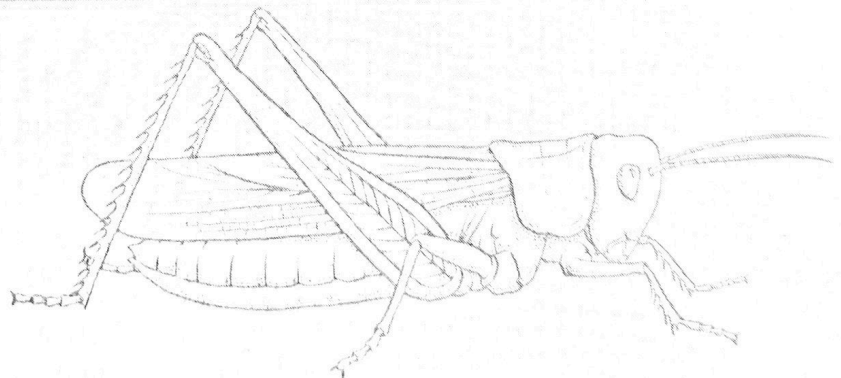


REVISED AND UPDATED BY JON F. HARRISON
AND LUTZ T. WASSERTHAL

INTRODUCTION

Gaseous exchange in insects occurs through a system of air-filled internal tubes, the tracheal system, the finer branches of which extend to all parts of the body and may become functionally intracellular in muscle fibers. Thus oxygen is carried in the gas phase directly to its sites of utilization. While the blood is not concerned with oxygen transport in most insects, some insects have now been shown to have hemocyanin, an oxygen-carrying pigment, in the blood. In terrestrial insects and some aquatic species, the tracheae open to the outside through segmental pores, the spiracles, which generally have some filter structures and a closing mechanism reducing water loss from the respiratory surfaces. Other aquatic species have no functional spiracles, and gaseous exchange with the water involves arrays of tracheae close beneath the surface of thin, permeable cuticle.

This chapter is divided into ten sections. Section 17.1 describes the tracheal system, its structure, distribution and development. Section 17.2 deals with the number, structure and distribution of the spiracles. Section 17.3 follows with cutaneous gas exchange; Section 17.4 treats respiratory pigments; and Section 17.5 describes gaseous exchange in terrestrial insects, considering diffusion and ventilation in resting and flying insects and control of ventilation. Section 17.6 addresses the gaseous exchange in aquatic insects, with oxygen uptake from the air and by gills. Section 17.7 gives attention to insects subject to occasional submersion. Section 17.8 refers to the gas exchange in endoparasitic insects. Section 17.9 is concerned with other functions of the tracheal system, and Section 17.10 with gas exchange in insect eggs.



17.1 Tracheal system

17.1.1 Tracheae

The tracheae are the larger tubes of the tracheal system, running inward from the spiracles and usually breaking up into finer branches, the smallest of which are about $2\text{ }\mu\text{m}$ in diameter. Larger tracheae are multicellular structures, while the smallest are formed by single cells. Tracheae are formed by invaginations of the ectoderm and so are lined by a cuticular intima that is continuous with the rest of the cuticle. The intima consists of outer epicuticle with a protein/chitin layer beneath it. A spiral thickening of the intima runs along each tube, called a taenidium (Fig. 17.1). In the taenidia the protein/chitin cuticle is sclerotized. The chitin microfibrils in

the taenidia run around the trachea, while between the taenidia they are parallel with the long axis of the trachea. A layer of resilin may be present beneath the epicuticle.

Some tracheae seem designed to remain expanded and to serve as conducting pipes, while others collapse easily, providing ventilation. The taenidia prevent collapse of the trachea if pressure within the tube is below air or hemocoel pressure (Section 5.2.1; Fig. 5.5). In the wing tracheae of some insects, the taenidia are themselves twisted or supercoiled, giving some elasticity to the wall of the trachea. The structural differences that account for collapsibility of some tracheae remain poorly known. Because the tracheal walls are relatively thick, it is thought that little oxygen passes through the walls; thus the tracheae serve primarily to transport oxygen from

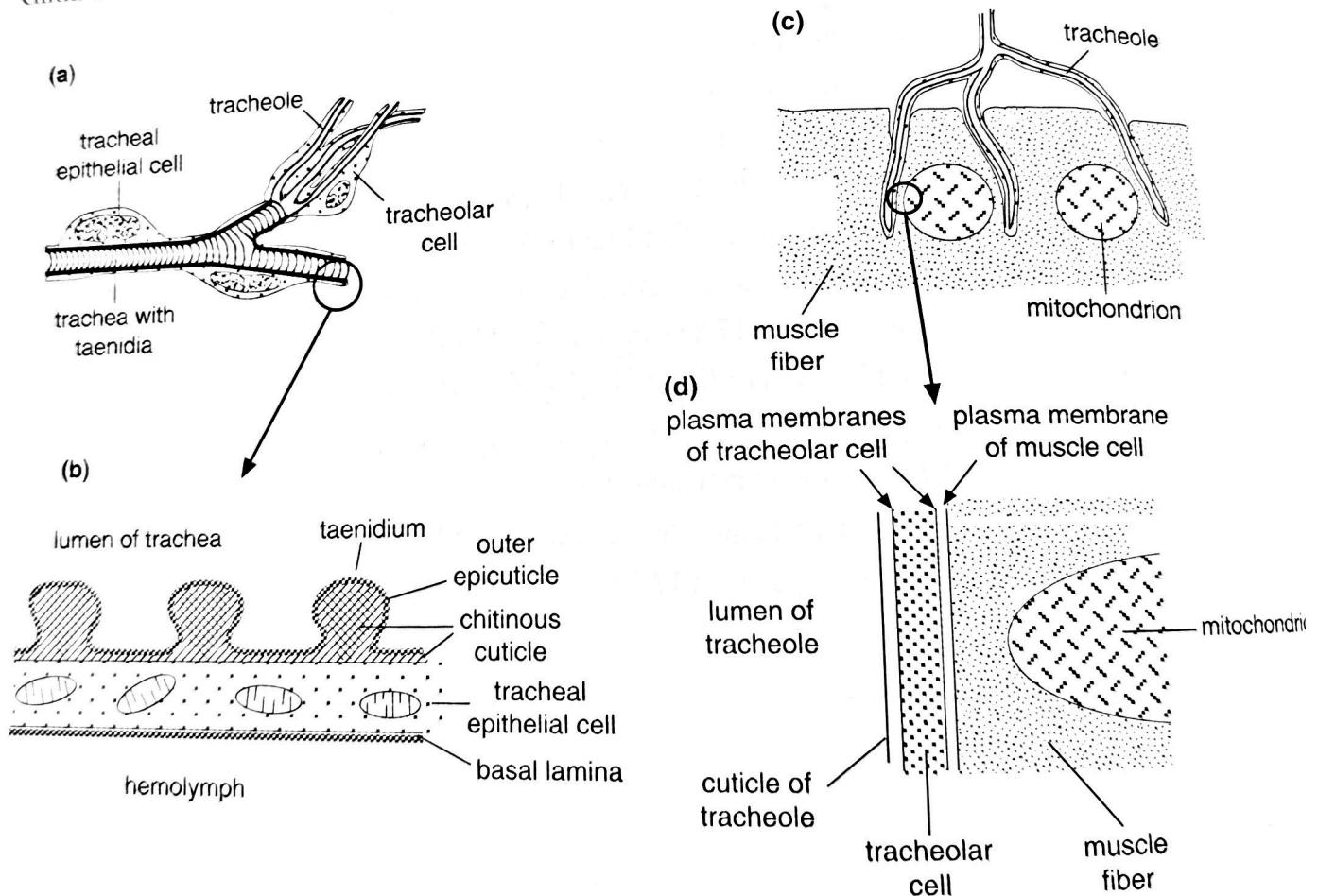


Figure 17.1 Tracheal system. (a) Diagram showing a trachea and tracheoles. (b) Longitudinal section of the trachea: tracheal lumen above, hemolymph below. (c) Diagram showing tracheoles indenting a muscle fiber. (d) Enlargement of the area circled in (c). Oxygen diffuses from the lumen of the tracheole (left) through the cuticle and the tracheolar cell to the mitochondrion in the muscle fiber.

the spiracles to tracheoles. However, because carbon dioxide is much more soluble in tissue, it is likely that a significant fraction of carbon dioxide transported does cross the tracheae. In some insects, the tracheae have an intimate contact with the ovaries by the cuticle between the taenidia, which bulges toward the oocyte. Wigglesworth called these "aeriferous tracheae" and suggested that they functionally replace tracheoles as the primary supply to the ovaries.

In some insects, the tracheae are expanded to form thin-walled air sacs (see Section 17.1.4) in which the taenidia are absent or poorly developed and often irregularly arranged. The air sacs collapse easily under pressure and serve as bellows for ventilation of the tracheal system; they also allow for tissue growth and increases in hemolymph volume. The distribution of air sacs is quite variable among insect species and with age. First-instar locusts virtually lack air sacs, while adults have many air sacs throughout the body. Similarly, caterpillars such as *Manduca sexta* lack air sacs while adult moths have many. *Drosophila* have air sacs in the head and thorax, but not in the abdomen. Many cockroaches and tenebrionid beetles apparently lack air sacs entirely.

17.1.2 Tracheoles

At various points along their length, especially distally, the tracheae give rise to finer tubes, the tracheoles. Tracheoles are blind-ended, air-filled

extensions of terminal tracheal cells, and are the primary site of gas exchange. They are usually less than $2\text{ }\mu\text{m}$ in diameter, tapering to $0.3\text{ }\mu\text{m}$ and lack the longitudinal seam found on larger tracheae. Some tracheoles retain their cuticular lining at molting, which is not usually true of tracheae. They are formed in "tracheolar cells," which are derived from the epidermal cells lining the tracheae (Fig. 17.2). The intima of tracheoles is some 16–20 nm thick and may consist only of outer epicuticle. It is thrown into taenidial ridges, but, unlike the taenidia of tracheae, these ridges are not filled with chitin/protein matrix. The thin walls and high surface-to-volume ratio of the tracheoles enable their high diffusing capacity. Most tracheoles are extracellular, and in flight muscles they generally run parallel to the muscle fibers. In some flight muscles they penetrate deeply into the muscle via invaginations of the plasma membrane (Fig. 17.1c,d).

17.1.3 Tracheal development

The development of the tracheal system in *Drosophila* has been a focus of intense interest by developmental biologists, and these studies have revealed many fundamental new insights. In flies, the tracheal system arises from sacs of 80 embryonic cells at each segment. Remarkably, the entire larval tracheal system develops from growth and branching of these 80 cells. At metamorphosis, some adult tracheae are formed from imaginal discs, while others

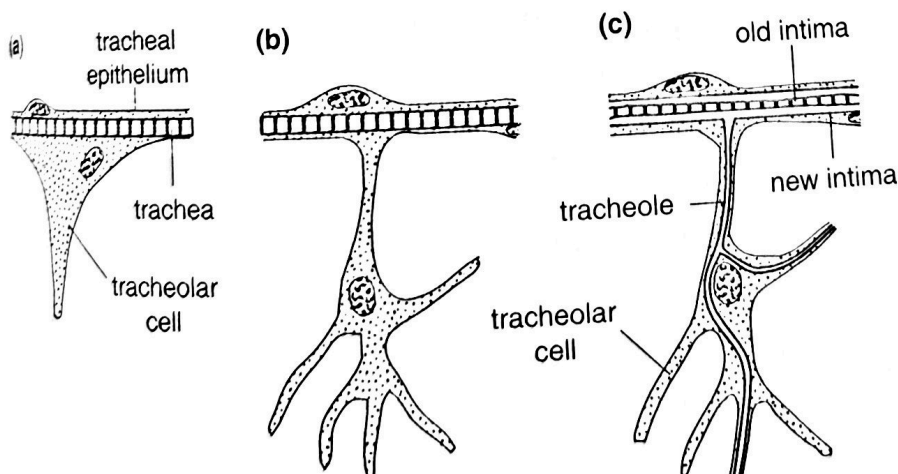
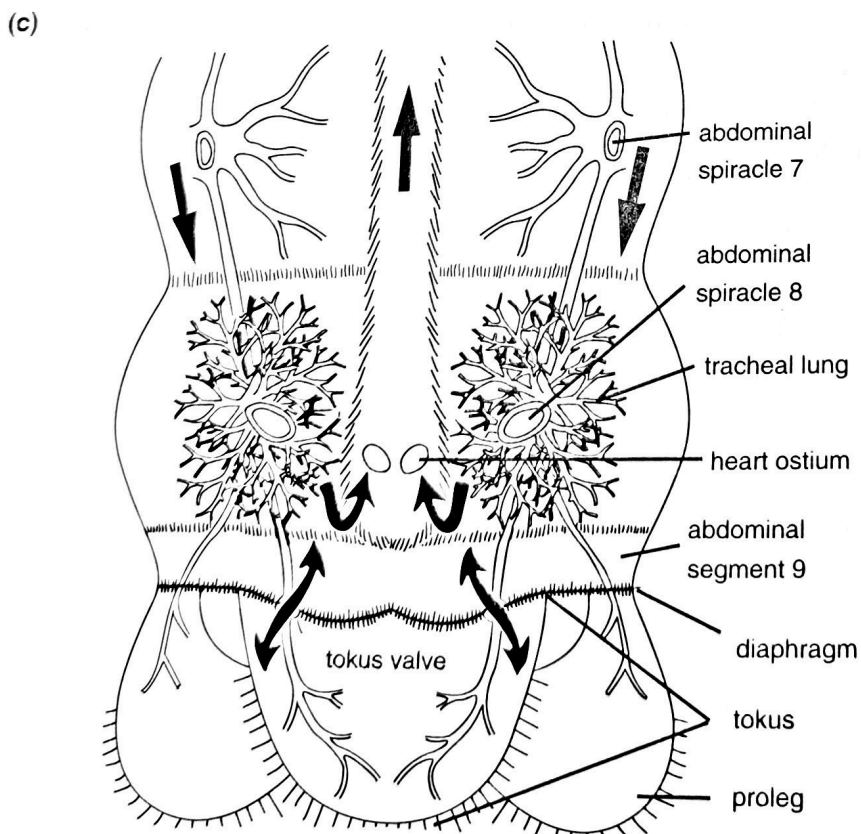
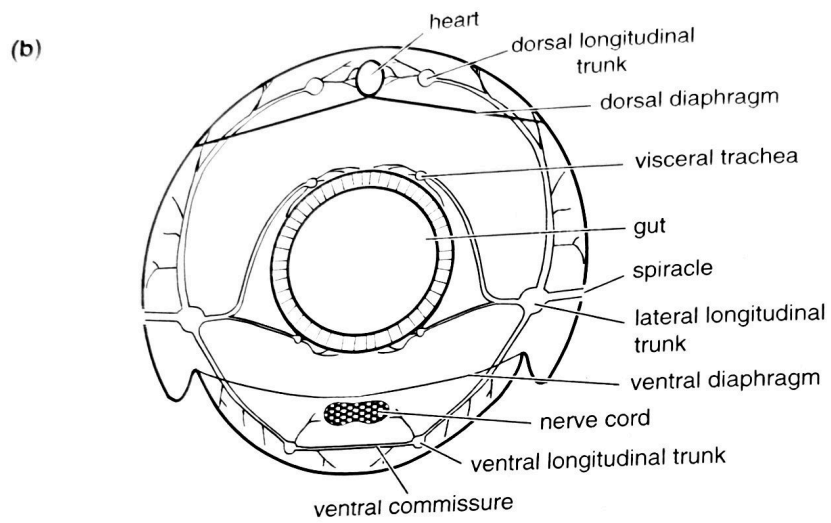


Figure 17.2 Development of a tracheole (after Keister, 1948). (a) Tracheolar cell developing from the tracheal epithelium. (b) Tracheolar cell with extensive cytoplasmic processes. (c) Tracheole within tracheolar cell and becoming connected to existing trachea at a molt.



from larval tracheal cells that re-enter the cell cycle and regain developmental potency. Each cluster of embryonic cells undergoes development and branching within that segment, leading to lateral symmetry. There are four major morphological steps in tracheal development: sac formation, and then primary, secondary and tertiary branching. The first tube is formed by invagination of the embryonic cell cluster, which retains an opening connected to the embryonic surface that will form the spiracle. Primary branches form at bud sites, with multiple cells migrating out that branch and form tubes. Secondary branches are formed by single cells that form a tube as they extend. Terminal branching, forming the tracheoles, occurs as cytoplasmic extensions of the secondary tube. These terminal branches grow toward target tissues, and then form internal air-filled tubes that connect with the air-filled lumen of the secondary branch (Fig. 17.2).

Our knowledge of the genes and developmental program of the *Drosophila* tracheae is improving. Specific genes are associated with each of the three morphological steps. In addition, branching is controlled by feedback between target tissues which secrete fibroblast growth factor (FGF, encoded by the gene *branchless*), which binds to a fibroblast growth factor receptor (FGFR, encoded by the gene *breathless*). Local hypoxia is sensed by hypoxia inducible factor (HIF) within the tracheoles, which respond by increasing the number of FGFR. Enhanced activation of this signaling pathway leads to increased branching and growth of the terminal tubes. This oxygen regulation of terminal cell branching likely is responsible for the matching of tracheole supply to the metabolic needs of the tissues.

Formation of the tracheal lumen occurs under the control of specific regulatory genes such as *ribbon* and *synaptobrevin*. Both the lumen of the tracheae and its length change during development, and these two processes are under different types of physiological and genetic regulation. Lumen expansion occurs primarily by changes in the apical (luminal) membrane.

As noted above, different branches and regions of the tracheal system have very different properties. How are these generated? Specific developmental genes are activated early in embryonic development, which label cells according to location. For example, the gene *Dpp* labels cells as dorsal or ventral; *Spitz/EGF* and *Wingless/Wnt* label cells as central or peripheral; and *Hedgehog* determines anterior/posterior differences. These location signals induce expression of regional and branch-specific genes that help determine the characteristics of particular tracheae. Substrate interactions with other local cells are also important in determining the direction of migration and morphology of tracheae.

Creation of a continuous tracheal system from the segmental systems of the embryo requires fusion of multiple tracheae across segments. This is accomplished by fusion cells, developmentally marked by the gene *escargot*, at the ends of primary branches in each segment. These fusion cells contact and fuse with each other, and form a continuous lumen.

17.1.4 Distribution of the tracheae within the insect

In those Collembola that have tracheae and in the Archaeognatha, the tracheae from each spiracle form a tuft which remains separate from the tufts of other

Caption for Figure 17.3 Tracheal system (from Snodgrass, 1935). (a) Tracheation of the thorax and first abdominal segment of a caterpillar, dorsal view. (b) Diagrammatic cross-section of the abdomen of an orthopteran showing the principal tracheae and tracheal trunks. (c) Tracheal lung in the posterior larval abdomen of the skipper *Calpodex*. The tracheae of the eighth segment finely branch and terminate freely in the hemolymph near the posterior heart ostia and in the tokus, a separate compartment (after Locke, 1998).

spiracles. In the majority of insects, however, the tracheae from neighboring spiracles join to form longitudinal trunks running the length of the body (Fig. 17.3). Usually there is a lateral trunk on either side of the body connecting the spiracles. In addition, there are often longitudinal trunks along the gut, and on either side of the heart and nerve cord. Tracheae branch from the longitudinal trunks to the nearby tissues. Transverse commissures often connect both sides at each segment.

The arrangement of tracheae tends to follow a similar pattern in each body segment, especially in larval forms and in the abdomen of adults. This basic arrangement is modified, however, in the head, where there are no spiracles, in the abdomen beyond the most caudal spiracle and in the thorax. In grasshoppers, the head is supplied with air from spiracle 1 (often on the prothorax, see Section 17.2.2) through two main tracheal branches on each side, a dorsal branch to the antennae, eyes and brain and a ventral branch to the mouthparts and their muscles (Fig. 17.4).

In many caterpillars the tracheae arising from the eighth abdominal spiracles differ from the tracheae of the other segments. They are finer, more numerous and have an especially thin-walled intima with a reticulate basal lamina. Through the transparent integument of the skipper butterfly, *Calpododes ethlius*, hemocytes have been observed to adhere transiently to these tufts on their passage through the posterior hemocoel into the posterior heart ostia (Fig. 17.3c). The fine tracheal ramifications probably serve as lungs for aerating the hemocytes, cells without permanent supply by tracheoles. In adults the tracheal system of the eighth segment is lost. However, the thin walls of many air sacs offer similar conditions for the gas exchange with adhering hemocytes.

The thorax of many flying insects contains multiple air sacs (Fig. 17.4). In insects such as locusts, these are compressed by changes in thoracic volume caused by contractions of the flight muscles, producing ventilation. Transport of oxygen to the head entails special problems as the head contains many highly metabolic tissues (brain, mandibular

muscles), yet the head lacks spiracles. Many insects have large, collapsible, longitudinal tracheae that extend from the thorax to the head; these are regularly ventilated. Locusts have two large tracheae from the first prothoracic spiracle that are likely sites of inspiration of air to the brain (Fig. 17.4).

The abundance of tracheoles seems to match the metabolic demands of the tissues, though there is little quantitative data. Tracheoles are most abundant in flight muscles. However, they are also particularly abundant in the central nervous system, especially in the neuropile, perhaps suggesting that synapses have a high demand for oxygen. In the ganglia of the locust central nervous system, every point in the neuropile is within 10 μm of a tracheole, but no tracheal branching occurs in the outer parts of the ganglion where the cell bodies are situated (Fig. 17.5).

Each flight muscle has a primary supply consisting of a large tracheal trunk or air sac running alongside or through the muscle (Fig. 17.6). If a trachea forms the primary supply it widens to an air sac beyond the muscle. From the primary supply, small, regularly spaced tracheae arise at right angles, running into the muscle. These form the secondary supply and they are often oval proximally, permitting some degree of collapse, and taper regularly to the distal end. Finer branches pass in turn from these tracheae into the muscles. In Odonata the terminal tracheolar branches run alongside and between the muscle fibers, but in close-packed and fibrillar flight muscle they sometimes indent the fiber membrane and the finest tracheolar branches can be closely associated with the mitochondria (Fig. 17.1c,d). Thus they are functionally within the muscle fiber, although anatomically they are still extracellular.

