INSECT PHYSIOLOGY

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Summary

Insect physiology is the study of how insects live and reproduce. This is a historic area of research that continues today. The study of insect physiology is usually divided into a systems approach. These systems are the same required by all animals. The major systems are: digestive, excretory, circulatory, immune, muscular, nervous, and reproductive. All of these will be discussed to some extent in this chapter. Additional information can be obtained from other sources for readers requiring a more in depth examination of each system.

1. Introduction

Insects are the most diverse of all organisms on earth. Their general body plan allows for this tremendous diversification in form. Insects are arthropods meaning they have an external skeleton that covers the internal tissues. The exoskeleton protects the internal tissue but also allows for sensory systems to function. Insect physiology is the specialized study of how insects live and reproduce. A discussion follows of how the organ systems function in insects. The first section will be concerned with a description of the exoskeleton and the molting process involved in growth and development followed by sections describing the major organ systems of insects.

2. Exoskeleton

The integument makes up the skeleton of insects and a considerable amount of research has taken place on this part of the insect. The insect exoskeleton is very strong yet flexible for movement and providing points for muscle attachment. The exoskeleton can be considered as a hollow cylinder, which on a per weight basis is stronger than a solid cylinder, but the hollow cylinder has a larger surface area for the same amount of weight. The exoskeleton is also highly variable and can be adapted for various functions. It can be very hard, for example mandibles, or very soft, for example integument of larvae. All external structures are made of cuticle, which produces the great diversity of insect species today. It is the site of sensory organs and also the source of color. The exoskeleton of insects is primarily made up of several layers that have been described in detail (Figure 1). These include the epicuticle, exocuticle, and endocuticle in order from outside to inside. The exoskeleton is produced by the underlying epidermal cells and is separated from the hemolymph by a basement membrane.

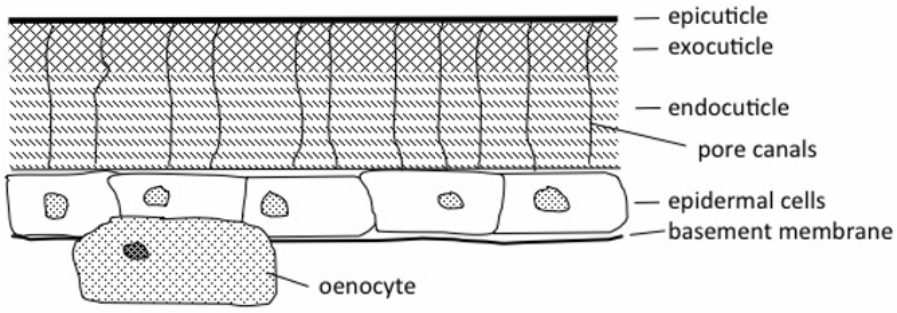

Figure 1. Schematic of the various layers of the insect cuticle.

The outer most layer, the epicuticle, is the thinnest layer with its primary function of water protection. Lipids are found on the surface of the epicuticle to provide protection against water loss in terrestrial insects and water gain in aquatic insects. Some insects have ordered blooms of waxes that provide additional water loss protection. Various types of lipids are present on the surface but most insects have hydrocarbons. The hydrocarbons and other lipids are constantly being transported to the surface through the underlying cuticle in pore canals. Oenocytes are a cell type usually found associated with epidermal cells that produce the hydrocarbons. These are transported to epidermal cells by a lipid carrier protein called lipophorin. Hydrocarbons and other molecules on the surface can also be used by insects in chemical communication with other insects and animals.

The exocuticle and endocuticle make up the majority of the thickness of the exoskeleton. These layers are primarily made up of a protein and chitin complex that is held tightly together with small molecular weight cross-linking molecules called diphenols. Chitin is the second most abundant biopolymer on earth. It is a polysaccharide composed primarily of *N*-acetylglucosamine molecules joined in a β 1-4 linkage. The most abundant biopolymer is cellulose, which is composed of β 1-4 glucose. Chitin chains are held together in a microfibril primarily by hydrogen bonding. This hydrogen bonding gives chitin some of its chemical properties, it is resistant to swelling in water, resistant to hydrolysis by bases and dilute acids, and to being dissolved by organic solvents. Chitin microfibrils are about 2.8-5 nm in diameter and are surrounded by a protein matrix in a helical fashion. The degree of cross-linking with diphenols results in a hard cuticle. The exocuticle has considerably more cross-linked protein than the endocuticle. The exocuticle can also be tanned resulting in a brown color. Sclerotization and quinone tanning are processes that harden the cuticle. Both use the diphenols *N*-acetyldopamine and *N*-β-alanyldopamine as a substrate for cross-linking. A polyphenol oxidase is the enzyme that initiates the cross-linking pathways.

