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Alimentary canal, digestion and absorption

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INTRODUCTION

The lumen of the alimentary canal is a portion of the external environment over which the insect has great control, and this control is exerted predominantly by the gut epithelium. The principal functions of the alimentary canal are to process ingested food, mostly by chemical modification but also, in some insects, by mechanical disruption, and then to assimilate the products of digestion. The alimentary canal also comes into contact with toxins and microorganisms, including pathogens, associated with the food. Some of these microorganisms become resident in the gut and are beneficial to the insect. It therefore has to combine three capabilities: to mediate chemical transformations involving enzymes capable of degrading the insect's tissues; to provide an absorptive surface for the assimilation of nutrients and water; and to protect against pathogens and noxious compounds. These diverse capabilities are achieved through regional differentiation of the alimentary tract and integration of structure and function at both the molecular and whole-organ levels. Furthermore, the insect alimentary tracts include spectacular examples of adaptation in structural organization and function to different insect diets and habits.

This chapter is divided into four sections. Section 3.1 describes the structural organization of the insect alimentary tract. It is followed by Section 3.2 on digestion and Section 3.3 on absorption of nutrients, ions and water, and concludes with Section 3.4 on the gut as an immunological organ. Throughout the chapter the variation in structure and function with insect phylogeny and diet is addressed.

3.1 The alimentary canal

3.1.1 General organization

The alimentary canal of insects is divided into three main regions: the foregut (or stomodeum), which is ectodermal in origin; the midgut (or mesenteron), which is endodermal; and the hindgut (or proctodeum), which again is ectodermal (Fig. 3.1). Unlike the multilayered gut wall of vertebrates, all parts of the insect gut consist of a single layer of epithelial cells, bounded by a basement membrane and striated muscle.

Since the foregut and hindgut are ectodermal in origin, the cells secrete cuticle that is continuous with that covering the outside of the body. The lining cuticle is known as the intima. It is shed and renewed at each molt. The midgut is not lined by cuticle, but most insects secrete a non-cellular layer called the peritrophic envelope which separates the epithelial cells from the food.

Usually the gut is a continuous tube running from the mouth to the anus, with regional differentiation and various evaginations, including diverticula in the foregut of some groups, and ceca in the midgut and the Malpighian tubules at the junction between midgut and hindgut in most insects. The connection between the midgut and the hindgut is occluded in some insects that feed on a fluid diet containing little or no solid waste material. This is the case for: some plant-sucking

Heteroptera, where the occlusion is between different parts of the midgut; larval Neuroptera which digest their prey extra-orally; and larvae of social Hymenoptera, which never foul the nest, and deposit a pellet of fecal matter at the larva-pupa molt.

3.1.2 The foregut

The cells of the foregut are usually flattened and undifferentiated since they are not generally involved in absorption or secretion. The cuticular lining tends to be unsclerotized, consisting only of endocuticle and epicuticle, but in many insects sclerotized spines or teeth project from its surface. The spines commonly point backwards, although the exact arrangement varies from species to species and in different parts of the foregut (Fig. 3.2a), and they are believed to assist with the backward movement of food toward the midgut.

The foregut is commonly differentiated into four regions: the pharynx, esophagus, crop and proventriculus.

The pharynx is concerned with the ingestion and backwards passage of food and has a well-developed musculature, which is described further in Section 3.1.6.

The esophagus is usually a simple tube connecting the pharynx and crop. It is often poorly defined in hemimetabolous insects, but in many adult holometabolous insects it is a long, slender tube running between the flight muscles and back to the abdomen. Exceptionally, the esophagus of

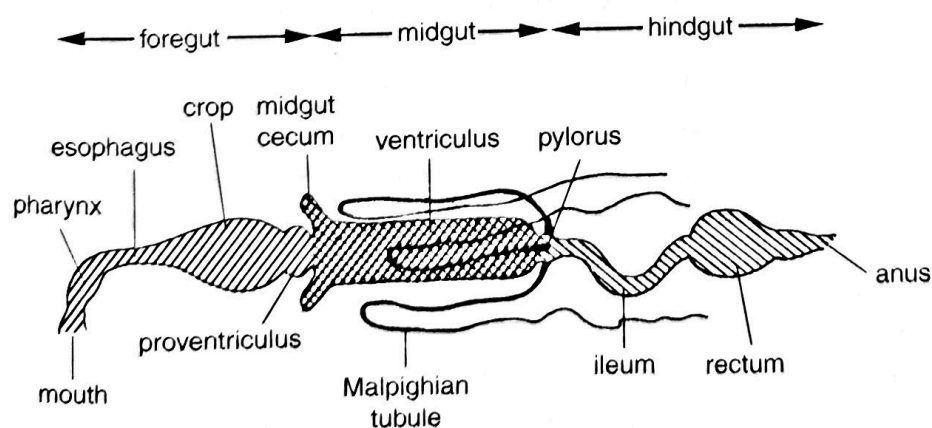


Figure 3.1 Alimentary canal. Diagram showing the major subdivisions in a generalized insect.

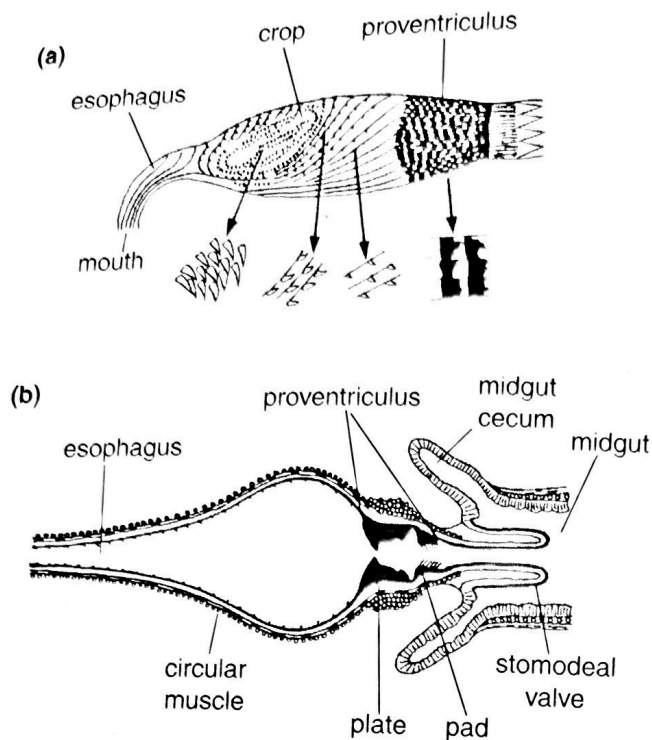


Figure 3.2 Adaptations of the foregut for mechanical disruption of food. (a) Sagittal section through the foregut of a locust showing the pattern of cuticular spines on the intima. Enlargements show details of the spines. In the proventriculus, the spines are replaced by larger sclerotized plates with backward-directed teeth at the posterior edges (after Williams, 1954). (b) Longitudinal section of the foregut of *Periplaneta* showing the development of the proventriculus to form a grinding apparatus (after Snodgrass, 1935).

some insects feeding on highly resinous plants bears single or paired diverticula, in which the resin is stored. Examples include caterpillars of *Myrascia*, which feed on Myrtaceae (Fig. 3.3d) and sawfly larvae in the genus *Neodiprion*, which feed on pines. In *Neodiprion* the diverticula have powerful circular muscles that enable the larva to eject the contents through the mouth in response to attack by natural enemies.

The crop is a storage organ which, in most insects, is an extensible part of the gut immediately following the esophagus (Fig. 3.3a); in adult Diptera and Lepidoptera it is a lateral diverticulum of the esophagus (Fig. 3.3b,c). The walls of the crop are folded longitudinally and transversely. The folds become flattened as the crop is filled, usually

permitting a very large increase in volume. In *Periplaneta*, however, there is little change in volume because when the crop does not contain it is filled with air. The effectiveness of the crop as a store, especially in fluid-feeding insects, depends on the impermeability of its cuticular lining to hydrophilic molecules.

The proventriculus is very variable in form. It often comprises a simple valve at the proximal end of the midgut, projecting a short distance into the midgut lumen. In grasshoppers (Acrididae) it forms a constriction just proximal to the midgut and can limit the movement of solid food from midgut to foregut, while permitting the movement of liquids in both directions. Ants have a specialized proventriculus that separates the partially digested food in the midgut from food in the crop which includes material used in trophallaxis. The proventriculus is also specialized in honey bees (Fig. 3.4), allowing them to retain nectar in the crop while passing pollen grains to the midgut, and modified in the Orthoptera, Blattodea and some Coleoptera to form a grinding apparatus comprising strong cuticular plates or teeth (Fig. 3.2b).

3.1.3 The midgut

The tubular part of the midgut is known as the ventriculus, which often exhibits marked regional differentiation at the anatomical level (e.g., in many Heteroptera) and at the cellular level (e.g., in *Drosophila*). Many insects bear ceca, usually at the proximal end of the midgut (Fig. 3.3). For example: crickets (Gryllidae) and some dipteran larvae have two ceca; Acrididae (grasshoppers) have six; the cockroaches and larval Culicidae (Diptera) have eight; and some Coleoptera and Heteroptera have many ceca that are variable in position.

The cells of the midgut are actively involved in the production and secretion of digestive enzymes and the absorption of nutrients. The majority of the cells are tall and columnar, with the membrane on the luminal side bearing microvilli that can increase

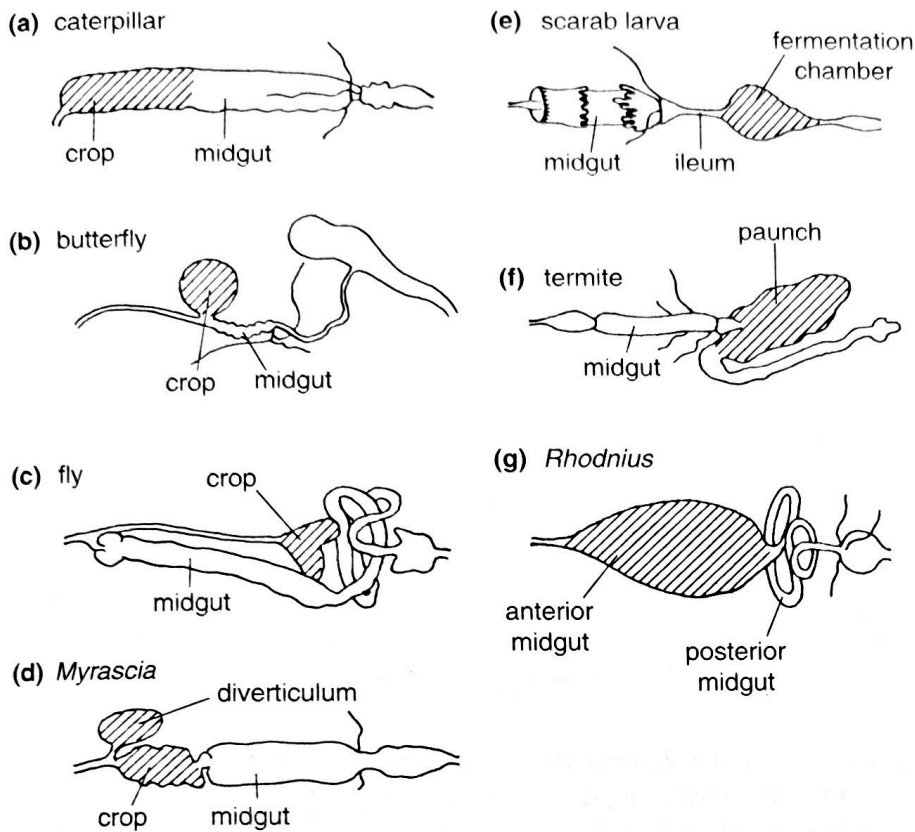


Figure 3.3 Storage in the gut. The different regions in which food or food components are temporarily stored are shown by hatching. (a)–(d) Storage in the foregut. (a) A typical caterpillar; (d) a caterpillar that feeds on resinous plants and stores the resin in the esophageal diverticulum. (e)–(f) Storage in the hindgut. (g) Storage in the midgut.