Major changes in tracheation occur at a molt and during the pupal period in endopterygote insects. This may involve changes in the functional spiracles as well as in tracheation. For example, the first-stage larva of *Sciara* (Diptera) has only a pair of posterior abdominal spiracles, the second-stage larvae has only a pair of thoracic spiracles and the fourth-stage larvae has eight pairs of spiracles.

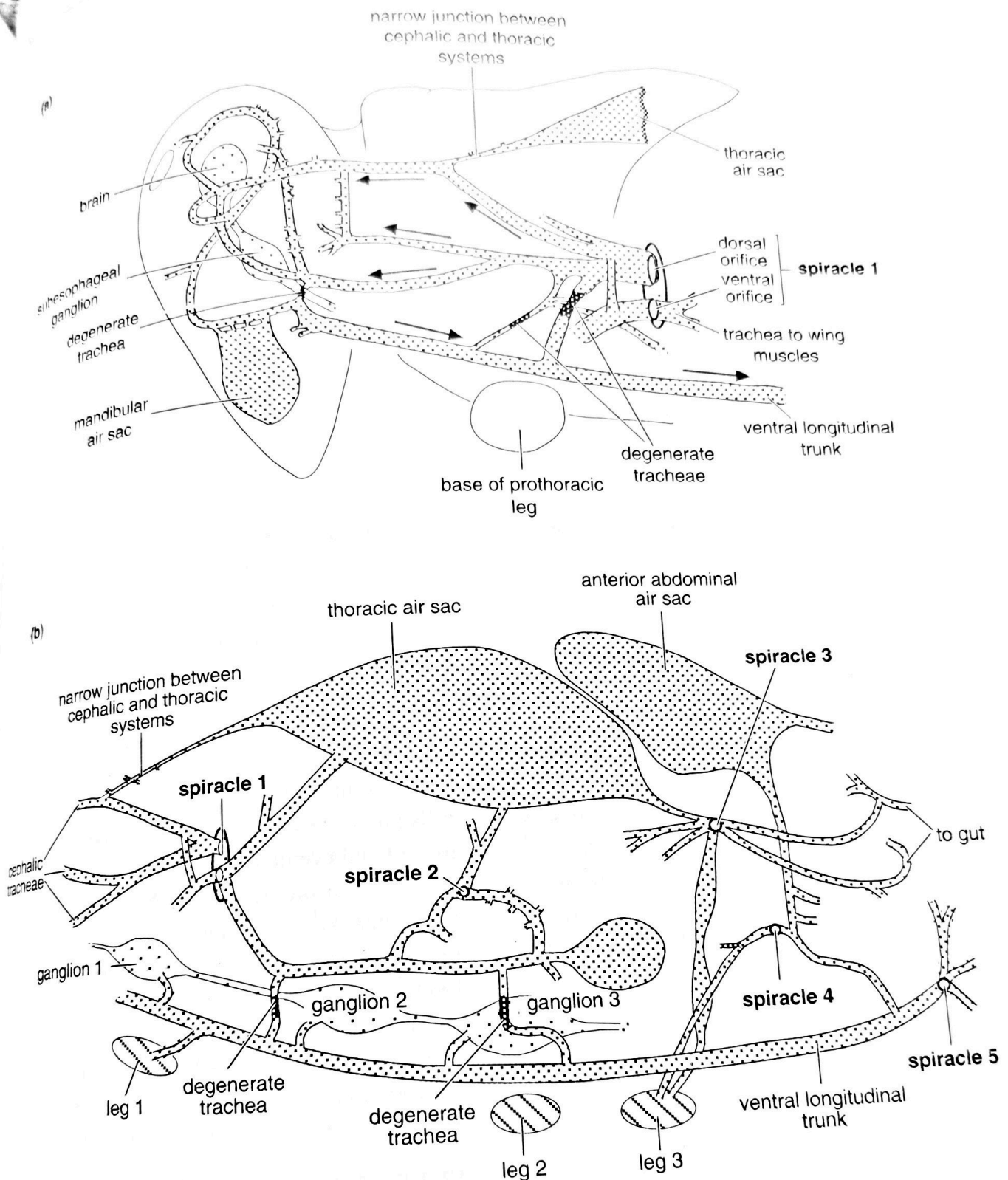


Figure 17.4 Tracheal system of a locust (*Schistocerca*) (after Miller, 1960b). (a) Supply to the head from spiracle 1; arrows indicate the probable direction of airflow resulting from abdominal ventilatory movements. (b) Supply to the pterothorax; notice that the thoracic air sac is largely isolated from other parts of the tracheal system by narrow or degenerate tracheae.

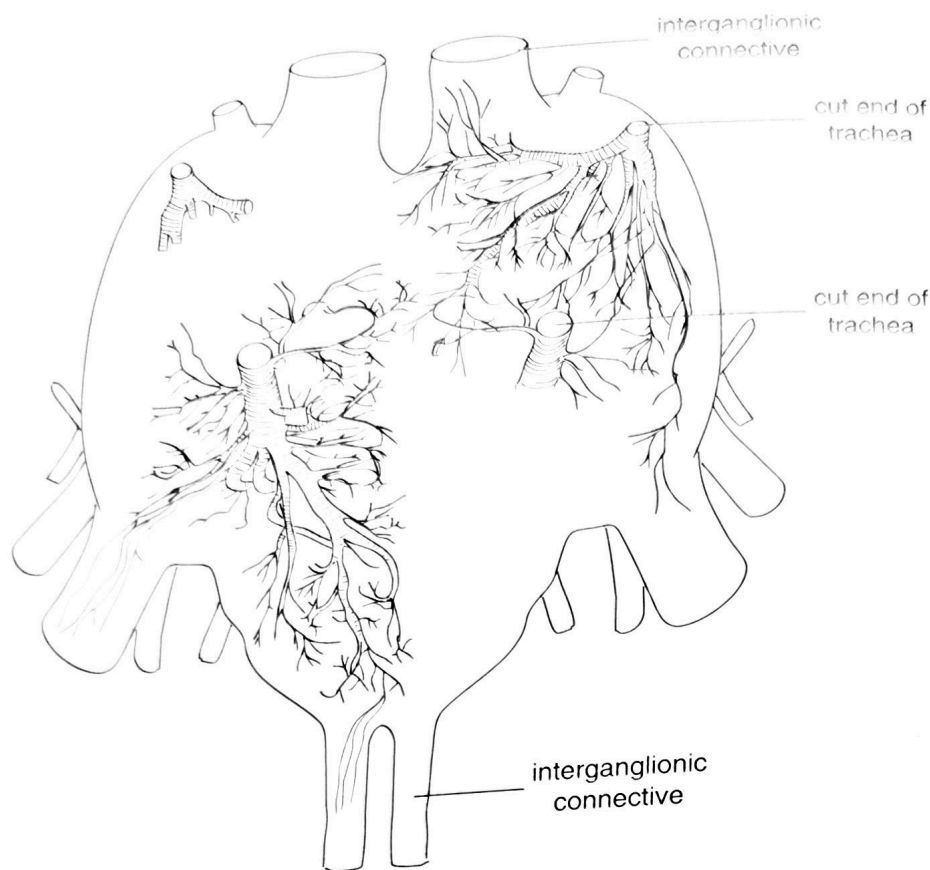


Figure 17.5 Tracheal supply to the metathoracic ganglion of the central nervous system of a locust. Two pairs of tracheae, arising from the ventral longitudinal tracheae, enter the ganglion on the ventral side. Notice that branching is largely restricted to the central region of the ganglion. The peripheral region, where the cell bodies of the neurons are present, does not have extensive branching (after Burrows, 1980).

Between molts, the relative tracheal volume decreases strongly because the tissues continue to grow without compensatory increases in the volume of the major tracheae. In *Schistocerca*, the air sacs also become compressed by growing tissues, decreasing absolute tracheal volume later in the instar. As a consequence, tissues become relatively less well supplied with oxygen with insect growth within each larval stage. Consistent with this hypothesis, late in the instar, metabolism and behavior of *Schistocerca* and *Manduca* are suppressed by very mild hypoxia, which is not the case early in the instar. Possibly sensing of tissue hypoxia is one of the triggers for initiation of the hormonal cascade that controls molting; *Manduca* and *Drosophila* molt at smaller sizes in hypoxia, and metabolic rates do not increase after the critical weight for molting, suggesting oxygen limitation (Fig. 17.7).

Changes in the distribution and number of tracheoles can occur between molts. For instance, in the event of damage to the epidermis, the epidermal cells produce cytoplasmic threads, which extend toward and eventually attach themselves to the nearest tracheole. These cytoplasmic threads, which may be 150 μm long, are contractile and drag the tracheole to the region of oxygen-deficient tissue (Fig. 17.8). During embryonic development and within an instar, hypoxia stimulates and hyperoxia suppresses tracheolar branching and proliferation.

17.1.5 Molting the tracheal system

Prior to the molt, the epithelial layer of the trachea increases in size, with increases in cell size, and in some insects, cell division, leading to an increase in tracheal diameter. A new cuticle is formed and

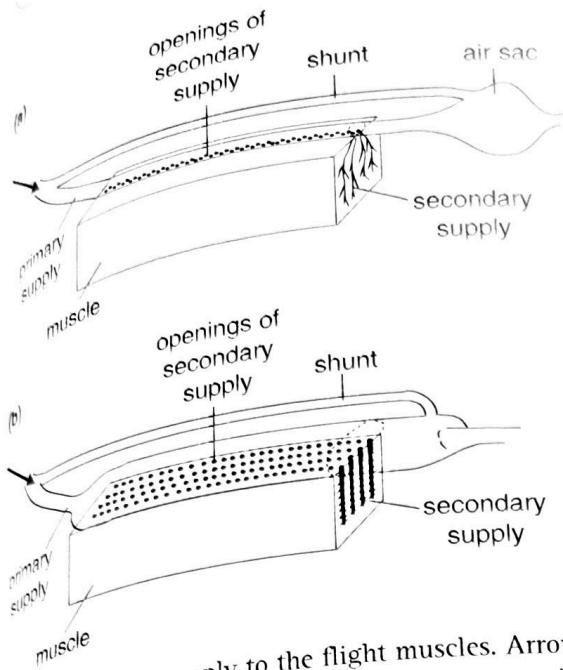


Figure 17.6 Tracheal supply to the flight muscles. Arrows indicate the inward flow of air (after Weis-Fogh, 1964). (a) Primary supply is a trachea ventilated by an air sac outside the muscle. (b) Primary supply is an air sac directly associated with the muscle.

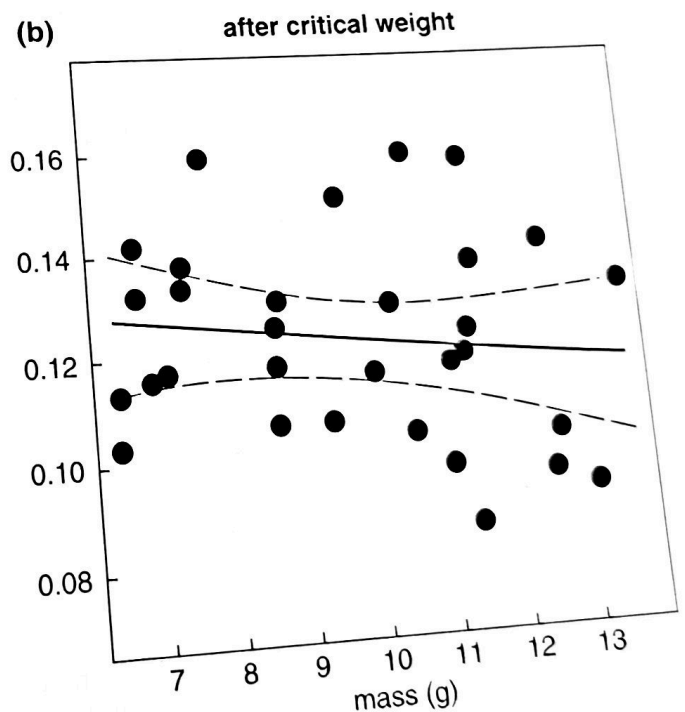
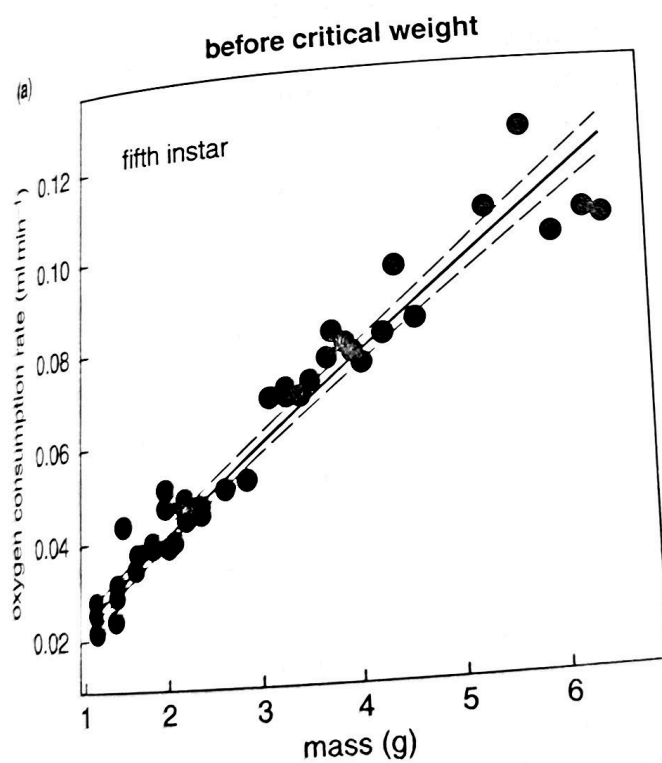


Figure 17.7 (a) Oxygen consumption rate increases linearly with mass before the critical weight, but (b) plateaus after the critical weight in *Manduca sexta* (after Callier and Nijhout, 2011).

example, in Elateridae and Scarabacidae among the beetles and in some Diptera.

The cuticular lining of the tracheoles appears to be shed in some insects and some stages, but not in others. In *Sciara* (Diptera) the intima is shed at larva-larva

molts, but not at the larva-pupa or pupa-adult molts, whereas in *Rhodnius* (Hemiptera) the linings of the tracheoles are never shed, and in grasshoppers even very small tracheoles shed their linings.

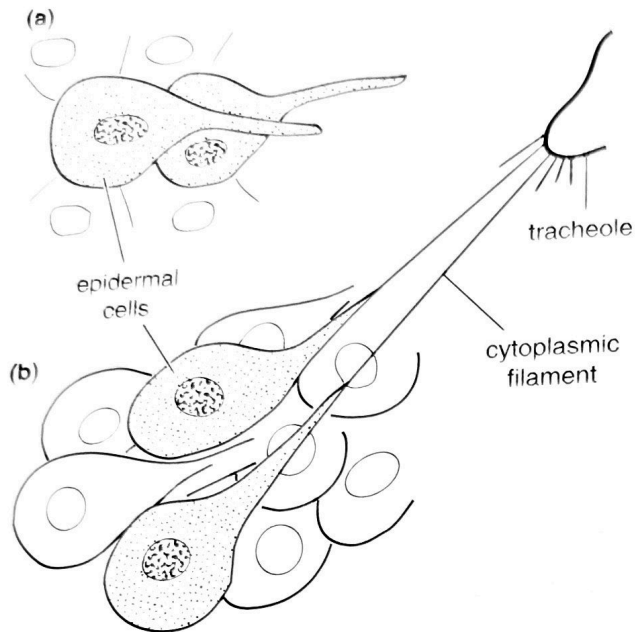


Figure 17.8 Change in the position of a tracheole (after Wigglesworth, 1959). (a) Two epidermal cells send out processes in the direction of a tracheole. (b) Cytoplasmic filaments from the epidermal cells attach to a tracheole and draw it toward the cells.

Pneumatization The tracheal system is liquid-filled in a newly hatched insect, and liquid fills the space between old and new cuticles before each ecdysis. The liquid is replaced by gas in a process known as pneumatization. In terrestrial insects, gas may enter via the spiracles, but in many aquatic species without access to air. Gas usually appears first in a main tracheal trunk and then spreads rapidly through the system, which becomes completely gas-filled in 10–30 minutes. Recent research on this process in insect eggs has supported two mechanisms by which it may occur. First, there is evidence for an important role for cavitation. Evaporation from the eggs creates a negative pressure, which draws air out of solution within the tracheae, creating a bubble inside the egg. Formation of the bubble coincides with the tanning of the tracheal cuticle, which apparently makes the inside of the tracheae hydrophobic. Second, there is evidence for active transport of fluid out of the tracheae, as liquid is progressively removed from smaller tracheae. It is not

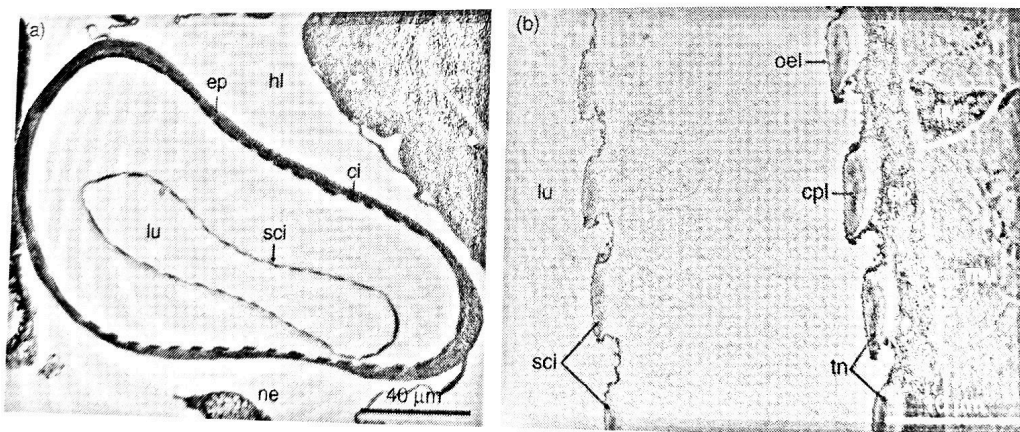


Figure 17.9 Images of molting tracheae in a grasshopper, *Locusta migratoria*. (a) Light micrograph of large leg trachea showing third-instar shed intima (sci) and its lumen (lu) within the larger fourth-instar trachea (ci = cuticular intima, ep = epithelial layer). (b) Electron micrograph of molting tracheae showing shed intima (sci) and the lumen of the third-instar tracheae (lu), next to the larger fourth-instar tracheal epithelia (tn = taenidia, cpl = cuticular protein layer, oel = epithelial layer, ep = epithelia, nu = nucleus, mu = muscle) (after Snelling *et al.*, 2011).

clear yet whether cavitation-type mechanisms can also function when tracheae are pneumatized after a molt. In many insects, some liquid normally remains in the endings of the tracheoles. During periods of high energy consumption the liquid is withdrawn from the system and air is drawn further into the tracheoles. Possibly, fluid reabsorption is due to increases in the osmotic pressure of cells when metabolic rate is high.

17.2 Spiracles

The spiracles are the external openings of the tracheal system, found on the thorax or abdomen (never the head). They are lateral in position, and in the Insecta there is never more than one pair of spiracles on a segment, usually on the pleuron. Often, each spiracle is contained in a small, distinct sclerite, the peritreme.

17.2.1 Number and distribution

The largest number of spiracles found in insects is ten pairs, two thoracic and eight abdominal; this occurs in dragonflies, grasshoppers, cockroaches, fleas and some hymenopteran and dipteran larvae. Some endopterygote larvae have only nine pairs of spiracles, with only one thoracic pair. Mycetophilid (Diptera) larvae have eight pairs of spiracles; one mesothoracic and eight abdominal. Many larval Diptera, especially *Cyclorhapha*, have only two pairs of spiracles, one mesothoracic and one posterior abdominal. Mosquito larvae have only one pair of spiracles; in some species these occur on the thorax and in others on the posterior abdominal segment. Many aquatic larval insects are apneustic, lacking functional spiracles.

Among other hexapod groups, some Diplura, such as *Japyx*, have 11 pairs of spiracles, including four pairs on the thorax, while the sminthurids (*Collembola*) have only a single pair of spiracles, between the head and prothorax, from which tracheae extend, without anastomoses, to all parts of the body. Most *Collembola* have no tracheae at all.

17.2.2 Structure

In its simplest form, found in some Apterygota, the spiracle is a direct opening from the outside into a trachea, but generally the visible opening leads into a cavity, the atrium, from which the tracheae arise. In this case the opening and the atrium are known collectively as the spiracle. Often the walls of the atrium are lined with hairs or filter lamellae (Fig. 17.10). In some Diptera and Coleoptera the spiracle is covered by a sieve plate containing large numbers of small pores. Hairs and sieve plates serve to prevent the entry of dust, microorganisms or, especially in aquatic insects, water into the tracheal system.

The spiracles of most terrestrial insects have a closing mechanism, which is important in the control of gas exchange and internal pressures. The closing mechanism may consist of one or two movable valves in the spiracular opening, or it may be internal, closing off the atrium from the trachea by means of a constriction. In most insects, closure results from the activity of one muscle; opening is produced by the elasticity of the cuticle associated with the spiracle, an elastic ligament or a second muscle. Even where there is an opener muscle, its activity is often associated with an elastic component so that, in the absence of any muscular activity, the spiracle opens.

