

Lipids

Lipid requirements include fatty acids and sterols. Reserve energy sources in the form of fat, mentioned previously, are derived from either sugars or dietary saturated and monosaturated fatty acids. Fatty acids for energy are not deemed essential because alternate sources of energy are available, but some butterflies and moths require polyunsaturated fatty acids for normal development.

In contrast to mammals, insects are unable to synthesize sterols. In addition to being essential components of cell membranes, a sterol is required for the production of ecdysone and other growth hormones. Foods vary in the predominant kind of sterol supplied to insects: animal foods have cholesterol, plants have β -sitosterol, and fungi have ergosterol. Regardless of the origin, the presumed biogenetic pathway to ecdysone involves converting cholesterol to 7-dehydrocholesterol. Cholesterol is ordinarily adequate in an artificial diet, but some insects with highly specific diets have apparently lost the ability to convert cholesterol: the fly *Drosophila pachea* (Drosophilidae, Diptera) requires the cactus sterol schottenol from its host *Lophocereus schotti*.

Vitamins and Growth Factors

Vitamins are essential for living processes, but they are not used for energy or basic structures. Insects at one time were thought to need only water-soluble vitamins. Among these, seven B vitamins are required for most insects (Table 5.1), and some species also require cyanocobalamin (vitamin B₁₂) and carnitine (vitamin B_r). Plants are usually a rich source of ascorbic acid (vitamin C), and most plant-feeding insects require vitamin C in their diet at concentrations higher than those required by vertebrates, apparently having lost the ability to synthesize it.

Among the fat-soluble vitamins are vitamin D (calciferol), involved in calcium metabolism; vitamin E (α -tocopherol), an antisterility factor for mammals; vitamin A (retinol) or precursors, involved in carotenoid visual pigments; and vitamin K (phylloquinone, etc.), essential for clotting mammalian blood. Vitamin D is not required because

insects do not have calcareous skeletons and are apparently unable to use it even as a sterol. The clotting of insect blood differs from that of mammals and does not involve vitamin K, but some experimental evidence exists that vitamin K stimulates growth in insects. Vitamin E deficiencies in insects have been difficult to demonstrate consistently, but both egg and sperm production, as well as larval growth, may be affected. A need for vitamin A has long been suspected because the visual pigments of insects are rhodopsins. Morphological and visual defects have been found in several species of insects reared on vitamin A-deficient diets.

The growth factors choline and *myo*-inositol are required as constituents of phospholipid molecules. Ribonucleic acid has been found to be required by some Diptera.

Minerals

Insects require approximately the same minerals as do vertebrates because minerals, of course, cannot be synthesized. In comparison to mammals, insects require much less calcium (not needed for the skeleton), less iron (few insects have hemoglobin, but iron is essential for many cellular processes), and much less sodium and chloride (many insects do not use these in homeostatic functions of blood). Although required in only trace amounts, zinc is apparently so critical in the moth *Heliothis virescens* (Noctuidae, Lepidoptera) that it is conserved by transferring it in the male semen to the female during copulation.

ANATOMY AND FUNCTION OF THE ALIMENTARY CANAL

The tubular alimentary canal of an insect passes through the body from its mouth to its anus, and it provides the special internal environment for food to be mechanically and chemically disintegrated and brought to the vicinity of absorbent cells. Despite the diversity in physical consistency and composition of food, the gut exhibits certain general anatomical features throughout the insects (Figs. 5.2, 5.3).

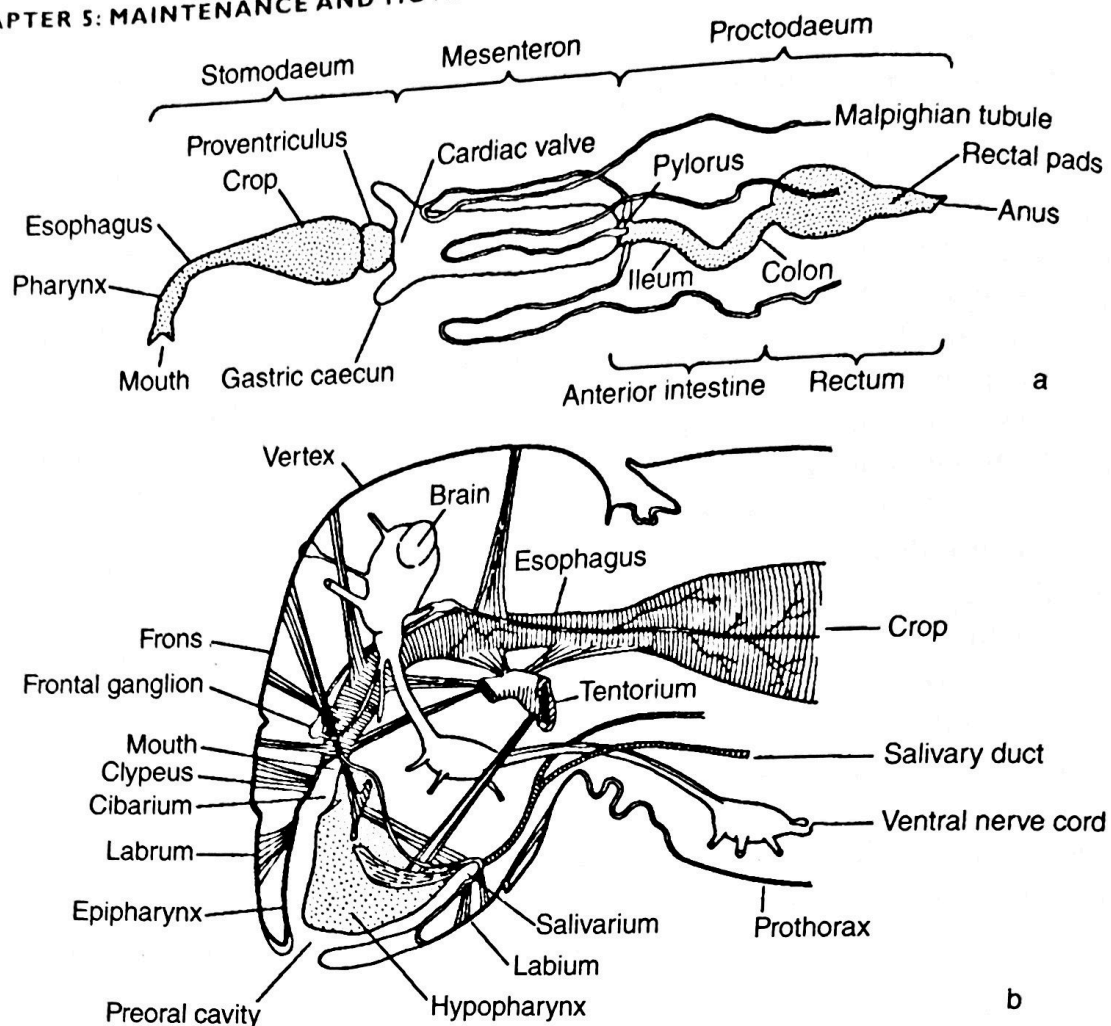


Figure 5.2 Alimentary canal: **a**, general structure; **b**, mouth and foregut.

Source: Redrawn from R.E. Snodgrass, *The Principles of Insect Morphology* - public domain.

Anatomy of the Alimentary Canal

The gut is attached at each end to the body wall but otherwise is held in place in the hemocoel largely by the pressure of adjacent organs and flexible tracheae. The three major divisions are (1) the **stomodaeum** or **foregut** (Fig. 5.2a), derived from embryonic ectoderm; (2) the **mesenteron**, or **midgut**, derived from endoderm, and (3) the **proctodaeum**, or **hind gut**, derived from ectoderm. Attached at the juncture of the midgut and hind gut are the filamentous excretory organs called **Malpighian tubules**. In the embryo, the Malpighian tubules arise from the anterior end of the developing hind gut.

The wall of the alimentary canal is composed of a single layer of cells separated from the hemolymph by a basement membrane. The foregut and hind gut are both lined with a thin cuticle, called the **intima**, that is continuous with the integumental

cuticle and is usually impervious to water. These portions of the gut are properly classed as organs of the integument, and the cell layer is homologous with the epidermis. The intermediate section, the midgut, is differentiated by its endodermal origin and lack of an intima. The cells of the midgut are called **epithelial cells**, or the **epithelium**. In place of an intima, however, a loose film, the **peritrophic membrane**, is secreted in most insects that, in addition to other functions, protects the delicate cells from abrasion by food particles and also compartmentalizes digestive processes.

Muscular connections between the gut and exoskeleton are limited to near the mouth and pharynx to provide a pumping action. Layers of circular and longitudinal muscles in the gut wall push the food along by peristaltic contractions. These are controlled by sensory and motor innervations from the stomodaeal nervous system (see Chapter 6)

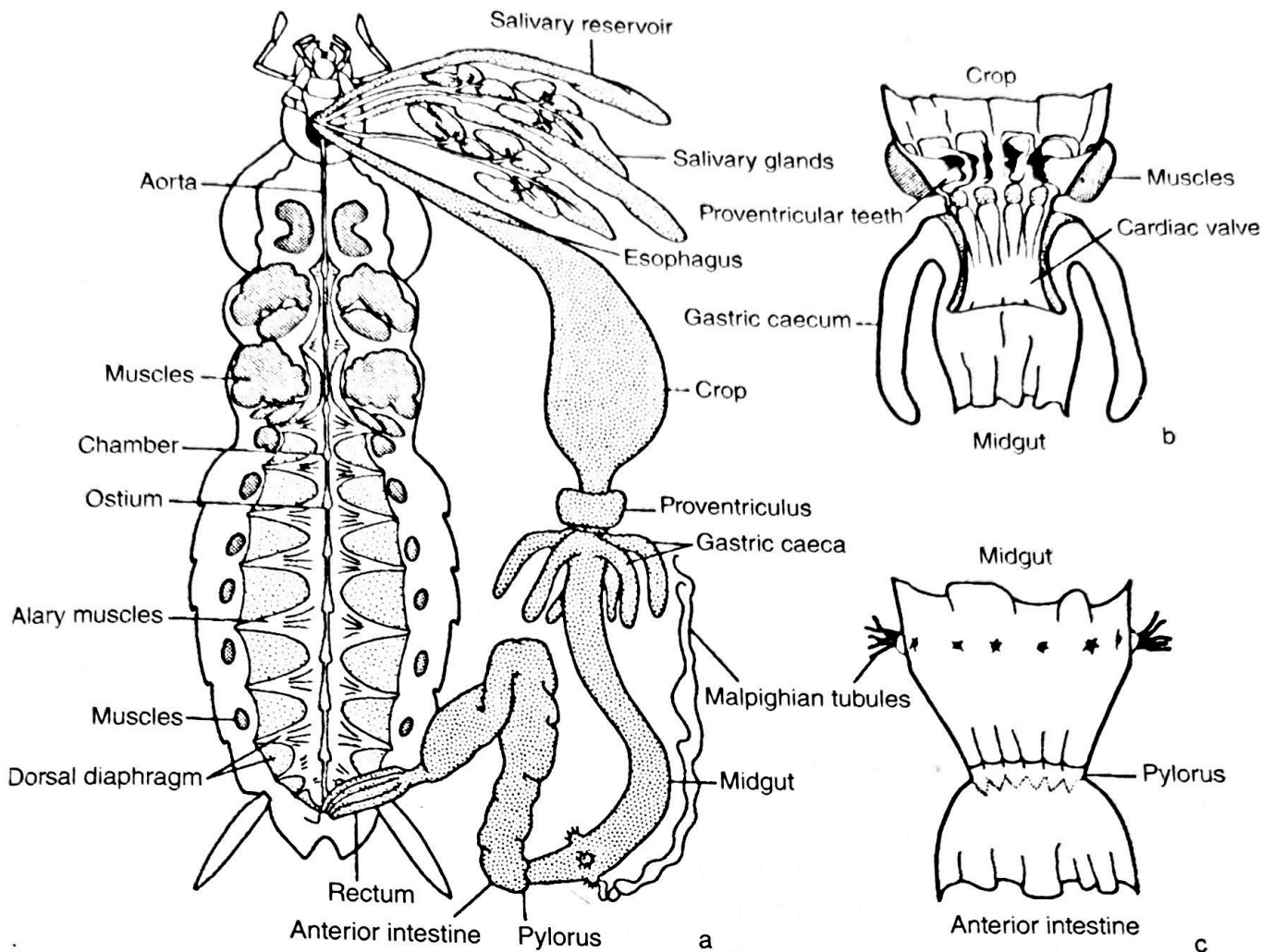


Figure 5.3 Alimentary canal and dorsal vessel of the roach *Periplaneta americana* (Blattidae): **a**, ventral view of dissected roach, with the fat body, reproductive organs, and most of Malpighian tubules removed; **b**, interior of proventriculus and cardiac valve; **c**, interior of pylorus.

Source: Barbara Boole Daly.

to the foregut and, in some insects, the midgut. The hind gut receives nerves from the posterior abdominal ganglia of the ventral nerve cord. Stretch receptors along the gut provide inputs to the nervous system that can affect development and reproduction as well as numerous other physiological processes. The scarcity of sensory nerves probably means that insects rarely suffer stomachaches. Tracheae from visceral trunks supply oxygen to the alimentary canal according to the metabolic needs of the different regions.

The true mouth is hidden from view and opens into the **preoral cavity** above the hypopharynx and below the epipharynx (Fig. 5.2b). The preoral

cavity directly in front of the mouth is the **cibarium**, where food is passed to the mouth. The preoral cavity also contains another chamber beneath the hypopharynx and above the labium. This is the **salivarium**, where the saliva is emptied into the cavity from the labial or salivary glands.

The first distinctive region of the foregut is the **pharynx**. This heavily muscled organ is situated behind the mouth. The **esophagus** is merely a tubular portion of the foregut leading to the saclike **crop** (Fig. 5.3a). The release of food from the crop is controlled by an intricate organ, the **proventriculus**. Like the pharynx, the proventriculus may have a heavy muscular coat. Internally, the cuticle is often

shaped into heavy teeth, plates, or spines that can act as a filter that regulates food passage or grinds the ingested food into smaller particles. In fluid-feeding insects and many larvae, it is a simple sphincter. The foregut may be expanded into a large sac or have an attached sac that is an outpocketing from the foregut for use as a storage organ from which fluids may be regurgitated (as in the case of honey bees) or slowly released into the midgut. The most posterior part of the foregut is invaginated in the midgut and called the **cardiac valve**, or proventricular valve (Fig. 5.3b). It may be heavily muscled to open and shut the connection between the foregut and midgut, or it may lack muscles but open passively in response to the positive pressure of incoming food from the foregut and close when the pressure difference reverses, to prevent food movement back into the foregut. Special secretory cells between the valve and the midgut wall are partly responsible for the formation of the peritrophic membrane in larval and adult flies (except blood-feeders) and some Lepidoptera.

The midgut may be a simple tube or variously modified. The epithelium may be differentiated into distinctive histological regions. The columnar cells are especially active since the secretory processes that yield the digestive enzymes often involve the eventual disintegration and rejuvenation of the cellular lining. Next to the lumen, the cells characteristically are densely covered with short, hairlike filaments, or microvilli. Secretory cells lost during enzyme production or by molting are replaced by regenerative cells. Substances may be accumulated in epithelial cells for storage.

In insects such as grasshoppers, true bugs, and larval scarab beetles, hollow, fingerlike pouches may be present at the anterior or posterior ends of the midgut. These are the gastric caeca, which shelter symbiotic bacteria in some species such as stink bugs (Hemiptera, Pentatomidoidea). At the junction of the midgut and hind gut are the Malpighian tubules. These serve as a convenient external marker to distinguish the posterior end of the midgut. Also in this area is the pyloric valve (Fig. 5.3c). The valvelike function is best developed in caterpillars and beetles.

The hind gut may have several recognizable sections: (1) an **anterior intestine**, composed of the **ileum**, which is larger in diameter, and a

narrow posterior portion, the **colon**; and (2) the **posterior intestine**, or **rectum**, which is the final section before the anus. The hind gut is lined with cuticle, which is usually impermeable. The **rectal pads** (see Fig. 5.8b), however, have a cuticular lining that is permeable to water, salts, sugars, and amino acid molecules. Together with the Malpighian tubules, the ileum and rectal pads form parts of the excretory system. In the rectum, the waste is dehydrated and often compressed by muscles into a pellet that bears the shape of the rectal walls.

Modifications of the Alimentary Canal

The alimentary canals of primitive insects such as Collembola as well as many larvae of Endopterygota are simple, direct tubes with but slight modifications even in the regions of the cardiac and pyloric valves. More advanced chewing insects, including Orthoptera, Odonata, Hymenoptera, and many Coleoptera, have an enlarged crop and proventricular specializations to handle particulate meals, plus frequent caecal pouches and enlarged rectal pads. The ingestion and passage of a fluid diet poses other engineering problems. Some fluid-feeders, such as some Diptera and Lepidoptera, possess lateral, bladderlike diverticula protruding from the crop that receive liquids. The midgut is enlarged in Siphonaptera and many Hemiptera (suborder Heteroptera). The most elaborate arrangement is the filter chamber of many Hemiptera (suborders Auchenorrhyncha and Sternorrhyncha), which is discussed later.

During the larval instars of Neuroptera and some Hymenoptera, the midgut is a blind sac, not connected to the hind gut until pupation. As a result, waste products are retained in the larval gut and excreted on emergence as adults. For insects such as larval bees that develop in a confined space, this has an obvious sanitary function.

The ileum is modified to contain symbiotic microbes in termites and larval scarab beetles. In the aquatic, immature stages of dragonflies, tracheated gills project into the rectum and acquire oxygen from water circulated in the enlarged rectum. Forceful contractions of the rectum also eject the water and propel the nymphs forward.

