization in to (a) the much sclerotized outer portion as exo-cuticle (b) the middle portion distinguishable by chemical tests as meso-cuticle, and (c) the unaltered inner portion as endo-cuticle. The exocuticle represents the first developmental stage of sclerotization and the mesocuticle represents the second developmental stage of sclerotization.

The process of sclerotization involves oxidation of tyrosine (a mono-hydric phenol) in to o-dihydroxyphenol. Finally there is formation of tanned protein, the sclerotin.

Sclerotization in insects occurs in 3 distinct ways:

(1) Sclerotization takes place uniformly over the entire surface of the outer portion of procuticile as the substrate for tanning reaction i.e. tyrosine is distributed throughout the whole area e.g. in puparium of higher Diptera (example Sarcophaga) (Fraenkell and Rudall, 1940, 1947; Denell, 1946, 1947, 1949). Sclerotization spreads inward from the epicuticle in to the outer portion of procuticile.

(2) Sclerotization is not uniform but localized to certain pre determined areas, destined to become sclerites, due to localized transport of the substrate for tanning reaction (i.e. tyrosine), controlled by the underlying epidermal cells eg. in

arthropods (including insects) in general. Sclerotization in these areas begins at the epicuticle-procuticle interface and spreads inward through the procuticle.

(3) Sclerotization (visible darkening) begins at the inner surface of the cuticle and spreads outward to give rise to a completely sclerotized cuticle i.e. all exocuticle eg. in antennae of honeybees (Richards, 1952).

Activity of epidermis in relation to moulting cycle

The epidermis consists of a single layer of cells interspersed with specialized large glandular cells viz., dermal glands (Verson's glands) and oenocytes, which originate from simple epidermal cells. All these cells show secretory activity in relation to secretion of substances for formation of cuticle and the moulting fluid.

Order of formation of cuticular layers in relation to moulting

Three inner layers of epicuticle, viz., the cuticulin, polyphenol and wax layers and the outer part of the procuticle are formed before moulting ; much of the inner part of the procuticle and the outermost layer of the epicuticle, viz. the cement layer are formed after moulting. Sclerotization also occurs after moulting.

Initiation of moulting is under hormonal control. The moulting hormone 'ecdysone', secreted by the prothoracic glands, determines moulting.

Moulting Cycle The different stages in moulting are

(i) The onset of moulting process is marked by increase in volume of epidermal cells, the appearance of numerous mitoses in the epidermis, resulting in the folding of the epidermis and its loosening from the cuticle.

(ii) Secretion of the moulting fluid by epidermal cells which digests most of the old cuticle

(iii) Secretion and deposition of part of the new epicuticle followed by a continuous secretion of procuticle.

(iv) Due to increased pressure the old partially digested cuticle splits and is shed resulting in ecdysis or moulting.

(v) The new cuticle completes its development. Expands and hardens (by sclerotization) to form the new exo- skeleton. At the same time the cement layer is secreted.

(vi) Material for the inner part of the procuticle is secreted either for a while or continuously until the beginning of the next moult.

The epidermal cells commonly increase in number and volume (thickness) before the formation of new cuticle. The loosening of the old cuticle from the underlying epidermal cells is the essential first step of the moulting cycle. This loosening is due to retraction of the epidermal cells from the old cuticle and due to the beginning of digestion of the endocuticle.

The moulting fluid is secreted by the general epidermal cells. Previously most authors thought that the dermal glands secrete this fluid until Wigglesworth (1948) concluded that the dermal glands secrete the cement layer and not the moulting fluid. The moulting fluid contains enzymes like protease and chitinase which digest the chitin-protein matrix of the endocuticle (Passonneau and William, 1953). The digested products of the endocuticle, representing up to 90% of the dry weight of the old cuticle, are subsequently reabsorbed by the epidermis and reused for the formation of new cuticle (Lafon, 1943).

During the moulting cycle and digestion of old cuticle, a thin white pellicle or membrane becomes visible beneath the old cuticle and above the new cuticle, called as ‘the ecdysial membrane’ by Wigglesworth. The function of this membrane is obscure.

Nutrition has been found to affect the moulting cycle and the cuticle development. In some blood sucking species like *Rhodnius* and Ticks a large blood meal provided the stimulus that starts the moulting cycle and in several species e.g. *Bombyx*, *Rhodnius,* etc. (Wigglesworth, 1948). Starvation of larvae may result in additional supernumerary moults and in this process the larva may become considerably smaller (Richards, 1951).