The metathoracic spiracle of grasshoppers is an example of a "one-muscle" spiracle (Fig. 17.11). It lies in the membrane between the meso- and

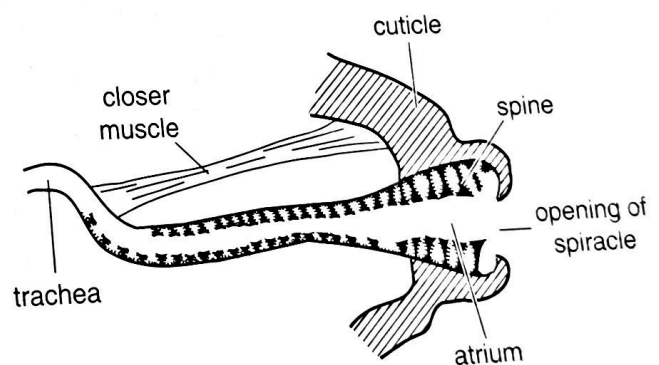


Figure 17.10 Section through a spiracle of a louse, *Haematopinus*, showing the atrium and branched spines in which dust accumulates (after Webb, 1948).

metathorax and has two movable semicircular valves, which are unsclerotized except for the hinge and a basal pad into which a muscle is inserted. This muscle, by pulling down on the valves, causes them to rotate and so to close. The spiracle normally opens because of the elasticity of the surrounding cuticle, but in flight it opens wider as a result of the slight separation of the mesepimeron and metepisternum. These two sclerites, which surround the spiracle, are normally held together by an elastic bridge, but, when the basalar and subalar muscles contract, the sclerites are pulled apart. This movement is transmitted to the spiracle largely through a ligament connecting the metepisternum to the anterior valve, making the spiracle open wide.

The mesothoracic spiracle of grasshoppers is a "two-muscle" spiracle (Fig. 17.12a). It is unusual in having two tracheae leading directly from the external opening. The dorsal opening is connected

with the head and prothorax and the ventral orifice is connected with the flight muscles and legs. A sclerotized rod runs along the free edge of the posterior valve, passing between the orifices and running around the ventral one. The closer muscle arises on a cuticular inflexion beneath the spiracle and is inserted into a process of the sclerotized rod, while the opener muscle, also from the cuticular inflexion, is inserted onto the posterior margin of the posterior valve. When the insect is at rest and the closer muscle relaxes, the spiracle opens to some 20–30% of its maximum as a result of the elasticity of the cuticle; the opener muscle plays no part. Contraction of the opener muscle occurs during slow, deep ventilatory movements and results in the spiracle opening fully.

Closure of abdominal spiracles usually involves a constriction method (Figs. 17.12b, 17.13). Commonly, the atrium is pinched between two sclerotized rods, or

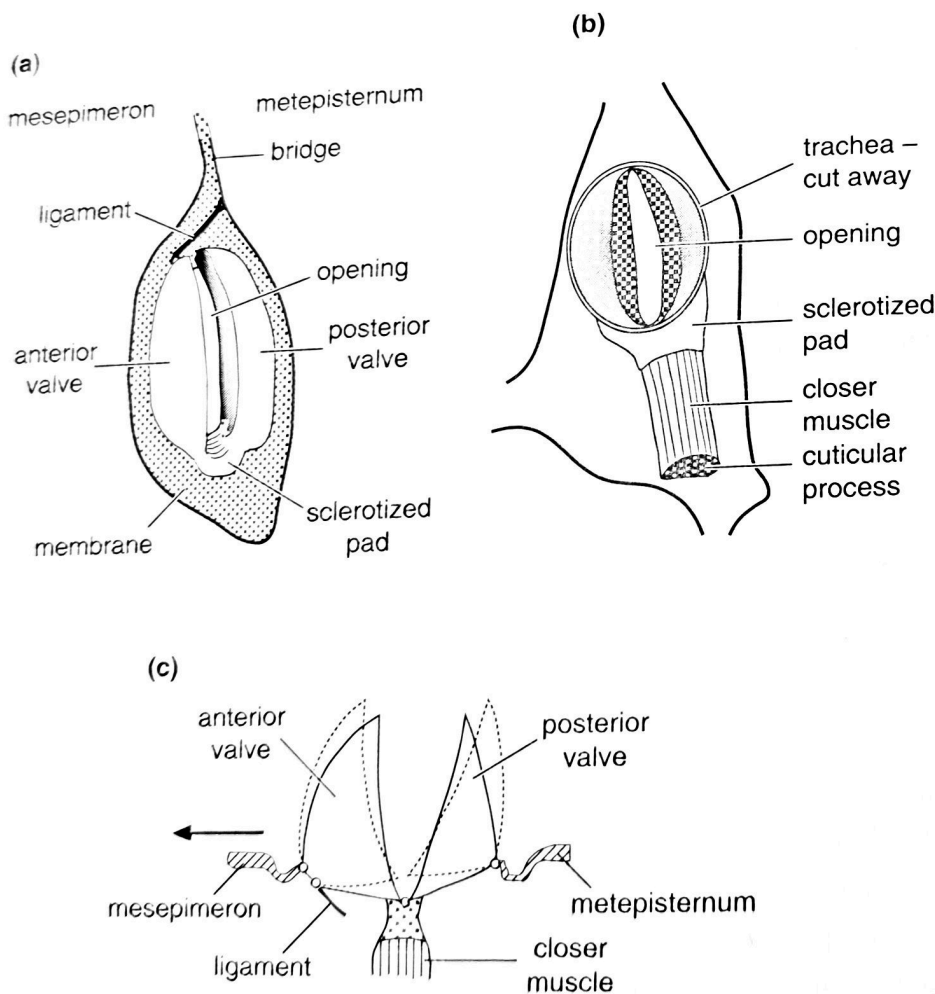


Figure 17.11 A one-muscle spiracle; second thoracic spiracle of a locust (*Schistocerca*) (after Miller, 1960a). (a) External view; (b) internal view; (c) diagrammatic section through the spiracle showing how movement of mesepimeron (arrow) causes the valves to open wide (dotted).

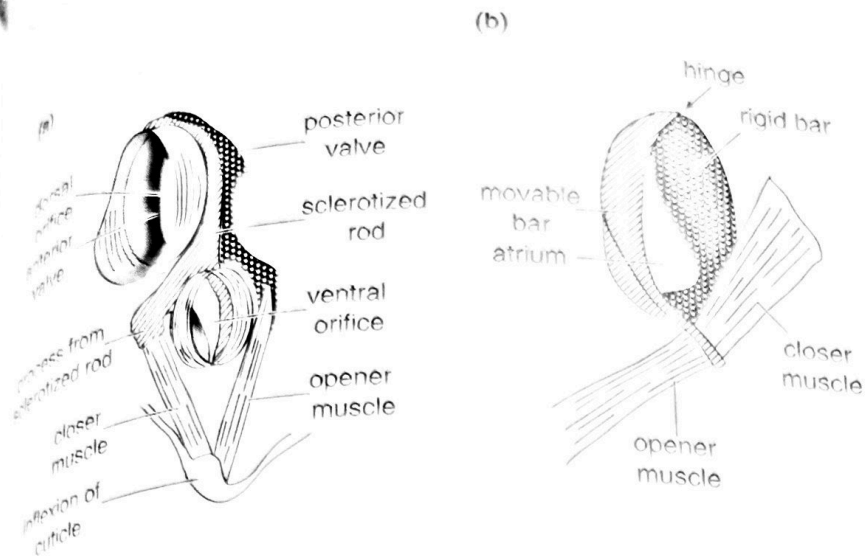


Figure 17.12 Two-muscle spiracles of a grasshopper. (a) First thoracic spiracle seen from inside (based on Snodgrass, 1935; Miller, 1960a). (b) Abdominal spiracle (after Snodgrass, 1935).

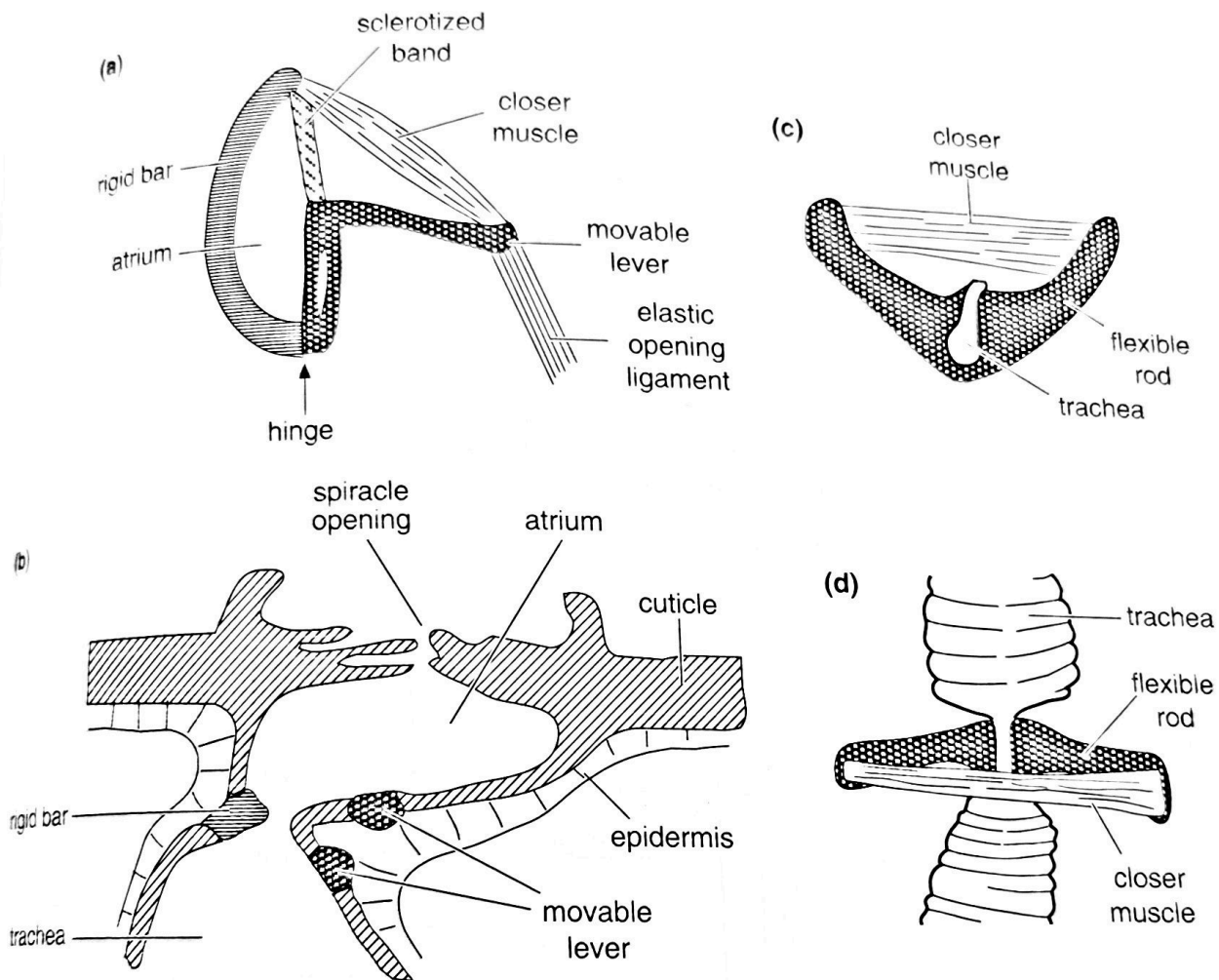


Figure 17.13 Closing mechanisms internal to the spiracle. (a), (b) Abdominal spiracle of a caterpillar seen from inside (a) and in horizontal section (b) (from Imms, 1957). (c), (d) Constricting mechanism on a trachea of a flea seen in horizontal section (c) and from above (d) (after Wigglesworth, 1965).

in the bend of one rod, as the result of the contraction of a muscle. In other instances, the atrium or trachea is bent so that the lumen is occluded.

17.2.3 Control of spiracle opening

In the insects that have been studied (primarily locusts, cockroaches and dragonflies), centers in the thoracic or first abdominal ganglia generate a respiratory rhythm that controls ventilation and coupled openings of the spiracles. These central

neural centers often override similar respiratory rhythms in the segmental ganglia. Motor neurons in the spiracle muscles in each segment arise in the ganglion of the same segment or that immediately in front. The closer muscles in dragonflies (Odonata), *Periplaneta* (Blattodea) and *Schistocerca* (Orthoptera) are innervated by two motor neurons whose axons pass along the median nerve and then bifurcate, sending a branch to either side, so that the two spiracles receive the same pattern of motor impulses (Fig. 17.14). Closer muscles may also be innervated

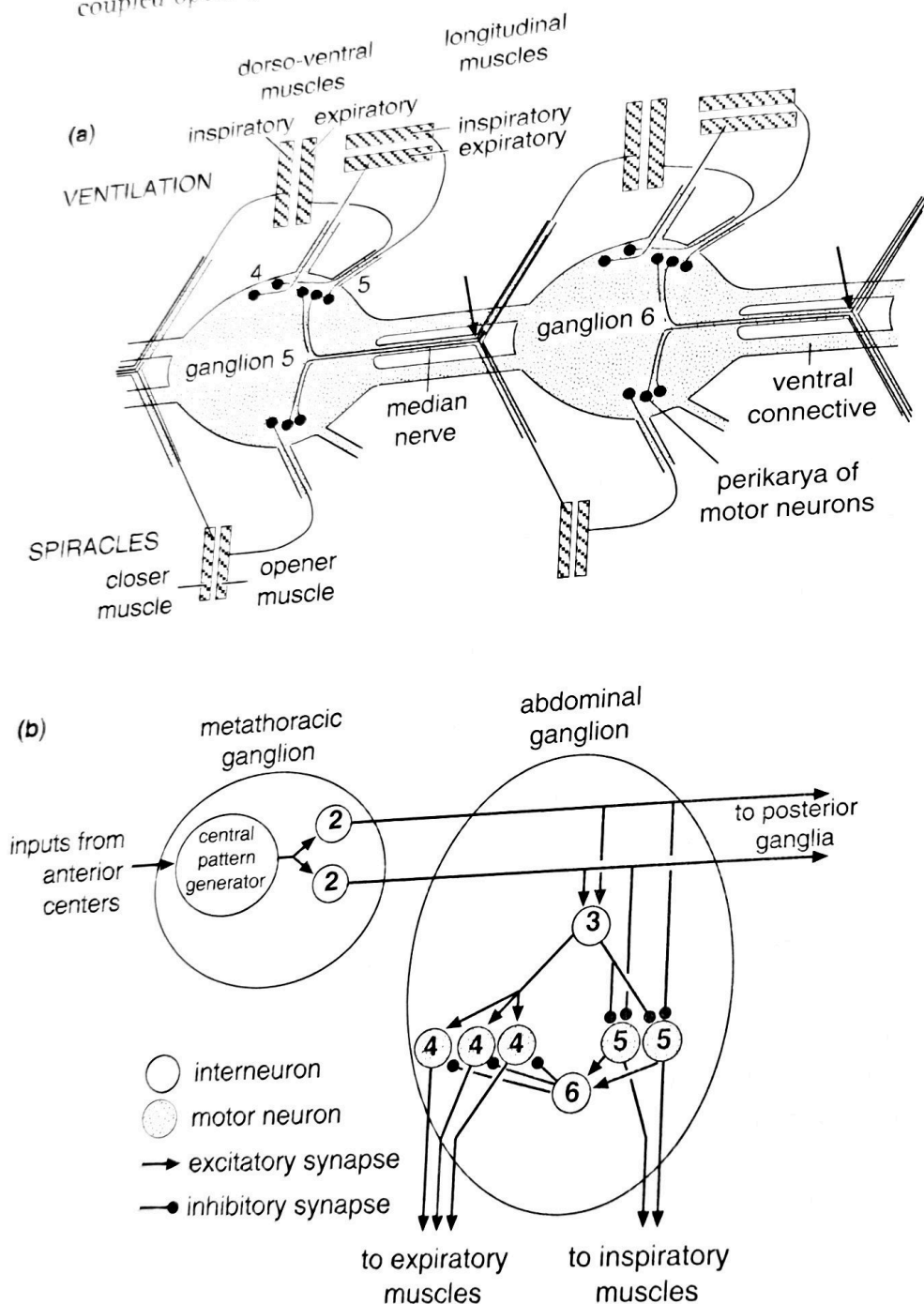


Figure 17.14 Control of spiracles and ventilatory movements in a locust (*Schistocerca*). (a) Innervation of ventilatory muscles (top) and spiracular muscles (bottom). The cell bodies of motor neurons controlling the muscles of one segment are not confined to the ganglion of that segment; some are in the ganglion of the next anterior segment. The axons in the median nerve divide (arrow) so that homologous muscles on the two sides are innervated by the same neurons. Notice that the ventilatory muscles and spiracular muscles are not controlled by the same neurons. Numbers adjacent to cell bodies in ganglion 5 relate to the cells shown in (b) (based on Lewis *et al.*, 1973). (b) Diagram showing the control of ventilatory muscles. The interneurons (2) are driven by a central pattern generator and extend to all the abdominal ganglia. Motor neurons are numbered to correspond with the same numbers in (a). See text for further explanation (based on Lewis *et al.*, 1973; Pearson, 1980).

by an inhibitory axon. The opener muscle, when it is present, is innervated from one or two neurons whose cell bodies are usually lateral in the ganglion and the spiracles of the two sides are independently innervated. In *Blaberus* and related cockroaches, a common inhibitory neuron innervates a number of abdominal spiracle opener muscles.

The closer muscle is caused to contract by the activity of its motor neurons, but the frequency of action potentials, determining the degree of contraction, may be altered by various factors acting on the central nervous system. Of particular importance are a high level of carbon dioxide and a low level of oxygen (hypoxia) in the tissues. Both of these conditions tend to arise while the spiracles are closed as carbon dioxide is produced and oxygen is utilized in respiration. Both conditions lead to a reduction in action potential frequency and so to spiracle opening. In two-muscle spiracles the action potential frequency to the opener muscle is increased by high carbon dioxide levels and hypoxia. Increases in the respiratory rhythm and spiracular opening can also be achieved by feed-forward regulation from the centers that control flight motor behavior.

Carbon dioxide acts directly on the closer muscle of one-muscle spiracles; with direct effects on muscle plus interference with neuromuscular transmission, the junction potential falls, muscle tension is reduced and the spiracle opens. The threshold of the peripheral response to carbon dioxide is set by the frequency of motor impulses from the central nervous system.

17.3 Cutaneous gas exchange

Some gaseous exchange takes place through the cuticle of most insects, but does not usually amount to more than a small percentage of the total gaseous movement. On the other hand, Protura and most Collembola have no tracheal system and must depend on cutaneous respiration together with transport by the hemolymph from the body surface to the tissues.

Cutaneous respiration is also important in many endoparasitic and aquatic insects, where it is coupled with an apneustic (no spiracles) tracheal system. Cutaneous respiration without an associated apneustic tracheal system can only suffice for very small insects with a high surface-volume ratio.

17.4 Respiratory pigments

Until recently it was thought that only a few aquatic insects specialized for extremely hypoxic environments had respiratory pigments. Larval midges of the genus *Chironomus*, and endoparasitic bot fly larvae in the genus *Gastrophilus* have hemoglobins that enable them to extract oxygen from extremely hypoxic media. Now it is clear that most insects have intracellular hemoglobin, and a wide variety of "lower" insects have hemocyanin in their blood. Our understanding of the function of most of these respiratory pigments remains limited.

Most insects that have been studied possess intracellular hemoglobin, primarily expressed in tracheal cells and fat body. Molecular analyses indicate that these proteins do bind oxygen, although their function remains controversial. In *Drosophila*, hypoxia exposure decreases expression of these hemoglobin genes, while hyperoxia increases expression, suggesting that these hemoglobins may function in protection against oxidative stress rather than as oxygen storage proteins.

Larvae of the genus *Chironomus* (Diptera) are the only insects yet known to have hemoglobin in the blood. *Chironomus* larvae live in burrows in the mud under stagnant water, which is commonly poor in oxygen. The hemoglobin of *Chironomus* has two heme groups, and is 50% saturated with oxygen at less than 0.1 kPa, compared to more than 3 kPa in vertebrates. A flow of water is directed through the burrow by dorso-ventral undulating movements of the body, and the current so produced provides food and oxygen. During such bouts of irrigation, the hemoglobin in the blood becomes fully saturated

with oxygen, and delivers oxygen to the tissues. During the intervals between ventilatory bouts, the oxygen of the surrounding medium is quickly used up, and hemoglobin releases oxygen; with an oxygen store able to last about nine minutes.

The third-stage larva of *Gastrophilus* is an internal parasite in the stomach of the horse. Larval *Gastrophilus* larvae contain intracellular hemoglobin concentrated in large hemoglobin cells. As in *Chironomus*, the hemoglobin has a very high affinity for oxygen. Four pairs of tracheal trunks run from the posterior spiracles and give off short branches at intervals along their lengths. Each branch breaks up into numerous tracheoles, which are functionally, if not structurally, within a hemoglobin cell. Within the horse's stomach, the larva receives only an intermittent supply of air in gas bubbles with the food, and the hemoglobin of the hemoglobin cells enables the larva to take up more oxygen than is needed for its immediate requirements. This oxygen is used later when air bubbles are no longer available, providing a store of up to four minutes.

Diving beetles of the genus *Anisops* also have intracellular hemoglobin that aids in oxygen storage. When *Anisops* dives, it carries with it a small ventral air store, which is continuous with air under the wings. All the spiracles open into the air store, and the spiracles of abdominal segments 5–7 are very large and covered by sieve plates. From the atria of these spiracles several tracheae arise, branching repeatedly to form "trees," the terminal branches of which indent large cells, called hemoglobin (or tracheal) cells, which are filled with hemoglobin. The hemoglobin becomes oxygenated when the bug is at the surface and deoxygenated during a dive. The oxygen released enables the insect to remain submerged for longer than would otherwise be possible, while at the same time affecting its buoyancy. When the insect first dives, it is buoyant because of its ventral air store, but as the store is used up this buoyancy is reduced until the density of the insect is roughly the same as water and it is able to float in mid-water. This phase is maintained by the

steady release of oxygen from the hemoglobin cells, resulting from the reduction in partial pressure of oxygen in the store. After about five minutes, however, the hemoglobin is fully unloaded and the insect, now with a tendency to sink, swims up to the surface and renews its air store.