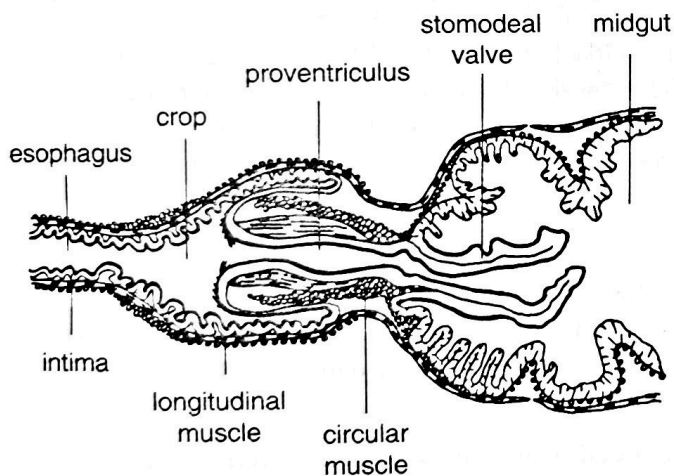


Figure 3.4 Foregut of a worker honey bee in longitudinal section showing the development of the proventriculus. The anteriorly directed part enables the insect to extract pollen grains from nectar in the crop; the posterior part, projecting into the midgut, forms a valve (after Snodgrass, 1956).

the surface area of the cell by up to two orders of magnitude (Fig. 3.5). These cells are called enterocytes (also sometimes termed columnar cells or principal cells), and they mediate the secretion of

digestive enzymes and absorption of nutrients. Other prevalent cell types in the midgut epithelium are enteroendocrine cells, which secrete hormones that regulate the function of the midgut, and intestinal stem cells (Fig. 3.6a), from which the differentiated cells are derived. The stem cells are often located in groups, known as *nidi*. In some beetles the *nidi* are restricted to crypts, which are visible as small papillae on the outside of the midgut (Fig. 3.6b).

The midgut of lepidopteran larvae has a further distinctive cell type, called the goblet cell, in which the luminal cell membrane is invaginated to form a flask-shaped cavity (Fig. 3.7a) bearing microvilli. The goblet cells mediate the alkalinization of the midgut via a $K^+/2H^+$ antiporter that is driven by an electrogenic V-ATPase (Fig. 3.7b). Midgut cells with a large central cavity also occur in Ephemeroptera, Plecoptera and Trichoptera, but their function is not fully established. The role of proliferation and

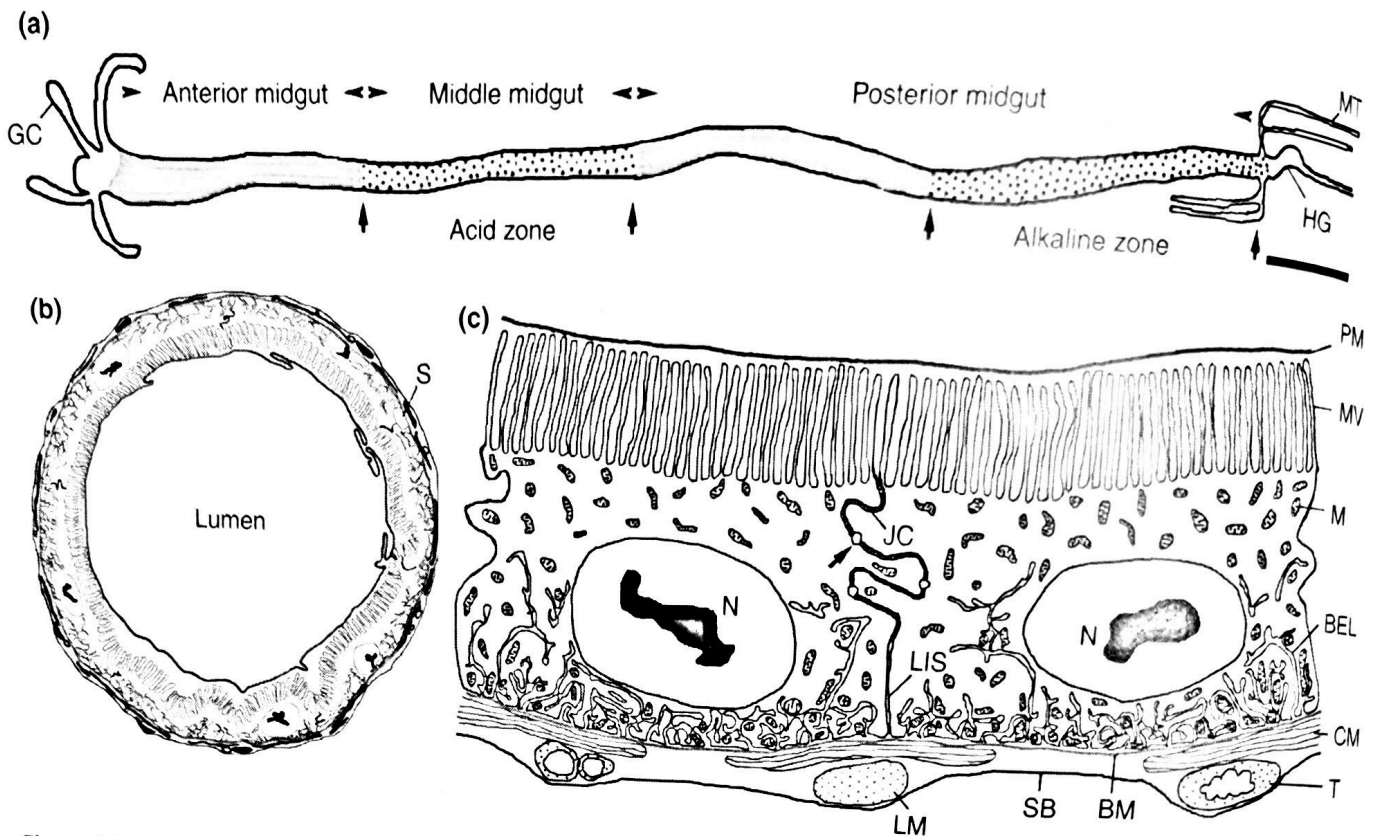


Figure 3.5 Larval midgut epithelium of *Drosophila melanogaster* showing various segments and the intraluminal pH zones. The luminal content of the anterior segment and the anterior part of the posterior segment is between neutral to mild alkalinity (pH >7 and <8); the middle segment is highly acidic (pH <3.0); and the posterior part of the posterior segment is highly alkaline (pH >10). HG, hindgut; MT, Malpighian tubules. (b) Schematic representation of a cross-section of the posterior region of the larval posterior midgut showing the arrangement of peritrophic membrane, epithelial cells, basement membrane, muscle fibers, regenerative cells or stem cells (marked S) and serosal barrier. (c) General organization of two adjacent epithelial cells, lateral dimensions compressed. The arrow denotes very small lateral intercellular spaces. BEL, basal extracellular labyrinth; BM, basement membrane; CM, circular muscle; JC, junctional complex; LM, longitudinal muscle; M, mitochondria; MV, microvilli; PM, peritrophic membrane; SB, serosal barrier; T, tracheole (reproduced from Shanbhag and Tripathi, 2009).

differentiation of the intestinal stem cells in shaping the midgut has been studied in various insects, especially Lepidoptera and *Drosophila melanogaster*. The midgut of adult *Drosophila* is produced at metamorphosis from stem cells present in the larval midgut, by differentiation regulated by EGFR (epidermal growth factor receptor) signaling, and the stem cells continue to divide throughout adult life, generating the enterocytes and enteroendocrine cells, as specified by Notch/Delta and Wingless signaling (Notch promotes differentiation, while expression of *wg* gene in the circular muscles underlying the midgut epithelium sustains the stem cell phenotype). The adult midgut epithelium has a turnover time of 1–2 weeks, with

cells continuously sloughed off into the gut lumen. This phenomenon is promoted by the presence of resident bacteria, which stimulate the production of reactive oxygen species by enterocytes, promoting stem cell proliferation and differentiation.

In *Drosophila* larvae, midgut growth is mediated mostly by increase in cell size. Lepidopteran larvae, in contrast, display bursts of stem cell division just prior to each molt, and the new cells intercalate into pre-existing midgut epithelium. The larval epithelial layer is replaced entirely at metamorphosis by a pupal epithelium (derived, as in *Drosophila*, from larval stem cells) and the pupal epithelium persists through adult life. The midgut epithelium of the larval Lepidoptera undergoes apoptosis and

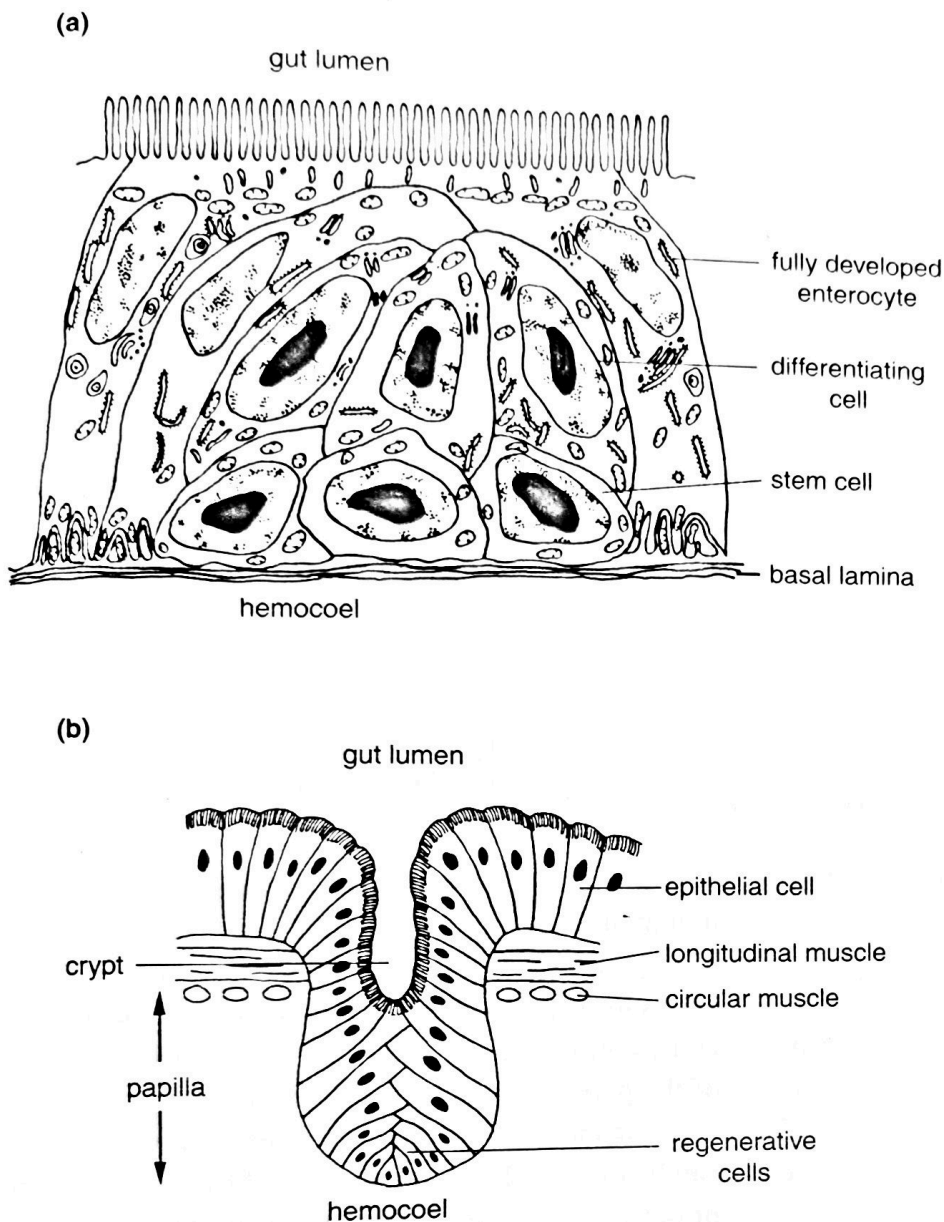


Figure 3.6 Stem cells of the midgut. (a) Group of stem cells (often known as a nidus) at the base of the midgut epithelium, showing the differentiation of enterocytes (after Fain-Maurel *et al.*, 1973). (b) Midgut crypt of a beetle extending through the muscle layer to form a papilla (after Snodgrass, 1935).

autophagy, so recycling nutrients, and the remnant is discarded as meconium at adult eclosion.