Salivary Glands

The enlarged integumental glands of the head function in a great variety of life processes, but they are discussed here because they contribute generally to ingestion, movement of food, and digestion. The largest and most common glands that secrete saliva are the paired **labial glands** (labeled salivary glands in Fig. 5.3a). The single, median salivary duct opens in the salivarium between the labium and hypopharynx. The glandular cells extend back into the thorax, or even far into the abdomen in some caterpillars, like clusters of grapes and may be differentiated into regions. In addition to saliva, the modified labial glands are responsible for the silk secreted by larvae of Lepidoptera, Trichoptera, and Hymenoptera. These fibrous proteins are used in the construction of cocoons, webs, and nets. Other glands of the head found in various insects are the **mandibular glands**, which are the main salivary glands of larval Lepidoptera and are also known to secrete the pheromone called **queen substance** in the queen honey bee (see Chapter 7); the **maxillary glands**, which are small and probably provide lubricants for the mouthparts; and the **pharyngeal glands**, which are best developed in worker honey bees for production of the food royal jelly, fed to larvae (see Chapter 7).

Functionally, the saliva of the labial glands moistens and lubricates the mouthparts, dissolves food, transports flavors to gustatory (taste) sensilla, contains enzymes that act on the food both before and after ingestion (Fig. 5.1), and also serves as an excretory organ. Aphids, mirids, a leafhopper, and a lygaeid bug are aided in the penetration of their piercing/sucking mouthparts into plants by a pectinase in the saliva. This enzyme hydrolyzes the pectin in the cell walls of plant tissues. Many sucking insects in the order Hemiptera secrete a salivary sheath around their feeding punctures. The sheath presumably functions to retain the turgor pressure of the plant's vascular system, thus enhancing the insect's sucking mechanism.

Bloodsucking insects, such as the biting flies and parasitic Hemiptera, facilitate ingestion by the inclusion of anticoagulins and antiagglutinins in the saliva. Blood clots would otherwise plug the fine canals of the insect's mouthparts. Digestive

enzymes are notably absent in the saliva of these insects, and some have a local anesthetic, perhaps to avoid irritating the host while they feed. Enzymes and other proteins in their saliva, however, act as antigens that invoke an immune response, which causes the irritation and swelling that follows the bites of mosquitoes and other blood-feeding insects.

The injection of saliva by insects into plant or animal hosts provides a natural entry point for pathogens. Complex cycles of transmission have been evolved by viruses and microbes that take advantage of the insect vector's feeding behavior.

Peritrophic Matrix (Membrane)

The peritrophic matrix or membrane (meaning "membrane around the food") is a thin (about 1 μm thick), tubular film surrounding the food and forming a loose lining inside the midgut and hind gut. The membrane is often multilayered or laminar. Structurally, it is a fibrous network, or an amorphous sheet of polysaccharide, including chitin, glycoprotein, and proteins. In most insects that have been examined, the membrane is produced from microvillate columnar epithelial cells distributed locally or generally in the midgut epithelium. In many fewer insects, secretory cells located in a ring or rings between the invaginated cardiac valve and the anterior end of the midgut form a "cardiac press." In certain Diptera, for example, the secretory cells in the cardiac press extrude a tubular membrane of several layers around the incoming food bolus as it passes through the cardiac valve. In Dermaptera, both methods have been reported, and in mosquitoes, the membrane is produced by the cardiac press in larvae and by the epithelium in adults. Regardless of the method, the transparent membrane continues to move posteriorly along with the food and is ultimately discarded, often still in place around the fecal pellets. The activation of the secretory cells is related to the hormonal controls of metamorphosis and in response to a feeding stimulus or gut expansion.

The fluid-feeding Hemiptera lack a peritrophic membrane, yet they have a unique, multilayered matrix on the apical surfaces of the midgut epithelial cells that may function like the peritrophic

membrane of other insects. The membrane presumably acts to protect the exposed midgut epithelium from abrasion by food particles and to lubricate and facilitate movement of food. The membrane proteins (peritrophins) also resemble mucus proteins (mucins) in chemical composition and in the kind of secretory cells that produce it, suggesting that mucus and the peritrophic membrane may have evolved from a common ancestor.

The peritrophic membrane covers the food and separates it from direct contact with the epithelium. It has minute pores that appear to be selectively permeable. The pores may act as a filter controlling the movement of enzymes into the ingested food and the movement of the products of digestion into the midgut cells.

Disease organisms that are ingested commonly invade the bodies of insects by penetrating the midgut and entering the hemocoel. These organisms include useful microbial agents for biological control of pests as well as agents of diseases harmful to humans that are carried by insect vectors. The peritrophic membrane is a critical barrier to invasion and may effectively exclude certain microbes. The pore sizes are usually smaller than bacteria, yet some bacteria, viruses, trypanosomes, and filarial worms are able to penetrate the membrane either mechanically, by action of their enzymes, or other as yet unknown methods.

Digestion

Digestion of food may begin extraorally, or outside the insect, but most digestion and nutrient uptake occurs in the midgut. The salivary labial glands and midgut epithelium are the primary sources of digestive juices. For solid food, mechanical breakdown begins with the mouthparts and may be continued with the cuticular proventriculus. The reduced size of food particles exposes larger surface areas to the action of digestive enzymes. The enzymes are hydrolases, commonly including proteases, lipases, carbohydrases, and nucleases. Thus, complex organic constituents in the food are reduced stepwise to a molecular structure that can be absorbed by the epithelial cells of the midgut.

As a tubular membrane enclosing the food within the tubular midgut, the peritrophic membrane is believed to have important functions in digestion by compartmentalizing digestive enzymes and food particles and channeling the flux of food flow. Enzymes initially break down the polymers in the food while it is covered by the membrane. Intermediate oligomers pass through the membrane to be finally digested into monomers next to the apical surfaces of the epithelial cells by cell membrane-bound enzymes and then immediately absorbed. In general, digestive enzymes are secreted by epithelial cells, pass through the peritrophic membrane, and begin hydrolyzing the food. The enzymes move posteriorly with the food and, on reaching the posterior midgut, pass back through the membrane. Fluids secreted by the epithelium in the posterior midgut move anteriorly between the membrane and the epithelium. This countercurrent flow of fluids outside the membrane carries forward the enzymes and products of digestion. Once in the anterior midgut, the enzymes pass again through the membrane to the food and repeat the cycle, reducing the need for enzyme production.

Extraoral digestion is widespread among the animal phyla and is common among predatory insects. Enzymes, especially proteases, in saliva or regurgitated from the midgut are injected into prey. The body of the victim becomes a container in which the tissues are liquefied and sucked out. The enzymes also are recovered, thus conserving protein, and continue their action after ingestion. Paralysis and extraoral digestion of prey are accelerated when assassin bugs (*Reduviidae*) inject hyaluronidase into prey. This enzyme probably dissociates the cells of the prey and hastens the action of digestive enzymes, which are concurrently injected. It is because of the enzymes that the bites of predatory Hemiptera are painful to people. The venomous saliva can also be defensively ejected at vertebrate enemies. The housefly ejects saliva on solid food, and the partly digested liquid is eaten. Enzymes such as amylase, which converts starch to maltose, or invertase, which converts sucrose to glucose and fructose, are commonly present in saliva.

Digestion in Orthoptera, Blattodea, and some Coleoptera occurs mainly in the crop with enzymes produced by the salivary glands or regurgitated from the midgut. In Diptera, Lepidoptera, and Trichoptera, digestion apparently is limited to the midgut.

The honey bee adds salivary invertase to sucrose-rich nectar that is carried back to the hive in the crop. The digested mixture of glucose and fructose is regurgitated in the hive, dehydrated, and stored in the comb as honey.

Digestion of Unusual Foods

Insects are exceptional in being able to digest some abundant, yet resistant, compounds. Structural proteins in vertebrate tissues, such as collagen of connective tissues or keratin in wool, hair, and feathers, are highly resistant to degradation by common proteases. This is evident when a dead bird or mammal decays and only the resistant tissues remain. The feces of blowfly larvae (Calliphoridae) that feeding on such corpses contain a collagenase that attacks the collagen and elastin of muscle tissues, resulting in extraoral digestion. Bird lice (Mallophaga) and the larvae of clothes moths (Tineidae), house moths (Oecophoridae), and dermestid beetles possess a keratinolytic enzyme that attacks keratin. The moths and beetles are household pests of woolen clothing and carpets. A strong reducing agent is able to break the sulfur bonds of keratin because poor tracheation in the insect's midgut provides a low oxidation-reduction potential. Once the peptide chains are free, the keratinase is effective.

Beeswax is ordinarily resistant to digestion, but the larval wax moth is able to utilize some wax components. At an optimal pH of 9.3 to 9.6 in the gut, the wax is hydrolyzed by enzymes originating from the larva's cells, from intestinal bacteria, or both.

Woody plant tissue is regularly exploited as a food source by larvae of some wood-boring beetles (some Anobiidae, some Scarabaeoidea, Buprestidae, Cerambycidae, Lyctidae, Bostrichidae, some Tenebrionidae, and others), termites, wood-feeding roaches, and silverfish. Cellulase, hemicellulase, lignocellulase, and lichenase have been

demonstrated in insects, some or all of which have been identified in members of the previous list. The most primitive families of termites house symbiotic flagellate protozoa and bacteria in the gut that are critical to digesting cellulose. Bacteria in the midgut caeca of Scarabaeoidea beetles are thought to digest cellulose. Some of these enzymes, however, are directly secreted by insects, such as more recently evolved termites and the larvae of some cerambycid beetles. Among wood-boring insects, the ability to digest cellulose to varying degrees permits partitioning of the food resource: some thrive only on the more easily digested, but scarce, starch and sugar in the wood (Lyctidae, Bostrichidae); others also use the hemicelluloses (Scolytidae); and a third group consumes the whole cell wall, minus the lignin (Anobiidae, Cerambycidae).

Xylem sap-feeding specialists such as spittlebugs (Cercopoidea, Hemiptera) and some leafhoppers (Cicadellidae, Cicadellinae, Hemiptera) must consume daily from 100 to 1,000 times their body volume because of the very low concentrations of nutrients, mainly amines in xylem sap. Their digestive efficiency for most amino acids is at least 99%.

Absorption

The midgut epithelium and the hind gut are the principal absorptive regions. Although digestion may take place in the crop, absorption is largely prevented by the cuticular intima. In the midgut, monosaccharide sugars, commonly glucose, rapidly pass through the permeable epithelial membrane without an active transport mechanism as long as the concentration inside the cells is lower than outside. A steep osmotic gradient is maintained by converting the simple sugar to the disaccharide trehalose. In the migratory locust, this conversion takes place in the fat body, which is near the midgut. In the silkworm, oligosaccharides may be absorbed and further hydrolyzed in the epithelium.

Amino acids are similarly absorbed by passive diffusion processes related to the uptake of water into the hemolymph. The blood-feeding kissing bug *Rhodnius* (Reduviidae, Hemiptera) can even absorb proteins that are not fully degraded to amino acids. The phloem and xylem sap in plants contains amino

acids in monomeric form (at concentrations of 3–50 mM) that need no digestion and are efficiently absorbed by these insects: up to 55% efficiency in aphids (Aphididae) and at least 99% efficiency in xylem sap-feeding leafhoppers (Cicadellidae).

The absorption of lipids is more difficult to analyze because these molecules might be able to pass through the cuticular intima of the crop or between epithelial cells of the midgut, in addition to the usual route via the epithelial cells. Lipids have been seen as droplets in cells of both the midgut and crop. Although cholesterol has been recently demonstrated to be absorbed by the crop of a cockroach, the midgut is the major absorptive region. The fats need not be fully degraded before absorption.

TRACHEAL SYSTEM AND GAS EXCHANGE

Oxygen for cellular respiration in insects is transported by an internal system of air-filled tubes, the **tracheae**, to within a few cell diameters of each cell (Fig. 5.4a). Respiratory pigments and blood are not involved in gas exchange in most insects. This is in contrast to oxygen transport in vertebrates, which depends on the pigment hemoglobin as an oxygen carrier and the rapid circulation of blood throughout the body. The respiratory system of terrestrial vertebrates has clearly evolved from that of aquatic ancestors, but the aerial systems of insects and certain other arthropods are thought to be peculiarly terrestrial adaptations that may have independently evolved in several arthropod stocks.

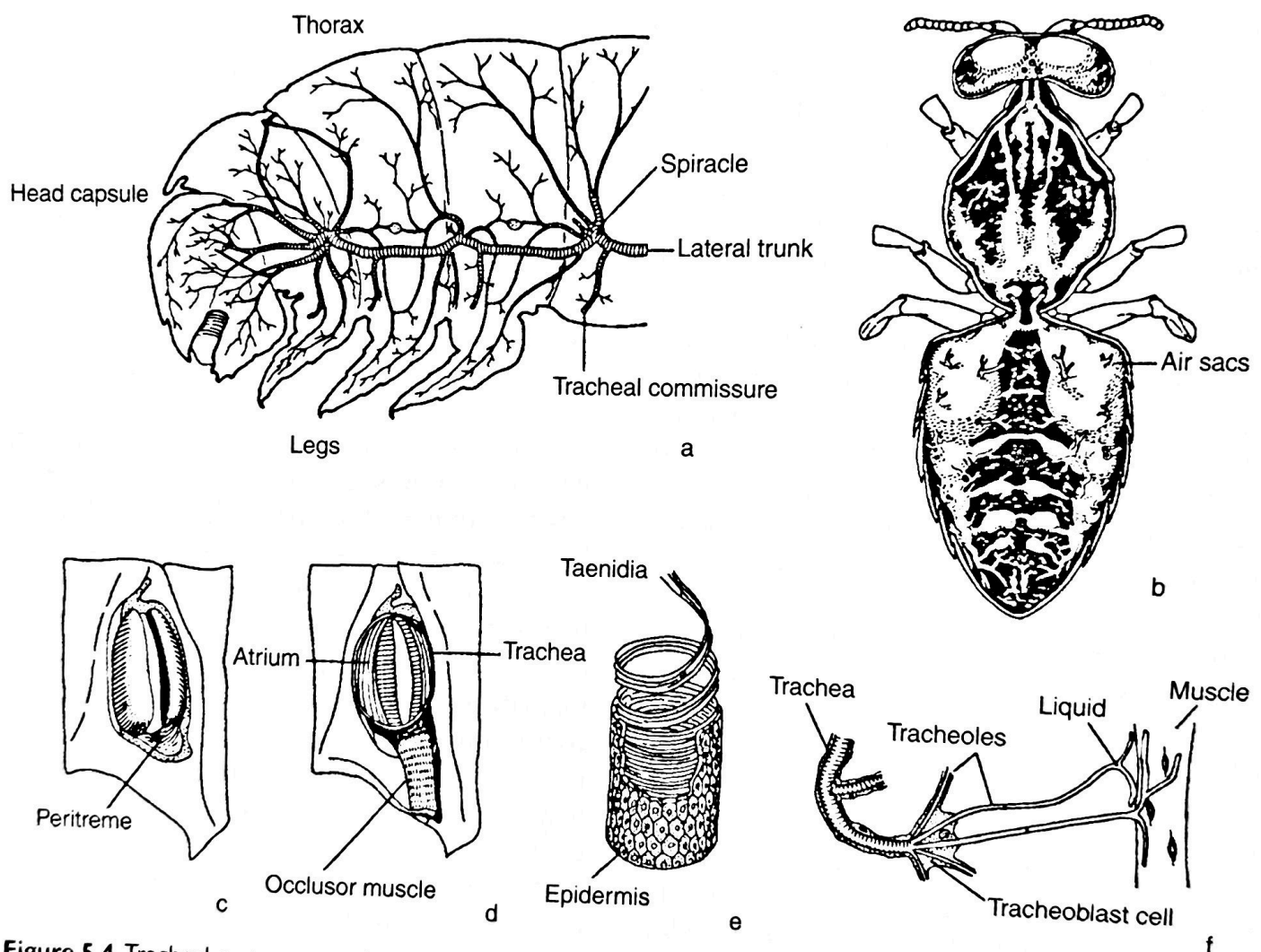


Figure 5.4 Tracheal system: **a**, tracheation of the head and thorax of a noctuid caterpillar; **b**, air sacs in a honey bee; **c**, external view of closing mechanism of a thoracic spiracle; **d**, internal view of same; **e**, structure of a trachea; **f**, structure of a tracheole.

Source: **a**, and **c–f**, Redrawn from R.E. Snodgrass, *The Principles of Insect Morphology* - public domain; **b**, Barbara Boole Daly.

Tracheae are highly branched and interconnected tubular invaginations of the integument that arise from paired, lateral openings, the **spiracles**, and ultimately terminate in fine fluid-filled tubules, known as **tracheoles** (Fig. 5.4f).

Small insects, such as Protura and most Collembola that live in damp places or some aquatic larvae of Chironomidae (Diptera), lack a developed tracheal system and exchange gases through the cuticle. In larger, free-living, terrestrial insects, the demand for oxygen cannot be met by the body surface, partly because the area is inadequate for sufficient transfer and the thicker cuticle permits only a negligible diffusion of oxygen. More importantly, a body wall permeable to oxygen diffusion would also be permeable to water loss; a molecule of water is smaller than a molecule of oxygen gas. The tracheal system solves this problem by creating an enormous internal surface of permeable cuticle where loss of water can be controlled. The fifth-instar larva of the silkworm has an estimated 1.5 million tracheoles.