Hemocyanins are copper-containing respiratory pigments that occur in the hemolymph of many invertebrates. Among insects, hemocyanins have been identified in Collembola, Archaeognatha, Dermaptera, Orthoptera, Phasmatodea, Mantodea, Isoptera and Blattaria. The functional significance of this hemocyanin has been best studied in the stonefly, *Perla marginata*. Hemocyanin accounts for about 25% of all hemolymph proteins in nymphal and adult stoneflies. In *P. marginata*, the hemocyanin is 50% saturated with oxygen at about 1 kPa, suggesting it can be useful in both oxygen transport and storage. Phylogenetic analyses confirm that the insect hemocyanins are related to crustacean hemocyanins.

17.5 Gaseous exchange in terrestrial insects

Oxygen passes through the tracheal system from the spiracles to the tissues and ultimately must reach the mitochondria in order to play a part in oxidative processes. There are thus two distinct phases in the transport of gases, one through the tracheal system, known as air-tube transport, and one through the tissues in solution in the cytoplasm, known as tissue diffusion.

17.5.1 Diffusion

The rate of diffusion of a gas depends on a number of factors. It is inversely proportional to the square root of the molecular weight of the gas, so that in air, oxygen, with a molecular weight of 32, diffuses 1.2 times faster than carbon dioxide, with a molecular weight of 44. The rate of steady-state diffusion (J , with units such as moles s^{-1}) of a gas depends on the area available (A), the distance over which

diffusion occurs (L), the diffusion constant for the molecule of interest in the particular media (D) and the concentration gradient for that molecule (ΔC):

$$A \propto D \times \Delta C / L$$

The diffusion constant (D , $\text{m}^2 \text{s}^{-1}$) varies with temperature (faster at higher temperature), molecular size (faster for smaller molecules) and differs dramatically between air and water (D is about 10,000 times larger in air than in water). The average distance traveled by a diffusing molecule increases with the square root of time; thus in tissues, diffusion works well over small distances (micrometers) but is generally inadequate at distances greater than a millimeter. Most tracheoles are within $50 \mu\text{m}$ of mitochondria (less than $20 \mu\text{m}$ in flight muscle), so diffusion works well. Many respiratory physiologists express gradients for oxygen and carbon dioxide in partial pressures, in this case the Krogh's constant, K (with units such as $\text{moles s}^{-1} \text{kPa}^{-1} \text{m}^{-1}$) is used instead of D . K is calculated by multiplying D by the solubility of the gas in the media; because solubility of gases decreases with temperature, K for tissues or water falls as temperature rises, unlike D . If the molecule of interest changes chemically in the media (e.g., oxygen binding to hemocyanin, or carbon dioxide forming bicarbonate), then K should be calculated by multiplying D by the capacitance of the media, which includes both physically dissolved and chemically bound molecules.

In some cases, $A \times D/L$ are combined into a single term called the diffusive conductance (G_{diff}), allowing an overall estimate of the diffusing capacity of the system:

$$J = G_{\text{diff}} \times \Delta C (\text{or } \Delta P)$$

The importance of diffusion in insect gas exchange remains a controversial topic. In the 1920s, Krogh calculated the partial pressure gradient for oxygen for a caterpillar by measuring oxygen consumption rate and the diameters and lengths of many of the tracheae. He concluded that diffusion should suffice for oxygen delivery, and for many years it was often stated that diffusion was the major mechanism of gas

exchange in insects. However, we now know that many insects, and perhaps all large insects, utilize convective ventilation of the tracheal system. Nonetheless, the fact that most insects can recover from complete anoxia suggests that diffusion can provide the minimal oxygen necessary to activate motor behavior in insects.

It is often stated that increasing body size necessarily leads to problems with diffusion due to the increasing diffusion distance down longer tracheae. However, as long as the cross-sectional area increases in proportion to tracheal length, steady-state diffusion rates can be maintained. In the abdominal transverse tracheae to the gut of locusts, the diffusive conductance does not increase as much as metabolic rate with age/size, and thus transport of oxygen by diffusion requires an increasing gradient for oxygen. In locusts, convective ventilation becomes increasingly more important than diffusion with age/size; perhaps this is a general trend for insects as size increases.

Due to its greater solubility, the permeability constant of carbon dioxide in the tissues is 36 times greater than that for oxygen, so that despite its higher molecular weight, carbon dioxide travels more quickly than oxygen through the tissues for the same difference in partial pressure. Hence a system capable of bringing an adequate supply of oxygen to the tissues will also suffice to take the carbon dioxide away. As carbon dioxide is more soluble, it is present in much higher concentrations in the blood and tissues than oxygen, generally in the form of bicarbonate. Carbonic anhydrase is required to quickly convert bicarbonate to carbon dioxide; this enzyme is reported to occur in the tissues but not the blood.

17.5.2 Convection

We now know that many (likely most) insects, large and small, ventilate their tracheal system by convective movements of gases. Convection is the bulk flow of fluid, driven by a pressure gradient. Given that diffusion can suffice for gas exchange

many insects, why is convection so common? Theoretical studies have demonstrated that use of convection rather than diffusion can decrease water loss rates, even in small insects. In addition, convection is likely important for allowing cells in different parts of the body to experience similar oxygen and carbon dioxide levels. Ventilatory convection generally occurs by discrete pumping movements. For a completely convective system:

$$V_{O_2} = f \times TV \times (\text{inspired } C_{O_2} - \text{expired } C_{O_2})$$

where V_{O_2} is the rate of oxygen consumption (in units such as moles s^{-1}), f is the ventilatory frequency, TV is tidal volume (volume of air per pumping event) and C_{O_2} is the concentration of oxygen in the inspired and expired air. For some insects (e.g., locusts exposed to hypoxia), it has been demonstrated that gas exchange through the spiracles is virtually completely convective. However, it is likely that for most insects gas exchange through the spiracles is a mixture of convection and diffusion.

Ventilatory convection can be accomplished by compression of the air sacs or tracheae. Some tracheae are circular in cross-section and resist any change in form because of their taenidia; these appear to be suitable to serve as conducting tubes for gas exchange. However, thanks to X-ray synchrotron imaging that allows visualization of the tracheal system of intact, living insects, we now know that many tracheae in a variety of insects compress substantially, apparently producing tidal ventilation. This phenomenon has been best studied in carabid beetles, in which longitudinal and secondary and tertiary tracheae of the head and thorax compress significantly (while abdominal tracheae do not). Combining measurement of such tracheal compression with high-resolution measurement of CO_2 emission has revealed that these tracheal compressions produce significant convection through the spiracles, but do not account for the majority of CO_2 emission (which may be diffusive). Because air sacs can be quite large, their compression and inflation can produce extensive convection.

Insects produce compression of the air sacs and tracheae in multiple ways. The best understood is abdominal pumping. Abdominal compression leads to a reduction of body volume and a rise in hemolymph pressure that causes expiration. Expansion of the air sacs and inspiration result from the reduction of pressure due to the muscular or elastic expansion of the abdomen. Changes in abdominal volume may be produced in various ways. In Heteroptera and Coleoptera, the tergum moves up and down (Fig. 17.15a); in Odonata, Orthoptera, Hymenoptera and Diptera, both tergum and sternum move (Fig. 17.15b), and this movement may be associated with telescoping movements of the abdominal segments (Fig. 17.15c); and in adult Lepidoptera, the movement is complex and involves movements of the pleural regions as well as terga and sterna.

Another mechanism for producing ventilation is by transfer of hemolymph back and forth between thorax and abdomen (Fig. 17.16). For example, in Lepidoptera and flies, septal structures such as connective tissue and fat body or air sacs at the front of the abdomen occlude the hemocoel. When the heart pumps forwards, it draws hemolymph from the abdomen and transfers it to the head and thorax. The effect of this is to cause expansion of air sacs in the abdomen and to compress those in the anterior compartment. When the heart reverses, it pumps blood into the abdomen and the changes in the tracheal system are reversed. During forward beating of the heart, air is sucked in through the abdominal spiracles and forced out through thoracic spiracles; when the heart reverses, the converse is true. The volume changes of the cephalic air sacs could be visualized in *Drosophila* and *Calliphora* using synchrotron X-ray videography (Fig. 17.16d,e). The hemolymph shift produces an alternating change in tracheal pressure and tracheal P_{O_2} . At the transition from backward to forward beating, the abdomen performs a pumping stroke, which exerts a pressure pulse restricted on the abdominal tracheal system at the moment when most hemolymph is accumulated in the abdomen and the hydraulic effect of the

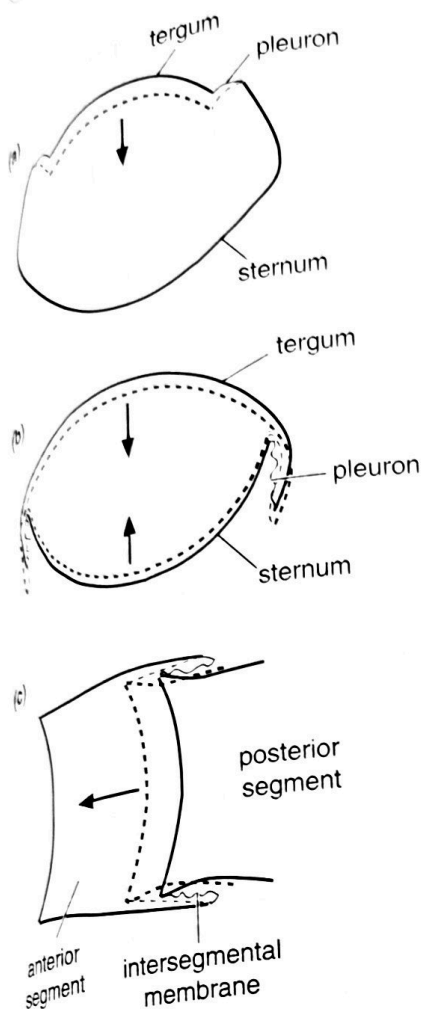


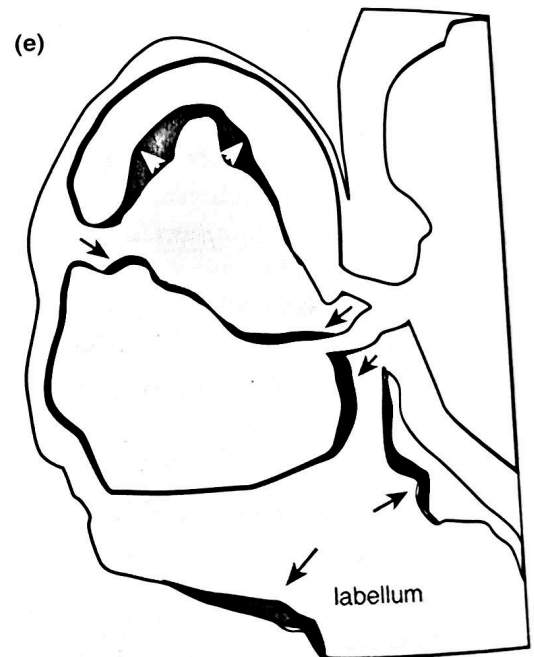
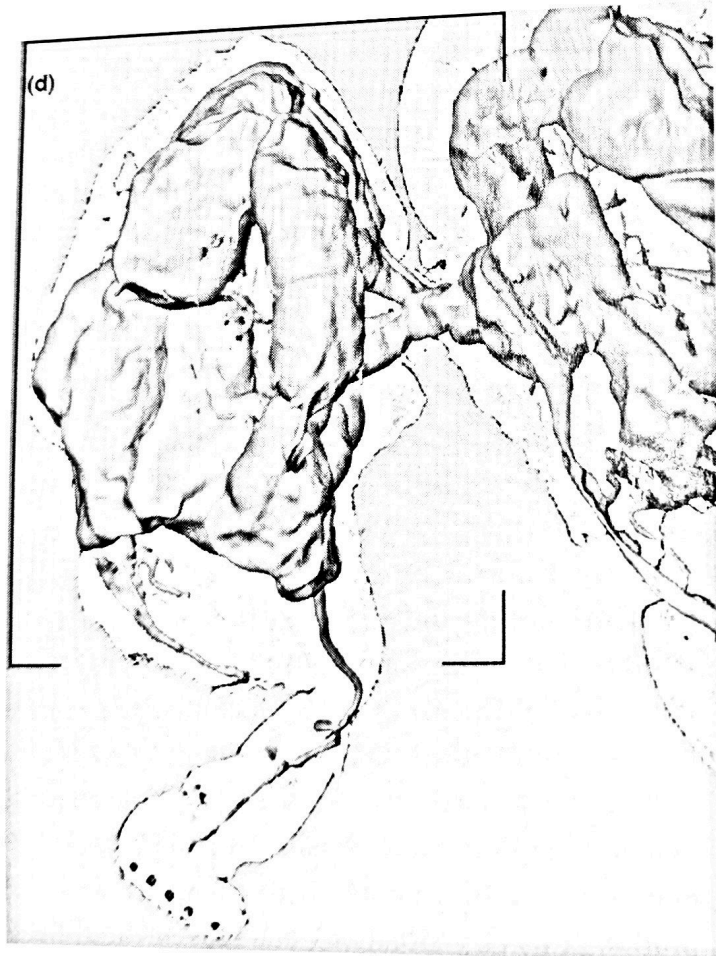
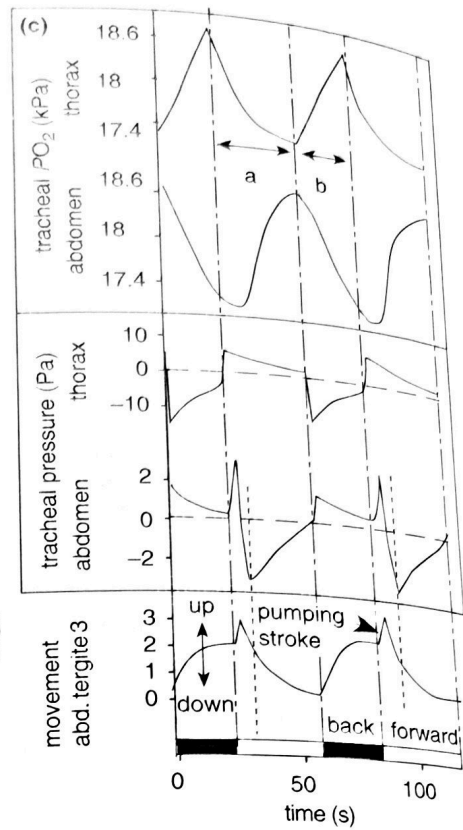
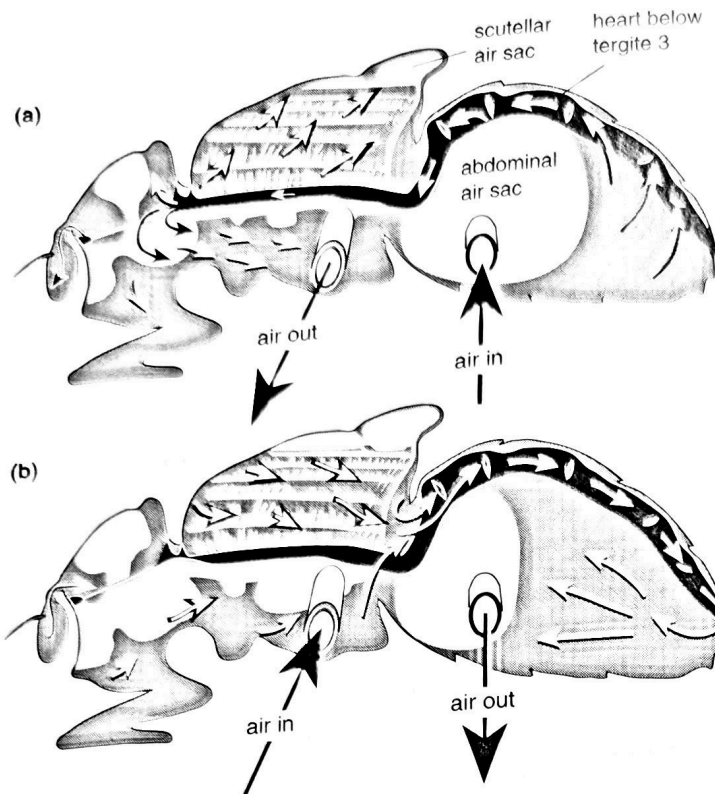
Figure 17.15 Ventilatory movements of the abdomen. Dashed lines represent the contracted position, compressing the abdominal air sacs and causing expiration. Arrows indicate directions of movement in the compression phase (from Snodgrass, 1935). (a) Dorso-ventral compression involving tergum and sternum, transverse section. (b) Dorso-ventral compression involving both tergum and sternum, transverse section. (c) Longitudinal compression by telescoping one segment within another anterior to it, horizontal section.

hemolymph is maximized, compressing air sacs and driving air through spiracles. In a resting blowfly at about 23°C, heartbeat reverses about twice each minute, in *Drosophila* about five times each minute.

Airflow may be tidal, moving in and out of the same spiracles, or unidirectional through the insect. Tidal airflow occurs in carabid beetles, *Periplaneta*

(Blattodea), in the wing tracheae of Lepidoptera and the elytral air sac of scarabaeid Coleoptera. In many insects, however, including other species of cockroach, opening and closing of certain spiracles is synchronized with the ventilatory pumping movements of the abdomen, so that air is sucked in through some spiracles and pumped out through others and a directed flow of air through the tracheal system is produced. This is a more efficient form of ventilation than tidal flow as much of the "dead" air, trapped in the inner parts of the system by tidal movements, is removed. In most insects the flow of air is from front to back and, in *Schistocerca* (Orthoptera), spiracle movements are always coupled with ventilatory movements. In many insects, however, the two activities may become uncoupled so that they are not always synchronized and the coupling may even be modified so as to produce a reversal of the airstream. In *Schistocerca*, spiracles 1, 2 and 4 are open during inspiration, but closed during expiration, when spiracle 10 opens (Fig. 17.17a). When the insect is more active, expiration takes place through spiracles 5–10. The spiracles for inspiration open immediately after the expiratory spiracles have closed and remain open for about 20% of the cycle while air is drawn in. Then, for a short time, all the spiracles are closed. The abdomen starts to contract while the spiracles are still closed, so the air in the tracheae is under pressure; this is known as the compression phase. Then the expiratory spiracles open and air is forced out. The expiratory spiracles are only open for some 5–10% of the cycle. During activity the frequency of ventilation and spiracular movements is increased. The periods for which the spiracles are open remain the same, but the intervals between openings are reduced and the compression phase eliminated (Fig. 17.17b). The net time for which the spiracles are open increases as a result.

A variety of mechanisms are known for producing ventilation of the head. *Schistocerca* sometimes exhibit protraction and retraction of the head on the prothorax (neck ventilation), and movement of the



-  shrunken air sacs during forward beating
-  air sac area replaced by blood
-  hemocoel and tissues

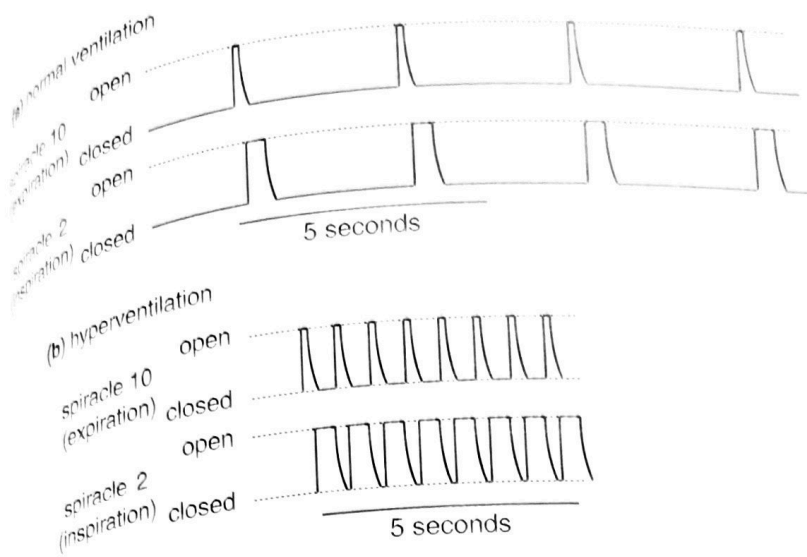


Figure 17.17 Spiracle activity during abdominal ventilation of a locust (after Miller, 1960a). (a) Normal ventilation; (b) hyperventilation.

prothorax on the mesothorax (prothoracic ventilation). *Protophila* ventilate their head and thorax during flight by pumping of the proboscis under certain flight conditions. Other mechanisms for producing ventilation during flight are covered below.

17.5.3 Discontinuous gas exchange

Although ventilation is often continuous, there may be extended periods during which all the spiracles are closed. The movement of oxygen into the tracheae and carbon dioxide emission then occur in discrete bursts when the spiracles open; relatively little gas exchange occurs while they are closed. This

phenomenon is known as discontinuous gas exchange (DGE) or discontinuous ventilation. It is common in adult insects when they are inactive at moderate to cool temperatures, that is, when their metabolic rates are low. The pupae of many insects also exhibit DGE.

The periodicity of ventilatory bouts varies from insect to insect and with environmental conditions. In diapausing pupae of the moth *Hyalophora*, the interval between spiracle openings is as long as eight hours. In some resting grasshoppers at 15°C, carbon dioxide is released about once per hour, but at 30°C the period of spiracle closure is reduced to about five minutes. In the beetle *Psammodes*, each cycle lasts

Caption for Figure 17.16 Alternating gas exchange in anterior body and abdomen caused by reversals of the heartbeat in the blowfly (*Calliphora*). The tracheal system is represented diagrammatically (after Wasserthal, 1996). (a) Forward beating of the heart draws hemolymph from the abdomen and pushes it into the head and thorax. This expands abdominal air sacs and reduces the volume of those in the head and thorax so that air is inspired through abdominal spiracles and expired through thoracic spiracles. (b) Backwards beating of the heart draws hemolymph from the head and thorax and releases it into the abdomen. This compresses the abdominal air sacs and expands those in the head and thorax so that air is drawn in through thoracic spiracles and forced out through abdominal spiracles. (c) Effect of heartbeat reversals and coordinated abdominal movements on tracheal pressure and oxygen partial pressure alternating in the scutellar and abdominal air sac (after Wasserthal, 2012 and unpublished). (d) X-ray tomograph of the distended cephalic air sacs in *Drosophila melanogaster*. (e) Periodic volume changes of the cephalic air sacs by hemolymph shift in *Drosophila*. Diagram based on X-ray videos, lateral view of the head as in (d) (modified after Wasserthal *et al.*, 2006). White: compressed air sac volume during accumulation of blood (black) during forward pulses of the heart. Arrows point at moving contours of shrinking air sacs and outwards-extending cuticle of the basal labellum by hemolymph increase during forward heartbeat (black).