The entire cuticle is not the same, even in the same insect. Some cuticle is sclerotized, some is tanned; some have different cross-linking diphenols in varying amounts, and along with different proteins and chitin content all contribute to giving cuticle its unique properties. Various parts of the cuticle have different levels of diphenols resulting in harder cuticle. *N*-β-alanyldopamine is found primarily in the hardest cuticle, for example the cutting edge of caterpillar mandibles. Zinc can also be found in the hardest cuticle. Adult leaf cutter ants have increased concentrations of zinc in the cutting edge of the mandible. The thickest hardest cuticle would have a thicker exocuticle while more pliable cuticle usually lacks the exocuticle. Pliable cuticle found between sclerites remains strong yet flexible due to the enodcuticle.

The insect cuticle is produced by underlying epidermal cells, which is only one cell layer thick. These cells are very active metabolically and will change shape as well as number with development. Before ecdysis there is an increase in mitosis to provide more cells for the following instar. Cell size changes from smaller during ecdysis to larger during the instar and before the next molt. Epidermal cells are separated from the hemolymph by a selectively permeable basal lamina or basement membrane. Microvilli are present on the cuticle side of epidermal cells, which contain plasma membrane plaques that are thought to be the actual site of synthesis of the chitin microfibril. These microvilli contain actin microfilaments, which are instrumental in producing movement of the microvilli. This movement is important in the laying down of the chitin microfibril when the cuticle is made. The chitin is then wrapped by proteins, which make up about 60% of the cuticle. A variety of proteins have been identified from insect cuticle and in general flexible cuticles contain proteins with lower isoelectric points (meaning an overall lower pH) and a higher glycine content, and more glycosylated proteins.

A special type of protein called resilin is found in hinge regions of the cuticle, such as the wing hinge. This protein is rubber-like in that when it is relaxed or not under stress it is compact, but under stress it can be pulled out, and then when the stress is released it

snaps back to its original configuration, much like a rubber band. Thus it always maintains its original shape. Its main function is for use in hinge regions between movable sclerites. Muscle stretches the resilin and when the muscle relaxes resilin pulls back the attached sclerites without muscular action, saving energy.

Exoskeleton color is produced in several ways. Browns are produced by the quinone tanning process. Melanization is the process of producing true black pigments in the cuticle. Color can also be due to pigments found in the epidermis or cuticle. An example of this is insecticyanin found in the epidermal cells of *Manduca sexta*, tomato hornworm, larvae, which is a combination of a blue pigment called biliverdin and a yellow pigment called lutein that is obtained in the diet. A lot of the bright colors are due to the reflective properties of the cuticle. Bright iridescent colors are produced as the result of interference by multiple thin films separated by material of slightly different refractive index results in the bright metallic colors. Scattering of light produces white colors and some greens and blues. Diffraction is the splitting of light into its component spectral colors. This is done by a series of fine grooves or ridges separated by spaces corresponding to a certain wavelength of light. The latter are structural colors produced by the microarchitecture of the cuticle.