Composition of cuticle and its properties

Cuticle is composed of the following constituents:
(i) Chitin (ii) Proteins (iii) Lipids (iv) Polyphenols (v) Cuticular enzymes (vi) Cuticular pigments (vii) Inorganic salts and (viii) Water.

1. **Chitin:** First named by Odier (1923), it is the major and best known constituent of insect cuticle, ranging from 25 to 55% (average about 33%) of the dry weight of cuticle. It is the nitrogenous polysaccharide made up of long chains of acetyl-glucosamine units. It is colorless, amorphous solid insoluble in water, alcohol, ether, dilute and concentrate alkalis, dilute acids and most other solvents (Campbell, 1929). It is soluble in concentrated mineral acids, being hydrolysed to lower sachharides of shorter chain lengths.

   The best test for chitin is known as “Van Wisselingh’s test”. When the material is treated with concentrated hot caustic alkalies the chitin is converted, by detachment of acetyl groups, into chitosan which gives a deep rose-violet colour when treated with 0.2% iodine in 1% sulphuric acid.

Nothing is known about chitin synthesis in insect body. One suggestion is that it arises from the transformation of glycogen.

Chitin is found in procuticle and not in epicuticle. Chitin test is negative for wing scales of butterflies and moths, small tracheae and tracheoles and insect egg shells.

2. **Cuticular Proteins** These usually range from 25 to 37% of dry weight of cuticle. These proteins have a water soluble fraction, arthropodin, and a water insoluble, alkali soluble fraction sclerotin.

   Arthropodin is highly soluble in hot water and is precipitated from aqueous solution by ethyl alcohol 45% and higher concentration. It is relatively high in tyrosine content; paper chromatography technique has shown the presence of other amino acids also like glycine, valine, leucine, serine, cystine, arginine, oroline, histidine, etc. (Iro, 1951).

   Sclerotin is a tanned protein, brown or amber in colour, and alkali soluble. It has lower nitrogen content, about 0.5% sulphur and about 3% carbohydrate. The carbohydrate group has been suggested to be linked to the protein molecule by the sulphur.

3. **Cuticular Lipids** These are mostly contained in the wax layers of the epicuticle, which average about 30 monolayers of wax molecules. The inner most monolayers have better oriented and more tightly packed molecules. The wax in the wax layers varies in characters from a soft grease as in cockroach and

pale yellow, soft and uncrystalline as in the larvae of *Athalia*, *Nematus* and Cabbage butterfly *Pieris* to hard white and crystalline as in *Tenebrio, Rhodnius,* etc. The cuticular waxes extracted from silk worm exuviae are found to be the mixture of long chain paraffin hydrocarbons and esters of long chain fatty acids and normal alcohols (Bergmann, 1938).

The grease layer of cockroach's epicuticle has been shown to consist of wax (melting point 55-60 °C). In other insects also perhaps this is the general method of secretion of insect waxes, the solvents being usually so volatile that their presence is not detected.

4. **Polyphenols** Polyphenols and their quinone derivates play a role in hardening and darkening (sclerotization) of the cuticle (Pryor, 1940). Tests with 'argentaffin reaction' which involves deposition of black silver precipitate from a solution of ammonical silver nitrate on reaction with O- dihydroxyphenols of cuticle have shown the presence of dihydroxyphenols in cuticles (Wigglesworth, 1948).

   Both tryosine and dihydroxyphenol alanine an amino acid (a monohydric phenol), and the enzyme tyrosinase are present in insect blood. Polyphenols appear to be derived from tyrosine due to its oxidation by tyrosinase as is suggested by a rise in tyrosine content in blood just before the process of sclerotization. Tyrosinase in the blood is inhibited by a dehydrogenase system (Dennell, 1949). Tyrosine in the cuticle is first oxidized by Hyrosinase to dihydroxyphenols which are further oxidized by Polyphenol oxidase to quinones. The quinones react with protein chains of the cuticle to form sclerotin which hardens and darkens the cuticle (Pryor, 1940). Both molecular (atmospheric) oxygen and oxidase are used for this oxidation.

5. **Cuticular enzymes** A number of enzymes have been found in relation to moulting cycle.