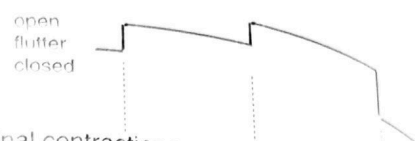
about 15 minutes (Fig. 17.18), while in some ants and the honey bee it is less than five minutes. DGE is best understood in lepidopteran pupae. During the closed phase, the spiracles are closed and little gas exchange occurs (Fig. 17.19). During this time, much of the carbon dioxide produced dissolves and is converted to bicarbonate in the hemolymph and tissues. Thus, the utilization of oxygen without replacement by gaseous carbon dioxide causes a reduction in tracheal system pressure. Next is the flutter phase, in which the spiracles rapidly pulse open and closed, allowing bulk inflow of air and oxygen, with reduced carbon dioxide emission. Next is the open phase, when the spiracles open wide; oxygen enters the spiracles and carbon dioxide and water vapor are emitted, often by diffusion. In some insects, such as *Psammodes* (Coleoptera), ventilatory movements of the abdomen are made during this period so that gases are actively pumped in and out of the system until the spiracles close again (Fig. 17.18b).

In pupal Lepidoptera, the pattern of spiracular behavior can be explained by effects of oxygen and carbon dioxide on the spiracles. At the end of the open phase, tracheal oxygen and pressure levels are near atmospheric. During the closed phase, the partial pressure of oxygen and the barometric pressure of the tracheal system drop linearly with time; the elastic abdomen compresses in response to the pressure gradient (Fig. 17.19). When the partial pressure of oxygen reaches about 4 kPa, the spiracles begin to flutter, and tracheal pressures rise to near atmospheric. Carbon dioxide accumulates up to a threshold of about 5 kPa, leading to spiracular opening.

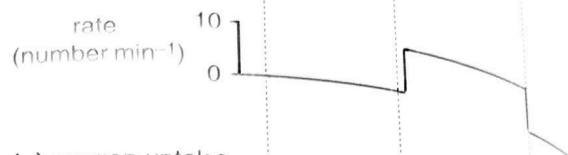
Many variations exist for DGE and its control. In some ants there is significant outward emission of CO_2 during the flutter phase. In grasshoppers oxygen has no effect on the duration of the phases of DGE, while hypoxia can either increase or decrease the duration of the flutter phase depending on species.

The functional significance of DGE in insects has been the subject of major controversy and investigation. There are at least three adaptive

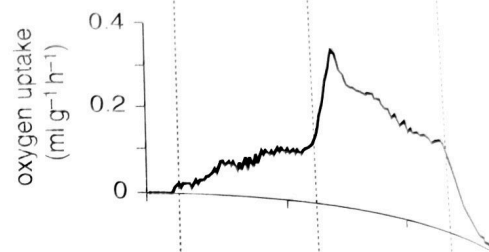
(a) spiracle activity



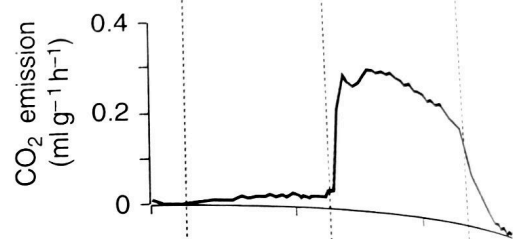
(b) abdominal contractions



(c) oxygen uptake



(d) CO_2 emission



(e) water loss

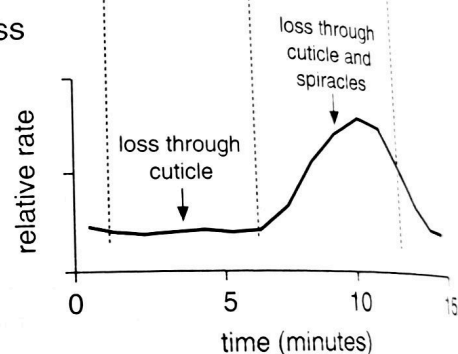


Figure 17.18 Discontinuous gas exchange in a beetle (*Psammodes*). Changes occurring in a single bout of ventilation (after Lighton, 1988). (a) Activity of the spiracles. During the flutter phase, they open slightly for brief periods and then close again. (b) Abdominal ventilation coincides with the open phase of the spiracles. (c) Oxygen uptake; some occurs during flutter but the rate is greatly increased when the spiracles open fully. (d) Carbon dioxide emission is largely confined to the open phase of the spiracles; very little occurs during spiracular flutter. (e) Water loss increases when the spiracles open.

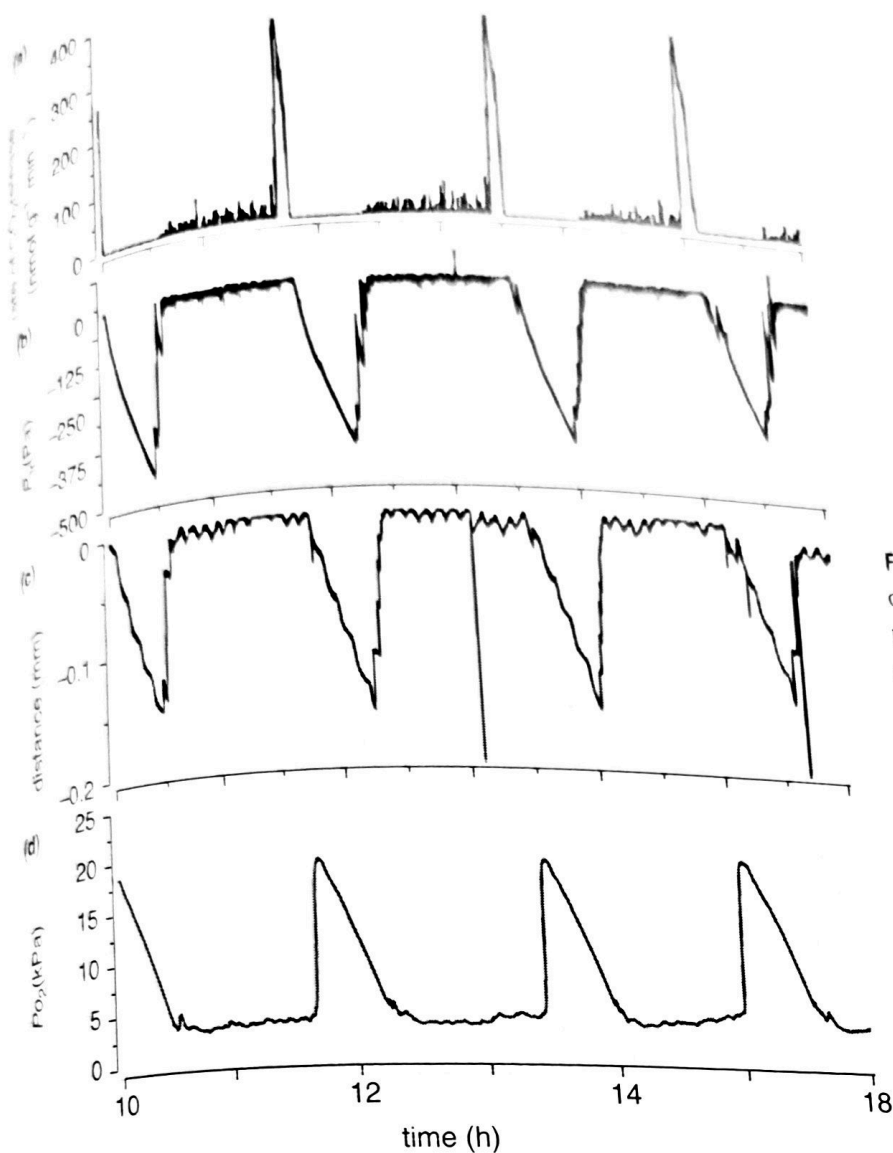


Figure 17.19 Discontinuous gas exchange cycle in *Atacus atlas* pupae. (a) Rate of CO_2 release vs. time; note the closed phase with zero CO_2 release flutter phase with low intermittent CO_2 release and then the open phase with a large burst of CO_2 emission. (b) Intra-tracheal pressure falls linearly with time during the closed phase, and then is near atmospheric during the flutter and open phases. (c) Abdomen length shortens during the closed phase, and remains at resting length during the flutter and open phases. (d) Intra-tracheal P_{O_2} falls linearly with time during the closed phase, remains near 3–4 kPa during the flutter phase and rises to near atmospheric during the open phase (after Hetz and Bradley, 2005).

hypotheses for the occurrence of DGE, including the reduction of respiratory water loss (hygric hypothesis), facilitation of underground gas exchange (chthonic hypothesis) and reduction of oxidative damage. In addition, it has been proposed that the occurrence of DGE need not be adaptive, but could be simply a consequence of having neurosensory systems for the control of ventilation and spiracular opening by carbon dioxide and oxygen (or for matching oxygen supply to demand). The idea that DGE is adaptive because it reduces respiratory water loss has been prominent since the

papers of Buck and Schneidermann and colleagues in the 1950s. There are two basic mechanisms by which DGE can reduce respiratory water loss. As a consequence of DGE, the spiracles are closed for most of the time, greatly reducing respiratory water loss. Prolonged spiracular closure leads to greater gradients for oxygen diffusion, allowing more oxygen uptake relative to water loss during the open phase (for either diffusion or convection). In addition, during the flutter phase, convective inward airflow can allow oxygen uptake with reduced outward diffusion of water. This adaptive argument for D

has been questioned on a number of grounds. First, the amount of water lost through the spiracles is usually very small relative to that lost via the cuticle. Second, in some comparative studies, the occurrence of DGE does not correlate well with expected exposure to desiccating conditions. Nonetheless, a variety of recent studies have supported the hypothesis that DGE reduces respiratory water loss, at least in some insects. In the caterpillar larvae *Erynnis propertius*, some individuals switch between continuous and discontinuous gas exchange, and use of DGE reduces water loss, without any change in metabolic rate. While some insects abandon DGE when acutely dehydrated (grasshoppers), acclimation to dry conditions reduces the duration of the open phase in cockroaches, and reduces water loss rate. Meta-analyses of 40 species in a phylogenetic context demonstrated that insect species from warmer habitats had longer DGE durations, especially when precipitation was low, consistent with the hygric hypothesis.

The hypothesis that DGE occurs to improve gas exchange underground is weakened by the fact that both hypoxia and hypercapnia tend to cause spiracular opening. The hypothesis that DGE functions to reduce oxidative damage is supported by the observation that some lepidopteran pupae regulate tracheal oxygen levels during the flutter phase at consistently low levels across a wide range of oxygen levels. Certainly, continuously open spiracles would expose insect tissues to levels of oxygen much higher than normally experienced by tissues of vertebrates, and higher tissue oxygen level is often correlated with increased mitochondrial reactive oxygen species production and oxidative damage.

There is also strong support for models that suggest that DGE arises non-adaptively from the properties of the control systems. For example, DGE is most common when the metabolic rates of insects are low (low rates of oxygen consumption and carbon dioxide production allow the spiracles to remain closed for longer periods of time), and DGE is

even observed in semi-aquatic insects at high humidities. In summary, it seems likely that DGE is likely to arise from interactions between internal gas levels and the neural system for control of the spiracles in most insects, but that reduction in respiratory water loss may be an important adaptive benefit of DGE in some insects.

17.5.4 Variation in gas exchange

Higher metabolic rates demand higher levels of oxygen intake. This is most obvious in flight, when metabolic rates can rise 5–30-fold. Flying insects achieve maximal mass-specific metabolic rates that are about double those observed in flying birds and mammals, likely due at least in part to the advantages of the tracheal respiratory system. Walking and running also require more oxygen than just standing still. The faster an insect runs, the higher its rate of oxygen consumption, and running vertically upwards requires more energy, and so more oxygen, than running on a horizontal surface. It is generally believed that very little anaerobic respiration occurs during locomotion of most insects, although grasshoppers do generate considerable lactate when jumping.

Feeding and digestion increase metabolic rates due to the costs of anabolism, gut peristalsis and active transport. The increase in metabolic rate with feeding, often termed the specific dynamic action, averages 3.3-fold higher than resting values in insects. Metabolic rate increases more than ten-fold in *Rhodnius prolixus* after a huge blood meal. Dietary characteristics can also affect metabolic rates; locusts consuming food high in carbohydrate have higher metabolic rates, probably to eliminate excess caloric intake (known as adaptive thermogenesis, or facultative, diet-induced thermogenesis). During extreme food restriction (e.g., during seasonal dearths), insects exhibit torpor or diapause accompanied by decreases in metabolic rates of up to 98%. In diapausing pupae of the flesh fly, *Sarcophaga*, peaks of oxygen consumption occur at

about four-day intervals when the temperature is constant at 25°C. Over a period of about 36 hours, oxygen consumption rises from a resting level of about $1.5 \mu\text{l h}^{-1}$ to $9\text{--}10 \mu\text{l h}^{-1}$ and then falls to the original level. The peaks in oxygen consumption coincide with peaks of protein synthesis and release. It is reported that there are no parallel changes in carbon dioxide emission, suggesting CO_2 is accumulating as bicarbonate or being incorporated into larger compounds. Similar variation in oxygen consumption occurs in the diapausing pupa of the cabbage butterfly, *Pieris*, and it is probably a common phenomenon.

Another important source of variation in metabolic rates among insects is body size. Across species, larger insects have higher metabolic rates (Watts) but lower mass-specific metabolic rates (Watts g^{-1}). On log-log plots, metabolic rates (Watts) scale approximately with body mass^{0.75}. This relationship also seems to hold for social insect colonies within species. During ontogeny, the pattern of metabolic rate with body mass is more variable. While it is usually true that older/larger insects have higher metabolic rates, the scaling exponents can be quite variable, probably because development of insects is associated with a wide variety of morphological and physiological variation.

Temperature also strongly affects metabolic rates, with most inactive insects showing exponential increases in metabolic rates with rises in temperature across moderate ranges of temperature (often doubling or tripling with a 10°C rise in temperature). However, some insects such as honey bees in flight, honey bee swarms and bumble bee colonies sometimes exhibit increases in metabolic rate at lower temperatures, apparently for thermoregulatory purposes.

Gas exchange in flight The massive increase in oxygen consumption that occurs when an insect flies requires a greatly increased airflow through the tracheae to the flight muscles. The size of the spiracles is closely matched to oxygen need during

flight. Experimentally sealing any thoracic spiracle causes a reduction in flight metabolic rate in *Drosophila*, and flight performance is tightly linked to the available spiracular area. During the flight of *Schistocerca*, abdominal ventilation increases in frequency and amplitude, but still only supplies about $50 \text{ ml air g}^{-1} \text{ h}^{-1}$, not sufficient to supply the needs of the flight muscles. However, the distortion of the thorax, and in particular the raising and lowering of the notal sclerites, produces large volume changes in the extramuscular air sacs of the pterothoracic tracheal system (Figs. 17.4b, 17.6), while changes in the diameters of shortening muscles may compress the intramuscular air sacs. This pterothoracic ventilation produces an airflow of about $350 \text{ ml air g}^{-1} \text{ h}^{-1}$, which is adequate for the needs of the flight muscles.

Thoracic pumping is also important in flight in Odonata and probably in Lepidoptera and Coleoptera. In Hymenoptera and Diptera, changes in the thoracic volume during flight are not very large and abdominal pumping is of greater importance in maintaining the air supply to the flight muscles. In a large cerambycid beetle, *Petrognatha*, there is evidence that a stream of air, resulting from the forward movement of the insect in flight, flows in through the forwardly directed second spiracles into large tracheae running directly to the third spiracles. This stream of air ventilates the primary supply to the flight muscles while the secondary supply is probably ventilated by muscular compression.

When *Schistocerca* starts to fly, the pattern of spiracle opening also alters (Fig. 17.20). At first, spiracles 1 and 4–10 close, but then they open and close rhythmically in synchrony with abdominal ventilation so that there is a flow of air via the head to the rest of the central nervous system. Increased abdominal ventilation may also improve the blood circulation and hence the fuel supply to the flight muscles. Spiracles 2 and 3 remain wide open throughout flight and although they show some incipient closures after a time these do not affect the airflow through the spiracles. These spiracles supply

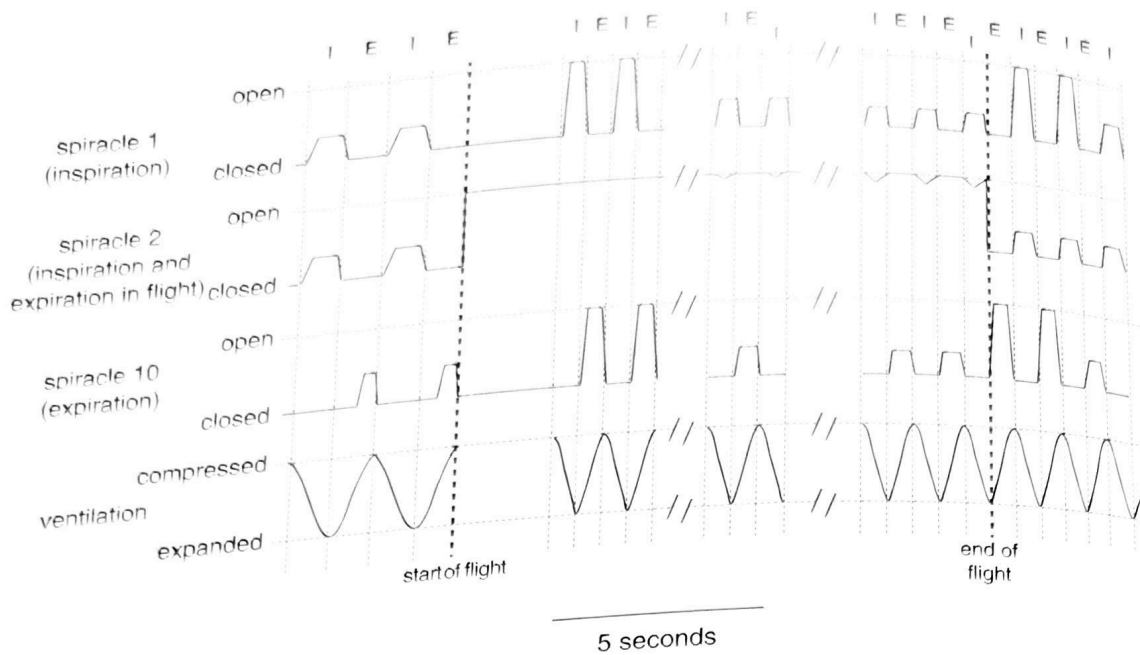


Figure 17.20 Spiracle activity and abdominal ventilation during flight of *Schistocerca*. I, inspiration; E, expiration (after Miller, 1960b).

the flight muscles, and, since the tracheal system of these muscles is largely isolated and the spiracles remain open all the time, there is a tidal flow of air in and out of them.

Hawk moths, with their powerful and long-lasting flight, have very high metabolic rates. The O_2 content of the flight muscles during steady flight exceeds even the resting level. This is facilitated by a unidirectional airstream with inspiration through the first spiracles and expiration through the second spiracles. The directed airflow is generated by the flight apparatus and abdominal up-and-down movements while inspiration is prevented through the posterior thoracic spiracles (Fig. 17.21). The different structure of the meso- and metathoracic spiracles reflects this unidirectional respiratory airstream: The inspiratory mesothoracic spiracle is protected by dense filter lamellae. The expiratory metathoracic spiracle is devoid of filter structures and has an external valve lip, which is directed outwards. During warm-up shivering of hawk moths, wingbeat amplitude and the deformation of

the pterothorax are small and the metathoracic spiracle does not become enclosed in the subalar cleft during incomplete downstroke. Ventilation pulses show the same mean pressure at the anterior spiracles and in the mesoscutellar air sacs. This tidal in- and outflow through both anterior and posterior thoracic spiracles occurs also in the steady flight of saturniid moths, with their low wingbeat frequency.

17.5.5 Control of ventilation

As in the control of spiracles, ventilatory movements are initiated by the accumulation of carbon dioxide and, to a lesser extent, the lack of oxygen in the tracheal system, acting directly on centers in the ganglia of the central nervous system. Each abdominal ganglion produces rhythmical sequences of activity controlling the movements. In *Schistocerca* and *Periplaneta* the metathoracic ganglion acts as a pacemaker and overrides the rhythms of the other ganglia.