2.1. Molting Physiology

The process of shedding the old cuticle and laying down of the new cuticle is called molting. It allows for growth in larval stages, i.e. each successive instar gets bigger, and allows for metamorphosis in the transition from larva to adult. The process begins when epidermal cells start to divide increasing in number, become close packed and columnar in form (Figure 2). The next step is apolysis where the old cuticle breaks away from the epidermal cells, forming a subcuticular space beneath the old cuticle. An inactive molting fluid or gel is secreted into the subcuticular space. Molting fluid contains inactive enzymes at first, which allows for the proper laying down of the new epicuticle. This is the first layer that is formed and protects the new cuticle as it is being formed. Molting fluid is inactive at first so it doesn't degrade the new epicuticle that is being made at this time. After epicuticle formation the molting fluid becomes active and starts to digest the old cuticle. However, only the un-sclerotized cuticle can be digested, i.e. only the endocuticle is digested with a lot of the products of digestion absorbed back into the epidermal cells. New cuticle is produced at this stage, which is undifferentiated cuticle, sometimes called procuticle. The insect now undergoes ecdysis, which is the actual shedding of the old cuticle. Through the action of the molting fluid the cuticle becomes very thin and weak along ecdysial lines. These lines are areas formed in the cuticle that do not have the hardened exocuticle, so the molting fluid digests away most of the cuticle along these lines. Most insects have an inverted Y shape along the head and prothorax. This starts a whole programmed behavior involved in shedding of the old cuticle. It includes swallowing air or water so that an increased internal pressure is developed and pumping of hemolymph into the thorax to exert even more pressure on the ecdysial lines. *Rhodnius* and blowflies (and most probably other insects) use special muscles that are made only for ecdysis, these will undergo programmed cell death or apoptosis after the ecdysial event. After splitting of the old cuticle the insect draws itself out, usually head and thorax first. All of the cuticular parts are shed, including the lining of fore and hind gut and lining of the major tracheae. The

old cuticle is called an exuviae. After ecdysis, there is continued internal pressure to expand the new epicuticle, and undifferentiated new procuticle into the new size and shape of the insect. Now the exocuticle can become hard due to quinone tanning and sclerotization. Hardening may take several hours and probably continues at least until the endocuticle is laid down. The endocuticle in some cases is continually produced resulting in a lamellate structure. Some epidermal cells could continue to produce endocuticle for days or even weeks depending on the type of cuticle and age of insect.

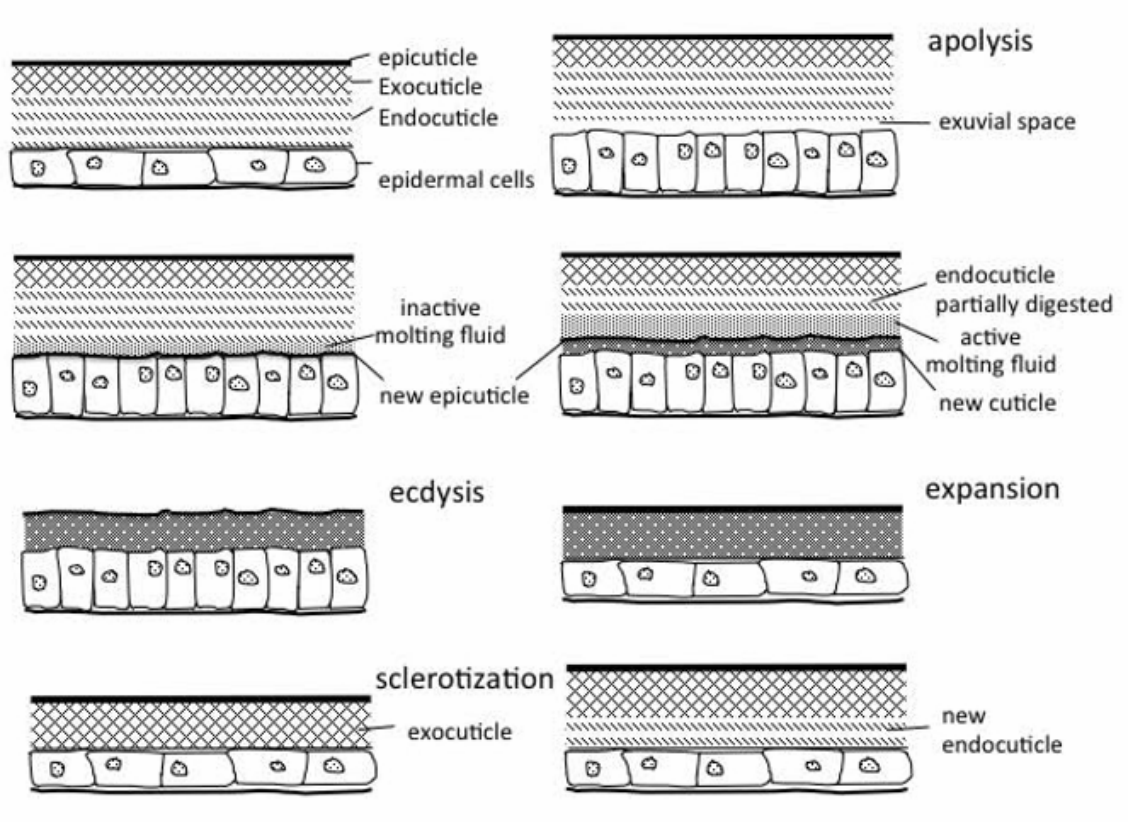

Figure 2. Schematic of the molting process.

