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 silkworm 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 textbook '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.

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.

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,

(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.

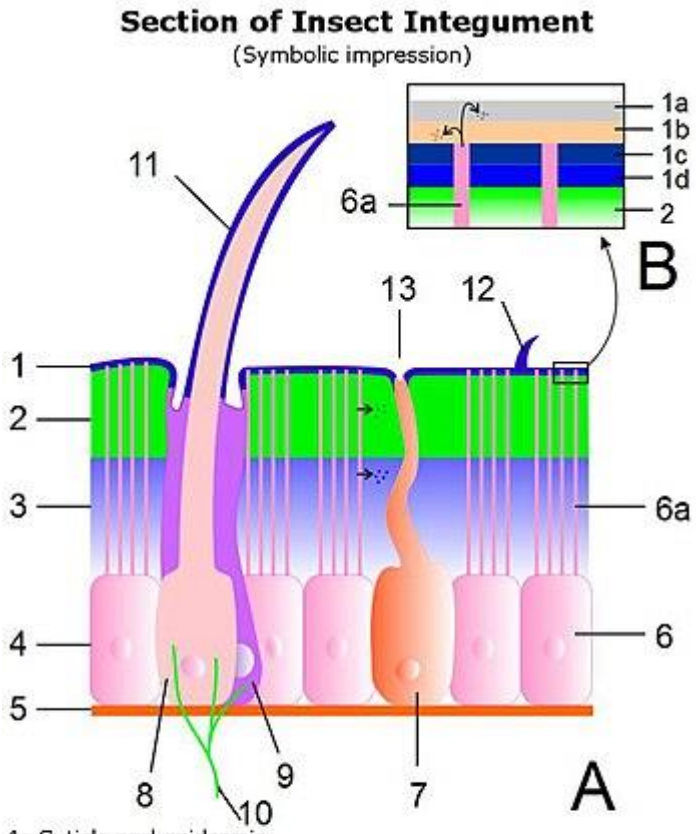
2. **Epidermis (or hypodermis):** It is composed of a single layer of epidermal cells which are differentiated in to (i) ordinary epidermal cells (ii) specialized epidermal cells forming the dermal glands and (iii) large occasional cells as well as the oenocytes and the dermal glands. that show cyclic 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. Oenocytes are large secretory cells present in the abdomen of insects known to synthesize very-long-chain fatty acids to produce hydrocarbons and pheromones that mediate courtship behavior in adult flies.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. 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. It is the water proofing layer of the cuticle. A few spp. livings 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 to10 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 into 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 pore canals, with their filaments. 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. 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. 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 into 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 mounting 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 exoskeleton. At the same time(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 chitinprotein 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 smaaler(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(average about 33%) of the dry weight of cuticle. It is the known constituent of insect cuticle, ranging from 25 to 55% nitrogenous polysaccharide made up of long chains of acetylglucosamine 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 hydrolyzed 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 eggshells.

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** Several 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 into 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 temporary.

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

7. Inorganic salts

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.

i. 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 somewhat lower (5o or 10oC) 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 sclerotization may be involve a considerable degree of water impermeability (resistance to water loss).

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 surface.

Penetration of insecticides through cuticle

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 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.