   The best known cuticular enzyme is “Tyrosinase enzyme system” responsible for oxidation of tyrosine. Some chemists consider that it has a single enzyme; others consider that there are at least two distinct viz. (i) tyrosinase (ii) polyphenol oxidase.

   For digestion of old cuticle the moulting fluid contains chitinase (for digestion of protein and carbohydrate part of cuticle).

   “Wax-synthesizing enzymes system” in the epidermal cells is responsible for synthesis of cuticular waxes.

6. **Cuticular Pigments** These are metabolic products of substances ingested with food. These can be separated in to definite chemical groups as follows.

a. **Melanins** These arise from oxidized polyphenols (Mason, 1948). They are dark-brown or black pigments located in the cuticle, usually in the exo-cuticle. Other cuticular colours are browns and yellows. These cuticular colours are permanent.

b. **Carotenoids** Carotenes, xanthophylls, carotenoid acids and xanthophyll esters, assumed to be derived directly from plant food sources, are subhypodermal colours, contained in the fat body and blood corpuscles. They are evanescent.

c. **Pterines or florescent pigments** These are most common pigments in insects, being red, orange, yellow and green. They are located in epidermis and are very evanescent

7. Inorganic salts: Lime is deposited in cuticle in a few aquatic insects, eg., larvae of some psychodids which occur in water rich in lime. Lime deposit in cuticle are also found in the larvae of Stratiomyidae and puparium of some fruit flies in which impregnation of puparium with lime has taken the place of phenolic tanning and in the egg shells of phasmids.

Penetration or diffusion of substances through the cuticle

Penetration is the actual passage of substances into and out of the organism through a membrane of a barrier layer. The amount of penetration under a particular set of conditions is called permeability. The most important physical properties of the cuticle are its rigidity, combined with flexibility and its impermeability, combined with permeability.

Permeability of insect cuticle has been studied in relation to penetration of water, gases, insecticides, etc.

(A) Penetration of water through cuticle

(a) The loss of water Terrestrial arthropods, including insects, being small, have a relatively large surface area and therefore must be protected from excessive evaporation or else they will soon become desiccated.

The resistance of cuticle of terrestrial insects to water loss is due to the following 3 causes-

1. **Thin wax layer** In terrestrial insects the waterproofing or resistance of cuticle to water loss by evaporation is largely due to the presence of a thin wax lipid layer in the epicuticle. This hypothesis is based on the following experimental evidences.

i. It the rate of transpiration from an insect is measured at various temperatures which corresponds to “the transition point” of the cuticular lipids. This Critical Temperature is characteristic of each species, according to the physical properties of its cuticular lipid. Thus, it is 30^0C in Periplaneta (Ramsay, 1935), 37^0C in cabbage caterpillar (Wigglesworth, 1945); or it may not occur until the insect has been killed by heat e.g. 49^0C in *Tenebrio* larvae and 57^0C in *Rhodnius* (Wigglesworth). This critical temperature is somewhat lower (5^o or 10^oC) than the actual melting point of the extractable waxes.

ii. If the wax layer is dissolved by chloroform, ether or peanut oil, the rate of transpiration is enormously increased even at ordinary temperatures. Similarly, the distruction of wax layer by detergents causes a great increase in the rate of transpiration.

iii. If the outer surface of the cuticle is abraded (scratched) with abrasive dusts like fine mineral dusts, the rate of transpiration is enormously increased. The rupture of the epicuticle by abrasive dusts not only accounts for death of insects by desiccation but also provides pathway for the entrance of insecticides.

2. **Sclerotization** The lipid layer should not be assumed to account for all the water retention or resistance to water loss, but sclerotization may also involve a considerable degree of water impermeability (resistance to water loss). Thus, the epicuticle and part of the exocuticle of certain beetles may be abraded (ground away) and yet the cuticle may remain relatively impermeable (Lafon, 1943).

3. Alternating Lipo-protein and protein zones impregnated with lipids Certain insects, like the layers of Sarcophaga, are said to be unaffected by abrasion as they have a relatively thick protein layer impregnated with wax rather then a discrete surface wax layer (Beament, 1948). This arrangement in these insects makes them resistant to water loss.