Diffusion, the random movement of molecules from a higher to a lower concentration, is a major process by which oxygen moves into and carbon dioxide moves out of respiring tissues. Diffusion is a millionfold more rapid in the gas phase than in tissue. Calculations of oxygen consumption, diffusion rates, and measurements of tracheae indicate that diffusion alone could supply small insects (less than 3 mm long) with active flight muscles. Ventilation, or moving drafts of air through the system, can greatly reduce the distance that oxygen must diffuse over a gradient from atmospheric concentration to respiring tissue. However, the tracheal system imposes maximum limits on the size that insects can attain. Even forced movements of air cannot overcome the size limitations of diffusion as the primary means of gas exchange. To illustrate by analogy of diffusion with sound communication, sound (330 meters per second) is as effective as electronic communication (300,000 kilometers per second) within a room, but it is not adequate for distances greater than tens or hundreds of meters. Similarly, diffusion aided by ventilation is adequate over distances measured in millimeters, but not over distances more than a few centimeters.

The passage of air into the tracheal system is regulated by the spiracles. In some apterygotes, such as the springtail *Sminthurus* (Sminthuridae, Collembola), the opening is simply a hole in the body wall. The rate of gas diffusion through this pore, as through the stomata of plant leaves, is a function of the perimeter rather than the cross-sectional area. Elaborate modifications of the spiracle therefore are possible without necessarily reducing the intake of air. In most insects, the tracheal orifice is recessed in the integument, creating a small chamber, or **atrium**, that communicates with the exterior. The atrium may be situated on a separate sclerite, the **peritreme**, and provided internally with filtering hairs or projecting, valvelike lips, closable by muscles (Fig. 5.4c). The latter are characteristic of thoracic spiracles and are not found on the abdomen. The atria of abdominal spiracles remain open, with a closing device located inside at the tracheal orifice. The opening is pinched shut by the action of an occluder muscle on one or two sclerotic bars. When the muscle relaxes, either the elastic bars spring open or they are pulled apart by a second muscle, the dilator. Spiracular muscles are innervated from their segmental ganglion or the ganglion immediately anterior. Tracheae leading from the spiracles may be connected longitudinally by **lateral trunks** and transversely by **tracheal commissures**.

The histological parts of tracheae are homologous to corresponding parts of the integument: an inner, cuticular intima secreted by a surrounding layer of epidermal cells that are bounded outside by a basement membrane. The intima is thin, permeable to gases, and marvelously flexible because of fine, spiral thickenings called **taenidia** (Fig. 5.4e). The epicuticle contains at least an outer layer and probably a hydrofugic (water-repellent) wax layer next to the lumen. Corresponding to the external procuticle is a chitin and protein layer, which is largely (if not entirely in smaller tracheae) involved in constructing the taenidia. At each molt, portions of the intima are routinely shed along with the exuviae. During metamorphosis of flying insects, the entire system is reorganized to accommodate the increased demand for oxygen by the flight muscles. New tracheal branches or fusions are developed,

and old tracheal pathways are not replaced. Yet the design of major trunks and anastomoses is consistent in taxonomically related forms and may be traced from the immature stages to the adult.

The tubular network of tracheae may be interrupted at intervals by enlarged dilatations, or **air sacs** (Fig. 5.4b). Taenidia may be discernible on the walls of some sacs as if the trachea is merely inflated, but other cavities are reinforced by **reticulate** thickenings. The sacs are collapsible, a feature of importance in ventilation and one that also permits internal organs to expand in the body cavity, as when the ovaries are enlarged by eggs or the gut is engorged with food. Associated with the tracheae in Diptera, Lepidoptera, and Hymenoptera are **peritracheal glands**, which consist of several cells each and are distributed pairwise in thoracic and abdominal segments. The cells are thought to secrete exuvial fluids during molting and possibly hydrofugic lipids that coat the intima.

Tracheoles

As they branch, tracheae become narrower in diameter and terminate in tiny subdivisions, the **tracheoles** (Fig. 5.4f). These tubules are up to 350 μm in length and 1 μm in diameter, tapering to a diameter as small as 0.1 μm . Whereas the tracheal tube is enclosed in a multicellular coat, the epidermis, the tracheole is intracellular, in a **tracheoblast cell**, and may be exempt from renewal at each molt. When tissues are experimentally deprived of tracheae or organs are implanted in a new host, tracheoles migrate to the affected tissues and establish an oxygen supply. The same processes are presumably involved in normal development and injury repair. Although the general surface of the tracheal intima probably is diffusible by gases, the tracheole is believed to be the major site of oxygen transfer. Organs of high metabolic activity are richly tracheated. For example, the flight muscles of fast-flying insects are enveloped in tracheae and air sacs. The profuse tracheoles indent the plasma membranes of muscles to bring oxygen to the mitochondria deep in the fibers.

Tracheoles are often filled with a lymphlike liquid. During the depletion of oxygen in surrounding cells, this liquid is withdrawn against a capillary

force by the tracheoblast cell, bringing gas to the tracheolar tip. The physical mechanism of withdrawal is not clear but is correlated with changes in osmotic pressure of surrounding tissues. A porous structure observed in the tracheolar outer layer may facilitate this process. Oxygen moves the final distance from the tracheoles to respiring cells by diffusing through tissue. The carbon dioxide produced by respiration exits by the reverse course, hastened by a greater solubility in water and a permeability through tissue 36 times faster than that of oxygen but diffusing slightly slower than oxygen in gaseous phase. The greater mobility of carbon dioxide in tissue favors some loss of this gas directly through the cuticle, especially at less sclerotized intersegmental membranes.

Ventilation

The distance gases must diffuse is shortened by ventilation of the tracheal system. Motions of the body or churning of internal organs of active insects incidentally move air through the interconnected major trunks. Air movement through the system is greatly accelerated by pumping movements of the telescoping abdominal segments, creating inspiratory and expiratory flows. When the spiracles remain open, air may enter and exit tidally through the same orifice. The closing valves attached to spiracles in most insects permit coordinated opening and closing of the tracheal orifices, resulting in unidirectional flow. Ordinarily, air is taken in through the anterior spiracles and is expelled via the posterior spiracles. Air sacs increase the volume of air pumped and may have other mechanical functions in ventilation since they are partly collapsible. During flight, abdominal pumping is inadequate to supply the flight muscles. Movements of the thoracic segments, in conjunction with open spiracles, ventilate the air sacs and tracheae surrounding the muscles. Large, quiescent insects like diapausing moth larvae and pupae as well as temporarily inactive insects may have discontinuous ventilation that can span up to several hours. This cyclic process begins by closing the spiracles while a slight vacuum develops as oxygen is consumed, then by partly opening and closing the spiracles to allow air to be sucked into the

system, and finally by fully opening the spiracles only briefly to release a burst of carbon dioxide.

X-ray cinematography of insects revealed that the major tracheal branches of a diversity of insect subjects rhythmically pulsate in successive contractions, which presumably increase air movement in the tracheal system. This finding challenges our current understanding of the importance of diffusion versus forceful ventilation in the gas exchange physiology of insects.

Ventilatory movement of air in and out of the insect's body increases water loss. Air in the tracheal system is near saturation even while outside the humidity may be quite low. The structure of the atrium and closing mechanisms reduce or prevent the diffusion of water vapor from the system. It is therefore advantageous for the spiracles to remain closed, with the occlusor muscle contracted, until breathing is demanded. Spiracles may be controlled separately or in concert by the central nervous system. Cellular respiration leads to lowered levels of oxygen and higher levels of carbon dioxide. Changes in both gases will reduce impulses from the central nervous system to the occlusor muscle (thus relaxing the muscle and opening the spiracle by its elastic hinge) or, where dilator muscles are involved, increase impulses to the dilator to stimulate opening. Occlusor muscles are also influenced directly by carbon dioxide and will relax when the local concentration is high.

Hemoglobin

Hemoglobin functions in the gas exchange of only three insects studied thus far: the bloodworm *Chironomus* (Chironomidae, Diptera), the backswimmer *Anisops pellucens* (Notonectidae, Hemiptera), and the horse-botfly larva *Gasterophilus intestinalis* (Gasterophilinae, Oestridae, Diptera). The hemoglobin of insects contains two heme groups, half as many as the vertebrate pigment. In each case, hemoglobin provides a temporary respite from oxygen deprivation. The bloodworm lives in stagnant or polluted water that is deficient in oxygen. Movements of the larva in its burrow refresh the cutaneous supply and charge the hemoglobin with oxygen, which is not released unless the tension in tissues is low. The bloodworm's hemoglobin

has a high affinity for oxygen, and the uptake of oxygen is more rapid than diffusion in the hemolymph. Hence, recovery from hypoxia is aided and aerobic respiration is sustained under poorly oxygenated conditions. The horse-botfly maggot is similarly situated in a liquid, oxygen-poor medium: a horse's stomach. Hemoglobin is in richly tracheated fat cells, permitting rapid uptake and storage of oxygen derived from passing gas bubbles. The bug *Anisops* utilizes hemoglobin not only to extend its period under water but also to sustain a favorable specific gravity that allows submerged floating.

Types of Tracheal Systems

The tracheal systems of insects vary depending on the medium in which they live and their activities. The structure, number, and position of spiracles vary taxonomically: the apterygotes exhibit the most diverse arrangements and provide some insight into the evolution of the system. As already mentioned, most Collembola and Protura lack tracheae altogether.

Three major types of respiratory systems are recognized. The **holopneustic** respiratory system (10 pairs of functional spiracles) is found in the immature stages and adults of many terrestrial insects, including Orthopteroidea, Hemipteroidea, and some Hymenoptera. The **hemipneustic** system is derived by the loss of one or more functional spiracles and is characteristic of larval Neuropteroidea. The **apneustic** system of submerged aquatic insects and most endoparasitic larvae has no functional spiracles but does have a closed tracheal system. Oxygen enters the body by cutaneous diffusion, either over the general body surface or in special, integumental gills. Tracheae within gills consist of very fine tracheae at the gill extremity that coalesce into larger tracheae leading to the main tracheal branches.

Gas Exchange in Aquatic Insects

Nearly all aquatic insects have a gas-filled tracheal system. The few exceptions are the young fly larvae of *Chironomus* (Chironomidae, Diptera) and *Simulium* (Simuliidae, Diptera) and some young aquatic caterpillars (Pyralidae, Lepidoptera). In

some groups, the system is **open**, and air can be taken inside the body. In other groups, the system is **closed** (apneustic) to the entry of air, and oxygen must be absorbed from water. In either case, many aquatic insects have the body surface well supplied with tracheae and are able to obtain at least part of their oxygen by **cutaneous gas exchange**.

In contrast with air, the supply of oxygen from water is sharply limited by a series of physical constraints. Oxygen dissolves in water in an amount inversely proportional to temperature (warm water has less oxygen than cold water). The concentration, even when saturated, is many times less than that in air, and movement by diffusion in solution is exceedingly slow. Immediately next to a submerged insect's body is a thin layer of still water called the boundary layer, in which oxygen is renewed only by the slow process of diffusion. Finally, the diffusion of oxygen through the cuticle is many times slower than in water.

Some insects, such as many larval Diptera, depend mainly on cutaneous gas exchange, but others have special **tracheal gills** that augment gas exchange. These are thin-walled outgrowths of the integument that are richly tracheated. Most commonly, gills occur on the sides of the abdomen. Oxygen in water diffuses through the cuticle and into the gas-filled tracheoles. Gills also are important in the elimination of carbon dioxide. Platelike gills are found in immature mayflies (Ephemeroptera) (Fig. 8.6a,b; color plate 2f) and damselflies (suborder Zygoptera, Odonata; Fig. 21.6b). Filamentous gills are also found in mayflies (Fig. 20.4b) and are characteristic of groups of, for example, aquatic flies, beetles, and caddisflies. The rectum of dragonflies (Odonata) is thin-walled and tracheated as a rectal gill. The flushing of water to and from the rectum refreshes the water adjacent to the gills. Movements of external gills such as those of mayflies (Ephemeroptera) have the same effects of refreshing water immediately along the gill surface to increase the rate of gas exchange with the water.

Adult and immature insects with an open tracheal system obtain their oxygen by frequent trips to the surface for air, by tapping air spaces in submerged plants, or by carrying a bubble or film of

gas with them while they are submerged. Some aquatic larval Diptera obtain air by returning to the surface (Fig. 5.5b, 5.6b). The spiracular opening is usually surrounded by **hydrofuge** areas or hairs that prevent entry of water into the spiracle.

Air spaces in plants are punctured by the specialized respiratory tubes of various unrelated insects: certain larval beetles (Donaciinae, Chrysomelidae; *Noterus*, Noteridae; *Lissorhoptrus*, Curculionidae) and flies (*Chrysogaster*, Syrphidae; *Notiphila*, Ephydriidae; *Mansonia* and *Taeniorhynchus*, Culicidae).

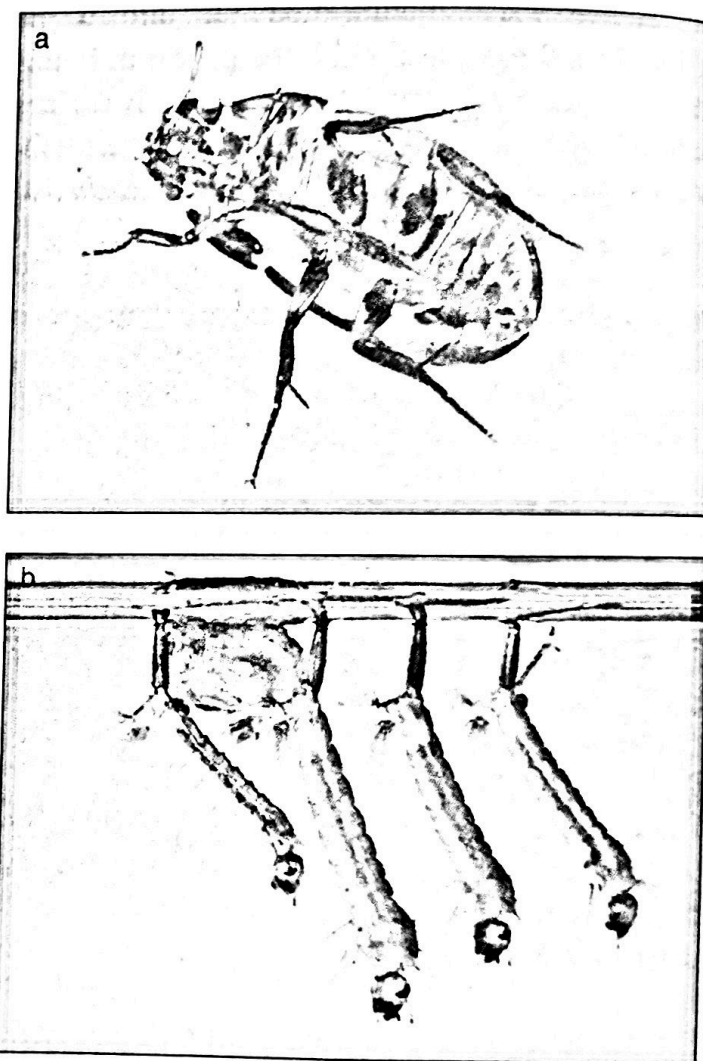


Figure 5.5 Examples of aquatic respiration. **a**, Aquatic beetle *Tropisternus* (Hydrophilidae) submerged with an air film that functions as a physical gill. **b**, Larvae and a pupa of a mosquito, *Culiseta* sp. (Culicidae), "hang" from the surface tension of water and obtain atmospheric air. The larvae have the metapneustic type of respiratory system with only the terminal spiracles open. The pupa has respiratory horns on the thorax.

Source: Edward S. Ross.

Bubbles of air are carried beneath the surface by many aquatic bugs and beetles (Fig. 5.5a) and some spiders. Such temporary air stores are held in place by special hairs or are carried in cavities beneath the wings or elytra. The spiracles are situated to open into the air bubble. All aquatic Hemiptera in North America that swim beneath the water carry air stores. The manner of obtaining air varies—for example, through a caudal siphon (Nepidae; Fig. 34.10b), caudal flaps (Belostomatidae), abdominal tip (Naucoridae, Notonectidae), or the pronotum (Corixidae). Aquatic beetles of the suborder Adephaga carry air under the elytra and renew the store by breaking the surface film with the tips of their elytra and abdomen. Aquatic members of the suborder Polyphaga have elytral air stores and usually also a coating of hydrofuge hairs on the ventral surface that holds a film of gas. Hydrophilid beetles break the surface with an antenna.

The bubble that is taken below serves not only as a store of atmospheric oxygen and a hydrostatic organ but also as a **physical gill**. The latter functions in the following manner. The gas in the bubble initially has the composition of air: about 20% oxygen, 79% nitrogen, and less than 1% carbon dioxide. The carbon dioxide produced by the insect rapidly diffuses into the water and is of negligible importance as a gas in the bubble. As oxygen is used by the insect, the content of oxygen in the bubble falls below that in the surrounding water. Oxygen will then diffuse into the bubble from the water. Nitrogen makes up almost 80% of the bubble volume and thus plays an important role in maintaining the bubble's volume. Because nitrogen is inert, the bubble maintains most of its volume even at low oxygen concentrations. A remarkable modification that utilizes the principle of the physical gill is the **plastron**. In this device, a thin film of gas is held on the body surface indefinitely by dense, water-repellent hairs or a fine cuticular meshwork (Fig. 5.6). The European bug *Aphelocheirus* (Naucoridae, Hemiptera) has fine, short hairs over much of the body surface. The density of water-repellent hairs is 200 to 250 million per square centimeter. The hairs are bent at the tips in an arrangement that supports the gas/water interface and resists compression of the gas

film. The bug and similarly equipped beetles of the families Elmidae and Dryopidae can remain submerged for months.