3. Metamorphosis

Insects undergo metamorphosis in transition from a nymph or larvae into a reproductive adult. In hemimetabolous insects the last instar nymph will undergo metamorphosis to the adult. In holometabolous insects the larvae will pupate and an adult will develop inside the pupal case. In both cases the endocrine system is controlling the molting and metamorphosis process. Factors that are involved in regulating molting include environmental factors such as temperature, photoperiod, humidity and physiological factors such as quantity and quality of food, presence or absence of nutrients in the food, and infection by microorganisms or parasitoids. Variations in these parameters can accelerate or delay the onset of molting or metamorphosis in complex ways that indirectly or directly stimulate the neuroendocrine system to start the molting process. The two main metamorphic hormones found in insects are ecdysteroids and juvenile hormone (JH). Ecdysteroids are the molting hormones of insects that will initiate the molting process. The presence or absence of juvenile hormone will determine what type of cuticle the next molt will produce. In the presence of juvenile hormone the next

cuticle will be another immature. In the absence of juvenile hormone the insect will develop into an adult.

The steroid hormone ecdysone is produced by an endocrine gland, called the prothoracic gland, found in the prothoracic segment of the thorax. It is released hours to days before the actual ecdysis to initiate the molting process. Ecdysone is produced and released from the prothoracic glands into the hemolymph. However, the active hormone is called 20-hydroxyecdysone, which is produced when ecdysone is acted on by the fat body and other tissues. The active 20-hydroxyecdysone will program the epidermal cells to start the molting process.

The production of ecdysone is regulated by a peptide hormone called prothoracicotropic hormone (PTTH) that is produced in neurosecretory cells of the brain. Several neurosecretory cells of the brain produce PTTH that is transported to the corpora cardiaca for release into the hemolymph. PTTH then travels to the prothoracic gland where it stimulates it to produce ecdysone. Ecdysone is not stored in prothoracic glands so it is synthesized and released. PTTH acts on the prothoracic glands to stimulate protein synthesis that is required for production of ecdysone. At the present time what is known about the regulation of ecdysone production is a complex phenomenon that could vary depending on the insect under study. However, extracellular Ca^{++} is absolutely required for signaling to increase ecdysone production. In some insects the innervation of prothoracic glands may have direct control of ecdysone biosynthesis, including inhibitory factors. But in general it is thought that PTTH is the main hormone regulating the production of ecdysone.

Ecdysone is released into the hemolymph from hours to days after stimulation by PTTH, depending upon the insect. Ecdysteroids are primarily acting on the epidermal cells to produce the changes necessary for a molt to occur; but is really acting on all of the tissues in the insect during the molting process. In hemimetabolous insects ecdysone is released prior to every molt. In holometabolous insects the larval to larval molt has one peak of ecysone before each ecdysis. However in the last larval instar before the pupal molt two peaks of ecdysone occurs. It is thought that the first peak is required to switch the state of commitment to produce pupal characteristics. This peak occurs in the absence of juvenile hormone. The second peak of ecdysone, usually involves an increased amount of ecdysone and induces the actual molting process that produces the pupae. Each peak is stimulated by PTTH secretion.

Ecdysteroid titer is critical for the various phases of molting to occur and along with the presence or absence of JH determine which type of cuticle the insect will secrete. However ecdysteroid titers do not act as simple triggers that set the molting cycle in motion, this is indicated by the long time period that the ecdysteroids act on the epidermal cells to induce molting. It is not known in detail how ecdysteroids are reprogramming the epidermal cells prior to the molt. It all happens at the DNA level. It involves the loss of mRNA for larval-specific proteins and the synthesis of new mRNAs and proteins that are required to permanently suppress larval specific genes. Ecdysteroids are also involved in alterations at the DNA level in preparing the nuclear machinery to produce the transcription of previously unexpressed pupa-specific or adult-specific genes.