The role of enterocytes in enzyme production and release is evident at the subcellular level. When they are synthesizing enzymes, the enterocytes have conspicuous stacks of rough endoplasmic reticulum and Golgi bodies. In most insects, synthesis appears to occur at the time of secretion into the gut lumen so that stores of enzymes do not accumulate in the cells (see Section 3.2.7). In some insects, including the blood-feeding dipteran *Stomoxys calcitrans* (stable fly), enzymes are stored, probably as inactive

precursors (zymogens), in membrane-bound vesicles in the distal parts of the cells, and then secreted within a few minutes of feeding.

Different routes of enzyme secretion have been reported. Membrane-bound vesicles may move to the periphery of the cell, fuse with the cell membrane and release their contents into the gut lumen by exocytosis. The gut principal cells have also been interpreted to display apocrine secretion, i.e., the pinching-off of cytoplasmic extensions of the cell, carrying their contents into the gut lumen and ultimately releasing them, but this putative process is

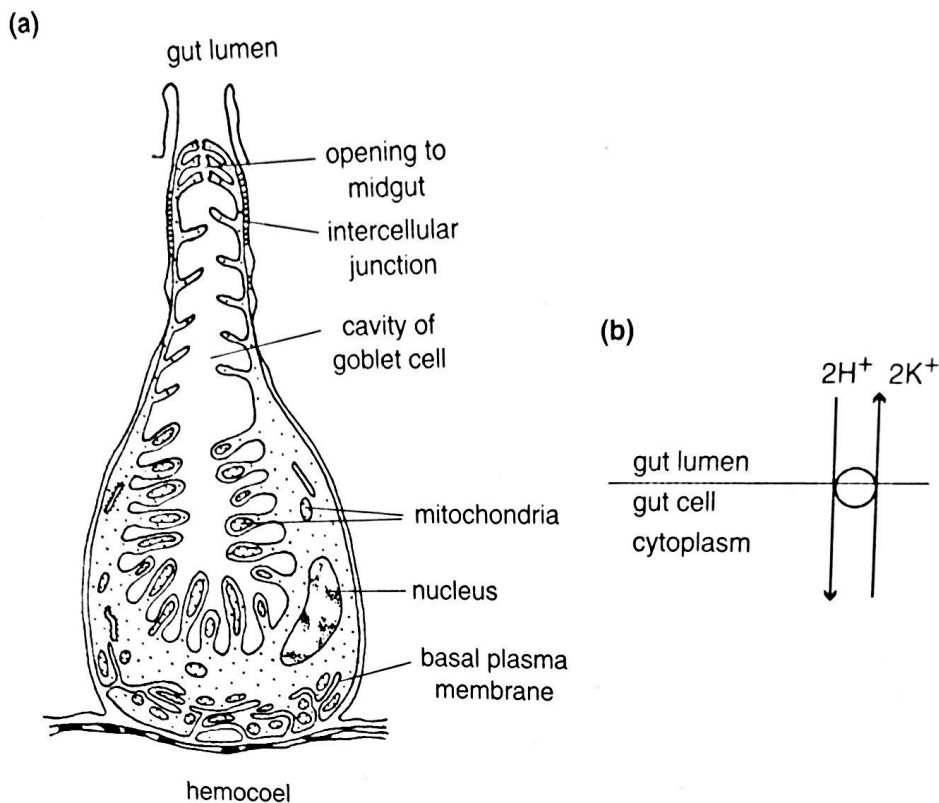


Figure 3.7 The goblet cell and its role in determining the physicochemical conditions in the gut lumen of the caterpillar midgut. (a) A goblet cell (after Cioffi, 1979); (b) ion exchange mediated by K^+/H^+ antiporter at the apical plasma membrane (after Lepier *et al.*, 1994).

contentious because the principal evidence is microscopical and could be an artifact of fixation methods. Intracellular vesicles containing enzymes may also be transported to the microvilli, from which they bud off before releasing their contents. Different enzymes may leave a single cell by different routes, and different methods of secretion may occur in one insect.

3.1.4 The peritrophic envelope

The midgut of most insects is separated from the main volume of the gut lumen by a non-cellular structure called the peritrophic envelope (also known as the peritrophic membrane or peritrophic matrix). The composition and mode of formation of the peritrophic envelope varies among insects, but it generally comprises a proteoglycan gel on a framework of chitin microfibrils.

The chitin chains in the peritrophic envelope are mostly α -chitin, which comprises antiparallel microfibrils and is aggregated into larger bundles that confer considerable tensile strength. The

dominant chitin synthetase in midgut cells of *Manduca sexta* is the product of the *chs2* gene, and is localized to the apex of midgut cell microvilli, consistent with its role in the production of the chitin of the peritrophic envelope. The proteins associated with the envelope vary in the ease with which they can be extracted: some are released by simple buffers or detergent, while others require strong denaturants (e.g., urea) to be isolated or are apparently covalently linked to the chitin microfibrils. Most research has been conducted on one group of proteins, known as the peritrophins, which can be extracted in urea. The peritrophins have chitin-binding domains and six, eight or ten cysteine residues that form disulfide bridges, creating a binding pocket in which hydrophobic residues form hydrogen bonds with the chitin microfibrils. (The peritrophins lack the RR motif characteristic of many chitin-binding proteins of the exoskeleton – see Chapter 16.) Some peritrophins are extensively glycosylated, with the carbohydrate accounting for up to 50% of the total molecular mass. These proteins are called

invertebrate intestinal mucins (IIMs), and they include proteins structurally similar to a group of proteins, the mucins, in vertebrate mucus.

The peritrophic envelope is akin to a molecular sieve, in which the pores are aqueous channels crossing the proteoglycan gel. The dimensions of these pores have been investigated by electron microscopy and permeability studies with compounds of known size. For example, studies with fluorescently labeled dextrans of different dimensions suggest that the peritrophic envelope of several larval *Lepidoptera* is permeable to 21–29 nm diameter molecules, and the envelope of *Orthoptera* to 24–36 nm molecules. The peritrophic envelope is permeable to inorganic ions, small organic molecules (sugars, amino acids, etc.), peptides and small proteins, but is an effective barrier to the passage of lipids, large proteins and polysaccharides.

The peritrophic envelope of many insects can be categorized as either Type I, produced by the entire length of the midgut, or Type II, produced by specialized tissues called cardia in the anterior midgut, but there is much variation within each type.

The Type I peritrophic envelope is found in *Coleoptera*, *Blattodea*, *Ephemeroptera*, *Hymenoptera*, *Odonata*, *Orthoptera*, *Phasmida*, larval *Lepidoptera* and adult *Diptera*. Production of the peritrophic envelope is not synchronized over the whole midgut but is made up of patches that join together. In some species the microfibrils forming the laminae are assembled at the bases of the microvilli, which form a template for the developing grid of chitin microfibrils (Fig. 3.8a,b), a process known as delamination. In an actively feeding locust a new lamina is produced about every 15 minutes, so the peritrophic envelope becomes multilaminar. The inner layers

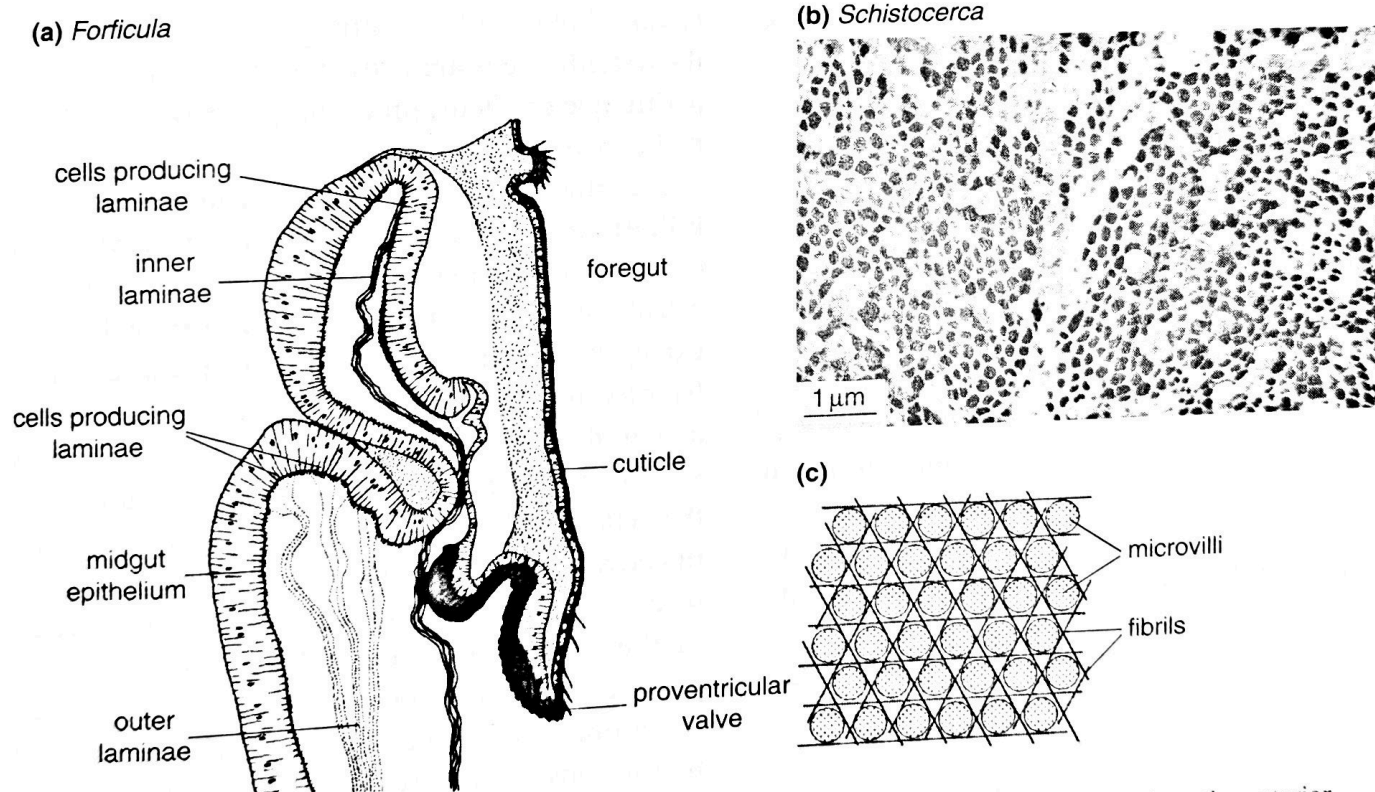


Figure 3.8 Peritrophic envelope. (a) Diagram showing the origin of the peritrophic envelope in cells at the anterior end of the midgut of *Forficula* (after Peters *et al.*, 1979). (b) Scanning electron micrograph of the peritrophic envelope of *Schistocerca*. The envelope has been washed so that only the chitinous lattice remains (after Chapman, 1985). (c) Diagram showing how the fibers which will form the lattice of the peritrophic envelope are laid down round the microvilli.

move back with the food as more laminae are produced, with the result that the envelope has more laminae in the distal midgut than in the proximal midgut. The robustness of the envelope varies from species to species. For example, among the Acrididae (grasshoppers), *Locusta*, which is graminivorous, has a thin, delicate envelope, whereas *Schistocerca*, a polyphagous species, has a much thicker and tougher envelope.