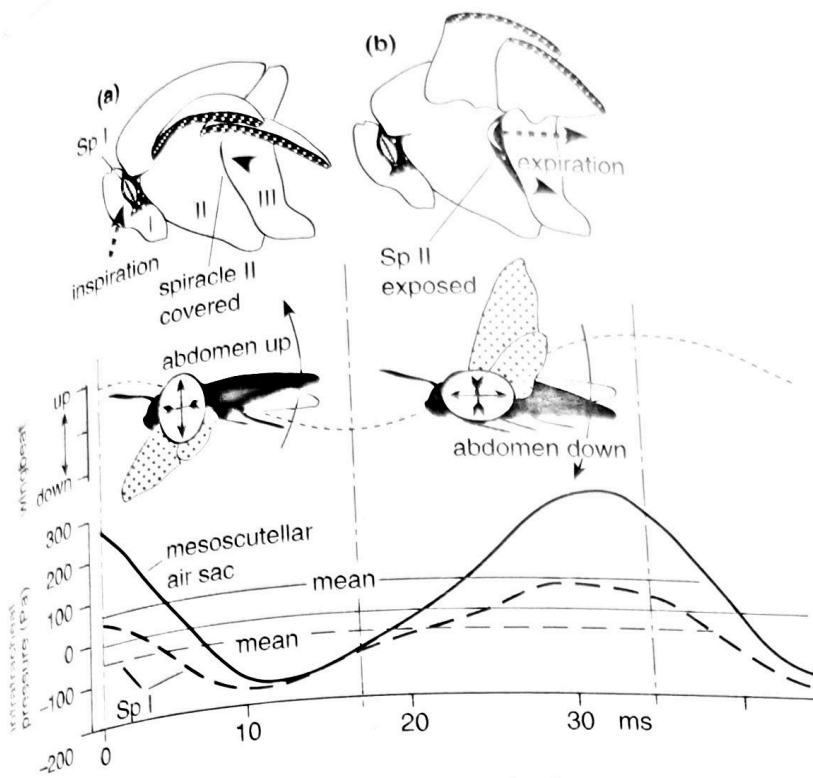


Figure 17.21 Unidirectional respiratory airstream in the flying hawk moth *Manduca* based on the pressure difference between anterior spiracles (Sp I) with a mean negative pressure and the mesoscutellar air sac with a mean positive pressure. Schematic lateral views of the thorax showing the deformation of the thorax (arrows) during (a) downstroke and (b) upstroke of the wings. This affects the adduction and retraction of the metathorax (arrowheads) enclosing or exposing the Sp II in the subalar cleft, and up-and-down moving abdomen. (a) Downstroke: volume increase of the metathoracic air sacs and decrease of intratracheal pressure. Inspiration through Sp I while Sp II are closed. (b) Upstroke: reduction of the metathoracic tracheal volume by flattening the thorax and increase of intratracheal pressure. Expiration through the open and exposed Sp II. I prothorax; II mesothorax; III metathorax (modified after Wasserthal, 2001).

In normal ventilation by *Schistocerca*, involving only dorso-ventral movements of abdominal sclerites, expiration is produced by tergosternal muscles innervated by motor neurons from the ganglion of the corresponding segment. Inspiration is produced by muscles inserted low on the tergum and innervated by axons in branches from the median ventral nerve. The perikarya of these motor neurons are in the ganglion of the preceding segment, so the alternation of inspiratory and expiratory movements is controlled from different ganglia (Fig. 17.14). Coordination is achieved by a coordinating interneuron, one in each ventral connective (cell 2 in Fig. 17.14b), which originates in the metathoracic ganglion and extends to the last abdominal ganglion. These interneurons are driven by a central pattern

generator, probably in the metathoracic ganglion, whose activity is regulated by inputs from the head and other parts of the body. Action potentials in the interneuron have an excitatory effect on a local interneuron (cell 3) and probably have a weak direct inhibitory effect on the inspiratory motor neurons (cell 5 in Fig. 17.14b). The local interneuron excites the expiratory motor neurons (cell 4) and strongly inhibits the inspiratory motor neurons (cell 5). Inspiration occurs when the intersegmental interneurons are silent and the inspiratory motor neurons become spontaneously active, at the same time inhibiting the expiratory motor neurons via another local interneuron (cell 6).

The rate of ventilation is altered by reducing the interval between inspiratory bursts and is affected by

sensory input from various sources. Centers sensitive to carbon dioxide are present in the head and thorax of *Schistocerca* and these modify the activity of the pacemaker, while the output is also modified by high temperature and nervous excitation generally. Proprioceptors may play some part in the maintenance of the frequency of ventilation. The coordination of the spiracles with the ventilatory movements is brought about by motor patterns derived from the ventilatory centers.

17.6 Gaseous exchange in aquatic insects

Aquatic insects obtain oxygen directly from the air or from that dissolved in the water. The former necessitates some semipermanent connection with the surface or frequent visits to the surface. Insects that obtain oxygen from water nearly always retain a tracheal system so that the oxygen must come out of solution into the gaseous phase. This is important because the rate of diffusion in the gas phase is very much greater than in solution in the hemolymph or tissues. Gaseous exchange with water takes place through thin-walled gills well supplied with tracheae, but in other cases a thin, permanent film of air is present on the outside of the body. The spiracles open into this film so that oxygen can readily pass from the water into the tracheae.

17.6.1 Aquatic insects obtaining oxygen from the air

Most aquatic forms obtaining air from above the water surface must make periodic visits to the surface, but a few have semipermanent connections with the air that enable them to remain submerged indefinitely. The larva of the hover fly *Eristalis* (Diptera) has a telescopic terminal siphon which can extend to a length of 6 cm or more in a larva only 1 cm long. By means of the siphon the larva can reach the water surface with its posterior spiracles, while the body remains on the bottom mud (Fig. 17.22).

Some other species obtain oxygen by thrusting their spiracles into the aerenchyma of aquatic plants. This habit occurs in larval *Donacia* (Coleoptera) and *Chrysogaster* (Diptera), and larvae and pupae of *Notiphila* (Diptera) and the mosquito *Mansonia*. With the exception of *Mansonia*, all of these species live in mud containing very little free oxygen. The functional spiracles are at the tip of a sharp-pointed post-abdominal siphon in larval forms (Fig. 17.23), and on the anterior thoracic horns of the pupae.

For most aquatic insects obtaining their oxygen from the air, however, periodic visits to the surface of the water are necessary to renew the gases in the tracheal system. Problems facing all insects that come to the surface are those of breaking the surface film and of preventing the entry of water into the spiracles when they submerge. The ease with which this is accomplished depends on the surface properties of the cuticle and, in particular, on its resistance to wetting. When a liquid rests on a solid or a solid dips into a liquid, the liquid-air interface meets the solid-air interface at a definite angle that is constant for the substances concerned. This angle, measured in the liquid, is known as the contact angle (Fig. 17.24). A high contact angle indicates that the surface of the solid is only wetted with difficulty; such surfaces are said to be hydrofuge. Under these

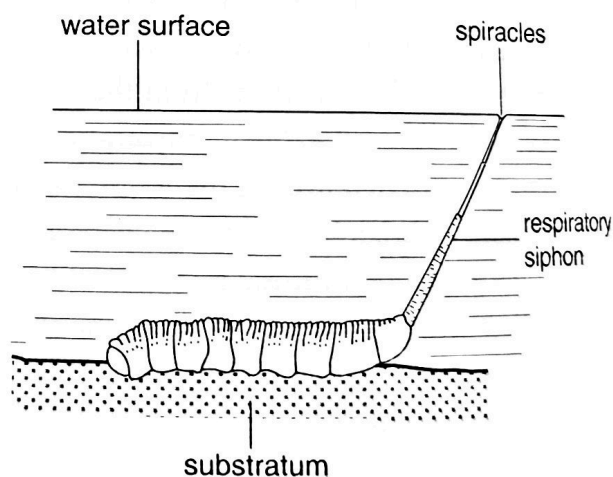


Figure 17.22 Semipermanent connection with the water surface. The larva (rat-tailed maggot) of the hover fly *Eristalis*, with its respiratory siphon partly extended (after Imms, 1947).

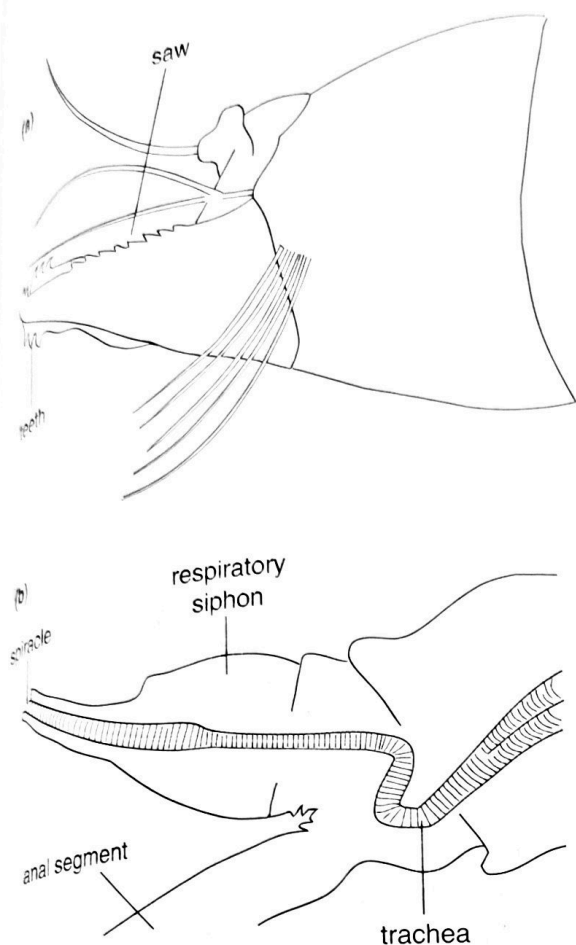


Figure 17.23 Semipermanent connection with the aerenchyma of a plant. The respiratory siphon of a mosquito larva (*Mansonia*) which connects with the aerenchyma of aquatic plants (after Keilin, 1944). (a) Lateral view showing saw which cuts into the plant tissue and recurved teeth which hold the siphon in place. (b) Longitudinal section showing a terminal spiracle and a trachea.

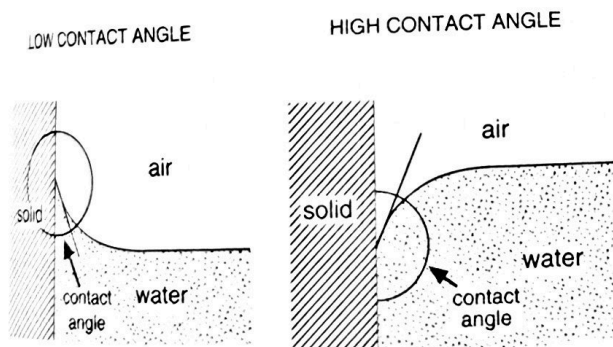


Figure 17.24 Diagrams showing low and high contact angles.

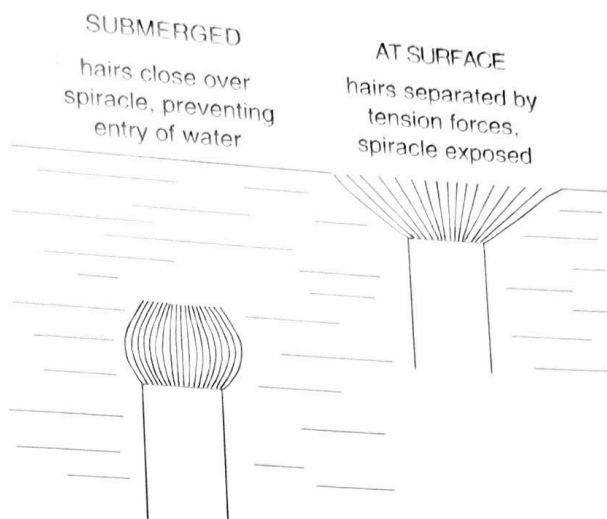


Figure 17.25 Diagrams showing the movements of hydrofuge hairs surrounding a spiracle when a *Stratiomya* larva (*Stratiomyidae*) is submerged and at the surface. The movement of the hairs is entirely passive, depending on physical forces acting between the hairs and the water (modified after Wigglesworth, 1965).

conditions, the cohesion of the liquid is greater than its adhesion to the solid, and so, when an insect whose surface properties are such that the contact angle is high comes to the air-water interface, the water falls away, leaving the body dry. The whole surface of the cuticle may possess hydrofuge properties, so it is not readily wetted at all, or these properties may be restricted to the region around the spiracles. In dipterous larvae, for example, perispiracular glands produce an oily secretion in the immediate neighborhood of the spiracle. Often, hydrofuge properties around the spiracle are associated with hairs, as in *Notonecta* (Hemiptera), or valves, as in mosquito larvae, which close when the insect dives, but open at the surface (Fig. 17.25), being spread out by surface tension.

In many of these insects, only the posterior spiracles are functional and they are often carried on the end of a siphon extending from the posterior body, as in larval Ephydriidae and Culicidae (Diptera). In these insects, only the tip of the siphon breaks the surface film. In water scorpions (Hemiptera, Nepidae), the spiracles are at the base of an air-filled tube.

An increase in the number of functional spiracles often occurs in the last-stage larva since this stage is commonly less strictly aquatic than earlier stages, leaving the water in order to pupate or, in hemimetabolous insects, to facilitate adult emergence. The number of functional spiracles never decreases from one instar to the next.

Gas exchange via air bubbles Some insects, such as mosquito larvae, can remain submerged only as long as the supply of oxygen in the tracheae lasts, but others have an extratracheal air store, carrying a bubble of air down into the water when they dive. The spiracles open into this bubble, so that it provides a store of air additional to that contained in the tracheal system, enabling the insects to remain submerged for longer periods than would be possible without it.

The position of the store is characteristic for each species. In *Dytiscus* (Coleoptera), it is beneath the elytra, and experimental removal of the hindwings increases the space beneath the elytra and enables the insect to remain submerged for longer periods. In *Notonecta* (Hemiptera), air is held by long hydrofuge hairs on the ventral surface as well as in a store under the wings and in a thin film held by small bristles over the dorsal surface of the forewing. The related *Anisops* (Heteroptera) has ventral and subelytral stores supplemented by oxygen loosely associated with hemoglobin in large hemoglobin cells just inside the abdominal spiracles (Section 17.4).

An air store also gives the insect buoyancy, so that as soon as the insect stops swimming or releases its hold on the vegetation, it floats to the surface. The position of the store is such that the insect breaks the surface suitably oriented to renew the air. *Dytiscus*, for instance, comes to the surface tail first and renews the subelytral air from the posterior end of the elytra. In *Anisops*, the aquatic bug first must swim down against the upward force of the large air bubble. However, as oxygen is consumed, the bubble decreases in size. For a considerable period

of time, the bubble is maintained at the size required for neutral buoyancy by release of oxygen from hemoglobin.

Physical gills. An air bubble provides an insect with more oxygen than that available at the time of the dive because it also acts as a temporary gill. When an insect dives, the gases in its air store are in equilibrium with the gases dissolved in the water, assuming that this is saturated with air. Normally, at the beginning of the dive, the bubble would contain approximately 21% oxygen and 79% nitrogen (Fig. 17.26). Carbon dioxide is very soluble, so there is never very much in the air bubble. Within a short period of diving, the P_{O_2} in the bubble is reduced, as the oxygen is utilized by the insect, and there is a corresponding increase in the proportion and p_{O_2} of nitrogen. As a result, the gases in the bubble and those in solution immediately surrounding it are no longer in equilibrium and movements of gases will occur, tending to restore equilibrium. Oxygen tends to pass into the bubble from the surrounding medium, because the oxygen tension in the bubble is reduced, while nitrogen tends to pass out of the bubble into solution, because the nitrogen tension in the bubble is increased. Thus more oxygen will be made available to the insect than was originally present in the bubble, which is, in fact, acting as a gill. This effect is enhanced by the fact that the oxygen passes into the bubble about three times more readily than nitrogen passes out into solution. As a result of this, the insect is able to remain submerged for longer periods than would be possible if it depended solely on the oxygen initially available in its store.

Nitrogen, as a non-respiratory gas, is essential for the air bubble to act as a gill as, in its absence, there is no change in partial pressure of P_{O_2} as the oxygen is utilized. For this reason an insect with a bubble of pure oxygen in water saturated with oxygen does not survive for very long unless it is able to come to the surface.

With inactive insects at low temperatures, when the rate of utilization of oxygen is low, the

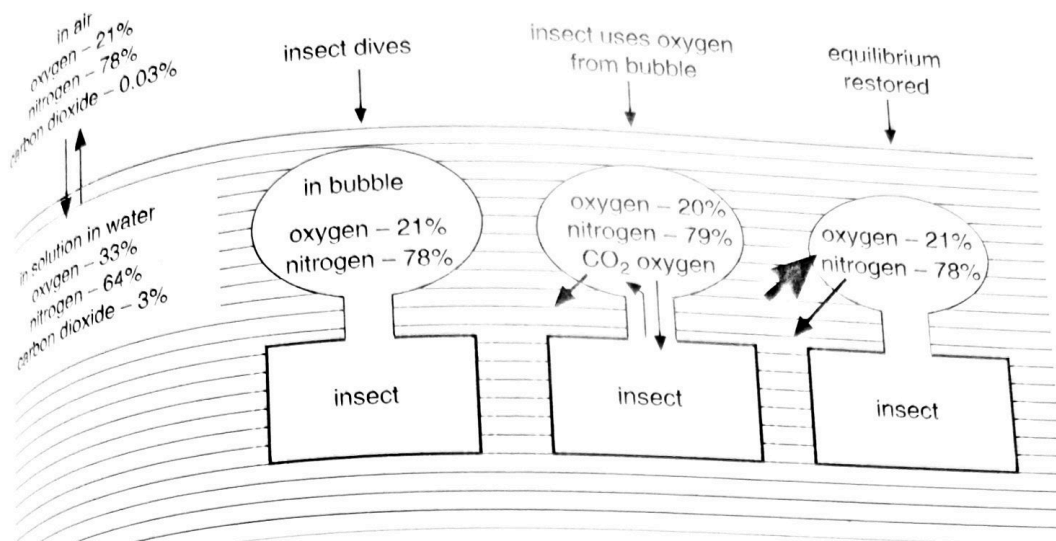


Figure 17.26 Diagram of an air bubble acting as a physical gill. As the insect dives, the gases in the bubble are in equilibrium with those dissolved in the water. As the insect uses oxygen, the equilibrium is perturbed. It is restored by the inward movement of oxygen and the outward movement of nitrogen. This is a continuous process; it is shown as two separate steps for clarity. Note that carbon dioxide produced by the insect is immediately dissolved in the water. Oxygen comes out of solution faster than nitrogen goes in. The bubble shrinks continuously as nitrogen goes into solution.

efficiency of the air store as a gill may be adequate for the insect to remain submerged for a long time. Thus *Hydrous* (Coleoptera) can remain submerged for some months during the winter, but in more active insects, with higher oxygen requirements, the bubble lasts for only a short time and the insect must come to the surface more frequently. If the temperature is above about 15°C, *Notonecta* (Hemiptera) uses oxygen so rapidly that the gill effect is of negligible importance and the insect soon surfaces to replenish its air store. At 10°C, however, it remains submerged for twice as long as would be possible with the initial oxygen supply, and below 5°C it can survive for a very long period without access to the surface.

The efficiency of the bubble as a gill depends on the oxygen content of the water adjacent to the gill. In water devoid of oxygen, or containing only a very little, the gas tends to pass out of the bubble into solution and will be lost to the insect. Even if the oxygen tension in the water exceeds that in the

bubble, the amount entering the bubble will depend on the difference in tension. Hence, the higher the oxygen tension of the outside water, the more effective will be the bubble as a gill, and this must be true of any type of gill. Consequently, the frequency with which an insect visits the surface will vary inversely with the oxygen tension of the water (Fig. 17.27).

17.6.2 Insects obtaining oxygen from the water

In all insects living in water, some inward diffusion of oxygen from the water takes place through the cuticle, and in many larval forms gaseous exchange takes place solely in this way. Cutaneous diffusion depends on the permeability of the cuticle and a lower oxygen tension in the tissues as compared with the water. In many larval forms the cuticle is relatively permeable and in *Aphelocheirus* (Hemiptera: Heteroptera), for example, the cuticle of

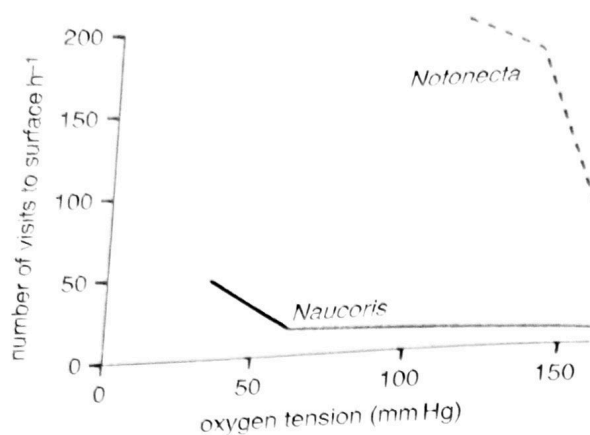


Figure 17.27 Because an air bubble carried by an insect acts as a physical gill, the time for which an insect can remain submerged increases as the amount of oxygen dissolved in the water (oxygen tension) increases. Consequently, the insect makes fewer visits to the surface at higher oxygen tensions. Data for *Naucoris* at 20°C; *Notonecta* at 17°C (data from de Ruiter *et al.*, 1952).

the last larval stage is about four times as permeable as that of the adult.

In very small larvae, such as the first-stage larvae of *Simulium* (Diptera) and *Chironomus* (Diptera), in which the tracheal system is filled with fluid, cutaneous diffusion into the hemolymph meets the whole oxygen requirement of the insect. In general, however, blood circulation is poor and the rate of diffusion through the blood slow, and diffusion into the blood would not suffice for most larger insects. Hence the majority of insects that obtain oxygen from water do have a tracheal system, although the spiracles are non-functional. This is called a closed tracheal system. As oxygen is used by the tissues of the insect, its partial pressure within the tracheae falls, creating a pressure gradient between the tracheoles just beneath the cuticle and the adjacent water. Under these conditions, oxygen from the water diffuses into the tracheal system, within which it can rapidly diffuse around the body to the tissues. An incompressible tracheal system is essential for this type of gas movement to occur as, otherwise, the tracheae would collapse under the pressure of water as oxygen was used.