The aquatic pupae of certain beetles (*Psephenoides*, Psephenidae) and flies (some Tipulidae, Blephariceridae, Deuterophlebiidae, Simuliidae, Empididae) have long cuticular processes developed from the peritreme and atrial region of one or more spiracles. These processes are called **spiracular gills**. The gill is associated with a plastron that is supported by a cuticular meshwork rather than by dense hairs. Likewise, the chorion of the eggs of some insects has a plastron that allows the embryo to respire when the egg is flooded or covered by a film of moisture.

The hair or cuticular plastron functions equally well when the insect or egg is out of the water because the spiracles or embryonic respiratory organs are then in direct contact with air. In water that is deficient in oxygen, however, the physical gill fails because oxygen no longer diffuses into the bubble. For this reason, insects with plastron gas exchange are typically slow moving or are found in swiftly flowing streams or other well-oxygenated waters.

Various adaptations for gas exchange similar to the foregoing can be observed among insects in semiliquid media: scavengers in decaying plant or animal bodies or animal waste, phytophagous insects that burrow in juicy fruits or other plant tissues, and endophagous parasites and parasitoids immersed in host blood. For example, small Hymenoptera larvae living as internal parasitoids of other insects may depend on cutaneous gas exchange but still retain tracheae inside their bodies.

ANATOMY AND FUNCTION OF THE CIRCULATORY SYSTEM

The circulatory system has multiple functions, including transportation of nutrients, waste products, and hormones; storage of nutrients and waste; hydrostatic pressure for molting and locomotion; and resistance to disease, wounds, and freezing. It does not function in the gas exchanges of carbon dioxide and oxygen.

Insect body fluid, called **hemolymph**, differs in many respects from vertebrate blood. It is a single fluid with cells, not divisible into blood and lymph

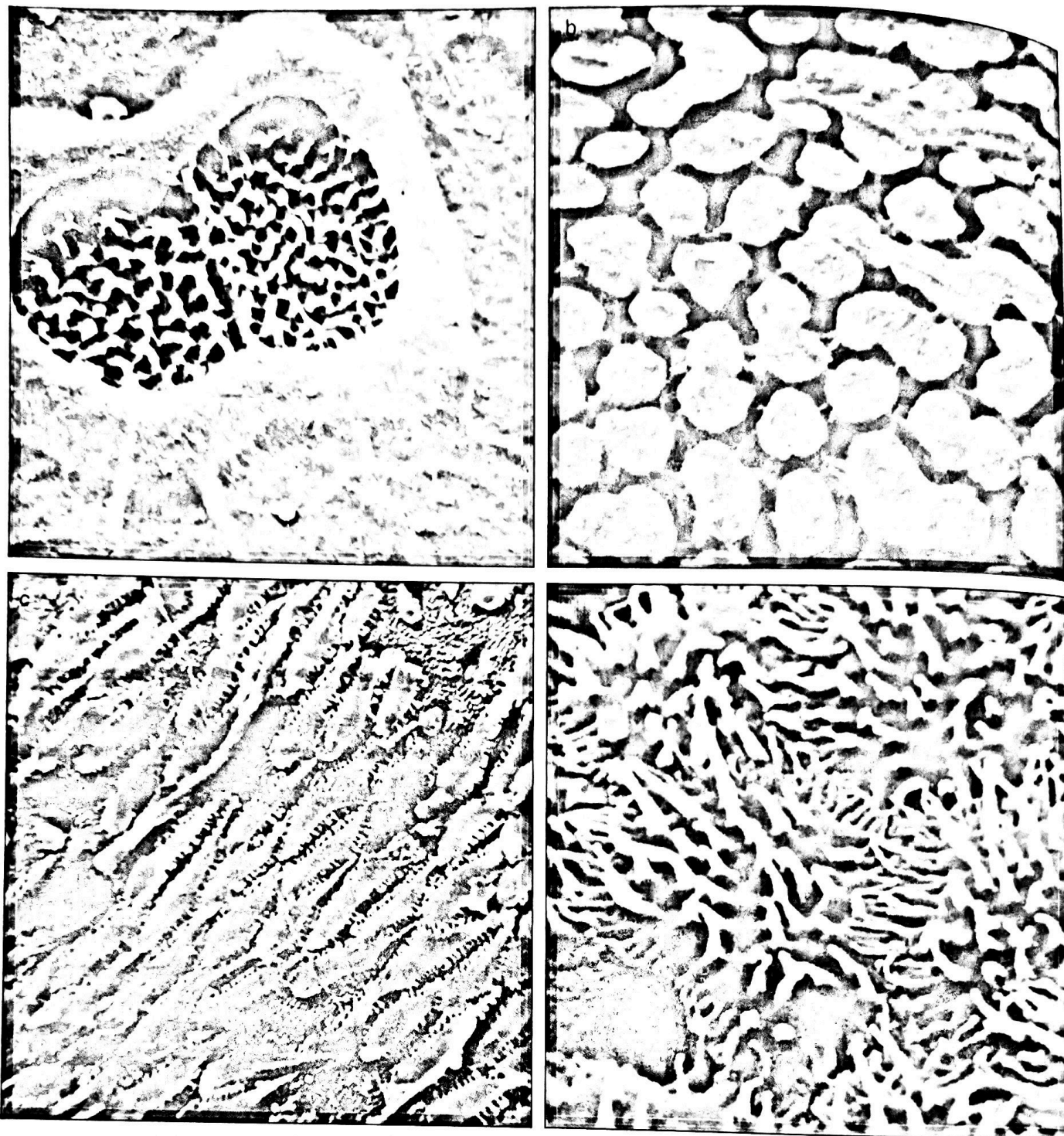


Figure 5.6 Examples of plastrons: **a**, plastron crater of eggshell of the fly *Musca sorbens* (Muscidae); **b**, respiratory horn of egg of the fruit fly *Drosophila melanogaster* (Drosophilidae); **c**, metasternum of the aquatic beetle *Tytelmis mila* (Elmidae); **d**, elytron of the aquatic beetle *Portelmis nevermanni* (Elmidae).

Source: Howard E. Hinton.

systems, that moves freely around the internal organs without a pressurized, closed network of blood vessels. The cells, or **hemocytes**, are highly variable in size, shape, number, and function. The plasma is biochemically unique in the regulation of osmotic pressure by amino acids and organic acids instead of inorganic ions. Neither hemocytes

nor plasma is significantly involved with the transport of oxygen and carbon dioxide in most species. Consequently, insect blood is rarely red (see the previous discussion of hemoglobin) but may be colored yellow, blue, or green by various pigments, including α -carotene, riboflavin, and chromoproteins, but probably not chlorophyll.

The blood is limited to the major cavity of the body, the hemocoel. Insect hemolymph, however, does not directly bathe the cells of the body, as is often claimed. The inner surface of the integument and all the organs are covered in a basement membrane of connective tissue. This membrane probably is interposed between the hemolymph and all other tissues. Thus situated, a "blood-cell barrier" is created that regulates the exchange of materials between the body cells and the hemolymph.

Dorsal Vessel

The circulation of the hemolymph is not haphazard but follows a pattern of flow. Motion is imparted to the fluid by movements of the body segments and of the gut and by the peristaltic contractions of the **dorsal vessel** (Fig. 5.7a). This is a tube developed mid-dorsally from the embryonic mesoderm and extending almost the full length

of the body. In the abdominal region, the vessel is closed at the posterior end but perforated by paired segmental openings, or **ostia**. Insects may have as many as 12 pairs. These have valvelike flaps projecting into the lumen of the vessel. The portion of the dorsal vessel with the ostia is called the heart. It is slightly inflated, especially so between the pairs of ostia. These dilations create the chambers of the heart. The heart is suspended mid-dorsally in the abdomen by a transverse membrane of connective tissue and thin muscles. This **dorsal diaphragm**, with its **alary muscles** (meaning "winglike" in shape), is not complete but is interrupted segmentally by openings. By this means, blood can pass from the vicinity of the gut or **perivisceral** cavity to the space above the heart, or the **pericardial** cavity. Also in the abdomen is a **ventral diaphragm**, formed by an incomplete sheet of connective tissue. This membrane extends

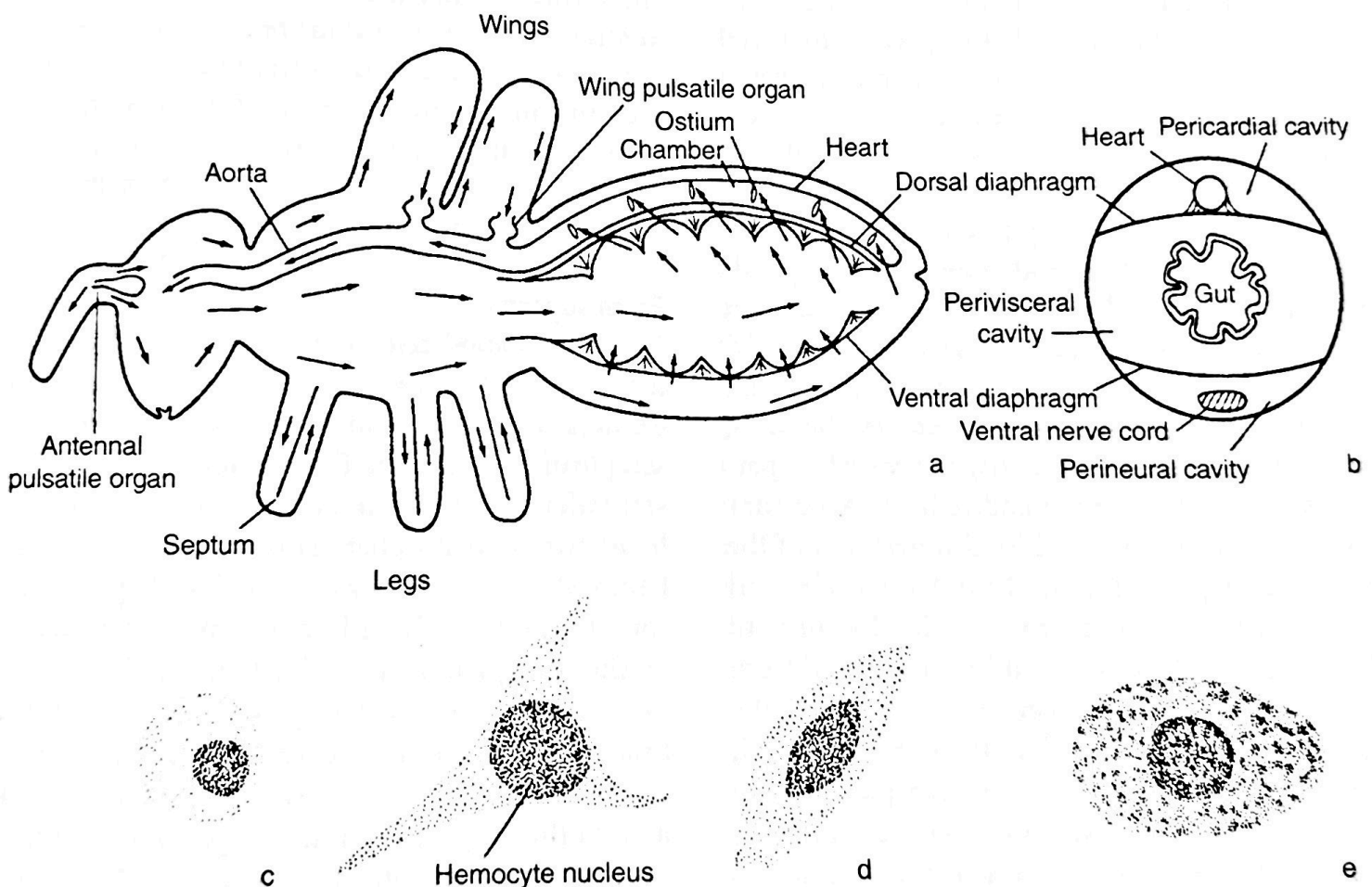


Figure 5.7 Circulatory system and hemocytes: **a**, diagrammatic longitudinal section of general structure and direction of circulation; **b**, cross section of abdomen; **c**, prohemocyte; **d**, plasmatocyte; **e**, granulocyte.

Source: **a**, modified from Weber, 1933; **b–e**, Barbara Boole Daly.

transversely, usually above the ventral nerve cord, and partly separates the perivisceral cavity from the ventral **perineural cavity**.

Movement of Hemolymph

Muscles embedded in the fibrous tissues of the heart wall provide the sequential contractions that impel the blood forward. As the wall of a chamber begins to contract (the systolic phase), the ostia close to block the rear chamber. Thus, blood moves forward to the next chamber. When the wall relaxes (the diastolic phase), the ostia open and the flaps admit blood from the outside and from the chamber to the rear. The flow of blood is normally toward the head, but reversals can be observed. The rate of heartbeat varies widely with age, species, state of nervous excitement, and environmental factors such as temperature. The initiation of contraction may come from an independent but diffuse "pacemaker" in the heart, or it may be partly or entirely controlled by the central nervous system.

The **aorta**, a continuation of the dorsal vessel, conveys blood to the head. This part of the vessel lacks ostia and is generally smaller in diameter. It is not supported by the dorsal diaphragm, but it may be suspended by a vertical sheet, or **dorsal septum**, in the thorax. The aorta opens beneath the brain and above the pharynx. Blood released here moves posteroventrad, entering the appendages of the head and the thorax. Flow into and out of these cylindrical structures is made possible by a septum that divides the appendage lengthwise into two channels. Blood also enters the wing veins and flows in an orderly, but variable, pattern throughout the wing and returns. **Accessory pulsating membranes** aid local movement of the blood. After passing around the leg muscles and flight muscles in the thorax, the blood returns to the abdomen. The movement here is upward from the perineural and perivisceral cavities through the dorsal diaphragm and back to the heart. Although there is almost no blood pressure, complete mixing time in an insect has been estimated at from 5 to 30 minutes. In humans, the time is 2 to 4 minutes.

The circulation of hemolymph can also provide **thermoregulation**. Bumblebees (Apidae, Hymenoptera) are among the first foraging bees

to appear in cool climates. In spring, overwintering adult female bumblebees warm up their flight muscles of the thorax with rapid contractions without wing movements. When the temperature of the flight muscles reaches a temperature at which flight can be sustained, the bee can fly in cool weather. If not dissipated, the heated thorax can rapidly reach lethal temperatures, but hemolymph moving through the dorsal vessel, as it passes along a contorted route through the thorax, absorbs excess heat from the surrounding muscle mass. The hemolymph empties from the dorsal vessel into the head, from which it then is shunted along the ventral thorax to the ventral abdomen. During flight the head and mouthparts and the ventral abdomen are cooled by air flowing over the insect. In the nest, the female can generate heat from the flight muscles and transfer the heat through hemolymph movements to her ventral abdomen. During the cool days and nights of the early spring months, she can use this method of heat transfer to speed the incubation of her first offspring by pressing her ventral abdomen against the brood cell of her first developing larva. This is far from the conventional view that insects are strictly "cold-blooded" organisms. The bumble bee displays remarkable control of not only its overall body temperature but the part of its body that is warmed.

Hemocytes

Unlike the blood cells of humans, the cells or hemocytes of insects vary greatly in size, cytological details, and the numbers that one can see in a sample of hemolymph. The naming of cell types is somewhat disputed, but common names are prohemocytes (small, spherical precursors of various hemocytes), plasmatocytes (variable shapes, from round to spindle-shaped), and granulocytes (containing conspicuous granules that are discharged in defensive reactions) (Fig. 5.7c–e). Hemocytes function in resisting disease and injury, in storage and distribution of nutrients, and probably in many other aspects of internal physiology. Most circulate with hemolymph movement, but some remain attached to specific tissues.

The cuticle of the body, gut, and tracheal system is the main barrier to the entry of disease organisms.

After entry, insects are able to resist the progress of disease by **humeral defenses** (due to substances within the hemolymph) and cellular defenses of **phagocytosis** (engulfing of foreign objects) and **encapsulation** (surrounding objects with hemocytes). Plasmatocytes and granulocytes protect against infections by phagocytizing viruses, bacteria, fungi, and protozoa. Immune reactions of the hemolymph also appear to regulate numbers of symbiotic microorganisms within insects. For example, symbionts of the human louse *Pediculus humanus* (Pediculidae, Phthiraptera) periodically move en masse from protected intracellular locations within a specialized organ (bacteriome) to quickly invade the base of the ovarioles and ultimately the developing eggs. Symbionts that do not succeed in penetrating the ovarioles are soon engulfed by hemocytes.

Foreign objects, microbes, and endoparasites too large to be engulfed by a single cell are immobilized or killed by the same kind of hemocyte in a process called encapsulation. A capsule of connective tissue is secreted as the inner layer of cells flattens over the object's surface and new cells are added outside. The capsule may remain clear or become darkened by melanization.

To act on foreign antigens, insects do not have the equivalent of mammalian lymphocytes or their serum proteins, the immunoglobins. However, insects do have induced humeral responses to parasite invasions. Lectins have been identified in insect hemolymph, are inducible by injury, and are known to agglutinate microbes. Also inducible by bacterial infection are defensive proteins such as the peptides called cecropins that disrupt the cell membranes of bacteria and other pathogens. Constitutive (not inducible) humeral defenses include the release of phenoloxidases and lysozymes (enzymes that weaken bacterial cell walls) into the hemolymph as a result of a cascade of enzymatic reactions. The clearance of bacteria from an insect's hemolymph can be quite rapid; most are cleared within an hour after experimental introduction into the hemocoel. This implies that the induced defensive reactions continue to destroy remaining pathogens with another defensive method to counter any rapid evolution of resistance in the population of invading pathogens.