The Type II peritrophic envelope is characteristic of Dermaptera, Isoptera, some Lepidoptera and larval Diptera. In larvae of the mosquito, *Aedes*, the most anterior 8–10 rings of cells in the midgut epithelium (just 300–400 cells) produce the entire peritrophic envelope, which comprises a single lamina about 0.7 μm thick with outer and inner granular layers bounding the chitin microfibrils. The earwig, *Forficula*, in contrast, has several separate rings of cells in the most anterior part of the midgut (Fig. 3.8c). The anterior rings produce up to four separate laminae in which the microfibrils form grids, and the posterior rings produce several more very thin laminae in which the microfibrils are randomly oriented. The complete peritrophic envelope of *Forficula* thus consists of several layers of the two types of lamina.

The peritrophic envelope functions as a barrier, separating the midgut cells from the ingested food. In this way it can protect the gut wall from damage by ingested material, including abrasive food particles, pathogens and certain toxins. In addition, the peritrophic envelope separates the gut lumen into two compartments: the endo-peritrophic space (between the peritrophic envelope and the midgut cells) and ecto-peritrophic space (in the gut lumen), so permitting compartmentalization of enzyme activities and the generation of a countercurrent flow of fluids that increases the efficiency of absorption (see Section 3.3.5).

The role of the peritrophic envelope in mechanical protection appears self-evident, and is illustrated by research on a mutant of *Bombyx mori* that cannot produce a larval peritrophic membrane: the midgut

epithelium of these insects was severely damaged. Chemical protection is illustrated by various phytophagous insects, including *Schistocerca* locusts and some larval Lepidoptera, in which the peritrophic envelope forms an effective barrier against tannins, a major constituent of many plants on which these insects feed. Tannin molecules are much smaller than the pores in the envelopes of these species, and how these molecules are retained by the envelope is not fully resolved. The peritrophic envelope of some insects, however, confers no protection against tannins. For example, the envelope of *Locusta* is permeable to tannic acid; when these insects ingest food containing tannic acid they incur major lesions in the midgut epithelium, resulting in high mortality. The peritrophic envelope also protects against many potential microbial pathogens. For example, young honey bee larvae, in which the envelope is not yet developed, are highly susceptible to American foulbrood – a disease caused by *Paenibacillus larvae* – but older larvae are resistant, partly because the bacteria are restrained by the well-developed peritrophic envelope, preventing their access to the midgut epithelium.

The critical importance of the peritrophic envelope is illustrated by experiments in which the envelope is experimentally eliminated by feeding insects on calcofluor, an inhibitor of chitin synthetase. For example, when the lepidopteran *Trichoplusia ni* was fed on calcofluor, most of the insects died as larvae, and the development time of the surviving larvae was more than doubled. It is no surprise, therefore, that the peritrophic envelope of phytophagous insects is the target of plant defenses. For example, maize plants resistant to *Spodoptera frugiperda* produce a 22 kDa cysteine protease that disrupts the peritrophic envelope of this insect species.

Peritrophic envelopes are apparently not produced by some insects. For example, they are absent from adult mosquitoes, except after a blood meal ingested by females, and unknown among nectar-feeding adult Lepidoptera. Exceptionally among insects, the Hemiptera and Thysanoptera lack a peritrophic

envelope but possess, instead, a lipoprotein layer called the perimicrovillar membrane, which lies external to the midgut cells. In most hemipteran groups, the perimicrovillar membrane forms complex folds, so appearing as multiple layers in cross-section, and extends both into the gut lumen and between individual microvilli of the midgut cells. In aphids it is attached to the apex of midgut cells by structures similar to septate junctions. The origin of the perimicrovillar membrane remains to be established definitively, but is proposed to be derived from membrane synthesized in the Golgi vesicles of midgut cells. In all species tested, the perimicrovillar membrane bears α -glucosidase activity.

3.1.5 The hindgut

The hindgut is usually differentiated into three regions: the pylorus, ileum and rectum (Fig. 3.1).

As with the foregut, the hindgut is bounded by cuticular intima, but the hindgut intima is generally very thin ($\sim 10 \mu\text{m}$) and much more permeable than that of the foregut.

The pylorus is the site of entry of the Malpighian tubules to the gut, and the pylorus is, therefore, the zone in which food residue from the midgut and secretions from the Malpighian tubules are mixed. In many insects, the pylorus is bounded by circular muscle and also acts as a sphincter, controlling the passage of food from midgut to hindgut and preventing reflux of the hindgut contents back into the midgut.

In most insects the ileum (also sometimes referred to as the intestine) is a narrow tube between the pylorus and the rectum. Sometimes the distal part of the ileum is recognizably different and is called the colon. In many insects, only a single cell type is present in the ileum. The cells have extensive folding of the apical plasma membrane with abundant closely associated mitochondria. The basal plasma membrane may also be folded, although the folds are less extensive than those apically (Fig. 3.9). Among insects that have symbiotic microorganisms in their

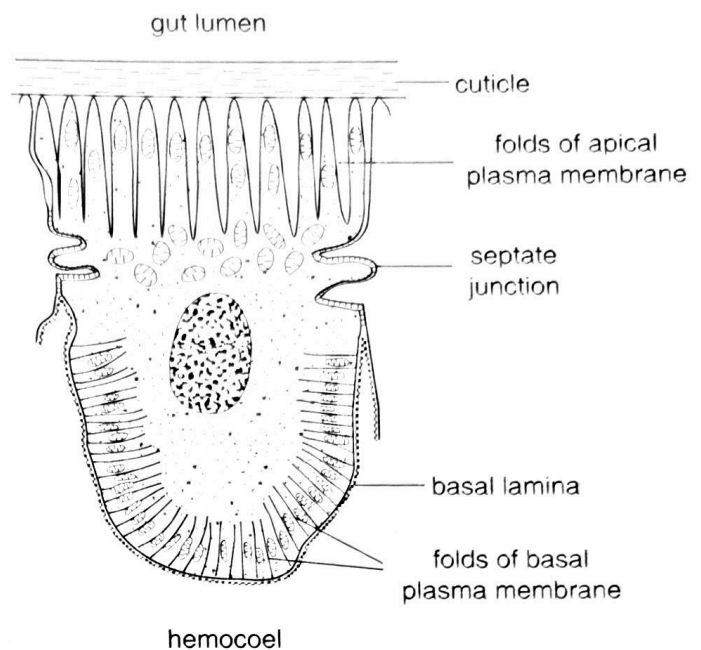


Figure 3.9 Cell of the ileum (part of the hindgut) of *Schistocerca* (based on Irvine *et al.*, 1988).

hindgut, the ileum is expanded to house the microbes, forming the “paunch” in termites and “fermentation chamber” in larval Scarabaeidae (chafers and dung beetles) (Fig. 3.3e,f). The cuticle lining these chambers is produced into elongate spines or bulbous lobes which probably serve for the attachment of microorganisms. Similar structures are present on the ileal cuticle of cockroaches and crickets that have a bacterial flora.

The rectum is usually an enlarged sac with a thin epithelium except for certain regions, the rectal pads, in which the epithelial cells are columnar. The rectal pads are the primary site of water absorption from the hindgut, and the cuticle in these structures is unsclerotized and much thinner than at other regions of the rectum, e.g., just $1 \mu\text{m}$ thick in cockroaches. There are usually six rectal pads arranged radially around the rectum (Fig. 3.10a), and they may extend longitudinally along the rectum or they may be papilliform, as in the Diptera. The epithelial cells in each rectal pad are isolated from the hemocoel by sheath cells and a basal cellular layer (Fig. 3.10b) or, less commonly, by a pad of connective tissue, as for example in the blowfly (Calliphoridae). Consistent

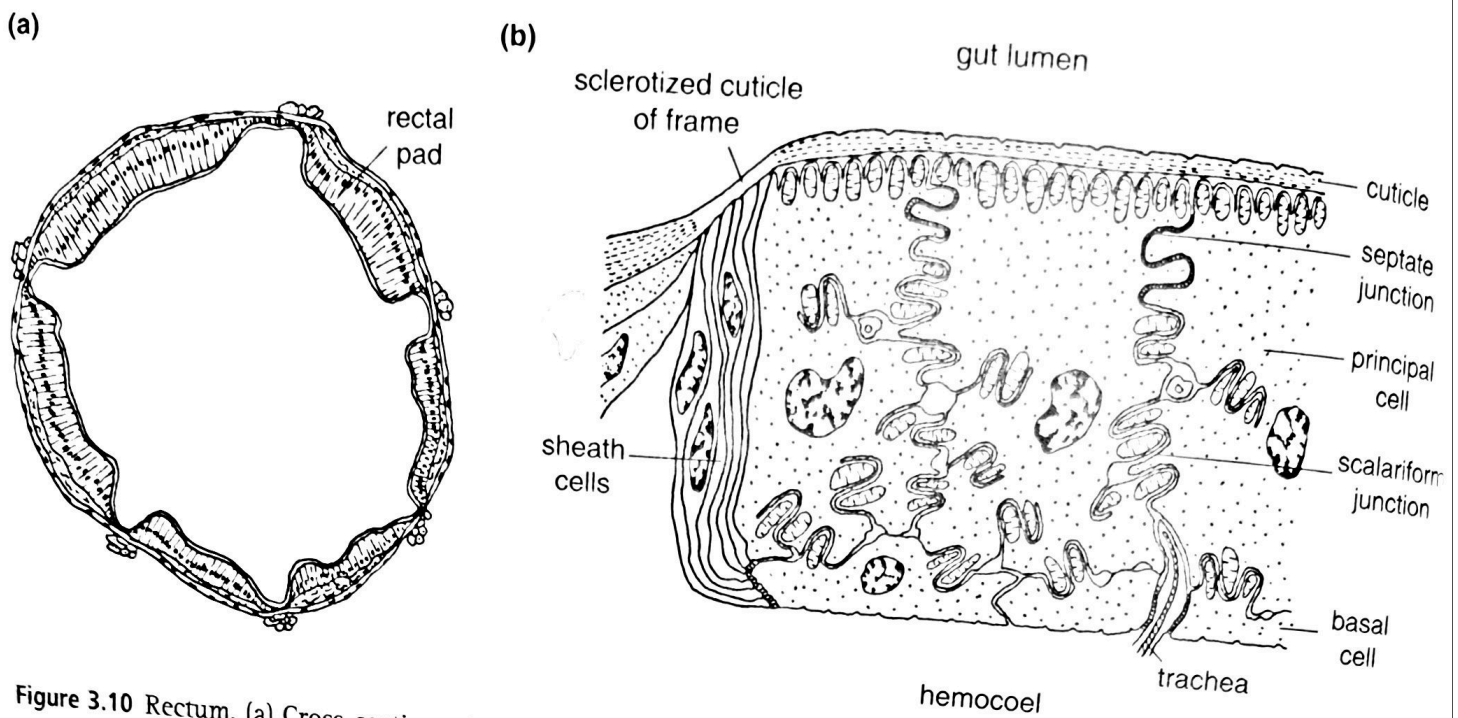


Figure 3.10 Rectum. (a) Cross-section of the rectum of a grasshopper showing the six rectal pads. (b) Section through a rectal pad (after Noirot and Noirot-Timothee, 1976).

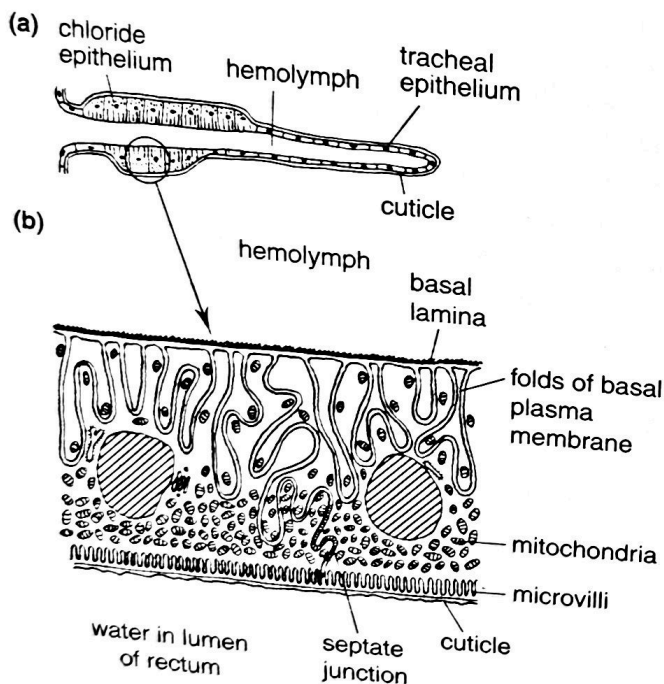


Figure 3.11 Chloride cells in the rectum of a dragonfly larva (Fig. 17.29 shows the position of the gills within the rectum). (a) Section of a rectal gill showing the position of the chloride epithelium. Note that tracheal epithelium refers to the epithelium of the gill through which gas exchange with the tracheae (not shown) occurs. (b) Details of the chloride cells. Notice that the luminal (water) side of the gill is at the bottom (after Schmitz and Komnick, 1976).

with the role of the rectal pads in water absorption, the cell membrane of the epithelial cells is thrown out into folds enclosing mitochondria, and the cells are extensively supplied with tracheoles.