Action of ecdysteroids on target cells involves binding to an ecdysone-receptor (EcR) in the nucleus. The ecdysteroids bound to EcR will form a complex with another protein called ultraspiracle (USP). The ecdysteroid-EcR-USP complex binds to an ecdysone response element or DNA-binding domain that lies within the promoter region of specific genes. This action will activate or inactivate gene expression.

Juvenile hormone (JH) is produced by the corpora allata (CA). The CA are small endocrine glands associated with the brain (see Figure 3). JH is not stored in the CA and so its biosynthesis is regulated. Insects differ widely in the mechanism by which they control their CA. In some, the CA is controlled by blood born hormones, an example is the Colorado Potato Beetle. In this beetle, if CA nervous connections are cut with the brain, the CA function normally. In many other insects a nervous connection between the brain and CA is necessary for normal CA function. It is known that the median neurosecretory cells of the brain will produce peptides that control CA. These are called the allatostatins and allatotropins. Allatotropins stimulate JH production and allatostatins inhibit the biosynthesis of JH in the CA.

Once JH is biosynthesized it is not stored in the CA but released into the hemolymph. The amount of JH in the hemolymph is also regulated. This involves an understanding of how JH is found in the hemolymph. Since JH is a lipophilic compound it can be found bound to various proteins and hemocytes in a nonspecific way and also in fats in general. It is also found bound to proteins called JH binding proteins (JHBP) that will only bind JH in a specific way. These JHBPs are important in regulating the levels of JH in the hemolymph.

The two enzymes that are involved in degradation of JH are JH esterase (JHE) and JH epoxide hydrolase (JHEH). These are also produced by the fat body. JH esterase is a specific esterase for JH whose role it is to clear the hemolymph of all JH in preparation for the pupal or adult molt. So JH esterase levels rise at the end of the last larval instar of holometabolous insects at the time when JH amounts decline in the hemolymph. This occurs at the same time when JH production by the CA stops. JH binding protein plays a role in the action of JH esterase. When JH esterase levels rise in the hemolymph JH bound to JHBP will be given up to the esterase. Thus when the levels of JH esterase rise during the last larval instar to get rid of JH in the hemolymph, even the JH bound to binding protein will be degraded. However in contrast, JH epoxide hydrolase will not act on JH when it was bound to JHBP. JH epoxide hydrolase's role is to hydrolyze the JH acid produced by the action of JH esterase to the JH acid diol.

Juvenile hormone is acting to determine which type of cuticle will be formed during the molting process. In the presence of JH larval cuticle will be formed due to the increase in ecdysteroid during the period prior to an ecdysis. In the absence of JH pupal and later adult cuticle will be formed in holometabolous or adult in hemimetabolous insects. The hemolymph titer of ecdysteroid is more critical than the titer of JH. The ecdysteroid response depends upon a certain physiological dose of the steroid. However the JH response does not necessarily respond to dose as long as the JH amount is above a certain critical level. So there is a threshold level of JH, above this level cells will respond below that level they will not respond. Also there are discrete JH-sensitive periods, that is, if JH is present above the threshold level then a current developmental

state is maintained, if JH is below the threshold level then there is a switch in developmental pathways. The JH-sensitive periods are the periods during which JH is exerting its influence. Outside of these periods cells are relatively unresponsive to JH.

In hemimetabolous insects there are JH-sensitive periods prior to each molt. If JH is present then the commitment is for another larval cuticle at the next molt. If JH is absent then there is transformation that occurs to develop adult characteristics at the next molt. It is thought that ecdysteroids are controlling the actual differentiation into the adult stage. In holometabolous insects there are JH-sensitive periods prior to each larval to larval molt. If JH is present then another larva will be produced at next molt. The last instar is more complicated due to the pupal stage. If JH is absent during the first JH-sensitive period then the next molt will be a pupal molt. If JH is present during the second JH-sensitive period then the next molt will be a pupal molt. So the last instar has two periods, one where JH is absent and one where JH is present.

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Biographical Sketch

Russell Jurenka, received a Ph.D. in Biochemistry from the University of Nevada Reno. He was a postdoctoral fellow at Cornell University – NYS-AES; and is a full professor of Entomology at Iowa State University, USA. He teaches Insect Biology to undergraduate students and Insect Physiology to graduate students. His main area of research is on pheromone biosynthesis and its hormonal regulation. Dr. Jurenka has published about 60 papers in the primary scientific literature and about 10 chapters in various books.