(b). The absorption of water: The asymmetry phenomenon or structure of cuticle allows the inward passage of water much more rapidly than the outward passage (loss) of water through the cuticle (Beament, 1948;1955; Husrt, 1941, 1948). Some terrestrial insects can take up water from the air even when it is well below saturation eg. *Tenebrio* larvae at 90% R. H. (Mellanby, 1932), prepupae of fleas at 50% R. H. (Edney , 1947) etc. The water penetrates the cuticle through the tufted external ends of the cytoplasmic filaments in pore canals which actively absorb water from the epicuticle (Hellandy, 1932 in *Tenebrio* larvae; Lees, 1946 in ticks etc.). In the eggs of grass hoppers, gryllids etc. water is absorbed through a hygroscopic plate or surface at one end.

(B) Penetration of gases through cuticle

The thin cuticle overlying all chemoreceptor and respiratory surfaces (gills in aquatic insects and tracheal walls in terrestrial insects) is adequately permeable to appropriate molecules of gases or vapors in order to enable important biological responses interrelation to functioning of chemoreceptor and the respiratory surfaces. In cutaneous respiration gaseous exchanges take place in different species.

(C) Penetration of insecticides through cuticle There is a gross relationship between the rate of penetration of insecticides and the speed of insecticidal action in different spp., and between that rate of transcription and insecticidal action (Wigglesworth, 1948).

Penetration of insecticides through the cuticle takes place in two ways.

i. **The penetration of insecticides by destructively affecting the structure of the cuticle** Insect cuticle has a heterogeneous set of barrier layers and if one or more of these is removed by solvent action or disrupted by detergents or interrupted by abrasive action, the efficiency of the cuticle barrier is lowered. Since the cement layer and wax layer on the outside of the cuticle seem to present formidable barriers to the entry of the insecticides, it is their removal, disruption or interruption by certain agents in the insecticidal formulations that most facilitate the entry of the insecticides. Mineral and vegetable oils, detergents and strong corrosive agents are effective in disrupting, and the mineral dusts and clays used as carriers or supplementary materials in insecticides formulations, are effective in interrupting (abrading) these barriers layers and thus enable the penetration of insecticides through both the epicuticle and procuticle.

ii. **Penetration of insecticides without destructively alerting the normal structure of the cuticle**: This is not well understood. Perhaps the following two mechanisms are involved in this:

a. **Absorption of insecticides in solution due to diffusion**

For this the solvent having a low solubility of toxins are most suited. It means that low solubility in particular solvents is favorable for absorption of insecticides in solution due to diffusion (Burt, 1945).

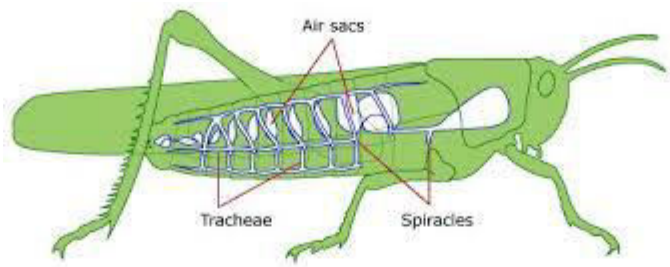
b. **Absorption of insecticides due to their being dissolved in lipid components of epicuticle**

The lipid components in the epicuticle may actually dissolve insecticides such as pyrethrum, DDT etc. and the quantity of lipid present in the epicuticle may be positively co-related with insecticide susceptibility (Klinger, 1936).

The thin walled areas of the cuticle, like setae, membranous intersegmental areas, and the chemoreceptor (particularly the tarsal chemoreceptors) are the most vulnerable areas of least resistance to penetration of insecticides.

RISPIRATION IN INSECTS

Respiration deals with the conveyance of atmospheric oxygen to the tissues and the elimination of carbon dioxide resulting from oxidation in the tissues. The skin of insects, due to their terrestrial existence, has become impermeable to water and thereby ill-adapted for respiration. The great majority of insects breathe by means of tracheal tubes, which usually open at the surface of the body through a number of spiracles, and convey air directly to the tissues.



The tracheal system

The tracheae are invaginations of the cuticle, which branch everywhere among the tissues. In most of the insects the tracheae unite freely to form longitudinal and transverse trunks. The number and arrangement of functional spiracles varies enormously in different groups of insects. With the exception of some collembolan living in wet places & larvae (maggot) of some insects (bee, many Diptera), the spiracles are almost always provided with closing mechanisms of varied designs.