The rectum is the site of respiratory gas exchange in some aquatic insects, notably larvae of the Anisoptera (dragonflies, Odonata), in which the proximal rectum is modified to form a branchial chamber. The gills are repeatedly branching filaments, with the terminal branches just 5–10 μm in diameter, and heavily invested with tracheoles. At the base of each gill are chloride cells, which actively take up Cl^- and other inorganic ions from the water as it is pumped in and out of the rectum during respiration. The chloride cells have microvilli at the apical margins beneath a very thin cuticle, and large numbers of mitochondria in the adjacent cytoplasm (Fig. 3.11).

3.1.6 Muscles of the gut

The muscles of the alimentary canal fall into two categories: extrinsic visceral muscles that arise on the body wall and are inserted into the gut, and intrinsic visceral muscles that are associated only with the gut. The intrinsic visceral muscles comprise circular and

longitudinal muscles. The circular muscles are not usually inserted into the gut epithelium, but are continuous all around the gut so that their contraction tends to produce longitudinal folding of the epithelium. The longitudinal muscles extend proximally–distally along parts of the alimentary canal. Ultrastructurally, the insect visceral muscles are striated (unlike vertebrate visceral muscles) because their myofilaments are regularly arranged. The extrinsic visceral muscles resemble typical skeletal muscles (see Chapter 10), but the intrinsic muscles have reduced sarcoplasmic reticulum and T-system and have 12 actin filaments around each myosin.

Extrinsic visceral muscles are localized in the foregut and hindgut and generally function as dilators of the gut. Those in the head form pumps which suck fluids into the gut and push food back to the esophagus. The muscles inserting at the pharynx arise on the frons and the tentorium, and those inserting at the cibarium arise on the clypeus. The pharyngeal muscles are antagonized by intrinsic circular muscles. The cibarium has no compressor muscles and compression results from the elasticity

of the cuticle lining of the cibarium. Insects with biting and chewing mouthparts generally force food toward the midgut by relatively weakly developed dilator muscles associated with the pharynx, while fluid-feeders utilize one or both of the cibarial and pharyngeal pumps. In most Hemiptera, Thysanoptera and Diptera, the cibarial pump is well developed, while the muscles of the pharyngeal pump are often relatively weakly developed (Fig. 3.12a); in adult mosquitoes, larval *Dytiscus* (Coleoptera) and probably also adult Lepidoptera and bees, both pumps are well developed (Fig. 3.12b,c,d). Exceptionally, in Hemiptera feeding on fluid foods under high hydrostatic pressure (e.g., plant phloem sap), the function of the extrinsic musculature and cibarium is modulated to act not as a pump but as a valve that controls and limits food uptake.

The extrinsic visceral muscles of the hindgut are usually present as dilators of the rectum. They are especially well developed in larval dragonflies that pump water over the gills in the branchial chamber of the rectum (see Fig. 17.29a).

The intrinsic muscles tend to be well developed in the foregut of most insects. The circular muscles are

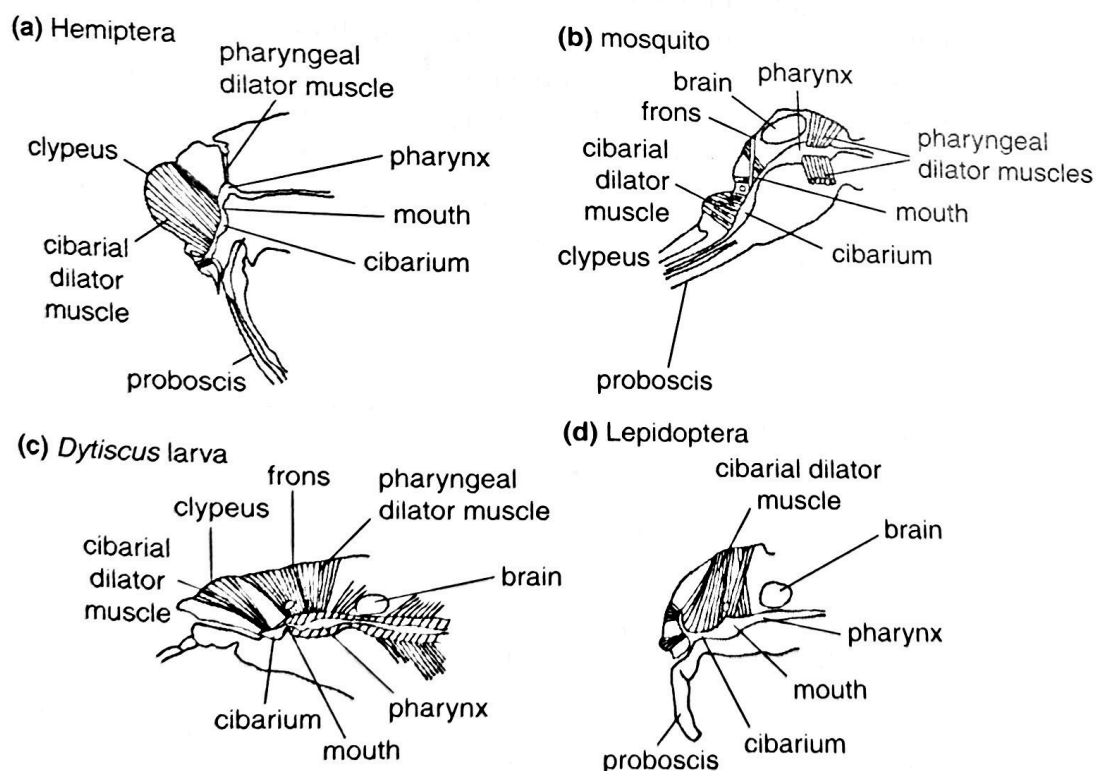


Figure 3.12 Foregut muscles in fluid-feeding insects. The cibarial muscles are well developed in all these insects, but the pharyngeal muscles are also important in larval *Dytiscus* and mosquito (after Chapman, 1985).

external to the longitudinal muscles, and they are well developed around the pharynx (acting as antagonist to the extrinsic muscles). They are also significant around the proventriculus where this forms a valve or has a grinding function. Generally, intrinsic muscles are poorly developed in the midgut. The main longitudinal muscles are outside the circular muscles, and appear to extend for the full length of the midgut without any insertions into it; they are inserted anteriorly into the posterior end of the foregut and posteriorly into the anterior end of the hindgut. The intrinsic musculature of the hindgut is complex in caterpillars, and probably in other insects. There are commonly strong muscles associated with the pyloric valve, where the Malpighian tubules join the gut. Other specific arrangements enable the insect to produce discrete fecal pellets from the cylinder of food passing along the gut. Usually the circular muscles are outside the longitudinal muscles.

3.1.7 Innervation of the gut

The muscles of the foregut and anterior midgut are innervated by the stomodeal (or stomatogastric) nervous system. The principal ganglion of this system is the frontal ganglion, which lies on the dorsal wall of the pharynx anterior to the brain (Fig. 3.13). It connects with the tritocerebrum on either side by nerves called the frontal connectives. In Orthoptera and related groups, a median nerve,

the recurrent nerve, extends back from the frontal ganglion beneath the brain to join with the hypocerebral ganglion. This ganglion is closely associated with the corpora cardiaca, and from it a nerve on each side passes to the ingluvial ganglion on the side of the crop. From this ganglion, nerves extend back to the midgut. The cell bodies of most the motor neurons controlling the muscles are in the ganglia of the stomodeal system, but a few are in the tritocerebrum. Central pattern-generator circuits in these ganglia coordinate the pattern of muscular contraction, which helps move food through the gut and plays a role in molting. The muscles and other tissues of the hindgut are innervated from the terminal abdominal ganglion. Various biogenic amines and neuropeptides are released from neuroendocrine cells innervating the gut, as well as from enteroendocrine cells, and serve to regulate gut motility and digestive enzyme secretion. It has been proposed that some of these neuroendocrine cells are chemoreceptive, detecting nutrients within the gut lumen and triggering release of digestive enzymes via neuropeptides. Multipolar cells, which function as stretch receptors, are present on the outside of the gut in many insects and are involved in monitoring gut fullness. They are most abundant on the foregut, but are also present on the mid- and hindgut. Trichoid and campaniform mechanoreceptors are present in the terminal region of the fermentation chamber of scarab larvae.

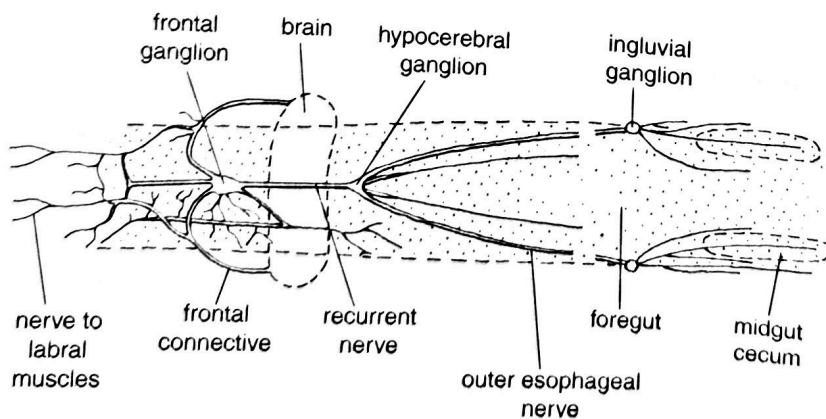


Figure 3.13 Stomodeal nervous system of a grasshopper (dorsal view) (based on Anderson and Cochrane, 1978, and R. Allum, unpublished).

3.1.8 The passage of food through the gut

Food is pushed back from the pharynx by the muscles of the pharyngeal or cibarial pump, and subsequently passed along the gut by peristalsis. These movements are controlled from the stomodeal nervous system and are modulated by various neurohormones. For example, when *Locusta* feeds after an interval of several hours without food, the solid food remains in the foregut while the insect is feeding. Backward movement of the food bolus begins shortly after the foregut becomes fully distended. This is at least partly controlled by a hormone from the corpora cardiaca.

In the females of blood-sucking flies that feed on both blood and nectar, the destination of the food is determined by its chemical composition, detected by the receptors on the mouthparts. Stimulation with sugars at high concentrations causes the meal to be directed to the crop, which in flies is a lateral diverticulum of the esophagus (Fig. 3.3c). Stimulation with ATP, ADP or dilute sugar solutions results in the meal going to the midgut. In the blowfly, *Phormia*, and probably also the cockroach, *Periplaneta*, crop emptying is regulated by the osmotic pressure of the hemolymph. At high hemolymph osmotic pressures, the food is passed more slowly to the midgut than at lower pressures. Hemolymph osmotic pressure is affected by that of the food because the sugars are rapidly absorbed. Consequently, meals of concentrated sugars are retained in the crop for longer periods than meals of dilute sugars.

In the midgut of most insects, the passage of food is aided by the peritrophic envelope which, as it moves down the gut, carries the enclosed food with it. Spines on the intima of the hindgut aid the backwards movement of the envelope in insects which possess them. Blood-sucking bugs, such as *Rhodnius* and *Cimex*, and flies, such as mosquitoes and stable flies, which take large, infrequent meals (Table 2.2) store food in the anterior midgut (Fig. 3.3g), where water is absorbed but no digestion occurs. The anterior midgut also acts as a temporary food store in plant-sucking bugs.