The risk of bleeding is greatly reduced among insects because of the exoskeleton and low blood pressure. However, most insects have hemolymph that form clots and repair wounds. Prohemocytes gather at a wound, causing local coagulation of the hemolymph. Later, epidermal cells and possibly hemocytes seal the wound and repair the cuticle.

Composition of the Plasma

The cell-free fluid, or plasma, of the hemolymph contains water, inorganic ions, and organic molecules, including metabolic wastes. The volume of hemolymph and water content varies widely—up to 94% of the body weight. In vertebrate blood, inorganic ions determine osmotic pressure, but as the evolution of insects is traced, the more advanced orders have inorganic ions replaced by amino acids and other small organic molecules. Thus, in Lepidoptera, Hymenoptera, and many Coleoptera, amino acids are the primary osmotic effectors. The high concentration of amino acids is a distinctive feature of insect hemolymph.

Organic molecules in the hemolymph include carbohydrates, amino acids, peptides, proteins, lipids, organic acids, organic phosphates, and glycerol. The main carbohydrate is the trisaccharide trehalose, although glucose and some other sugars have been found in quantity in the honey bee and certain flies. Glycogen and lipids are largely stored in the fat body. Depending on the stage of the life history, the large amounts of amino acids in the hemolymph support growth and metamorphosis. Insect hemolymph has a remarkable variety of peptides, polypeptides, and proteins. These function in a wide array of physiological processes, including immunity, regulation of growth, development, reproduction, metabolism, and homeostasis. The most abundant proteins, especially in larvae, are storage proteins (heximerins) synthesized by the fat body. Transport proteins are necessary for the movements of hydrophobic compounds. Lipophorins are the most abundant transport proteins and bind with lipids to shield them from water. Insects that use lipids as fuel for flight release diglycerol from the fat body under the influence of adipokinetic hormone. Juvenile hormone is a lipid transported through the hemolymph by speci-

carrier proteins. Organic acids, especially those associated with the tricarboxylic acid cycle, are most prevalent in larval endopterygotes. Acid-soluble organic phosphates are synthesized and found at high concentrations in certain moths. Levels of waste end products of protein metabolism in plasma—including uric acid, allantoin, allantoic acid, urea, and in some immature aquatic insects, ammonia—are sometimes high. Glycerol is accumulated in the plasma of certain insects as an antifreezing agent.

ANATOMY AND FUNCTION OF THE EXCRETORY SYSTEM

Excretory processes expel or render harmless metabolic wastes and other substances that are harmful; at the same time, they maintain an appropriate balance of water, ions, and other components of the hemocoel (Fig. 5.8a). Excretion thereby plays the key role in physiological **homeostasis** (i.e., the maintenance of a constant internal environment) by regulating body chemistry. In contrast to defecation (i.e., physical expulsion of undigested food and excreted waste), excretion involves the movement of waste molecules across one or more plasma membranes. The most critical excreted products are nitrogenous compounds resulting from the breakdown of proteins and nucleic acids. The processes of excretion occur mainly in the alimentary tract. Most of the excretory products in insects are regulated by the Malpighian tubules and the rectum acting in concert.

Nitrogenous Waste

Nitrogenous end products may be discussed in three groups: (1) ammonia, (2) urea, and (3) uric acid, allantoin, and allantoic acid.

Ammonia is a direct product of the oxidative deamination of amino acids. Containing 82% nitrogen, it is an efficient waste product, but it is also highly soluble in water and highly toxic. Terrestrial animals must conserve water; hence, ammonia is usually transformed to the less toxic urea (e.g., in mammals, amphibians, and some reptiles) or uric acid (e.g., in birds). Among insects, ammonia is the primary waste of species that ingest large quantities of water, such as the xylem sap-feeders and the aquatic immature stages of some dragonflies

(Aeshnidae), alderflies (Sialidae), and blowflies (Calliphoridae). The rapid flow of water through the gut in such insects rapidly dilutes and eliminates excreted ammonia to avert its toxicity without the energy requirement for synthesizing less toxic forms of nitrogen excretion products.

Urea, containing 46% nitrogen, is the primary waste of carpet beetles (Dermestidae). Most insects excrete uric acid, one of its breakdown products (allantoin and allantoic acid), or a combination of these substances. These are the least soluble, as well as the least toxic, end products, and they contain less nitrogen (32%–35%). Some uric acid is derived from the breakdown of nucleic acids, but the bulk of these wastes must be synthesized by energy-consuming pathways.

Waste Storage and Reuse

In certain insects, some of the nitrogenous waste is not expelled but is stored in a harmless state or utilized elsewhere in the body. Uric acid is deposited in special **urate cells**, or **urocytes**, of the fat body in the roach *Periplaneta americana* (Blattidae, Blattodea), adult *Culex* (Culicidae, Diptera) mosquitoes, the silkworm *Bombyx mori* (Bombycidae, Lepidoptera), and the wasp *Habrobracon juglandis* (Braconidae, Hymenoptera). The rate of accumulation of uric acid thus stored provides an index of aging but can be heavily influenced by the amount of food consumed. Adult males of *Blattella germanica* (Blattellidae, Blattodea) store uric acid in accessory sex glands in amounts up to 5% of the insect's weight. This is discharged as a white covering on the spermatophore during mating. The white color of crystalline uric acid is used as a pigment in the epidermis of some insects or conspicuously in the wing scales of butterflies in the family Pieridae.

Waste Elimination

Most nitrogenous waste is eliminated from the insect's body as part of regulatory processes involving other substances of small molecular size. The organs responsible are the **Malpighian tubules** (named for the 17th-century insect anatomist Marcello Malpighi) and the hind gut, especially the **rectum**. The filamentous, blind tubules insert and empty at the anterior end of the hind gut.

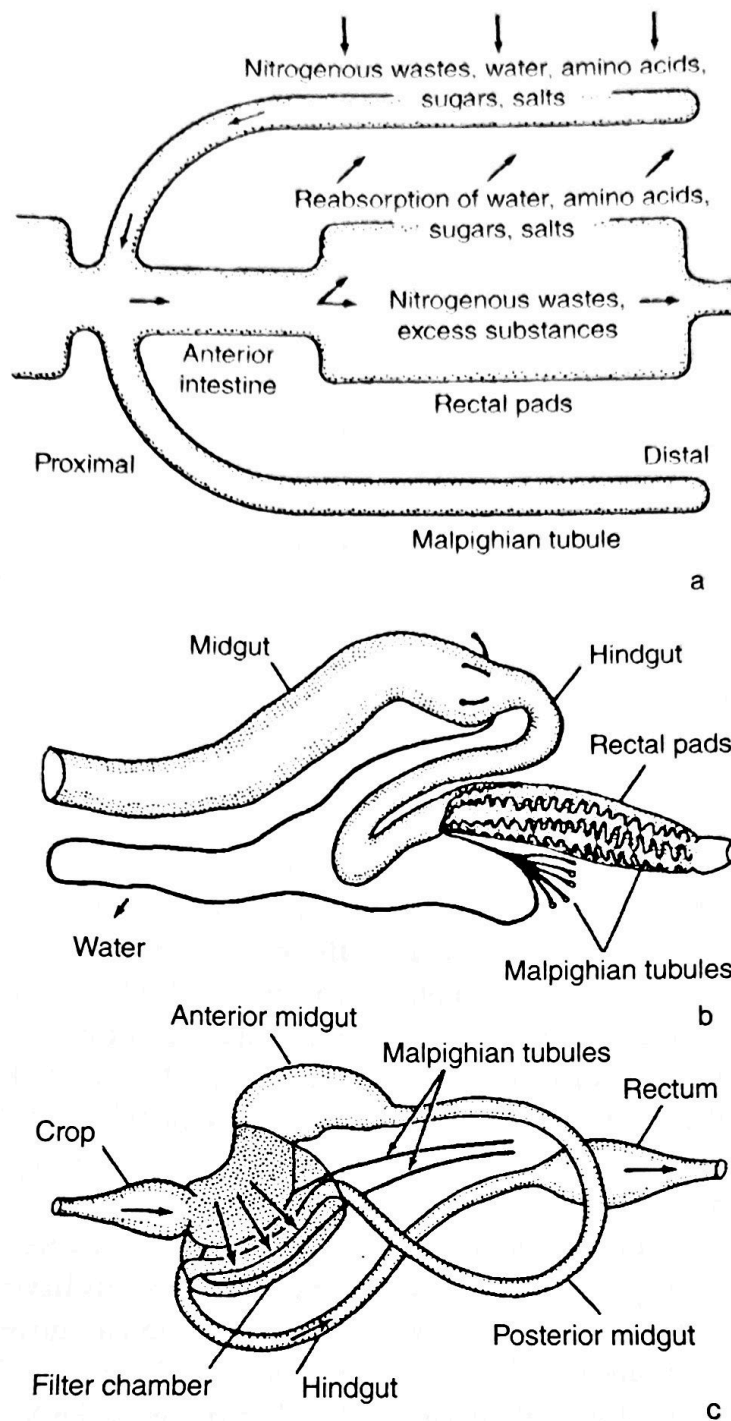


Figure 5.8 Excretory systems: **a**, generalized diagram of flow of nitrogenous wastes in the insect excretory system; **b**, cryptonephric system of *Tenebrio molitor* larva (Tenebrionidae), with five of the six tubules partly removed; **c**, diagram of filter chamber of Homoptera.

Source: **a, b**, Barbara Boole Daly; **c**, modified from Weber, 1933.

The walls of the Malpighian tubules and rectum are both one cell in thickness. Thus, an internal and an external plasma membrane are situated between the hemolymph and ducts leading to the

exterior. The cells of the Malpighian tubules are richly tracheolated and bounded by a basement membrane. The plasma membrane next to the hemolymph is deeply and complexly infolded; the

luminal membrane is microvillate and associated with long mitochondria. The tubules are usually free in the hemocoel and may move by means of slender muscles placed lengthwise in a spiral fashion. Malpighian tubules are lacking in Collembola, *Japyx* (Japygidae, Diplura), thrips, and most aphids; are **rudimentary** in certain Protura, Diplura, and Strepsiptera; and number only two in Coccoidea and some larval parasitoid Hymenoptera. Other insects usually have multiples of two, commonly having a total of six. Fifty or more tubules are formed in some Ephemeroptera, Plecoptera, Odonata, Orthoptera, and aculeate Hymenoptera. Grasshoppers (Acrididae, Orthoptera) may have hundreds.

The composition of the hemolymph is regulated in the following manner (Fig. 5.8a). Water, salts, sugars, amino acids, and nitrogenous wastes pass into the lumen of the Malpighian tubules, beginning at the tip or distal end. The filtrate then moves through the tubule to the hind gut and rectum. Along this route, useful components are selectively reabsorbed back into the hemolymph while the waste and surplus are voided. Potassium ions, sodium ions, or both are transported across a steep electrochemical gradient from hemolymph to lumen. The movement of these ions by a cation pump and the transport of chloride ions are linked to the secretion of urine in the lumen. Water, sugars, amino acids (except proline, which is transported), and nitrogenous wastes are thought to be passively carried along with this flow, while some other substances are actively transported. The rates of passage of solutes in the hemolymph into the tubules are typically at least 10 times less than the uptake of solutes in the vertebrate kidney glomerulus. This is because of the relatively limited area for permeation in the insect's tubules. This slow permeation of molecules into the tubule means that less energy is required to reabsorb needed solutes back into the hemolymph. Glucose and trehalose may be returned to the hemolymph at once from the tubule. The fluid is nearly iso-osmotic with the hemolymph when it is initially secreted.

Selective reabsorption may begin in the tubule and the anterior parts of the hind gut, but the major site of activity is the rectal pads in most insects. Active transport by transmembrane transporter

proteins moves potassium, sodium, and chloride ions against an osmotic gradient. Here, the molecules must move from the lumen of the rectum, through the rectal cuticle, through or between the rectal cells, and finally, to the hemolymph. The recovery of useful amounts of water, amino acids, salts, and sugars is linked to this process, but the molecular pathways remain unclear. Undoubtedly, aquaporins transport water into and out of the tubules (see Box 5.1).

In larvae and adults of Coleoptera and larval Lepidoptera, the distal ends of the Malpighian tubules may be held in contact with the rectum. This **cryptonephric** arrangement of the tubules has been carefully studied in the mealworm *Tenebrio molitor* (Tenebrionidae, Coleoptera). The adults and larvae live in dry, stored grains where conservation of water is required. The rectal complex is specially developed to dehydrate feces before elimination. Water is even extracted from air around the feces if the humidity is greater than 88%. The six tubules are held in contact with the rectum by a multilayered, perinephric membrane and an outer membrane (Fig. 5.8b). This arrangement allows the potassium pumps in the tubules to generate an osmotic gradient strong enough to draw water from the rectum across the rectal cells and into the tubule. The fluid in the tubule is moved proximally into the free portion of the tubule, where it is passed to the hemolymph. The waste is eliminated as a dry powder.

Insects that feed directly on the sap in the vascular tissues of plants have the opposite problem: excessive fluid and dilute nutrients. In the Cicadoidea (Hemiptera, suborder Auchenorrhyncha), the basal portions of the Malpighian tubules, the posterior end of the midgut, and the beginning of the hind gut are folded into and surrounded by the anterior midgut (Fig. 5.8c). The area of contact is enclosed in a membrane and is called the **filter chamber**. This structure concentrates ingested plant sap by shunting water from the anterior midgut to the hind gut. As a result, the density of nutrients in the absorptive regions of the midgut is increased. Cations from the hind gut are actively transported from the hind gut contents into the distal ends of the Malpighian tubules. As pressure builds up within the tubule, the tubule fluid moves

BOX 5.1 How Do We Know This? The Functioning of Malpighian Tubules

The anatomy of Malpighian tubules and the chemical composition of the fluids within them immediately suggested their function as important excretory organs. Physiological experiments can manipulate the chemical content of hemolymph and measure the resulting changes in the hemolymph and tubule fluid. Ion-specific microelectrodes can measure changes in ion concentrations over time within various regions along the lumen of a tubule in response to the fluids surrounding the tubule. These and other experiments demonstrated that Malpighian tubules have a regulating effect on hemolymph chemistry by selective uptake and output of water, salts, and other chemicals. The bloodsucking bug *Rhodnius prolixus* (Reduviidae, Hemiptera) is a favorite model insect for Malpighian tubule studies because of its large size and its incredible rates of urine production following a blood meal that is typically more than its own weight. Experimental severing of nerves to stretch receptors on the outer gut wall demonstrated that those stimulating these nerves not only serve to alert the insect to stop feeding when full but also trigger the neurosecretion of a **diuretic hormone** that mediates rapid urine formation by the Malpighian tubules. In only 30 minutes, *Rhodnius* can eliminate urine equivalent to its own hemolymph volume.

The advent of genomic methods has revolutionized studies of many physiological processes, including Malpighian tubule function. Because of the long-standing use of *Drosophila melanogaster* as a model system, with its many investigators worldwide, extensively characterized and mapped mutant genes, and complete sequences for entire *D. melanogaster* genomes, we probably know more about this fruit fly's Malpighian tubule than we do about any other epithelial tissue known to science. Another advantage offered by studies using *D. melanogaster* is the ability to make and introduce specific mutations by injecting transposable elements (P-factors) to incorporate specific genes into the germ line of the freshly fertilized egg. The function of a known gene is inferred from creating a "knockout" of the gene, often by inserting a marker gene into a targeted gene to disable it as well as to confirm the introduced gene's location. The resulting phenotype is evidence of the gene's function. A more recent approach is to selectively reduce

the expression of an individual gene through the use of interfering RNA (RNAi). Yet another approach is to measure changes in concentrations of mRNA to determine the level of gene expression under varying conditions and along different sections of a tubule. If the coding gene for the transcription of the mRNA is known or deduced, usually by homology with other known genes, the function of the protein coded for by the RNA can be determined. What all of these studies increasingly show is that the Malpighian tubule is far more complex than first imagined. This epithelial tissue contains a huge variety of transporter proteins embedded within the membranes of the epithelial cells of this tissue. The many unidentified RNAs transcribed within the tubule epithelium suggest that we have a long way to go to more completely describe the function of the Malpighian tubule.

Just as the many interconnected *Drosophila* researchers contribute to basic biology that benefits medical advances, medically based research provides new understanding of insect physiology. Specialized pores in animal cell membranes, constructed by proteins called aquaporins, directly transport water into the cell. These were first described in 1988 and earned their discoverers a Nobel Prize in 2003. By using molecular methods to determine the location of similar proteins or their corresponding mRNAs in insects, aquaporins have been discovered in insect organs and tissues that one would expect to need rapid and efficient water movement into or out of cells. In insects, aquaporins occur within tissues of rectal pads and the distal ends of Malpighian tubules (Fig. 5.8) flattened along the external rectal wall of insects that extract water from feces in the rectum as well as in the filter chambers of sap-feeding insects that bypass large amounts of water from the anterior foregut to the hind gut. Water movement across insect membranes, as in other animals, has long been assumed to be driven by osmotic gradients created by the active pumping of cations such as potassium, sodium, and calcium, but aquaporins can transport water directly across membranes with astonishing rapidity—about a billion water molecules per second through each pore. How much water movement each method creates and how they are coordinated are unresolved questions.

toward the hind gut. Water in ingested sap moves osmotically from the proventriculus into proximal parts of the Malpighian tubules because the proximal parts contain increased salt concentrations. Following the discovery of water-transporter membrane proteins, the aquaporins, we presume that these too are involved in water transport by the filter chamber (see Box 5.1). From the lumen of the tubules, the fluid is expelled into the hind gut. The cuticle-lined hind gut usually is impervious to water movement except for the rectum, where cations in the urine again may be recycled. Thus, the bulk of the liquid is excreted, bypassing the rest of the midgut where nutrients are concentrated, digested, and absorbed.