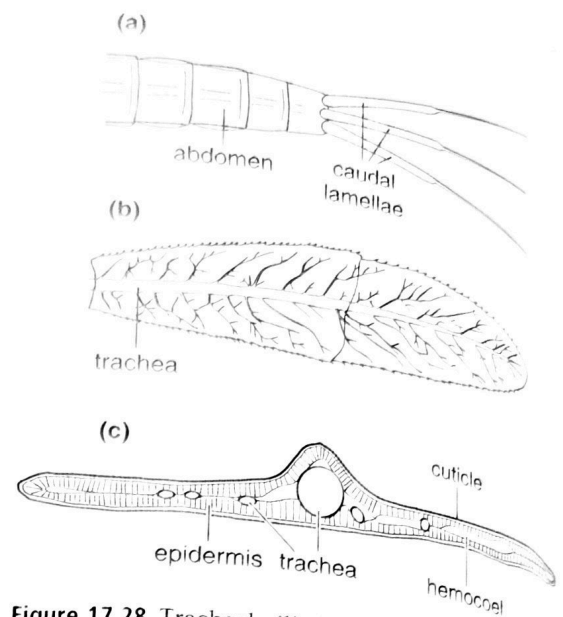


Figure 17.28 Tracheal gills (caudal lamellae) of a damselfly larva (Zygoptera). (a) Dorsal view of the posterior end of the abdomen. (b) One lamella showing the tracheae; only the major branches are shown (after Gardner, 1960). (c) Transverse section of a lamella (after Tillyard, 1917).

Tracheal gills In some insects, such as *Simulium* larvae (Diptera), there is a network of tracheoles just beneath the general body cuticle, but often there are leaf-like extensions of the body, forming gills. These are covered by very thin cuticle with a network of tracheoles immediately beneath and are known as tracheal gills. In most larval Zygoptera there are three caudal gills (Fig. 17.28); larval Trichoptera have filamentous abdominal gills, while, in larval Plecoptera, the position of the gills varies from species to species.

Larval Anisoptera (Odonata) have gills in the anterior part of the rectum, known as the branchial chamber (Fig. 17.29). Water is drawn in and out of the chamber by the activity of muscles largely unconnected with the gut. The hemocoel in the posterior part of the abdomen is isolated from the rest of the body by a muscular diaphragm across the fifth abdominal segment. Because of this, contraction of dorso-ventral muscles in the posterior abdominal segments exerts pressure on the branchial chamber.

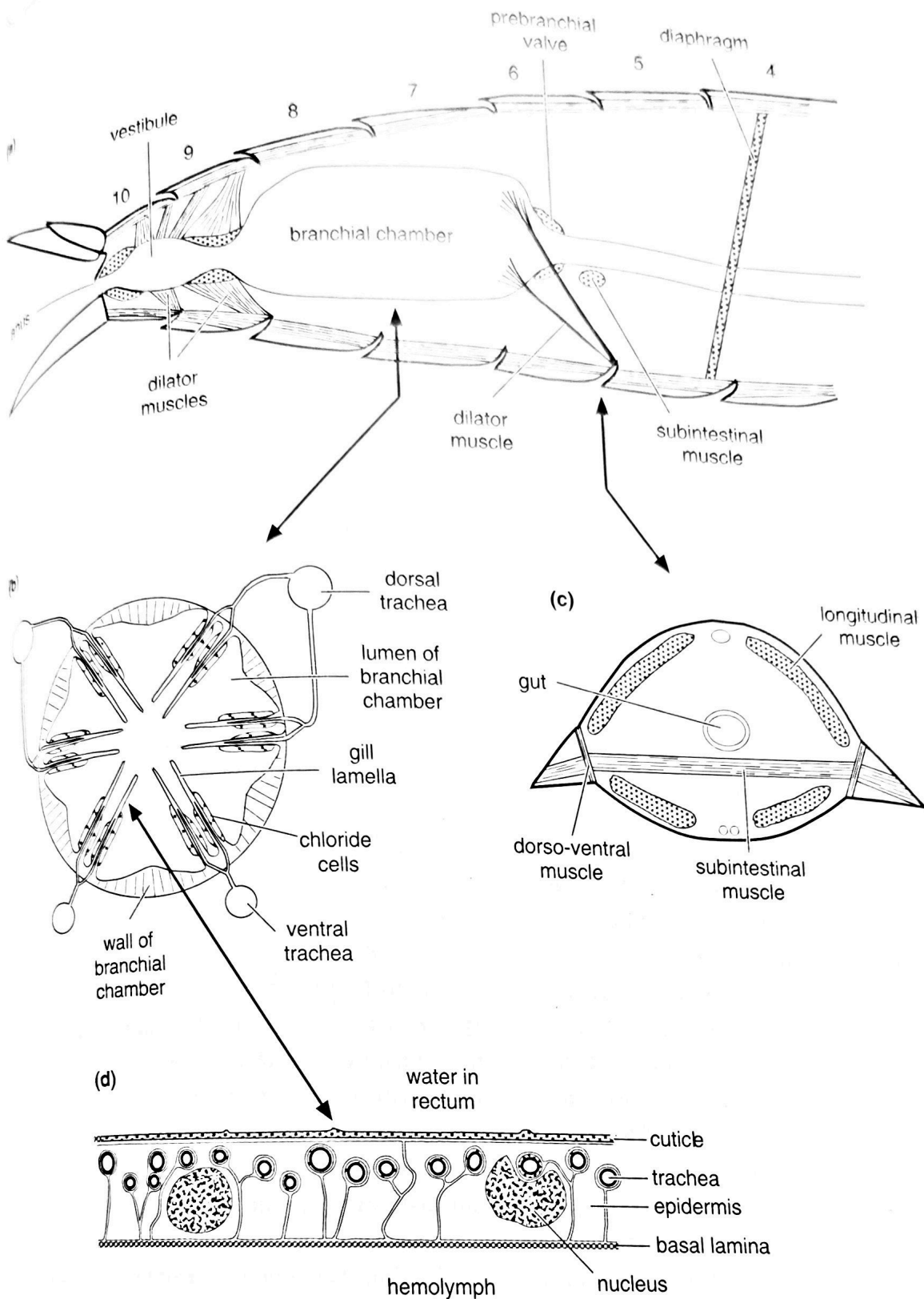


Figure 17.29 Tracheal gills in the rectum of a dragonfly larva (after Tillyard, 1917; Mill and Pickard, 1972). (a) Longitudinal section through the abdomen; numbers indicate abdominal segments. (b) Transverse section through the branchial chamber. (c) Transverse section through abdominal segment 6 showing the subintestinal muscle. (d) Detail of gill epithelium showing the tracheae close beneath the cuticle (after Schmitz and Komnick, 1976).

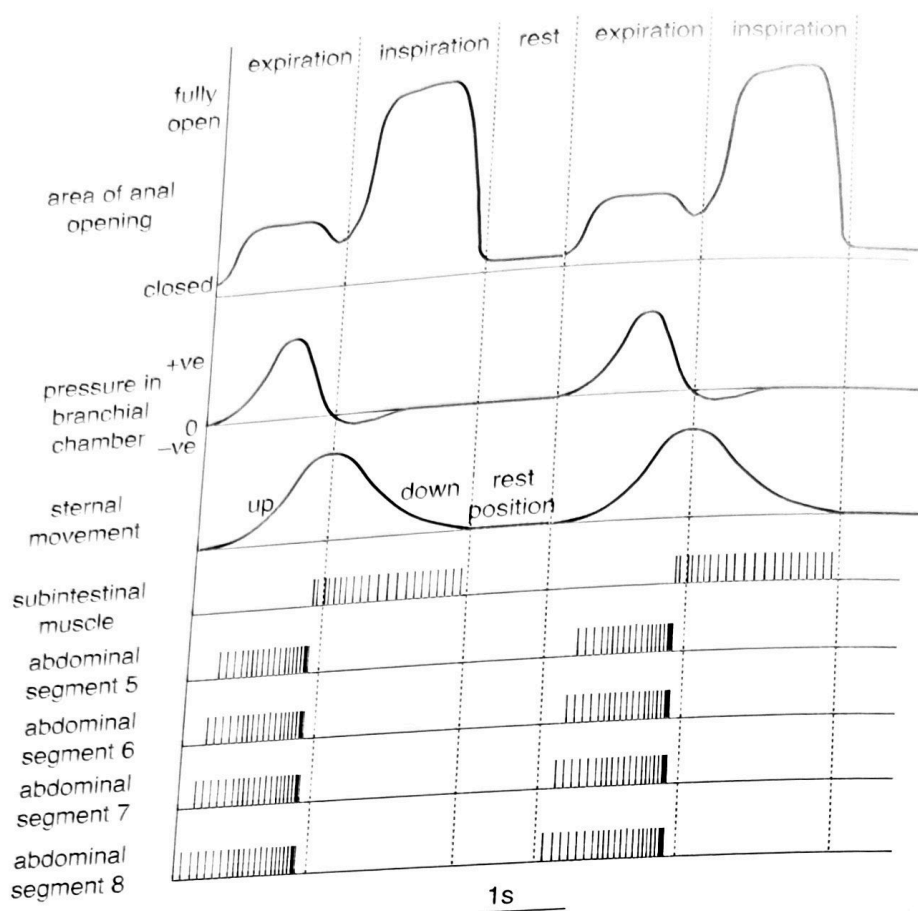


Figure 17.30 Rectal ventilation in a dragonfly larva. Lower traces show action potential frequency in the subintestinal muscle and in the dorso-ventral muscles of successive abdominal segments. As the dorso-ventral muscles contract they raise the sterna and compress the branchial chamber and water is forced out. When the subintestinal muscle contracts, the sterna are lowered, pressure in the chamber becomes negative and water flows in through the widely open anus (after Mill and Pickard, 1972).

Water is forced out through the partially open anus and drawn in again when the volume of the abdomen is restored, partly by the elasticity of the cuticle and partly by contraction of muscles in the diaphragm and of a transverse subintestinal muscle. During inspiration the anal valves are wide open (Fig. 17.30). A muscular prebranchial valve presumably prevents water from moving forwards into the midgut. Some 85% of the water in the rectum is renewed at each cycle of compression and relaxation, and the frequency of pumping in *Aeschna* varies from about 25 to 50 cycles per minute. At the higher rates the interval between inspiration and expiration is reduced.

In general, although a good deal of gaseous exchange probably takes place through tracheal gills (in *Agrion* [Odonata] larva 32–45% of the oxygen absorbed normally takes this route) some insects are able to survive without gills under normal oxygen

tensions. Where the oxygen tension of the water is low, however, gills are of importance as they considerably increase the area available for gaseous exchange.

Plastron respiration Some insects have specialized structures holding a permanent thin film of air on the outside of the body in such a way that an extensive air–water interface is always present for gaseous exchange. This film of gas is called a plastron and the tracheae open into it so that oxygen can pass directly to the tissues.

The volume of the plastron is constant and usually small as it does not provide a store of air, but acts solely as a gill. The constant volume is maintained by various hydrofuge devices spaced very close together so that water does not penetrate between them except under considerable pressure. Excess external pressures that may occur during the normal life of

the insect are resisted. Such pressures may develop through the utilization of oxygen from the plastron so that the internal pressure is reduced, or through the insect being in deep water and therefore subjected to high hydrostatic pressure.

In adult insects the plastron is held by a very close hair pile in which the hairs resist wetting because of their hydrofuge properties and their orientation. The most efficient resistance to wetting would be achieved by a system of hairs lying parallel with the surface of the body. *Aphelocheirus* (Hemiptera) approaches this condition in possessing hairs that are bent over at the tip (Fig. 17.31a); in other insects, such as *Elmis* (Coleoptera), the hairs are sloping (Fig. 17.31c). The ability of sloping hairs to withstand collapse depends on their being slightly thickened at the base and also on their close packing. As the air in the plastron is compressed, the closely packed hairs become pressed together so that their overall resistance to further compression increases.

In the adult *Aphelocheirus*, the plastron covers the ventral and part of the dorsal surface of the body. The hairs which hold the air are 5–6 μm high and about 0.2 μm in diameter (Fig. 17.31a). They are packed very close together, at about $2\,500\,000\text{ mm}^{-2}$, and are able to withstand a pressure of about 400 kN m^{-2} before they collapse. Hence, this is an extremely stable plastron that will only be displaced by water at excessive depths. The spiracles open into a series of radiating canals in the cuticle and these connect with the plastron via small pores. These canals are lined with hairs so that the entry of water into the tracheal system is prevented. The basal rate of oxygen utilization at 20°C is about $6\text{ }\mu\text{l h}^{-1}$ and this is readily provided by the plastron, so that, except in poorly oxygenated water, the insects need never come to the surface.

The plastrons of other insects are generally less efficient than that of *Aphelocheirus* as they have a less dense hair pile from which the air is more readily displaced. However, a number of insects with a hair density of 3×10^4 to $1.5 \times 10^5\text{ mm}^{-2}$ have plastrons that are usually permanent and adequate for the

insect's needs. In these cases, the plastron is often supplemented by a less permanent macroplastron, consisting of a thicker layer of air outside the plastron and held by longer hairs than the plastron, as in *Hydrophilus* (Coleoptera) (Fig. 17.31b), or by the erection of the plastron hairs, as in *Elmis* (Fig. 17.31c). The macroplastron is an air store and acts as a physical gill. It becomes reduced in size and finally eliminated when the insect is submerged, leaving only the plastron. The hairs holding the macroplastron are relatively long and flexible, so as the gas bubble is reduced they tend to clump, leaving patches of exposed cuticle liable to wetting. To avoid this, these insects groom their hair pile and, in *Elmis*, there are brushes on the legs for this purpose. These brushes are also used to capture air bubbles and add them to the macroplastron. Insects that only have a plastron do not make grooming movements of this type.

17.6.3 Ventilation in aquatic insects

Close to a solid surface, water movement becomes laminar with little mixing between successive layers. The region of laminar flow is called a boundary layer and, in general, is greater in depth in still or slowly moving water than in fast-flowing, turbulent water. This has important consequences for the availability of oxygen at any respiratory interface. In turbulent water, oxygen is moved by convection and tends to be uniformly distributed. Movement across the boundary layer, however, depends on diffusion. As a result, water adjacent to an air bubble or a gill will tend to become depleted of oxygen because the rate of diffusion into the layer adjacent to the gill surface may not match the rate at which oxygen is taken into the gill. As movement into the gill is also a passive, diffusive process, it is obviously important to maintain the concentration of oxygen in the boundary layer at a high level. This is achieved by the insect making ventilatory movements that renew the layer of water adjacent to the gill.

In many insects this is achieved by movements of the gills themselves. In *Ephemera* (Ephemeroptera) a

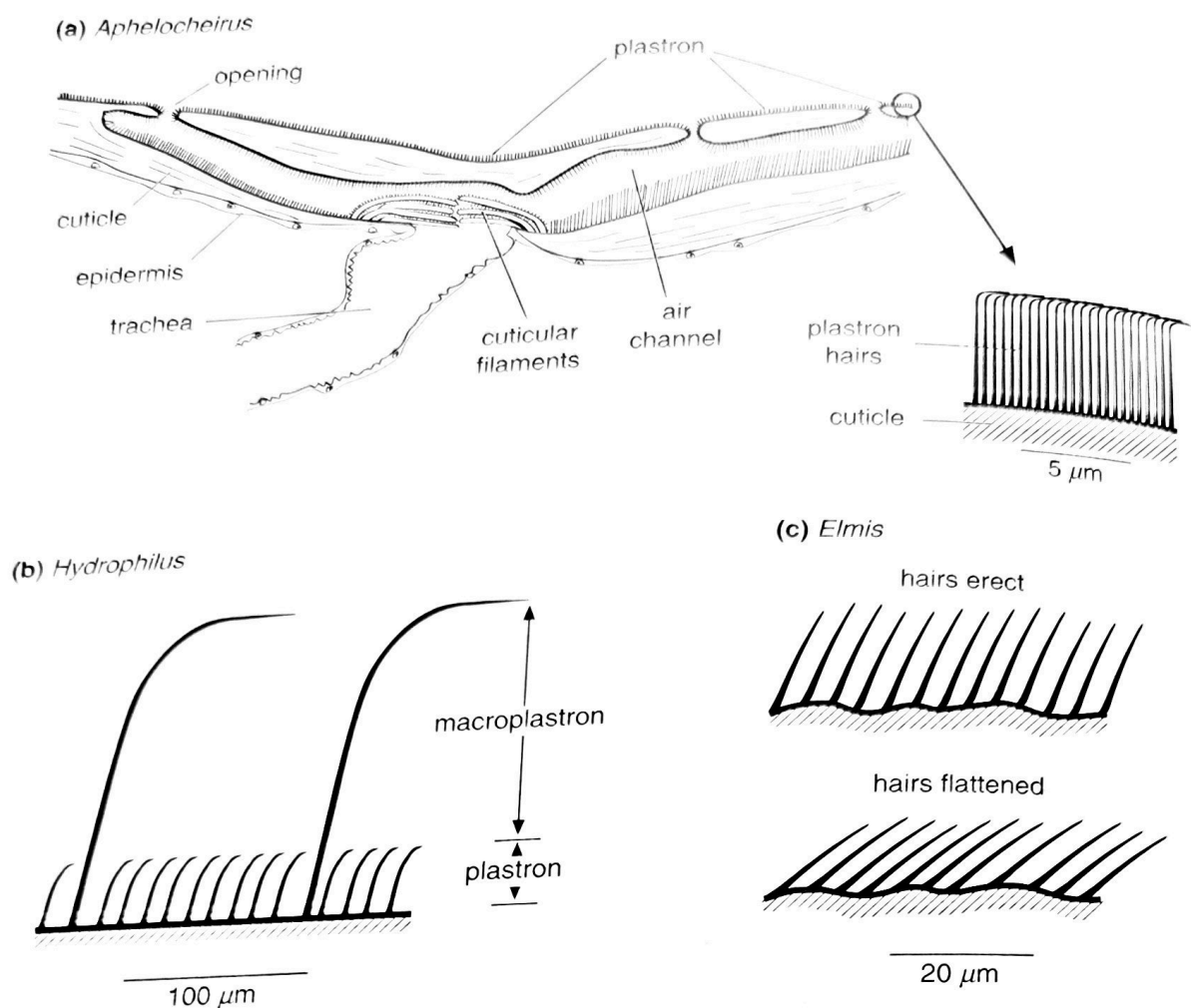


Figure 17.31 Plastron. (a) Plastron of *Aphelocheirus* (Hemiptera). Section through a spiracular rosette showing the junction of a trachea with a system of channels in the cuticle connecting with the plastron. Below right, detail of the plastron (after Thorpe and Crisp, 1947). (b) Macroplastron of *Hydrophilus* (Coleoptera) (after Thorpe and Crisp, 1949). (c) Macroplastron of *Elmis* (Coleoptera) showing the hairs erect, forming a macroplastron (above) and with the hairs compressed so that only the true plastron remains (after Thorpe and Crisp, 1949).

good deal of gaseous exchange takes place through the gills, but in *Cloeon* (Ephemeroptera) they function almost entirely as paddles, pushing water over the real respiratory surface on the abdomen. The larva of *Corydalis* (Neuroptera) ventilates the gills using rhythmic movements of the tubercles on which the gills are mounted. Anisopteran (Odonata) larvae achieve the same result by pumping water in and out of the rectum. Zygopteran larvae also pump water in and out of the rectum, and, in most species, this is

associated with ionic regulation (see Chapter 18). In *Calopteryx* (Odonata: Zygoptera), however, pumping activity is enhanced in water deficient in oxygen. The flow of water from the rectum probably stirs the water around the caudal gills, but it is possible that some gaseous exchange also occurs in the rectum.

Some insects using an air bubble as a gill or that have a plastron also ventilate. For example, the bug *Naucoris* (Hemiptera: Heteroptera) holds onto vegetation and makes swimming movements with

its legs, directing a current over its air bubble; *Notonecta* (Coleoptera) ventilates its plastron with its middle legs.

By changing the frequency with which these movements are performed, the insect is able to maintain its oxygen uptake at a constant level even in water containing small amounts of dissolved oxygen. For example, in *Ephemera*, the gills beat actively in water containing little oxygen and more slowly when the oxygen tension is high (Fig. 17.32). If the oxygen content of the water is very low, however, oxygen consumption falls. Similar changes occur in the rates of ventilatory movements made by a variety of other aquatic insects.

Control of these ventilatory movements appears to depend on similar phenomena to those occurring in terrestrial insects. The ventilatory movements of *Convolvulus* are produced by hypoxia, while those of *Notonecta* are probably stimulated by a high concentration of carbon dioxide in the tracheal system. At least in the former, there is evidence for central pattern generators in the ganglia of abdominal segments 2 and 3, with that in segment 3 being dominant. This drives the interneurons that activate or inhibit the motor neurons.

For insects living in fast-flowing water, active ventilatory movements are not necessary, but their body forms and orientation in the current may be

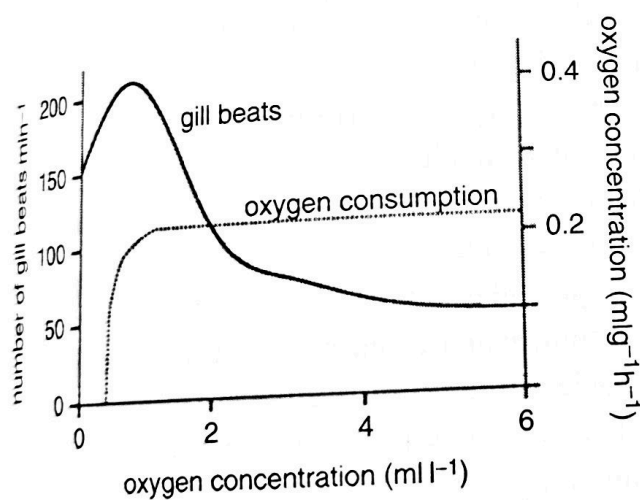


Figure 17.32 Ventilation. The relationship between gill beat and oxygen uptake in relation to the oxygen content of the water in a mayfly larva (*Ephemera*) (after Eriksen, 1963).

important in maintaining the oxygen supply. The cocoons of black flies (Diptera: Simuliidae) are oriented so that their posterior, pointed ends face upstream while the spiracular gills, extending from the open end of the cocoon, are pointed downstream or upwards. The cocoons lie within the boundary layer of water flowing over the stones to which they are attached, and the flow around the cocoon produces vortices around the gills, ensuring a steady supply of oxygen (Fig. 17.33a).