The movements of the hindgut are important in the elimination of undigested material. In *Schistocerca* the ileum usually forms an S-bend, and at the point of inflexion the muscles constrict the gut contents. This probably breaks the peritrophic envelope and the food column so that a fecal pellet is formed. Contractions of the distal part of the ileum and the rectum force the pellet out of the anus. In grasshoppers the feces are enclosed in the peritrophic envelope, but in caterpillars the envelope is broken up in the hindgut.

The time taken for food to pass through the gut is very variable. In a grasshopper with continuous access to food, liquids from the food reach the midgut and are absorbed within five minutes of the start of a meal, but solid particles may take 15 minutes or more. The food from one meal has usually left the foregut in about 90 minutes, being pushed back by newly eaten food, and by this time some of the meal is present in the hindgut and may appear in the feces (Fig. 3.14). In the absence of more food, the foregut is completely empty in about five hours and the midgut is empty after about eight hours. In *Periplaneta* solid food passes through the gut in about 20 hours, but some can still be found in the crop after some days of starvation.

Termites and larval scarab beetles feeding on wood and other intractable plant material retain food fragments for long periods in the hindgut, where their microorganisms digest the cellulose (see Section 3.2.4).

3.2 Digestion

3.2.1 General patterns in insect digestion

Digestion refers to the chemical transformation of large and complex molecules in food to smaller molecules that can be absorbed across the gut wall for insect nutrition. Digestion is mediated principally by enzymes, the profile of which has coevolved with the diet of the insect and the

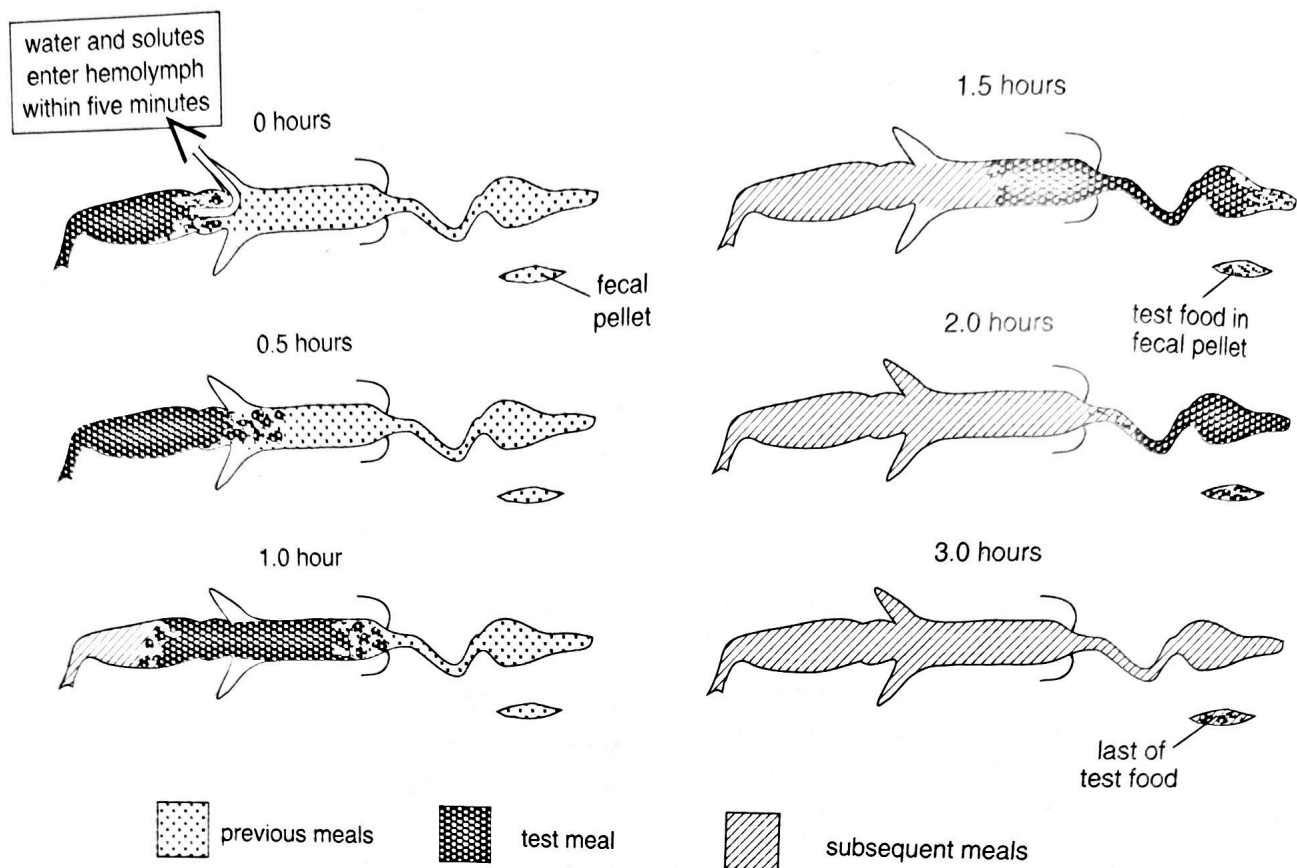


Figure 3.14 Movement of food through the gut of a grasshopper with continuous access to food (after Baines *et al.*, 1973).

physicochemical conditions (especially pH) in the insect gut. All insects have intrinsic digestive enzymes – i.e., enzymes produced by the insect. These may include proteinases, carbohydrases and lipases. In some insects, however, refractory materials, especially plant cell wall material, are not digested by intrinsic enzymes, but broken down by microbial fermentation. The incidence of digestion by intrinsic enzymes versus microbial fermentation cannot be inferred exclusively from the insect diet, beyond the generality that insects on digestively tractable diets, e.g., nectar or animal flesh, use intrinsic enzymes exclusively. Among species using plant material, some digest and assimilate the easily utilized compounds (sugars, starch, protein, etc.) using intrinsic enzymes and void the remainder, including the cellulose. Others utilize the plant cell wall material by one or both of intrinsic degradative enzymes in the midgut and microbial fermentation in the hindgut. The capacity to degrade refractory

plant material is more widespread among insects feeding on wood or detritus (e.g., termites, silverfish and woodroaches) than among insects, such as caterpillars and grasshoppers, feeding on living plant tissue (leaves, grass, etc.).

Digestion by intrinsic enzymes occurs principally in the midgut of most insects (Section 3.2.3). Nevertheless, digestion prior to ingestion by salivary enzymes is important in some groups (Section 3.2.3), and some insects (notably Orthoptera and carabid beetles) have appreciable enzymatic activity in the foregut, presumably mediated by ingested salivary enzymes and the forward movement of enzymes from the midgut. Microbial digestion is almost invariably restricted to the hindgut, distal to insect digestion and absorption. In this way, the insect host can prevent microbial consumption of food items utilizable by the insect host.

The key conditions influencing digestive function are temperature, pH and redox potential.

Enzymatic reactions increase with increasing temperature, from undetectable activity in cold conditions to a maximum, above which the enzyme is progressively denatured and activity declines rapidly to zero (Fig. 3.15). The maximum activity of most insect digestive enzymes *in vitro* is in the 35–45°C range, even in insects that are intolerant to such high temperatures. In other words, the thermal limit of many insects is determined by factors other than the thermal profile of their digestive enzymes. The activity of digestive enzymes in insects is very low or undetectable at temperatures lower than 10°C.

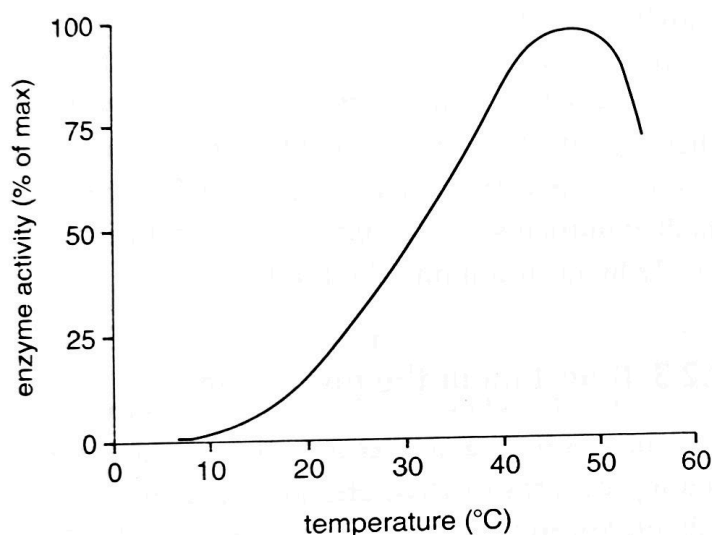


Figure 3.15 Enzyme activity in relation to temperature. The activity of the α -glucosidase of a locust (after Evans and Payne, 1964).

The pH of the gut varies along the length of the insect gut. In many insects the pH range is relatively small – e.g., between 5.8 and 7.3 in the midgut of *Locusta* and other Orthoptera. Insects generally do not have a strongly acidic gut region equivalent to the vertebrate stomach. Exceptionally, the middle midgut region of the cyclorhaphous Diptera (including *Drosophila*) is pH 3–4 (Fig. 3.5), and this has been interpreted as an adaptation to lyse bacteria in the diet of these insects. At the opposite extreme, the midgut pH of larval Lepidoptera is always above 8 and can exceed 12 units, at least in part through the uptake of H^+ by goblet cells (see Section 3.1.3 and Fig. 3.7). The hindgut is usually slightly more acid than the midgut, partly due to the secretions of the Malpighian tubules. The digestive enzymes of insects are most active only within a limited range of pH, and many have their optimum at pH 6–7, but in lepidopteran larvae optimal activity occurs at about pH 10 (Fig. 3.16), corresponding with the midgut pH. As well as affecting the activity of the insect's own enzymes, the pH of the gut influences the potentially harmful effects of some ingested compounds. For example, the capacity of the fall webworm, *Hyphantria cunea*, to feed on the cyanogenic leaves of black cherry, *Prunus serotina*, without apparent harm has been attributed to the high pH of the gut contents, inhibiting the conversion of the plant cyanogen to cyanide.

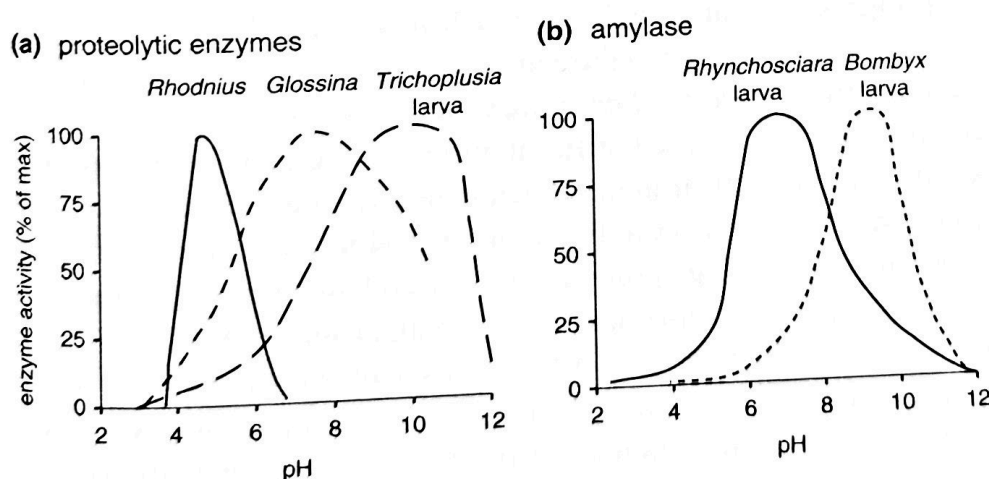


Figure 3.16 Enzyme activity in relation to pH. (a) Proteolytic activity of *Rhodnius*, a blood-sucking bug; *Glossina*, a blood-sucking fly; larva of *Trichoplusia*, a leaf-eating caterpillar. The endopeptidases of *Rhodnius* are cathepsins, those of *Glossina* and *Trichoplusia* are trypsins. (b) Amylase activity of the larva of *Rhynchosciara*, a fly larva feeding on decaying plant material; larva of *Bombyx*, a leaf-eating caterpillar.