The Malpighian tubules have an unusual function in spittlebugs (Cercopidae, Hemiptera), where they add a substance to prolong the durability of bubbles ("spittle") that spittlebug nymphs expel from their anus to surround their bodies. This presumably protects them from some natural enemies. All leafhoppers (Cicadellidae, Hemiptera) and some treehoppers (Membracidae, Hemiptera) expel unusually shaped particles called brochosomes.

Malpighian tubules produce these peculiar particles, which fall in two general functional categories. Leafhoppers spread or "anoint" excrement containing spherical brochosomes over their bodies, perhaps as a water repellent. The adult females of a few species of leafhoppers use their hind legs to capture their excrement containing high concentrations of elongated, rodlike brochosomes in a large drop and then manipulate it until it evaporates to a paste, which they transfer to a spot on the outer wings. The brochosomes appear in the excrement just before egg-laying, and the females spread the stored brochosomes as a powder over plant surfaces containing their freshly inserted eggs. The brochosomes appear to irritate and distract tiny wasp parasites that attack the leafhopper eggs by sticking to the parasite's antenna and body hairs.

MUSCLES AND LOCOMOTION

Insects move from place to place in virtually every natural medium on the earth's surface except solid rock, ice, or rarely, salt water. On the surface of the

ground, they wiggle, hop, walk, or run, often with surprising agility. By their feeding and burrowing activities, they move through the flesh of animal hosts, the tissues of plants (including solid wood), and the leaf litter, soil, or subsoil of the earth. In the aquatic environment, some skate on the surface, propelled by their legs or by chemicals that alter the surface tension. Under the surface, they swim with the legs or wings, with body movements, or by jets of water expelled from the anus. The best speeds, the longest distances, and probably the safest trips are achieved in the air. Yet compared to locomotion on the ground or in water, flight consumes much more energy and requires a lightweight body equipped with wings and muscles for flying together with keen senses and a nervous system for coordination.

Insects were the first animals to evolve flight and are still the only invertebrates that fly actively and can control the duration and direction of their flights. The only other invertebrates to fly are the ballooning spiders and some mites, which, however, are passive drifters. Flight has clearly had a major influence on the diversity and abundance of insects. As shown by the fossil record, insects had wings and presumably were flying in the Pennsylvanian Period (323 to 290 million years ago; see Figure 15.3), over 150 million years before the first flying reptiles (Pterosauria) appeared in the Jurassic (208 to 146 million years ago). Much later, the birds and mammals (bats) each independently evolved flying mechanisms.

Insect Muscle

The power for locomotory and other movements is supplied by the muscular system. Internal organs, such as the gut, heart, or Malpighian tubules, also have sets of muscles. All muscles of insects are **striated**; in other words, each fiber has a cross-banded appearance when viewed under the microscope (Fig. 5.9a). In contrast, vertebrates and many other invertebrates have both the striated type and a second type, smooth muscle, which is composed of single spindle-shaped cells.

The muscular system of insects differs in several important respects from that of vertebrates. The most conspicuous difference is the relation to the

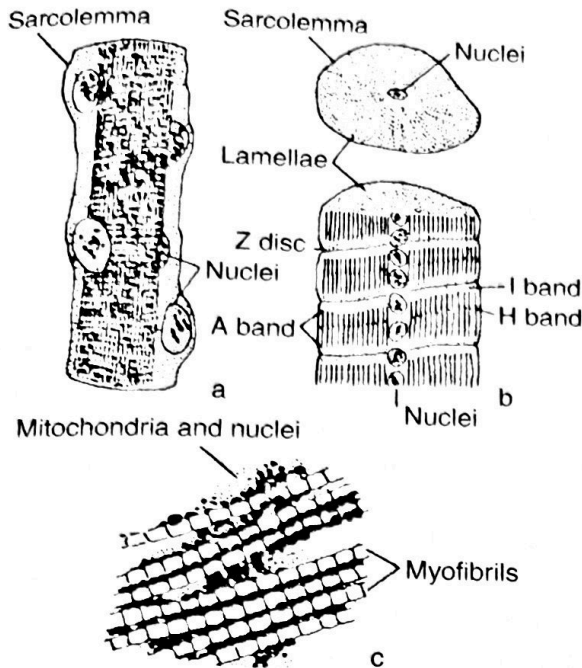


Figure 5.9 Types of muscles: **a**, larval; **b**, tubular; **c**, fibrillar.

Source: Redrawn from R.E. Snodgrass, *The Principles of Insect Morphology* - public domain.

skeleton: the muscles of insects are inside the skeleton, while those of the vertebrates are outside. As a consequence of the tubular construction, which resists bending, and of the general absence of calcium salts, the insect skeleton compared to the vertebrate skeleton is relatively lighter and stronger and provides greater space and attachment areas for the muscles. The attachment of muscles to the skeleton is not by connective tissues, as in the vertebrates, but by **tonofibrillae**, which are special secretions of the epidermal cells whose attachments may extend through the cells into the cuticle as far as the epicuticle. If so, the muscles remain attached to the old cuticle even while molting is in progress, thus allowing movements during this critical period. At the point of attachment, the cuticle may be invaginated into a tendonlike apodeme. When composed of normal cuticle, such apodemes lack elasticity, but when the special cuticular protein resilin is present, a highly elastic tendon is formed. Although insects might be assumed to have fewer muscles than larger animals such as vertebrates, some insects have more than two or three times

as many separate muscles than do humans, largely owing to the more numerous body segments and appendages.

Insects may appear to perform feats of unusual strength, but the power per gram of muscle is about the same as that of vertebrates. A muscle's power is proportional to its cross-sectional area. The explanation for insects' seemingly greater strength lies in the effective use of lever systems and the small body size. As the body of an organism decreases in size, the volume or mass decreases as with the cube root of length, whereas the cross-sectional area decreases as the square root. Thus, muscle weight (a function of muscle volume) decreases faster with miniaturization than does muscle power (proportional to cross-sectional area). The amount of work per gram achieved by insect muscles can greatly exceed that of vertebrates because some insect muscles can contract much faster, leading to relatively more energy consumed and work accomplished on a per-weight basis. Reductions in the size of insect muscles involve mainly reductions in the number of fibers rather than reductions in the size of the fibers. A species of small insect may have only a single fiber functioning in the position occupied by hundreds of fibers in a species of large insect.

Structure of Muscles

Muscle cells have multiple nuclei (**syncytium**). Furthermore, each muscle is the product of many cells that share a common plasma membrane and an outer sheath, the **sarcolemma**. This outer covering may be invaginated into the muscle in certain places, forming a system of intracytoplasmic **intermediary tubules** (called the IT or T, for transverse, system). Tracheoles may reach deep into the interior of the muscle without penetrating the sarcolemma by entering these tubules. This is one feature that enables insect flight muscles to have the highest rates of oxygen consumption per gram for any animal locomotory muscle. When the muscle is stimulated by an excitatory nerve, the T system allows the depolarization of the cell membrane to pass from the surface to the deeper regions of the muscle.

Within the muscle are the contractile **myofibrils** traversing the length of the muscle. These are

striated and may exist as flat sheets (lamellae) or as threadlike fibers. Under great magnification with an electron microscope, each fibril is seen to be composed of protein filaments of two sizes. The thick filaments are myosin, and the thin filaments are actin. In cross section, each actin filament of an insect muscle is usually situated between two myosin filaments. In vertebrate muscle, the actin filament is between three myosin filaments. When the muscle contracts, the two sets of filaments slide past each other. The distribution of the two kinds of filaments also provides an explanation for part of the banded, or striated, appearance. The I bands represent only actin filaments, the H band represents only myosin filaments, and the A band represents both together. At regular intervals, both thin and thick filaments attach to a disklike structure called the Z disk. The interval between disks is called a sarcomere.

Dispersed among the myofibrils are numerous nuclei and mitochondria and various amounts of a sarcoplasmic (endoplasmic) reticulum. Mitochondria and tracheolar endings are more numerous in muscles that have a high degree of activity, such as flight muscles. Individual muscles are grouped together into a "muscle unit," which has a separate tracheal supply. A single muscle unit or several units may be separately innervated by branches of one, two, or three motor axons (see Chapter 6), making a motor unit.

Finally, a single motor unit or several motor units may be grouped closely together into a functional "muscle," which is anatomically discrete from other such units, has definite areas of attachment, and exerts a pulling force on the attachments in a definite direction. Unlike vertebrate muscles, insect muscles lack connective tissues among the fibers. Ordinarily, a **skeletal muscle** has one of its attachments, or its origin, on an immovable part of the skeleton and its insertion on a movable part, such as a segment of an appendage or an articulating sclerite. Some are bifunctional—under certain circumstances, the structures at either end may be moved. For example, some thoracic muscles function during walking by moving the leg bases and also function during flying by moving sclerites at the opposite ends of the muscles. The large indirect flight muscles move large portions of the thoracic skeleton simultaneously. **Visceral muscles**, which

move various organs such as ovaries or alimentary organs, usually attach to other visceral muscles.

Innervation and Contraction of Muscles

Although all insect muscle is striated, evolutionary changes in structure and function have led to several distinctive kinds of muscle. Perhaps the most primitive locomotory muscles are **tubular muscles**, characterized by the tubelike form of myofibrils being arranged in sheets around a central canal that is filled with nuclei (Fig 5.9b). **Larval muscles**, known from immature bees and flies, are distinguished by minute myofibrils with the nuclei located externally beneath the sarcolemma (Fig. 5.9a). **Visceral muscles** are composed of parallel or anastomosing fibers similar to larval muscles in construction, but they have larger myofibrils and are associated with the internal organs of adult insects. **Microfibrillar muscles** have small myofibrils measuring 1.0 to 1.5 μm in diameter that are found in the flight musculature of some orders. **Fibrillar muscles**, so called because their coarse fibers are easily sheared and separated when dissected, have large myofibrils measuring 1.5 to 5.4 μm in diameter (Fig. 5.9c). They make up the flight muscles of the orders Hymenoptera (most species), Diptera, Coleoptera, and certain Hemipteroidea (some Psocoptera, Thysanoptera, Heteroptera, and some other Hemiptera).

Insect muscle cells or units may be innervated (connected to the nervous system) by more than one neuron, or a single nerve output process (called an axon) may branch out to attach to the same muscle fiber in more than one place. This contrasts with vertebrate muscles, which have a single end-plate connection. Insects usually have two motor axons, rather than the one that is typical of vertebrates, attached to each muscle: one "fast" and another "slow" in its rate of contraction. Some motor nerves inhibit rather than stimulate contraction. The rapidity of nerve transmissions and the number and type of motor nerves used result in differences of insect muscle response ranging from slow and precise to rapid and powerful. Vertebrates achieve such muscle contraction differences by varying which muscles or units are stimulated.

Normally, skeletal muscle contractions are **synchronous** with each nerve impulse. Visceral

muscles may be innervated by typical neurons or by special neurosecretory cells that secrete neurotransmitter substances not closely restricted to nerve junctions. The major flight muscles of several groups of insects contract several times for each nerve impulse. Fibrillar muscle, called **asynchronous** (or resonating) muscle, is pivotal to the flight abilities of many "advanced" insect groups, such as the flies, beetles, and wasps as well as many true bugs (Heteroptera). The asynchronous type has evolved independently from the synchronous type in 7 to 10 different lineages of insects. Asynchronous muscles allow greater speed of contraction, enabling higher wingbeat frequencies. They also make possible greater mechanical power output by having a higher ratio of contractile fibers and energy-providing mitochondria. The wing muscle of active fliers can contain 30% to 40% mitochondria by volume. After contraction, the shortened asynchronous muscle is deactivated and can then be restretched to its original length with less force than produced by the contraction. Stretching reactivates the muscle contraction. The positive net difference in work output between the contraction and stretching phases is the net amount of work used for powering movements. Asynchronous muscle is more efficient and powerful than synchronous muscle at the high contraction frequencies of advanced fliers. Its greater efficiency results from less energy being used for nervous control of muscle activation, largely due to the amounts of calcium recycled in muscle activation. It is more powerful partly because it contains a higher proportion of power-producing fibrils within the sarcoplasmic reticulum.

Walking, Jumping, and Swimming

Hexapods are distinct among the arthropods for having three pairs of locomotory appendages. In terrestrial locomotion, each leg must function in two ways: (1) to support the body's weight above the substrate and (2) to thrust against the substrate for propulsion. The best explanation for the evolutionary choice of six legs lies in the fact that a minimum of three legs can provide support in a stable tripod arrangement while the other three legs are stepping forward (Fig. 5.10). The insect walks by shifting from alternate tripods of leg

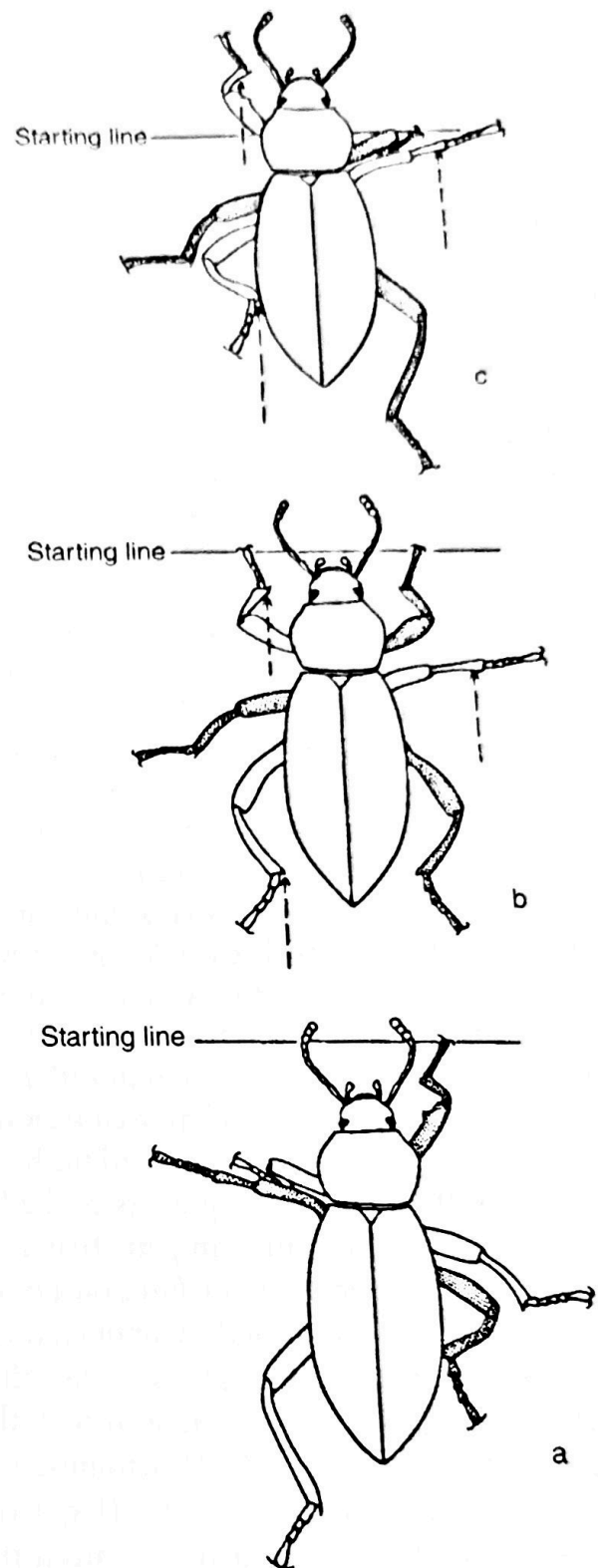


Figure 5.10 Diagram of walking movements of the beetle *Eleodes dentipes* (Tenebrionidae). Shaded legs are supporting the beetle as the unshaded legs step forward in the sequence a to c. At c, the unshaded legs assume support of the body and the shaded legs begin stepping forward.

Source: Barbara Boole Daly.

contact with the substrate. The sequence or rhythm of leg movements changes as the insect increases speed. Depending on the gait, more than three or less than three legs may be on the substrate at once. At high speeds, cockroaches run by alternate steps of only the hind legs. The rhythmic movements of insect legs originate in the central nervous system and are modified by feedback from sensory receptors in the legs. The segmental ganglia control their respective legs but are coordinated by intersegmental nervous coupling.

The forelegs, which are in front of the center of gravity, exert their force in a pulling direction, while the middle and hind legs, which are near or behind the center of gravity, exert a pushing thrust. The propulsive thrust of the hind legs is greatly developed as a jumping device in Orthoptera (Figs. 29.1, 29.3), many Auchenorrhyncha (Fig. 34.13a,b), fleas, and flea beetles (Alticinae, Chrysomelidae). Jumping is also accomplished in other ways: click beetles snap their bodies at the joint between the prothorax and mesothorax, and springtails utilize an abdominal appendage (Figs. 18.2, 18.3b, 18.4).