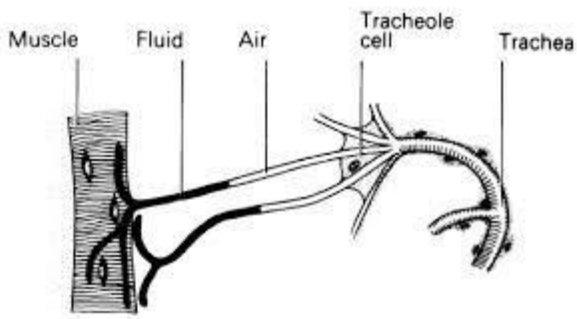
Structure of tracheae

The histological structure of the tracheae resembles that of the body surface from which they are derived. They consist of a matrix of discrete epithelial cells and a thin but complex cuticular lining. The cuticle is thrown into folds, which typically run a spiral course round the tubes for a short distance before a new fold begins. Often the margins of the folds fuse to form a thread, called spiral filament or taenidium, or a rather dense deposit of chitin and protein may be laid down between the folds.

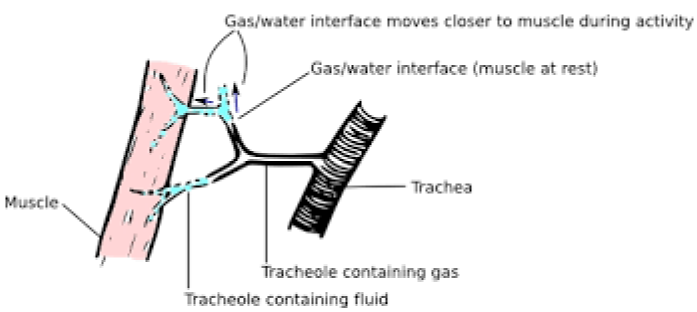
Types of tracheae

1. **Circular tracheae:** They are circular in cross-section and prevented from collapsing by their spiral folds.
2. **Elliptical tracheae:** They are found in *Dytiscus* grubs or mosquito maggots. The spiral thread tends to atrophy hence the tubes collapse when the air pressure within them is reduced. Saccular dilatations occur along their course, e.g. Melolontha, and such dilatations may be enlarged by the fusion of the matrix of adjacent branches to form great air sacs. These air spaces are generally flattened and often collapsed.

Tracheole: Typically, the trachea, at the end is divided into a number of small branches having a diameter of 2-5 μm. Such branching is observed at the end of trachea, where a large stelate cell, the tracheal end cell or transition cell is present. Within this cell the trachea breaks up abruptly into a number of tracheal capillaries or tracheoles, which are less in thickness and are characterized by the absence of a spiral fold. At their ultimate termination they are about 0.2 μm in diameter.



Movement of fluid in the tracheal endings: After death the tracheoles quickly fill with fluid, which creeps along them from the tissues. This has led to an old standing controversy as to whether, during life, the endings contain air or liquid. Endings are easily affected by the fluid in which they are immersed. Examination of living insects under microscope by transmitted light has shown that there is much variation in different tissues and different insects. In the abdomen of the flea, for example, the column of air in the tracheoles ends abruptly while they are still quite large and beyond that they contain fluid; whereas among the muscles of the legs they contain air as far as they can be resolved with the microscope.



Capillary force draws the liquid upwards from the endings of the tracheoles. This force is opposed by an equal force holding the fluid in the tissues. It is probable that imbibitions by the colloid substance of the tracheole wall and the cytoplasmic layer around it, is responsible for holding the liquid back.

Transport of oxygen to the tracheal endings

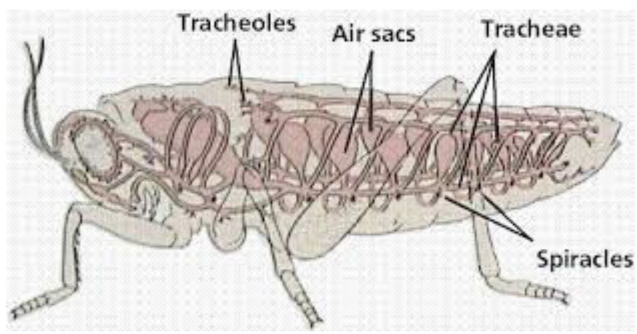
Site of oxygen uptake In most insects, under ordinary conditions, comparatively little respiration takes place through the skin. Most of the oxygen taken up enters the tissues through the walls of the tracheoles. The tracheoles are present in abundant quantity in organs having high oxygen requirement, such as the wing muscles, ovaries, etc. The tracheal walls are permeable to gases; however it is difficult to say as to how much of the oxygen consumed passes through them. Tracheoles are better spread among the tissues to be supplied with O_2 as compared to the trachea, hence most of O_2 is supposed to be supplied through tracheoles.