Redox potential is a measure of the oxidizing or reducing conditions in a system. A high positive potential indicates strongly oxidizing conditions; a high negative potential indicates strongly reducing conditions. Correlated with the small size of the alimentary tract and aerobic condition of the gut epithelium, the gut lumen of most insects is believed to have a positive redox potential. Some lepidopterans, including *Manduca* larvae and the clothes moth, *Tineola bisselliella*, sustain reducing conditions in the midgut, probably by the secretion of antioxidants, especially ascorbate and reduced glutathione; and the redox potential of the hindgut fermentation chambers are invariably negative, for example ranging from -50 to -230 mV in the paunch of termites, and ca. -100 mV in the scarabeid beetle, *Pachnoda ephippiata*.

3.2.2 Extra-oral digestion

For many insects, not all the digestive activity occurs within the gut lumen. The food can be partially or completely digested even before it is ingested, by digestive enzymes secreted in saliva or regurgitated from the midgut onto the food. Digestion before ingestion is termed extra-oral digestion or extra-intestinal digestion.

All heteropteran bugs feeding on solid food display extra-oral digestion. Because these insects are only capable of ingesting liquid food, they inject saliva containing potent digestive enzymes into their food, converting it to liquid, which is then sucked up. For example, when the reduviid bug *Platymeris* injects saliva into a cockroach prey, the cockroach stops struggling at 3–5 seconds and is motionless by 15 seconds as a result of the rapid digestive function of the digestive enzymes in the *Platymeris* saliva. The bug then ingests the liquefied prey. The enzymes in the saliva of heteropterans vary with diet, but include proteases, especially serine proteases, and amylases. Other insects for which extra-oral digestion by salivary enzymes is important include adult Asilidae and Empididae among the Diptera.

The enzymes responsible for extra-oral digestion by predaceous beetles, such as *Dytiscus* and the Lampyridae, and also by larval Neuroptera, are injected into the prey from the biting mandibles with which these insects capture their prey (Fig. 2.5). Beetles have no salivary glands – the secreted enzymes are presumably derived from the midgut. Adult carabids in the tribes Carabini and Cychnini release enzymes onto their prey as they masticate, such that the food is well digested before the fluid contents are ingested.

Proteolytic enzymes persist in the excreta of larva blowflies and so the meat in which they live is partially liquefied before it is ingested.

Heliconiine butterflies and the eucalypt nectar fly, *Drosophila flavohirta*, collect pollen but are unable to ingest it because of the form of their mouthparts. They regurgitate fluid onto the pollen, which is believed to release nutrients that the insects can then imbibe; nutrients may be extracted from the pollen simply by diffusion into the fluid.

3.2.3 Digestion in the gut lumen

Most insects have a similar array of enzymes that digest proteins, carbohydrates and lipids in the midgut. Nevertheless, the enzymes produced reflect the type of food eaten by each species and stage. For example, insects feeding on plant seeds and stored products rich in carbohydrates generally have high amylase activities; insects feeding on protein-rich animal products, such as animal flesh, blood or wool, have high proteolytic activity; and digestive proteases are undetectable in adult Lepidoptera feeding on nectar.

The localization of digestive enzymes relates to their mode of action. Enzymes mediating initial stages in digestion of food macromolecules (e.g., proteases, α -amylase) are localized in the endoperitrophic space and associated with the peritrophic envelope; enzymes mediating the intermediate and final stages of digestion occur in the ectoperitrophic space

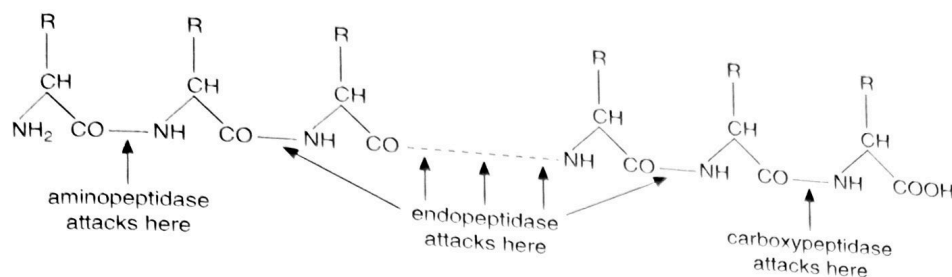


Figure 3.17 Sites of hydrolysis of proteins by different classes of proteases.

(e.g., acetylglucosaminidase) and are associated with the surface of the gut epithelial cells (e.g., maltase, dipeptidases). This pattern is consistent with the permeability of the peritrophic envelope to peptides, amino acids, oligosaccharides, etc., but not to macromolecules such as proteins and polysaccharides. The role of the perimicrovillar membrane of the Hemiptera (which do not produce a peritrophic envelope [Section 3.1.4]) in digestive function is less well understood, beyond the observation that the two chief digestive enzymes, sucrase and aminopeptidase, in the distal midgut of aphids are borne on this membrane.

3.2.4 Protein digestion

The digestion of proteins involves endopeptidases, which attack peptide bonds within the protein molecule, and exopeptidases, which remove the terminal amino acids from the molecule (Fig. 3.17). Within these general categories, the enzymes are classified according to the nature of their active sites and the sites at which they cleave protein molecules.

Three types of endoproteases have been demonstrated in insects: serine proteases (e.g., trypsin and chymotrypsin), which have serine at the active site; cysteine (thiol) proteases, including the B and L families of cathepsins, with cysteine residues at the active site; and aspartic (carboxyl) proteases with aspartate residues at the active sites. The exopeptidases are metalloproteases and fall into two categories: carboxypeptidases that attack peptides from the $-\text{COOH}$ end, and aminopeptidases that attack the chain from the $-\text{NH}_2$ end.

Insect groups vary in their complement of digestive proteases. Among the Coleoptera, cysteine

proteases are dominant. The pH optimum of these enzymes, at 5–7 units, matches the conditions in the midgut of these insects. Larval Lepidoptera and many Diptera tend to have serine proteases, especially trypsin and chymotrypsin, which have a high pH optimum. Trypsin cleaves peptide linkages involving the carboxyl groups of arginine and lysine residues, and chymotrypsin, which is less specific, preferentially cleaving bonds involving the carboxyl groups of tyrosine, phenylalanine and tryptophan. The only insects that definitively have digestive aspartic proteases are the cyclorrhaphous Diptera, including the housefly and drosophilid flies. Their digestive proteases are members of family A1 of aspartic proteases, and characterized by the presence of two catalytic aspartic residues, activity at low pH and inhibition by pepstatin. Sequence analysis demonstrates that they are related to cathepsin D, a major intracellular aspartic protease (also in family A1) localized to the lysosome and widely distributed across the animal kingdom. The dominant digestive cathepsin D of the housefly, *Musca domestica*, (CAD3) lacks two features of the lysosomal cathepsins: an eight-amino acid signature sequence, known as the proline loop, and glycosylation sites. Apart from the cyclorrhaphous Diptera, the only other cathepsin D with a known digestive function is the vertebrate stomach pepsin. Cathepsin Ds have been described in the midgut of various other insects, but they are almost certainly lysosomal, playing a role, for example, in gut remodeling, especially at metamorphosis, and do not contribute to digestion.

Many insects have multiple proteases. For example, 11 proteases, mostly serine proteases, mediate the digestion of the blood meal by the mosquito *Anopheles gambiae*, presumably reflecting

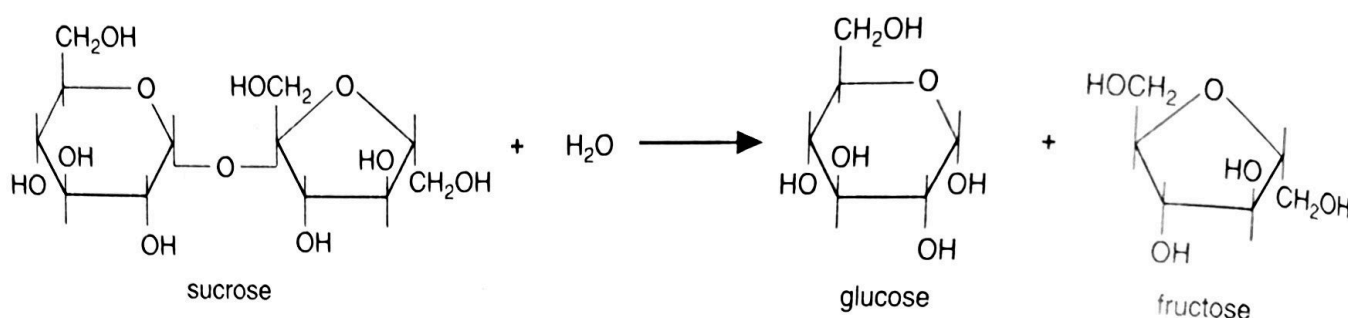


Figure 3.18 Hydrolysis of sucrose by the α -glucosidase, sucrase, to the constituent monosaccharides, glucose and fructose.

the complexity of the ingested proteins, and larvae of *Helicoverpa armigera* (Lepidoptera) have at least nine digestive serine proteases, all detectable in the proteome of the endoperitrophic space – i.e., associated with the food bolus enclosed within the peritrophic envelope. One factor proposed to contribute to the diversity and complexity of digestive proteases in herbivorous insects is the high incidence of proteinase inhibitors (PIs) in plants. PIs against serine, cysteine and aspartic proteases are known; and they all lead to high mortality of susceptible insects. However, many insects adapt readily to chronic ingestion of plant PIs by increased feeding, increased production of susceptible enzymes (so saturating the inhibitory effect) and synthesis of alternative PI-resistant proteinases. In this way, multiple proteinases with different susceptibilities to PIs can confer resistance to PI-based plant defenses. It has also been proposed that cysteine proteases have been recruited for digestive function in insects feeding on legume seeds and other plant tissues that are defended by serine proteinase inhibitors.

Wool, hair and feathers contain large amounts of a single protein, keratin, which has multiple disulfide linkages. To break down the keratin, various insects, including the clothes moth (*Tineola*), carpet beetles (Dermestidae) and numerous biting lice (Ischnocera), exploit a complex mixture of digestive proteases, including a highly active cysteine desulfhydrase released into the gut lumen. This enzyme generates hydrogen sulfide from cysteine, contributing to the strong reducing conditions in the gut which, in turn, promote the breaking of disulfide bonds in the keratin.

3.2.5 Carbohydrate digestion

Carbohydrates are generally absorbed as monosaccharides (glucose, fructose, etc.), so disaccharides and polysaccharides in food require digestion. Starch and glycogen, the main storage polysaccharides of plants and animals, respectively, are digested by amylases that hydrolyze α -1,4-glucosidic linkages. There may be separate endo- and exo-amylases, acting on starch internally or terminally. Other common carbohydrases are the α -glucosidases and β -glucosidases, which hydrolyze specific disaccharides and oligosaccharides. Examples include a sucrase (α -glucosidase) (Fig. 3.18) that breaks down sucrose in the midgut of phloem sap-feeding aphids, a β -galactosidase in the digestive juice of the palm weevil, *Rhynchophorus palmarum*, and a maltase (α -glucosidase) in the gut lumen of the larval housefly, *Musca domestica*. These enzymes generally mediate hydrolysis because water is the typical acceptor for the sugar residues, but sugars at high concentrations can act as acceptors, resulting in the formation of oligosaccharides. Thus, the aphid sucrase can mediate the formation of trisaccharide and longer-chain oligosaccharides, which are commonly voided in the honeydew, and the palm weevil galactosidase can, similarly, catalyze transgalactosylation reactions.