During walking, a stepping motion involves movements of the whole leg as well as movements of individual leg segments. The whole leg is moved by a complex musculature that originates inside the thorax and exerts pulling forces on the basal rim of the coxa. By the positions and insertions of the muscles, the whole leg can be moved forward, backward, away from the body, or toward the body. Movements of the individual segments of the leg are made by muscles operating in pairs that arise in one segment and exert a pulling force on a more distal segment. One member of the pair operates as a levator, raising the distal segment, and the other member operates as a depressor, lowering the distal segment. As shown by the skeletomuscular mechanism of a grasshopper's hind leg (Fig. 5.11), the femur is moved by a levator muscle from the coxa (1) and two depressor muscles, one from the coxa (2) and the other from the thorax (3). The tibia is moved by a levator muscle (4) and a depressor muscle (5), both originating in the femur. The tarsus is moved by a levator muscle (6) and a depressor muscle (7) that originate in the tibia and insert on the base of the tarsus. The individual

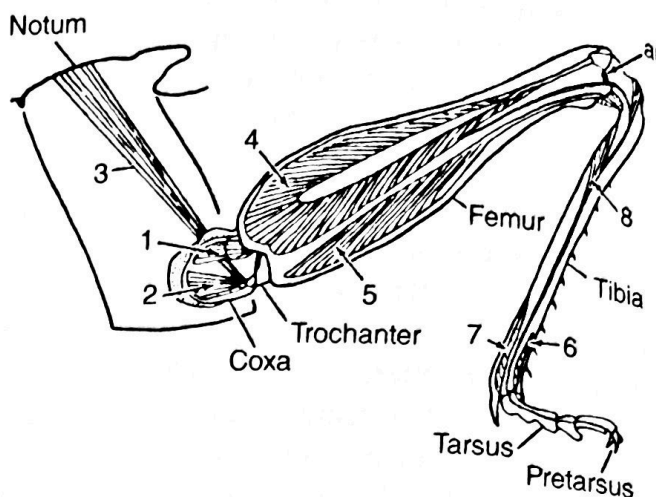


Figure 5.11 Skeleton and muscles of the hind leg of a grasshopper. Numbers 1–8 are muscles described in the text; ar, articulation of femur and tibia.

Source: Redrawn from R.E. Snodgrass, *The Principles of Insect Morphology* - public domain.

tarsomeres do not have muscles. The pretarsus has only a depressor muscle (8) that originates in the femur and tibia and inserts by means of a long tendon on the base of the pretarsus.

Modifications of hind legs enable impressive jumping ability in many insects. Enlarged hind femora of grasshoppers (Fig. 5.11; see figures in Chapter 29), for example, house large muscles whose slow contractions compress the elastic cuticle in the femur-tibial joint much like compressing a spring or drawing a bow. The sudden release of energy propels the insect for jumping. The jumping mechanism of a flea is illustrated in Fig. 44.2. Vermiform (Figs. 42.5c) or eruciform (Figs. 4.4d, 43.5c,d) larvae can crawl through coordinated, wavelike, successive contractions of the body segments. External spines along the body may help provide traction. Lepidopteran and Hymenopteran (suborder Symphyta) larvae have abdominal prolegs (Fig. 41.5), which are unsegmented but leglike modifications of the body wall equipped with hooked spines called crochets (Fig. 17.2b).

Insect tarsi adhere to surfaces by the action of tarsal claws or adhesive pads (Fig. 2.11c,d). Adhesion is facilitated in some cases by molecular forces (Van der Waals forces) between the substrate and the numerous, very fine hairs on the feet or by lubricating fluids applied from the tips of fine hairs.

Aquatic insects that live in flowing water face the problem of adhering to exposed substrates. Larvae of black flies (Simuliidae, Diptera) anchor their abdomens to rocks or logs in fast-flowing portions of streams with anal suckers or hooks at the end of the abdomen as they extend their fanlike mouthparts to capture small particles of food suspended in the current. Some aquatic larvae such as stoneflies (Plecoptera) and mayflies (Ephemeroptera) that crawl among rocks in swift currents have highly flattened bodies, both to crawl into tight spaces and to minimize drag from the moving water.

Insect tarsi and legs can be modified to a paddle shape for swimming (Figs. 2.16d, 34.10c, 39.5c,d). The paddles are usually formed by hairs projecting from the tibia or tarsi. Because of the viscosity of water relative to the size of the legs, the hairs develop adequate traction for swimming. An advantage of hairs in swimming (and for flying in minute insects, as described later) is that the hairs can be extended to increase the effective area for propulsion, and then streamlined along the leg so that the net drag caused by bringing the leg forward is less than the opposite force for the propulsion stroke.

Flight

The historical origin and evolution of wings are discussed in Chapter 16. Here, we are concerned with the skeletomuscular and aerodynamic mechanisms of flight (see Box 5.2). Insect wings are not homologous with appendages, as they are in birds and bats. There are no muscles in insect wings, although the wings have trachea and circulating hemolymph. Wings are powered entirely by muscles within the thorax that either deform the mesothoracic and metathoracic segments of the thorax to move the wings (**indirect flight muscles**) or by directly deforming of the basalar and subalar sclerites near the wing base that act as a lever to move the wings (**direct flight muscles**).

Lengthwise in the thorax, the **dorsal longitudinal indirect flight muscles** extend between skeletal phragmata. Their contraction raises the center of the flexible notum. The **dorsoventral indirect flight muscles** operate as antagonists and depress the notum by their contraction. Mechanically, the wing base rests on the pleural wing process in the same manner as a lever on a fulcrum (Fig. 5.12a). Thus, contraction of the longitudinal

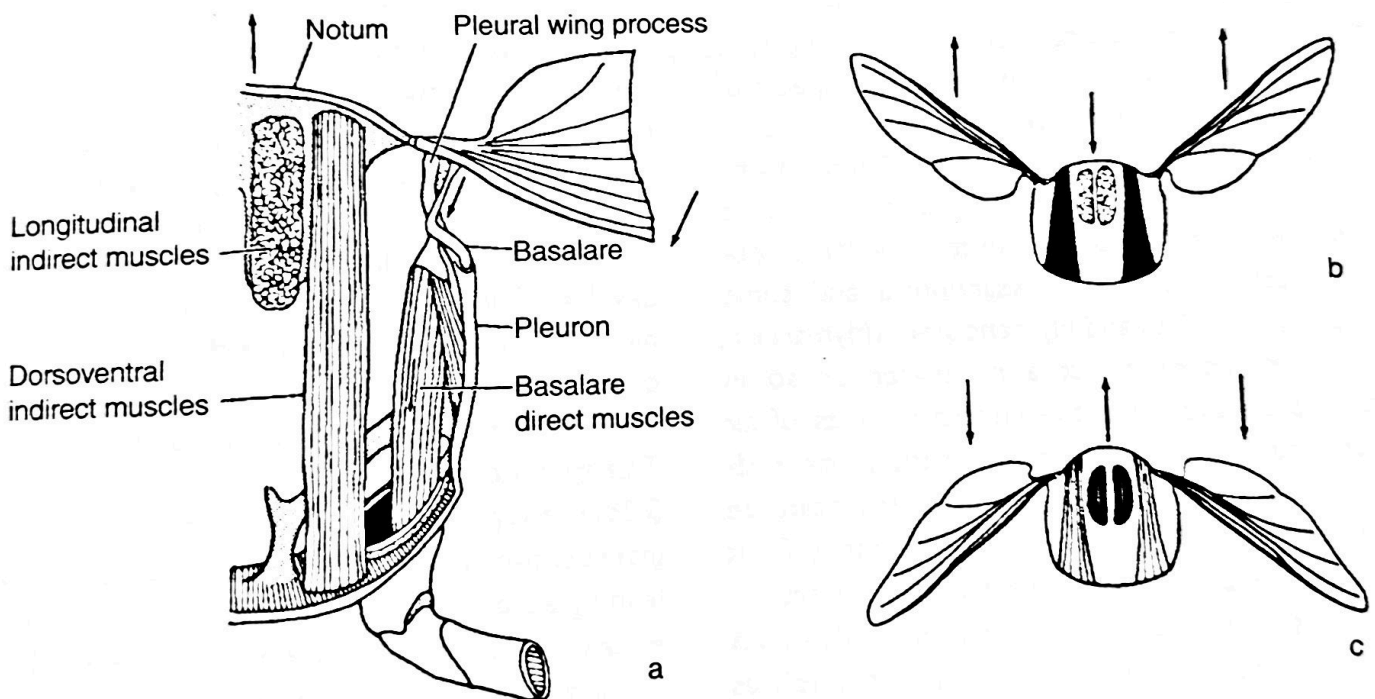


Figure 5.12 Flight mechanism: **a**, cross section of a mesothoracic segment during the downstroke and pronation of the wing (view is of the left side of the insect as seen from in front); **b**, wing upstroke and supination (contraction of dorsoventral indirect muscles indicated in black); **c**, wing downstroke and pronation (contraction of longitudinal indirect muscles indicated in black).

Source: Redrawn from R.E. Snodgrass, *The Principles of Insect Morphology* - public domain.

BOX 5.2 How to Study Insect Flight

How can bumble bees fly? Legend has it that engineers in the 1930s calculated that, according to conventional aeronautical theory, bumble bees should not be able to fly. The point of this story was that insects must have used some aeronautical principles unknown at the time. Since then, we have made major advances to date in understanding how muscular, aerodynamic, and neural processes operate to achieve insect flight.

Most of the lift on the wings of aircraft is attributed to the Bernoulli principle of lowered static pressure created by the higher velocity of air over a wing's upper surface creating lift for flight. You can easily demonstrate this lifting force by blowing across the top of a strip of paper held horizontally, which causes the paper to rise. This example, and the studies of airfoils in wind tunnels or on cruising aircraft, describe steady-state conditions. Insect flight, however, is the epitome of constant changes that enable flight. Many insects, especially those with large wings, use their momentum to glide between wing strokes, and the Bernoulli principle can account for some of the lifting forces in this flight with large wings and slow wingbeats. But due to their small size and type of wing movements, insects utilize other aspects of other aerodynamic forces for flight.

To begin with, at the size scale of most flying insects—a few millimeters—the viscosity of air is a far more influential force than it is for larger objects. Minute insects like Thysanoptera and some Coleoptera (Ptiliidae) and Hymenoptera (Mymaridae, Trichogrammatidae), about a millimeter or so in length, are so small that the viscosity effects of air predominate in flight. Their tiny paddle- or rod-shaped wings are fringed with fine bristles that utilize drag forces on the wings rather than lift, so their flight is analogous in part to swimming through the air.

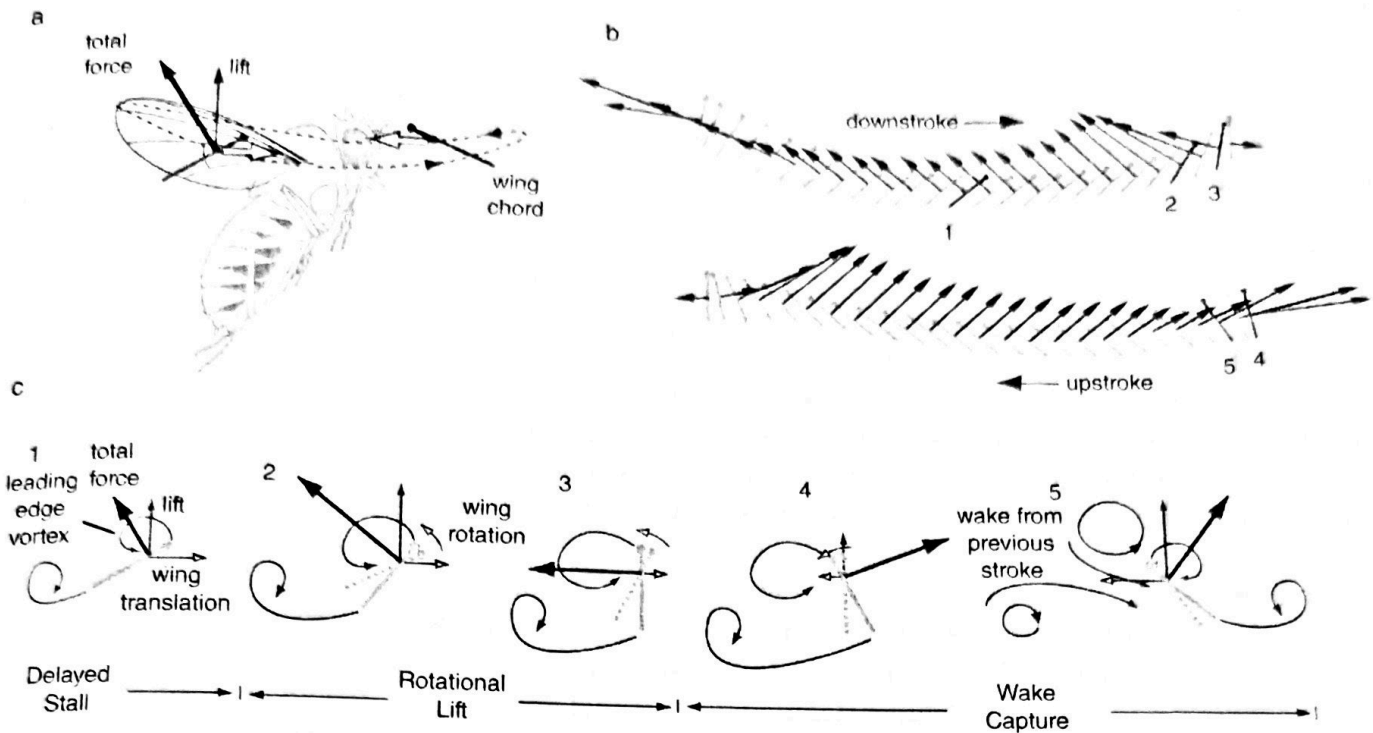
Engineers use a scaling factor called the Reynolds number (Re) to calculate how fluid viscosity relates to the size and speed of an object moving in either a gas or liquid fluid. Re is a dimensionless number that is the ratio of inertial forces (velocity $\times$ size) to viscous

forces (force/area $\times$ time). Examples of Re range from over 10^8 for a whale to 10^5 for a typical baseball pitch and 10^{-4} for human sperm. Insects have an Re of about 10 for the smallest and 10^4 for the largest. Engineers can adjust the dimensions of aircraft models to have the same Re , and thus similar flight dynamics, as the full-size aircraft as a way to predict and test aerodynamic characteristics by working with a model. Similarly, in experiments to study insect flight at a scale and speed that was easier to observe, researchers used oil instead of air as the model fluid, thus enabling the much larger 25-cm wingspan of model insects submerged in oil to have the same Re as the real insect in air. Other experiments utilize smoke plumes in generated airflows coupled with high-speed photography from different perspectives of a flying insect. These experiments showed that vortices and downdrafts coming off the moving wing produced forces significant for providing lift (Box Fig. 5.2c). You can visualize a vortex as the visibly swirling water coming off the blade of a paddle or oar during a stroke.

In part because of their small size, insect wings can develop significant lifting forces at very high angles of attack (α), the angle between a wing's horizontal axis and the oncoming air (relative wind). In excess of an angle of attack of about 15° , a conventional fixed aircraft wing typically loses smooth flow over the wing, resulting in turbulent airflow and a sudden loss of lift, called a stall. Many insect wings develop lift at an angle of attack two to three times higher (α of 30° – 45°) by using the upwelling vortex of air that separates from the leading edge of the wing at these high angles of attack to provide lift. This type of lift, also called “delayed stall” (Box Fig. 5.2c1), is rapidly transient in fixed-wing aircraft, but insect wingbeats are so rapid that after shedding the leading edge vortex at the end of power stroke, the wing rotates and generates another vortex. The rotation of the wing produces another lifting force, rotational lift (Box Fig. 5.2c2,c3), much like a tennis ball hit with backspin rises because of the faster airflow across its top side. In addition, in some conditions

(Continued)

BOX 5.2 (Continued)



Box Figure 5.2 Aerodynamic mechanisms of insect flight. **a**, In hovering flight, the fruit fly wing tip's path (dotted line) is formed by forward and rearward movements (white arrows), with a high angle of attack (angle between wing chord and flight direction). The wing rapidly rotates between strokes to reverse the wing surface for downstroke (dorsal surface up) and upstroke. The total wing force (thick black arrow) is shown with the lift component (part of wing force that is opposite gravity) as a vertical arrow. **b**, The magnitude and direction of the wing's force (black arrows) and the orientation of the wing to flight direction change continually throughout flight (wing chord, or wing axis, is shown by gray lines with circles at leading edge). **c**, Curved lines show air flow generated by wing movements (as in b). The vortex generated at the wing's leading edge creates delayed stall (1). Rapid wing rotation at the end of the wing stroke provides rotational lift (2–3). In wake capture, the swirling wake generated by a previous wing stroke (4) is captured by the wing's next stroke (5) to aid lift.

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insects can use a mechanism called “wake capture” (Box Fig. 5.2c4,c5) to conserve much of the energy that would otherwise be dissipated in vortices by positioning its wings to capture some of the lifting energy of its wake.

A method to study insect free flight is to make high-speed motion pictures of insects flying through smoke plumes to record air movements about the wings. One such study of a butterfly (Srygley and Thomas, 2002) showed the versatility of this insect to use all of the mentioned methods of generating flight

forces, in addition to the “clap-and-peel” method of slamming the wings together on the forward stroke and retrieving the wings by peeling the stiff leading edge of the wing apart. The more flexible parts of the wings open in a billowing manner to generate lift. The method of producing lift or thrust often varied from one wing stroke cycle to the next. These and other studies show that insects use no single method to generate flight forces. Some methods act simultaneously, while others alternate depending on the type of insect or phase of flight.

indirect muscles causes an upward movement of the thoracic notum, which lifts the wing base and forces the rest of the wing downward (Fig. 5.12c). Likewise, contraction of the dorsoventral indirect muscles depresses the notum, which lowers the wing base and forces the wing upward (Fig. 5.12b).

A simple up-and-down movement of flat wings would not, however, generate the forward thrust needed for flight. For propulsion, the wings must twist at the base so that the surface of the wing is at an angle to the body. On the downstroke, the leading (anterior) edge of the wing twists ventrally (pronates). On the upstroke, the leading edge twists in the opposite direction (supinates). Located at the anterior edge of the wing base is a small sclerite, the **basalare**, and at the posterior base of the wing is the **subalare** (Fig. 2.15a). Each sclerite has a direct muscle from the pleuron. Contraction of the **direct basalare muscle** depresses the leading edge of the wing to produce **pronation** (Fig. 5.12a). Similarly, contraction of the **direct subalare muscle** depresses the trailing edge, producing **supination**.