Vortices create regions of low pressure and, if the water is already saturated with air, gas will readily come out of solution, facilitating oxygen uptake by gills. In the extreme, the reduction in pressure may result in air coming out of solution as bubbles. In the pupae of Blephariceridae (Diptera), bubbles of gas appear on the gill surfaces, and this phenomenon of "out-gassing" appears to maintain an air bubble around the adult beetle *Potamodytes* (Fig. 17.33b). It is possible that the facilitation of gas exchange by reduction in water pressure is a widespread phenomenon in aquatic insects living in flowing water.

17.7 Insects subject to occasional submersion

It is relatively common for terrestrial insects to fall into water, but in general this does not occur sufficiently regularly for special respiratory adaptations to have evolved. Insects living close to the edge of water are, however, subject to more frequent alternations of submersion and emergence and so tend to be adapted for respiration in air or water. This is true for insects living intertidally, where submersion occurs regularly, and for those living at the edges of streams, where submersion is much less regular.

Some intertidal beetles, such as *Bledius* and *Dichirotrichus*, effectively avoid submergence by living in burrows and crevices where sufficient air is trapped to enable them to survive aerobically for

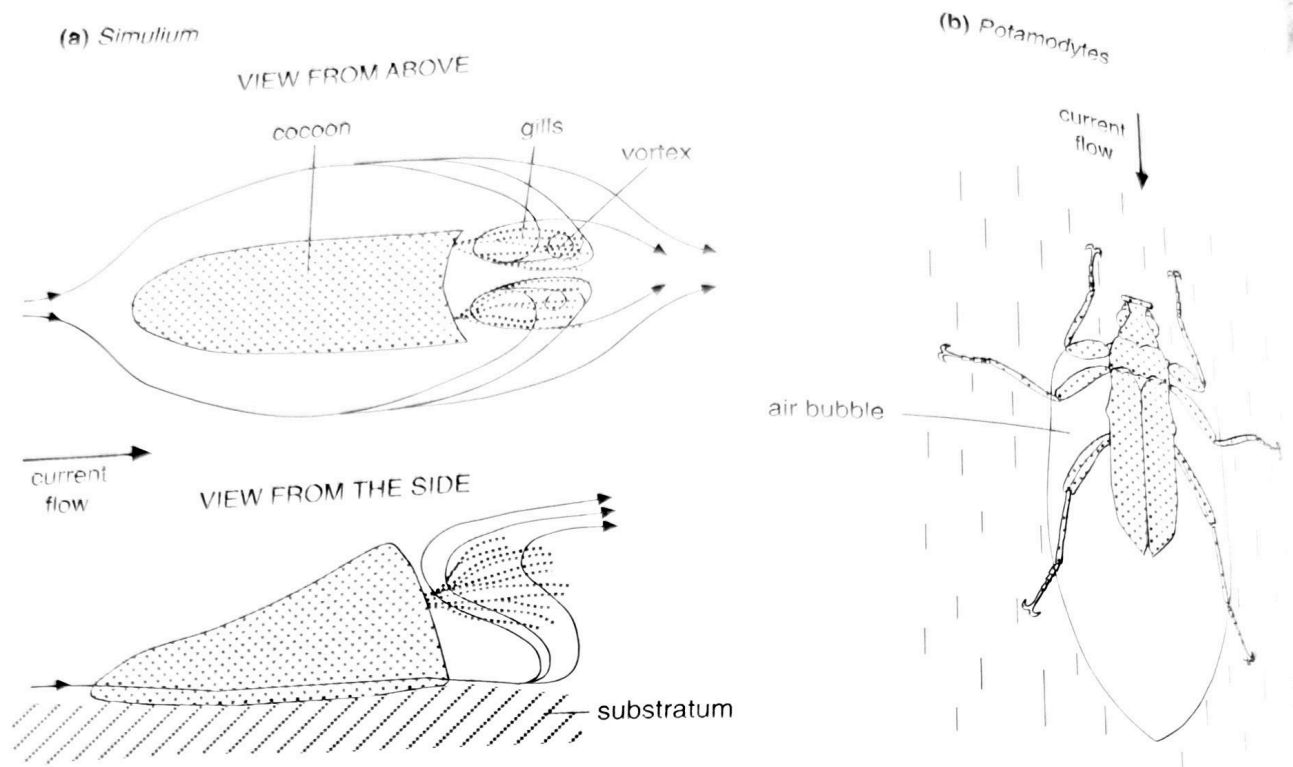


Figure 17.33 Insects in fast-flowing water are oriented with respect to the current. The orientation produces vortices in the flow. These result in regions of reduced pressure so that gases tend to come out of solution. (a) Pupal cocoon of a blackfly (*Simulium*) from above and the side. The orientation of the cocoon in the water current results in the production of vortices around the gills (after Eymann, 1991). (b) The beetle, *Potamodytes*, holds onto the substratum facing into the current. The flow round the legs and body creates regions of low pressure and gases come out of solution to form an air bubble round the posterior part of the insect (after Stride, 1955).

several hours. If they do become submerged they become quiescent as a result of anoxia. The time for recovery when returned to air is then proportional to the period of anoxia. Lice living on aquatic mammals live in the layer of air trapped in the fur, so that these, too, probably encounter no particular respiratory problems.

17.7.1 Spiracular gills

A spiracular gill is an extension of the cuticle surrounding a spiracle and bearing a plastron connected to the tracheal system by aeropyles. In water, the plastron provides a large gas-water interface for diffusion, while in air the interstices of the gill provide a direct route for the entry of oxygen, and water loss is limited because the gill opens into

the atrium of the spiracle. Thus, in air, water loss through the spiracles is scarcely greater than in terrestrial insects.

Spiracular gills occur in the pupal stages of flies and beetles living intertidally or at the edges of streams. They also occur in the larvae of a few Coleoptera and in *Canace* (Diptera). Where they occur in pupae, the basal structure of the gill is modified to permit respiration by the pharate adult (Fig. 17.34). The spiracular gills of most dipteran pupae are prothoracic and connect with the prothoracic spiracles. In the crane fly, *Taphrophila* (Diptera), for example, there is a single gill on each side of the thorax with many branches (Fig. 17.34a). The whole gill is about 1.5 mm long. Blackfly pupae (Diptera: Simuliidae) also have two prothoracic gills. In this case, each has at least two main branches and in some species

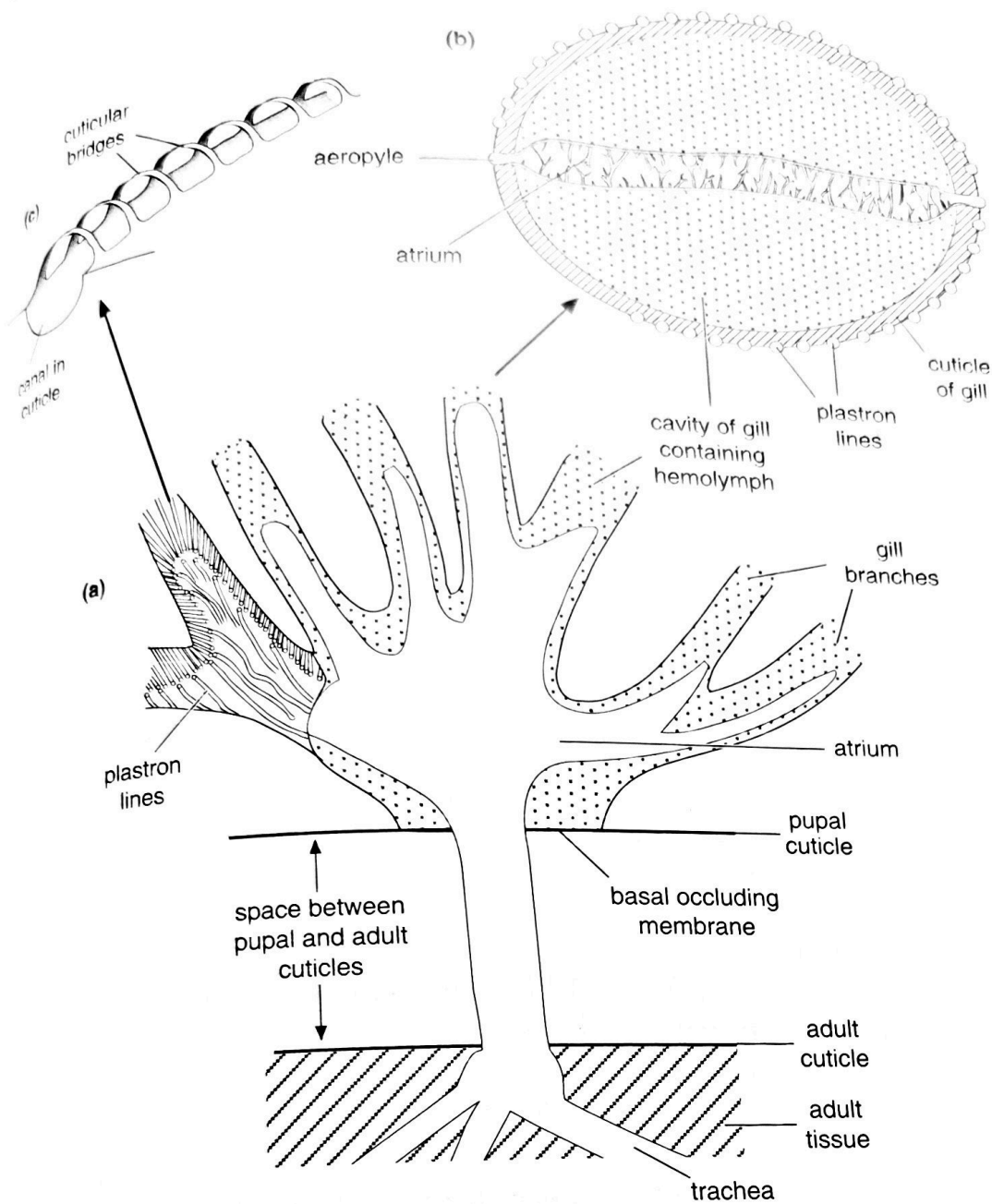


Figure 17.34 Spiracular gills of a pharate adult crane fly (*Taphrophila*) (after Hinton, 1957). (a) Diagram showing basic structure and connection of gill to the tracheal system; the two left-hand branches represent the gill as seen from the outside; the remainder have the upper layer of cuticle removed to show the extent of the atrium. (b) Transverse section through a gill branch. (c) Detail of a plastron line.

is abundant secondary branching. In the pupae of *Psephenidae* (Coleoptera), however, the gills are associated with the abdominal spiracles and in *Psephenoides volatilis* (Coleoptera) they are on abdominal segments 2–7. Each gill has 4–10 long, slender branches. Abdominal spiracular gills also

occur in the larvae of beetles of the genera *Torridincola*, *Sphaerius* and *Hydroscapha*.

The form of the plastron differs in different insects. In *Eutanyderus* (Diptera) and many other species it is held by hydrofuge cuticular struts running at right angles to the surface of the gill. These struts branch at

the apices and the branches of adjacent struts anastomose to form an open network. An extensive air-water interface thus exists in the interstices of the network. In these insects the plastron extends all over the gills and connects with the atrium of the spiracle at the base of each gill.

In *Taphrophila* (Diptera), the spiracular atrium extends into each gill and its branches, and where the atrium meets the wall of the gill it opens to the outside through a series of small pores, the aeropyles, about $4\text{ }\mu\text{m}$ in diameter (Fig. 17.34b). The atrium is flattened in cross-section, with the two walls connected by cuticular struts so that it does not collapse even if the gills dry, and the hydrofuge properties of the struts prevent the entry of water into the atrium. Running from each aeropyle, on the outside of the gill, is a shallow canal about $4\text{ }\mu\text{m}$ wide, which is crossed at intervals of about $1.0\text{ }\mu\text{m}$ by cuticular bridges about $0.5\text{ }\mu\text{m}$ wide (Fig. 17.34c). The lining of the canals is strongly hydrofuge, so that in water each holds a long cylinder of air, known as a plastron line, which is not easily displaced because of the cuticular bridges. The plastron lines provide a relatively large air-water interface over which gaseous exchange occurs.

For the plastron to function efficiently it is important that the gills are turgid when the insects are submerged. In many species of tipulid (Diptera) and in *Psephenoides* (Coleoptera) the gills are rigid, but in other species turgor is maintained by the high osmotic content of the gills, which results from the hemolymph and epidermal cells that they contain. At the pupa-adult apolysis the pupal cuticle becomes separated from that of the adult, but the gills become cut off from the rest of the tissues by a basal occluding membrane so they are still lined by an epithelium and contain hemolymph (Fig. 17.34a). The epithelium within the gills disintegrates and the cells form loose, irregular clusters in the middle of the gill. This isolated tissue is important because it is able to repair damage to the surface of the gill, forming cuticular plugs in any holes that are

produced, and this facility is retained even after the gills have been strongly desiccated. Gill repair is important as a damaged gill loses its high internal osmotic pressure and becomes flaccid and inefficient when in the water.

The gills of pupal *Simulium* (Diptera) are fully expanded in water as they have an opening at the base into which water can freely pass. A thin membrane at the base of each gill in the newly ecdysed pupa bursts due to the intake of water resulting from high internal osmotic pressure. This provides the opening for subsequent water movement into or out of the gill, ensuring that the shape of the gill is independent of the hydrostatic pressures exerted on it.

The efficiency of a plastron depends on its surface area. In spiracular gills the area of the interface varies from $1.5 \times 10^4\text{ }\mu\text{m}^2\text{ mg}^{-1}$ net body weight in the pupa of *Eutanyderus* to $1.9 \times 10^6\text{ }\mu\text{m}^2\text{ mg}^{-1}$ net weight in the pupa of *Simulium*. These areas are presumably large enough to permit efficient absorption of oxygen from well-aerated water. As with other plastrons, the plastron of a spiracular gill resists displacement by high hydrostatic pressures.

17.8 Gas exchange in endoparasitic insects

Endoparasitic insects may obtain their oxygen directly from the air outside the host or by diffusion through the cuticle from the surrounding host tissues. In many ichneumonid and braconid (Hymenoptera) larvae, the tracheal system of the first instar is liquid-filled and, even when it becomes gas-filled, the spiracles remain closed until the last instar. Thus, these insects and the young larvae of most parasitic Diptera depend entirely on cutaneous diffusion. In braconid larvae the hindgut is everted through the anus to form a caudal vesicle. This is variously developed in different species, but in some, such as *Apanteles*, it is relatively thin-walled and closely

associated with the heart (Fig. 17.35) so that oxygen passing in is quickly carried around the body. In these insects the vesicles are responsible for about one-third of the total gaseous exchange. When the tracheal system becomes air-filled, networks of tracheoles may develop immediately beneath the cuticle, thus facilitating the diffusion of gases away from the surface. In *Cryptochaetum* (Diptera), a parasite of scale insects, there are two caudal filaments, which in the third-instar larva are ten times as long as the body and are packed with tracheae. These filaments often get entangled with the host tracheae and so provide an easy path for oxygen transfer.

Other insects, and particularly older, actively growing larvae with greater oxygen requirements, connect with the outside air by penetrating the host's

body wall or respiratory system. The majority of these insects use the posterior spiracles to obtain their oxygen. Chalcidid (Hymenoptera) larvae are connected to the outside from the first instar onwards by the hollow egg pedicel, which projects through the host's body wall. The posterior spiracles of the larva open into the funnel-shaped inner end of the pedicel and so make contact with the outside air. Many tachinid (Diptera) larvae that are parasitic in other insects tap the host's tracheal supply or pierce its body wall from within using their posterior spiracles. The host epidermis is stimulated to grow and spreads around the larva, almost completely enclosing it, and secreting a thin, cuticular membrane over its surface. The larva of *Melinda* (Diptera), parasitic in snails, respire by sticking its posterior spiracles out through the respiratory opening of the snail.

Parasites of vertebrates also often use atmospheric air. The larva of *Cordylobia* (Diptera) bores into the skin and produces a local swelling, but it always retains an opening to the outside into which the posterior spiracles are thrust. Similarly in the larva of the warble fly, *Hypoderma*, the warble opens to the outside, but here the larva bores its way out to the surface from within the host's tissues.

17.9 Other functions of the tracheal system

Apart from gaseous exchange, the tracheal system has a number of other functions. The whole system, and the air sacs in particular, lower the insect's specific gravity. In aquatic insects it also gives some degree of buoyancy and in the larvae of the phantom midge, *Chaoborus* (Diptera), the tracheae form hydrostatic organs enabling the buoyancy to be adjusted.

The tracheal system in some insects has a major role in hemolymph circulation (Section 5.2).

Air sacs, being collapsible, allow for the growth of organs within the body without any marked changes in body form. Thus, at the beginning of the final-stage

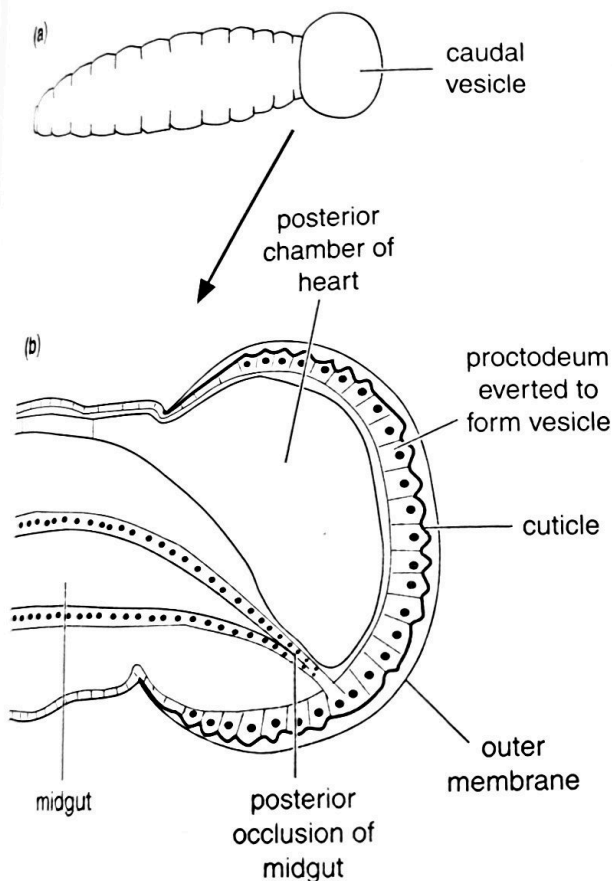


Figure 17.35 Caudal vesicle of a parasitic hymenopteran larva (*Apanteles*) (from Wigglesworth, 1965). (a) Whole larva showing the caudal vesicle. (b) Longitudinal section of caudal vesicle.

larva, the tracheal system of *Locusta* (Orthoptera) occupies 42% of the body volume, but by the end of the stage it only occupies 3.8% due to the growth of other organs causing compression of the air sacs. The air sacs also permit changes in gut volume as a result of feeding. In *Locusta*, the increase in crop volume following a meal is accompanied by a corresponding decrease in the volume of the thoracic air sacs.

In nocturnal moths tracheae form a reflecting tapetum beneath the eye (Section 22.1.2), and tympanal membranes are usually backed by an air sac which, being open to the outside air, allows the tympanum to vibrate freely with a minimum of damping (Section 23.2.7).

Expansion of the tracheal system may also assist when an insect inflates itself after a molt. Thus, in dragonflies, spiracle closure, preventing the escape of gas from the tracheae, accompanies each muscular effort of the abdomen during expansion of the wings.

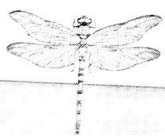
The air sacs of some insects insulate the thorax, and therefore the flight muscles, from the abdomen. This makes it possible for thorax and abdomen to have significantly different temperatures and gives the insects the capacity to regulate thoracic temperature (Section 19.2).

An important general function of tracheae and tracheolar cells is in acting as connective tissue, binding other organs together.

Finally, the tracheal system may be involved in defense. In the cockroach *Diploptera*, quinones, which probably have a defensive function, can be forcibly expelled from the second abdominal spiracle; and in *Gromphadorrhina* (Blattodea) sounds are produced by forcing air out through the spiracles.

17.10 Gas exchange in insect eggs

Gas exchange in insect eggs occurs by diffusion through wax and protein layers. The eggs of many terrestrial insects are subject to occasional flooding and those of many species have a chorionic plastron. Physiological studies suggest that the egg chorion is a high-resistance structure, designed to minimize water loss while allowing adequate oxygen delivery. Older and warmer *Manduca sexta* eggs have lower internal oxygen levels. The oldest, warmest eggs are nearly anoxic internally, and survival and development in these eggs is increased by hyperoxia, indicating oxygen-limitation of development. It seems likely that similar trade-offs between reduction of water loss and uptake of oxygen occur in the eggs of many terrestrial insects, especially those from warm, dry habitats.



Summary

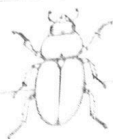
- Gas exchange in insects is facilitated by a tracheal system, an air-filled cuticle-lined invagination. While most insects appear to have intracellular hemoglobin, and some groups of insects have hemocyanin in the hemolymph, the dominant pathway of gas exchange is via the air-filled tracheae. The tracheal system provides a lightweight, high-capacity oxygen-delivery system that enables the highest rates of gas exchange in animals, and recovery from anoxia by diffusion.
- Most insects have spiracles with closing mechanisms that are actively regulated by the nervous system; these respond to oxygen and carbon dioxide levels, and often are coordinated to allow unidirectional flow.

- Most insects utilize convection to move air through the larger parts of the tracheal system. Usually, convection is accomplished by skeletal muscle-driven body volume changes or hemolymph transfers between compartments; these raise local pressures, compressing tracheae and air sacs. Inspiration may be due to passive elasticity or active increases in body volume.
- Tracheal system branching and growth is controlled by interactions between growth factors secreted by the tissues and receptors on the tracheal cells. Local hypoxia stimulates increases in growth factor receptors on the tracheae, increasing diameters, branching and proliferation.
- Aquatic insects commonly modify their tracheal system to enable oxygen uptake from the water, such as via a thin cuticle (gills) or structures that hold air against a spiracle (plastrons or bubbles). Gas exchange across gills is enhanced by pumping mechanisms that move water across the gill.



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