Mechanism of oxygen absorption It is generally supposed that passage of oxygen from the tracheoles in to the tissues takes place by physical diffusion. But some scientists hold the view that the tracheal epithelium and particularly the tracheal end cells, play a more active part. In many insects like Orthoptera, Odonata, etc. the cellular matrix of the tracheae is filled with granules of pigments, which are supposed to be helpful in increasing oxidations or in storing up oxygen.

1. **Diffusion theory** Greater part of the uptake of oxygen occurs in the tracheoles, and the main problem in insect respiration is the supply of oxygen to these endings. It was assumed long back that many insects must be dependent on diffusion. When we take into consideration the average diameter and length of the tracheae, the oxygen consumption of the insect and the diffusion constant of oxygen, it becomes clear that the diffusion

alone can supply the tissues with those quantities of oxygen actually consumed.

The spiracles contain sphincters, the chief function of which is to protect the insect from water loss. When the spiracles open for oxygen uptake, there starts the loss of water. Hence, the spiracles remain closed during the period when O_2 requirement is not there, being opened for the time only just enough to supply the insect with sufficient oxygen.



The control of respiration by the opening and closing of the spiracles, which is regulated by nerves, is termed as diffusion control.

2. **Ventilation control** Many insects have a system of mechanical ventilation of the larger tracheal trunks which is referred to as ventilation control. Muscular movements of insects, and the contraction of the heart, intestine, malpighian tubes, etc, by pressing upon the tracheae, assist their ventilation, and this is done more or less in proportion to the needs of the animal.

In many insects, particularly in active adult forms, specialized movements of body wall occur, which serve the purpose of ventilating the tracheal system. Some examples of such movements include peristaltic waves over the abdomen as in *Tipula*, dorsoventral flattening of the abdomen in many forms like grasshoppers & beetles, telescopic movements of abdominal segments in Hymenoptera & Diptera.

The respiratory movements partially renew the air in the tracheae by alternate compression and expansion of the tracheal system. Such movements create change in pressure which is transmitted from the body wall by haemolymph.

Functions of air sacs:

1. The chief function of air sacs is to increase the volume of the tidal air, which is renewed at each respiration.
2. They serve to lower the specific gravity of the insect, and to that extent they assist in flight.
3. In some insects, vast air sacs provide space into which the abdominal organs can grow without influencing the outward form of the abdomen, e.g. in muscid flies (*Calliphora* sp)
4. Help in heat conservation in those insects which generate high temperature during flight.
5. Assistance in haemolymph circulation.

6. Formation of the tympanic cavity of the hearing organs of various insects.

The elimination of carbon dioxide:

While the supply of O_2 to the tissues is mainly through the tracheal system, by diffusion through the smaller tracheal branches combined in many insects with mechanical ventilation of the larger trunk, the elimination of CO_2 takes place through both the body surface and the linings of the tracheae.

In the skinned larvae, co_2 is given off by the posterior half of the body which may be due to the backward circulation of air through the tracheal system, as in grasshopper or due to the co_2 carried backwards by the circulating blood or due to the fact that in quiet respiration only certain spiracles are functional. In heavily sclerotized insects the co_2 escapes from the soft inter-segmental membranes.

Respiration in aquatic insects:

1. **Cutaneous respiration:** In certain larvae, although the tracheal system is developed in the usual manner by ectodermal invaginations, it becomes entirely cut off or closed from the exterior. During the early stages of larvae of this type e.g. *Chironomus, Simulium*, etc. the system may remain filled with fluid and the respiration takes place by diffusion of gases through the skin.

2. **Blood gills:** In some larvae certain regions of the integument are often very thin and project from the body surface (*Chironomus*), or are evaginated from the rectum (*Simulium*), as delicate blood filled sacs, devoid of tracheae or provided with only a few small branches. These are often called blood gills, through which the gaseous exchange is supposed to take place.