On meeting the resistance of the air and depending on the structure of the wing veins and flexibility, the wings may bend and fold in a regular pattern of deformation during each stroke. In the grasshopper, the hind wing traverses a slightly greater amplitude and leads the forewing, thus avoiding its turbulence. The stiff forewings of beetles (elytra) are held open, often without moving during flight. The elastic properties of the thorax and antagonistic muscles are greatly enhanced by special deposits of the elastic protein resilin on the pleural wing process.

The general design of the flight mechanism of the grasshopper is found throughout the Orthopteroidea and some Neuropteroidea. Exceptions are the Odonata, Blattodea, and Mantodea, where the downstroke is powered by direct muscles rather than the longitudinal indirect muscles.

In summary, with the exceptions just noted, the orders discussed here have two sets of synchronous muscles, the indirect and direct muscles, which provide the power and the twisting of the wings. Both the mesothoracic and metathoracic segments are usually so equipped. The wingbeat frequency and, consequently, the speed of flight in all these

orders are limited to the rate at which the nervous system can send impulses to each of the participating muscles in a coordinated fashion—about 50 per second.

A second major type of flight mechanism is found in the Diptera, Hymenoptera, Coleoptera, and some Hemipteroidea. These orders have a high wingbeat frequency that depends on flight muscles contracting asynchronously with many contractions per nerve impulse. The wingbeat frequencies of bees and flies is on the order of 100 to 300 beats per second. With wings shortened experimentally and at a high temperature, the midge *Forcipomyia* (Ceratopogonidae, Diptera) was rated at a frequency of 2,218 strokes per second.

In these orders, the main source of power for the wings resides in one thoracic segment and is often limited to one set of longitudinal and one set of dorsoventral muscles operating antagonistically, such that one set relaxes (stretches) when the other set contracts. Flies have elastic parts of the thoracic box that are stretched during one part of the wing stroke, storing energy, and then quickly released by a “click” mechanism to assist the next movement. The complex path of the wing is now determined mechanically by the shapes of the interconnected sclerites at the base of the wing and the adjoining thoracic processes. You can demonstrate this for yourself in a freshly killed bee or fly. Depress of the mesonotum and see the wings automatically supinate as they are forced upward, or raise the mesonotum with a pin and watch the wings pronate as they are forced downward. The direct muscles of these insects serve to modify the wing stroke over many beats. Search Internet for terms “insect flight slow motion” for action flight videos.

The insect nervous system has also evolved some impressive adaptations for flight. Day-flying insects have much larger compound eyes and associated connections with the brain to transport, process, and respond to visual information useful for flight. The flicker fusion rate (speed with which the brain renews an image) of a housefly is almost 20 times faster than that of humans. The hind wings of Diptera have evolved to become tiny stubs (halteres) that vibrate rapidly to provide information about changes in orientation to the horizon, much

as applying force to a gyroscope causes a predictable rotational reaction force. Complex pattern-generating neural circuits set up a pattern of nerve inhibition and excitation to rapidly coordinate the activation of upstroke and downstroke muscles.

Additional adaptations for flight include providing robust ventilatory air movements in the tracheal system to provide oxygenation and to expel carbon dioxide as well as providing energy for the oxidative energy producing metabolism of the flight muscles. Hemolymph circulation provides ways to dissipate heat from the relatively massive and highly energetic flight muscles, as discussed later.

THERMOREGULATION

Insects are active within wide ranges of temperature. The snow scorpionflies *Boreus* (Boreidae, Mecoptera; Fig 8.5), snowfly *Chionea* (Tipulidae, Diptera), snow "fleas" *Achorutes* (Poduridae, Collembola), and winter stoneflies *Allocapnia* (Nemouridae, Plecoptera) are active at low temperatures on snow. *Boreus* dies of heat stress if kept for long in the human hand. At the other extreme, the firebrat *Lepismodes inquilinus* (= *Thermobia domestica*; Lepismatidae, Thysanura) prefers 12 to 50 °C and dies at 51.3 °C in a short time. At unfavorably high ambient temperatures, an insect is severely limited in the extent to which it can cool itself. Cooling by the evaporation of water quickly leads to desiccation unless water is freely accessible. Nectar-gathering bees regurgitate fluid from their crop ("honey stomach") and hold it within their mouth while flying so that the evaporation of the fluid cools their heads, absorbing heat from the adjacent thorax. The tsetse fly *Glossina morsitans* (Glossinidae, Diptera) feeds while exposed to sun on the hot skin of African mammals. The opening of the spiracles at temperatures above 39 °C permits evaporative cooling within the tracheal system to lower the body temperature by about 2 °C. This is possible because the meal contains ample liquids to restore lost water. Honey bees transport water to their hives, deposit it in the combs, and vigorously fan with their wings so that evaporative cooling takes place. Most insects, however, escape heat by seeking shade or cooler places.

Ectothermy

Although insects usually cannot lower their temperature below that of ambient, many can actively regulate their temperature above that of the environment. Insects are able to increase their body temperature well above ambient by absorbing heat from the sun. Organisms that derive heat almost entirely from their environment are called **ectothermic**. Butterflies assume distinctive postures while basking in the sun. "Dorsal baskers" spread their wings and point their heads away from the sun. The skippers (Hesperiidae) commonly spread only the hind wings, leaving the forewings vertical. "Lateral baskers" keep both wings vertical over the body and orient to present one side to the sun's rays.

Endothermy

Insects that elevate their temperature through metabolic activity are **endothermic**. Large flyers such as bumble bees and sphinx moths (Sphingidae, Lepidoptera) heat their thorax to temperatures optimal for flight muscle performance. With their wings folded back across the body to disengage the flight muscle contractions from moving the wings, these insects contract both the dorsoventral and the horizontal indirect flight muscles at the same time, rapidly building up heat in the thorax and enabling them to fly even in very cool weather. If not dissipated, the heated thorax can rapidly reach lethal temperatures, but hemolymph moving through the dorsal vessel as it passes along a contorted route through the thorax absorbs excess heat from the surrounding muscle mass. The hemolymph empties from the dorsal vessel into the head, from which it then is shunted along the ventral thorax to the ventral abdomen. During flight, the head and mouthparts and the ventral abdomen are cooled by air flowing over the insect. In the nest, the female can generate heat from the flight muscles and transfer the heat through hemolymph movements to her ventral abdomen. During the cool days and nights of the early spring months, she can use this method of heat transfer to speed the incubation of her first offspring by pressing her ventral abdomen against the brood cell of her first developing larva. The dorsal abdomen is insulated by a covering of hairs (setae), but the ventral

abdomen is bare, facilitating heat transfer. Without this added heat for incubation to develop the first brood, there would not be enough time to rear subsequent broods within the brief warm season of the subpolar climates these bumble bees inhabit.

The complexity and efficiency of the bumble bee's **thermoregulation** belies the simplistic view that insects are strictly "cold-blooded" organisms. The bumble bee displays remarkable control of not only its body temperature but what part of its body is warmed. Vertebrates shift some blood flow to the peripheral circulation for cooling during strenuous activity at the cost of diverting oxygen availability to the muscles. In contrast, the heat transfer mechanisms of insects are separate from gas exchange mechanisms, allowing high flight muscle activity, for example, to be unrestricted by oxygen demand.

Most insects are small and have a high surface:volume ratio, so radiation and convection rapidly dissipate heat produced by the flight muscles. Very small flying insects thus have flight muscles that operate even at cool temperatures.

Many endothermic insects that operate in cool climates have a covering of insulating scales (moths) or hairs (bumble bees). Modification of tracheal trunks into air sacs can also function as insulators between the thorax and abdomen to retard the loss of heat from the thorax.

PROTECTION FROM COLD

Insects must adapt to the temperature regimes of their location and habitat. They may become immobilized at temperatures above freezing and, if kept in this state, will die from desiccation because they are unable to restore lost water by drinking. Insects living in temperate or colder regions, such as the Arctic or high mountains, risk freezing when air temperatures drop below 0 °C. Yet some insects do survive subzero weather, and others live normally after being frozen. Freezing is not the only cold temperature stress for insects, either. Tropical insects can often be easily killed by brief exposures to temperatures of 4 °C.

Some insects avoid low air temperatures by virtue of their habitat or behavior. Aquatic insects and those in deep, moist soil escape because freezing usually occurs only at the water or soil surface. In

winter, honey bees cluster together in a mass in the combs of their hive. Even when air temperatures outside are well below 0 °C, they maintain 20 to 30 °C inside the cluster. Heat is derived from the metabolism of stored honey. Winged adult insects that migrate, such as the monarch butterfly, travel south in autumn to warmer regions and return northward in spring.

Immature stages, however, are often on the soil surface, on plants, or on other objects and must endure exposure to cold weather. Diapause is a physiological state that is associated with overwintering (see Chapter 4). In the next section, we will describe other processes that are usually combined with diapause to ensure survival even in the Arctic.

Susceptibility and Tolerance to Freezing

Freezing injury to insects is most obvious when it results from ice formation. Ice crystals may disrupt cell membranes and alter the concentrations of intracellular fluids by initially concentrating water into the growing ice crystals. These shifts in water concentration in turn set up osmotic imbalances.

The supercooling point (temperature of crystallization) of a liquid is the temperature at which it turns to ice. Although 0 °C is ordinarily considered the freezing point of water, insect fluids and cell contents may not freeze down to -20 °C or lower because of **cryoprotectants** that reduce the supercooling temperature. Glucose, trehalose, low-molecular-weight lipids, and sorbitol in the hemolymph act as cryoprotectants. No other natural chemical, however, equals glycerol to prevent freezing. Glycerol has been found in most overwintering, freezing-tolerant insects, especially larvae and pupae. Glycerol extends the temperature range of supercooling without freezing to retard the rate of freezing and to reduce the size of crystals. Both actions reduce freezing injury to tissues. When the insect is indeed frozen, glycerol presumably reduces the deleterious osmotic effects and prevents the intracellular freezing that is fatal.

A second strategy that insects use to avoid freezing injury is to avoid having ice nucleation centers (nucleators) that accelerate ice formation. Mineral particles, and especially bacteria, act as catalysts

for ice formation. The presence of nucleators in the gut or hemolymph of an insect raises the supercooling point. On the other hand, the addition of dissolved substances in water lowers the points of freezing and supercooling. Even without special antifreeze substances, the body fluids of overwintering insects can often be supercooled to -20°C . Thus, many insects survive subzero weather by evacuating their guts in autumn and supercooling without harm, to temperatures above the supercooling point. When the supercooling point of the insect is reached, an ice crystal will form internally around a nucleator.

Studies of overwintering adults of the Arctic ground beetle *Pterostichus brevicornis* (Carabidae, Coleoptera) provide an example of the ecology, behavior, and physiology of an insect in a cold environment. The beetle is a scavenger that lives in forest litter in circumpolar regions. Near Fairbanks, Alaska, summer is spent in the upper 2 to 3 cm of surface litter that is warmed to 10 to 14°C by air and intermittent sunshine. Only a few centimeters below the surface, the temperature of litter drops to 0 to 1°C because of the underlying permafrost. After the first frost in autumn and independent of

photoperiod, the adult beetles migrate by the thousands into the old galleries of wood-boring beetles in decaying tree stumps. The beetles continue to move toward the interior of the stump, favoring regions of -1 to 2°C , until immobilized and finally frozen by the approaching winter.

In winter, the stumps may be exposed to -40°C for several weeks and may reach -60°C for shorter periods. Temperatures in the interior of the stumps lag behind the outside temperature. Stumps also are often covered by an insulating blanket of snow. Thus, the hibernating beetles are buffered against rapid temperature changes. When beetles are kept at 5°C , no glycerol is detectable in the hemolymph. Without glycerol, the freezing point of hemolymph is -2°C and the supercooling point is -7.3°C . Synthesis of glycerol is stimulated by exposure to 0°C for more than 24 hours. Glycerol then continues to accumulate at temperatures below freezing (Fig. 5.13). A sudden drop in temperature to -15°C or more is fatal. Beetles inside stumps, however, are protected against such rapid changes. If given time to synthesize glycerol, the beetles become freezing-tolerant.

Other adult insects are generally not known to be freezing-tolerant. In this respect, the carabid is

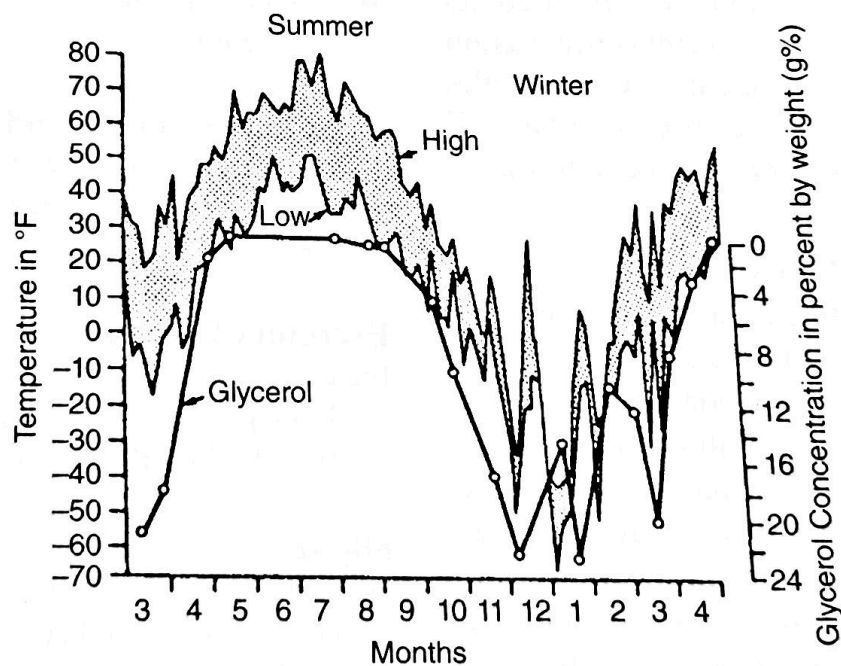


Figure 5.13 Seasonal variations in the average glycerol concentration in hemolymph of the Arctic carabid *Pterostichus brevicornis*, and high-low ambient temperature near Fairbanks, Alaska. Note that the glycerol scale is inverted.

Source: Daly authors (data from Baust and Miller, 1970).

unique, but the annual cycle of glycerol production and loss resembles that known in less detail for overwintering larvae and pupae of other insects. It is also interesting to note that the carabid is able to digest food, develop eggs, and exhibit other signs of physiological activity while in a cold-hardened and frozen state. Although only the adults have thus far been analyzed, the beetles also overwinter as larvae or pupae. Some individuals overwinter twice and live up to 36 months.

Beetles start hibernation with a water content at about 62% of body weight, abundant fat, and the guts filled with the summer diet of small insects and other arthropods. During the first few months of hibernation, digestion and excretion continue until the gut is emptied. Body weight declines as the gut is cleared, fat is consumed, and water content drops about 10%. Then, in January and February, whenever temperatures exceed -3 or -4 °C, the beetles begin to crawl about and feed. The diet is now rotten wood containing bacteria and fungi. The ovaries become active, and eggs start to develop. Fat droplets in fat cells fluctuate in abundance as a result of the renewed intake of food (increasing fat) and the growth of eggs (decreasing fat). The water content of the beetle's body begins to rise as water in the stump thaws and becomes available. In the spring, the glycerol concentration declines steadily. After one day at 21 °C, the beetles have completely lost their tolerance to freezing; all die if returned to below-freezing temperatures.

Cold Hardiness and Shock Proteins

A change in tolerance to cold as a result of prior exposures to low temperature is called cold hardening. This process may take months to complete in some species and only minutes in others. The season or generation of the insect may determine how quickly and to what extent it may be conditioned to withstand cold temperatures. This adaptation appears to be widespread and important for survival in insects of temperate to arctic climates. Molecular methods have revealed proteins associated with cold hardening. Not only cold but, interestingly, heat and other stresses, such as poisons and desiccation, can induce the expression of

shock proteins, often called "heat shock proteins." Studies to date, mostly in bacteria and vertebrates, indicate that these proteins (chaperonins) act to preserve protein assembly and folding to prevent their denaturation by temperature changes. This should be a fertile field for further investigations.

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1. 在 $\triangle ABC$ 中, $\angle A = 60^\circ$, $\angle B = 45^\circ$, $AB = 10$, 求 AC 的长.

解: 在 $\triangle ABC$ 中, $\angle A = 60^\circ$, $\angle B = 45^\circ$, $AB = 10$.
由正弦定理得: $\frac{AC}{\sin B} = \frac{AB}{\sin C}$
因为 $\angle C = 180^\circ - \angle A - \angle B = 75^\circ$,
所以 $\frac{AC}{\sin 45^\circ} = \frac{10}{\sin 75^\circ}$
所以 $AC = \frac{10 \sin 45^\circ}{\sin 75^\circ}$

2. 在 $\triangle ABC$ 中, $\angle A = 120^\circ$, $\angle B = 30^\circ$, $BC = 10$, 求 AB 的长.

解: 在 $\triangle ABC$ 中, $\angle A = 120^\circ$, $\angle B = 30^\circ$, $BC = 10$.
由正弦定理得: $\frac{AB}{\sin C} = \frac{BC}{\sin A}$
因为 $\angle C = 180^\circ - \angle A - \angle B = 30^\circ$,
所以 $\frac{AB}{\sin 30^\circ} = \frac{10}{\sin 120^\circ}$
所以 $AB = \frac{10 \sin 30^\circ}{\sin 120^\circ}$