3. **Tracheal gills:** There are many modifications of the closed tracheal system which help in obtaining oxygen from water. The skin is often supplied with a rich net work of fine capillaries as in *Simulium*. This network may be most abundant where the cuticle is thin, as in Trichoptera. Then in various regions of the body wall or within the rectum, there may be evaginations well supplied with trachea which form true tracheal gills. But cutaneous respiration is still important in most aquatic larvae even when efficient tracheal gills are present.

4. **Spiracular gills:** There is another type of gill in aquatic insects in which there is no secretory activity. These are termed 'spiracular gills', tube gills or cuticular gills, e.g. in larvae of *Teichomyza* and in the pupae of *Simulium*. They are filamentous outgrowths of the ectoderm and are covered with a layer of cuticle. They provide enormous surface area for diffusion of oxygen inwards from the surrounding water.

5. **Surface breathing hydrofuge structures:** Most of the aquatic insects depend on the atmospheric air for respiration. Many structural adaptations are present in insects which facilitate the contact of certain spiracles with the atmosphere, e.g. the terminal respiratory tube of *Nepa*, modified antennae of *Hydrophilus* and its allies which come head first to the surface, *Dytiscids* which raise tail first, the respiratory siphons of *Eristalis* bearing the spiracles at the apex.

But a problem common to all these insects is the breaking of the surface film of the water so that the spiracles may be exposed to the air above. This problem has been solved in all cases by the provision of region of the body wall whose physical properties are such that they have a greater affinity for air than for water. Thus, when the insect reaches the surface film, the water falls away and leaves the cuticle dry.

6. **Air stores of aquatic insects:** Besides retaining a film of air immediately around the spiracles, many aquatic insects are able to carry bubbles or films of air on other parts of the body. In the larvae of *Hydrocampa* and on the ventral surface of *Notonecta* and many other insect, there is a fine pile of erect

'hydrofuge' hairs, which hold between them a layer of air, like velvet when immersed in water. Sometimes the hairs are sent over at the tips.

7. **Plastron respiration :** In some cases, e.g. in the beetles *Hemonia*, the air film is so held by the hydrofuge hairs that it can't be replaced by water. Condition then approximate to the closed tracheal system, and the insect becomes independent of the atmospheric air. This very thin firmly held layer of gas is termed plastron. The volume of gas in it is negligible but the volume remains constant. Functionally it resembles a tracheal gill.

8. **Aquatic plants as source of oxygen**: Many aquatic insects obtain oxygen from aquatic plants. Free bubbles of gas given off by plants are frequently taken up by the hydrofuge surfaces of insects. Many insects obtain their oxygen from the intercellular air spaces of water plants, either by biting into the air containing tissues or by inserting a specially modified respiratory siphon into the air containing cells.

Respiration in endoparasitic insects;

1. **Cutaneous respiration**: The respiration in endo-parasitic insect larvae shows similarity with the respiration of aquatic forms. In the first instar larvae of many Hymenopterous parasites, the tracheae are filled with fluid and non- functional. The exchange of gases takes place directly between the tissue fluids of parasite and host. The spiracles become open and functional at the time when the larvae is about to quit its host.

2. **Blood gills and tracheal gills**: Many Braconid and Ichneumonid larvae posses a tail of varied form which resemble blood gills of aquatic larvae. Such blood gills perform locomotor, absorptive and excretory functions but their respiratory function is of minor importance. The caudal vesicle of Braconids is the site of rather more active oxygen uptake then rest of the body surface. The tails of Agromyzid *Cryptochaetum* are well supplied with fine tracheae and are important in oxygen absorption. The carbon dioxide is given off more or less equally over the general body surface.

3. **Respiration of atmospheric air** : Many parasitic Hymenoptera e.g. *Blastothrix* are metapneustic, breathing only through the hindmost pair of spiracles, in this case the pedicel of the egg protrudes externally through the body wall of the host and functions as a kind of respiratory tube into which the larva inserts its posterior spiracles in order to breathe the

atmospheric air. The remains of the egg may serve this purpose throughout the first three larval stages.

4. **Obtainig air contained in host tissues**: There are parasitic larvae which tap the tracheal system of their host. This is very common among Tachinids, where the trachea is ruptured by the larva, its epithelium again spreads in wards to form a cuticular siphon or sheath around the parasite.