A very abundant potential source of carbohydrate for plant-feeding insects is cellulose (Fig. 3.19), which is a β -1,4-glucose polymer. Cellulose is the dominant component of plant cell walls, accounting for 20–30% (by weight) of primary cell walls, and

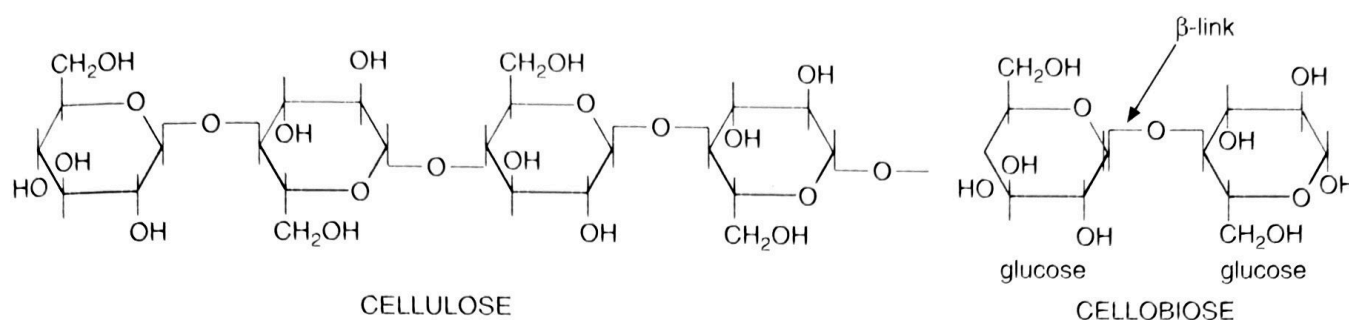


Figure 3.19 Cellulose (β -1,4-glucose polymer) and its disaccharide hydrolysis product, cellobiose.

more than 50% of secondary cell walls, in which the cellulose microfibrils are bounded by hemicelluloses and lignin to form lignocellulose. Cellulose is degraded by the combined action of three sets of enzymes: endoglucanases (EC 3.2.1.4), which attack the bonds between glucose residues within the chain; exoglucanases (EC 3.2.1.74 and 3.2.1.91), which attack bonds near the ends of the cellulose molecule; and β -glucosidases (EC 3.2.1.21), which hydrolyze cellobiose (Fig. 3.19).

Three potential sources of cellulase activity have been proposed for insects: intrinsic; microbial symbionts located in a fermentation chamber; and enzymes ingested with the food. Until comparatively recently, it was believed that animals lack the genetic capacity to produce cellulases, and that all cellulose degradation in insects is mediated by microorganisms. It is now realized, from compelling genomic and enzymological evidence, that various insects and other animals produce digestive cellulases. Even so, the importance of cellulolytic microbial symbionts for lower termites is well established, and the contribution of microbes to cellulose digestion in other insects is increasingly recognized. Ingested cellulases are currently considered not to be generally important, although this source of digestive function may be significant for certain species, including the longicorn beetle, *Monochamus marmorator*.

Unambiguous evidence for intrinsic cellulase activity has been obtained for Blattodea (cockroaches), Isoptera (termites), Coleoptera

(longicorn and mustard beetles) and Orthoptera (crickets). In addition, genome sequencing projects have identified candidate genes annotated as cellulases in a total of 27 species from six orders (Table 3.1). Cellulases of glycoside hydrolase family 9 (GH9) have been reported in all insect orders represented, and Coleoptera additionally have GH5 and GH49. The genetic capacity to produce cellulases is not, however, universal among insects. The genomes of all *Drosophila* and mosquito species that have been sequenced to date lack an identifiable cellulase gene.

In the lower termite, *Mastotermes darwiniensis*, intrinsic cellulase activity accounts for approximately 25% of the total activity in the gut (partitioned equally between the midgut and the foregut/salivary glands). The remainder is mediated by protists, specifically trichomonads and hypermastigotes, in the paunch of the hindgut (Fig. 3.3f). The protists engulf fragments of plant material and ferment the cellulose, producing short-chain fatty acids (SCFAs), such as acetate, carbon dioxide and hydrogen. The SCFAs are absorbed in the hindgut and provide a large proportion of the respiratory substrate used by the insect. These organisms may constitute more than 25% of the wet weight of the insect. Because the cellulose degradation takes place within the protist cells, free cellulase activity is negligible in the gut lumen. Woodroaches (family Cryptocercidae), which comprise the cockroach sister group of the termites, also bear cellulolytic hypermastigote protists.

The microbiota of the higher termites (family Termitidae, accounting for 75% of all termites) comprises entirely bacteria, which have traditionally been considered to lack any cellulolytic capability (with the implication that all cellulose digestion in higher termites is mediated by intrinsic cellulases in the midgut). However, recent data suggest that hindgut bacteria in *Nasutitermes takasagoensis* may have cellulases. Cellulolytic bacteria have also been isolated from the gut of larval bark beetles, *Ips pini* and *Dendroctonus frontalis*, and the longicorn beetle, *Saperda vestita*.

Some insects gain access to cellulose digestion by associating with cellulolytic fungi, which they maintain in their immediate environs (e.g., their nest) and carry with them when dispersing, in pockets known as mycangia, in their cuticle. Termites of the subfamily Macrotermitinae cultivate fungi of the genus *Termitomyces* in fungus gardens, formed from feces containing chewed but only partially digested plant fragments. The fungus grows on this comb, producing cellulolytic enzymes, and the termites then feed on the fungus and the comb. Similarly, the leaf-cutter ants and other Attini maintain the fungus *Attomyces*, which composts plant fragments provided by the ants; and the ants, especially the larvae, feed on the fungal mycelium. In this way, these ants are proxy herbivores, as elegantly revealed by stable isotope analysis (Fig. 3.20). The ambrosia beetles (weevils of the subfamilies Platypodinae and Scolytinae) and larval Cerambycidae and wood wasps (Siricidae) excavate tunnels in trees, in which they cultivate fungi. The fungi digest the lignocellulose, especially of the xylem vessels, and concentrate nitrogen; and, as with the termites and ants, the insects feed on the fungus. All these insects exploit the degradative capacity of a fungal biomass that is considerably greater than could be accommodated within their guts.

The β -1,3-glucanases comprise a further group of carbohydrases abundant in the digestive tract of various insect herbivores and detritivores, including Collembola, Trichoptera, Blattodea, Orthoptera,

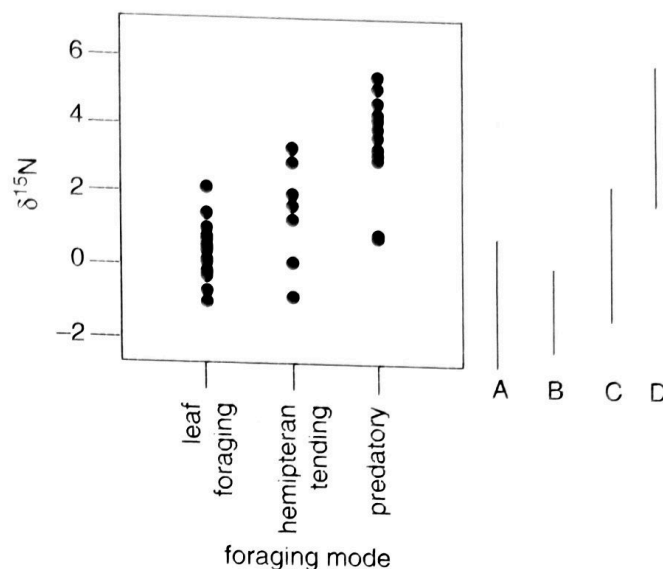


Figure 3.20 Mean $\delta^{15}\text{N}$ values (‰) of ant species in a Borneo rainforest, classified according to predominant feeding mode and calibrated against the range of $\delta^{15}\text{N}$ of plants (A), hemipterans (B), chewing herbivorous insects (C) and arthropod predators (D). (Redrawn from Fig. 2 of Davidson *et al.*, 2003, omitting species with mixed or uncertain foraging strategies.)

Coleoptera and Diptera. As with cellulases, these enzymes are apparently absent from vertebrates. The β -1,3-glucanases contribute to the degradation of hemicelluloses in plant cell walls and also to β -1,3 and β -1,6-glucans in fungal cell walls. They are, therefore, potentially important in releasing the contents of fungal cells for access by other digestive enzymes, as well as in the degradation of carbohydrate polymers.

3.2.6 Digestion of lipids

The enzymes mediating lipid digestion of insects are lipases (EC 3.1.1.3). These enzymes cleave the carboxylester linkages in triacylglycerols (TAGs), diacylglycerols (DAGs), galactolipids and phospholipids. Lipolysis occurs predominantly in the proximal midgut of most insects, yielding free fatty acids, glycerol, partial acylglycerols and lysophospholipids, which are absorbed into the midgut cells. (Lysophospholipids are phospholipids in which one of the two O-acyl groups at positions

sn-1 or sn-2 is replaced by a free alcohol.) As in vertebrates, the digestive lipases of insects belong to two families: the neutral lipases and acidic lipases (represented by the pancreatic and gastric lipases, respectively, of vertebrates). However, there is no direct correspondence between the digestive lipases of insects and vertebrates, and the lipase genes in insects have diversified independently among and even within different insect orders.

Based on the sequence and expression pattern of lipase genes, *Drosophila melanogaster* has been deduced to have at least three major neutral lipases (CG6271, CG6277 and CG6283) and possibly one acidic lipase (CG8093) that are secreted into the gut lumen, where they mediate lipid digestion. Multiple lipases have also been implicated in lipid digestion by other Diptera, including the mosquito *Anopheles gambiae*, as well as Lepidoptera, Coleoptera and Hemiptera. For example, lipid digestion in the midgut of larval *Epiphyas postvittana* (an apple moth, Lepidoptera) is mediated by six functional neutral lipases and three acidic lipases. The sequence of the digestive neutral lipases suggests that they can hydrolyze phospholipids and galactolipids, but not TAGs; and TAG hydrolysis may be mediated exclusively by the acidic lipases. For the phytophagous Lepidoptera, at least, this profile of lipase specificity correlates well with the dominant lipids in leaf tissue, which are the phospholipids and galactolipids of chloroplast thylakoid membranes. Although TAGs are minor lipids in green plant tissues, they are the major lipids in plant seeds and other storage organs and also in animal tissues. The lipases responsible for TAG digestion in seed-feeding and carnivorous insects have yet to be studied in detail.

3.2.7 Variation in enzyme activity

The enzymatic activity in the gut is a crucial factor determining the nutrient availability for the insect, and it is therefore tightly regulated according to the diet composition and nutritional requirements of the insect. The regulation of enzyme activity is

particularly apparent in female mosquitoes that feed on both sugar-rich nectar and protein-rich vertebrate blood. Within hours of taking a blood meal, the digestive protease activity in the mosquito midgut increases rapidly, reaching its maximum after about two days and then declining; protease activity is undetectable after a sugar meal.

In many insects, digestive enzyme activity does not increase consistently with increasing substrate availability in the diet. Several factors can contribute to this effect. The first is technical: enzyme activity assays do not generally discriminate between activity available for digestion and stored enzyme in cells. For example, the midguts of starved insects often have very high enzyme activity, even though the digestive activity is negligible. A second important issue is that the digestive function is not invariably regulated to maximize digestion of all ingested foods. When an insect is feeding on a nutritionally unbalanced diet, such that one dietary component is in excess, the enzymes mediating the degradation of that dietary component can be down-regulated. For example, locusts *Locusta migratoria* feeding on diets with excess protein or carbohydrate display reduced activity of digestive α -chymotrypsin and α -amylase, respectively (Fig. 3.21).

Some digestive enzymes are apparently synthesized constitutively, while others are produced in response to the presence of substrate in the midgut (a process known as secretagogue or prandial regulation), or under the control of hormones. The pattern of enzyme production varies among both insect species and enzymes within one insect. For example, *Aedes aegypti* mosquitoes have three trypsin genes expressed in the midgut. The synthesis of two trypsins, known as the late trypsins, is under secretagogue regulation, although the detail is complex. Initial production (within three hours of feeding) is from a preformed mRNA in response to protein in the blood; subsequent production (8–10 hours after feeding) comes from *de novo* trypsin gene expression induced by amino acid products of trypsin-mediated digestion of blood proteins. The

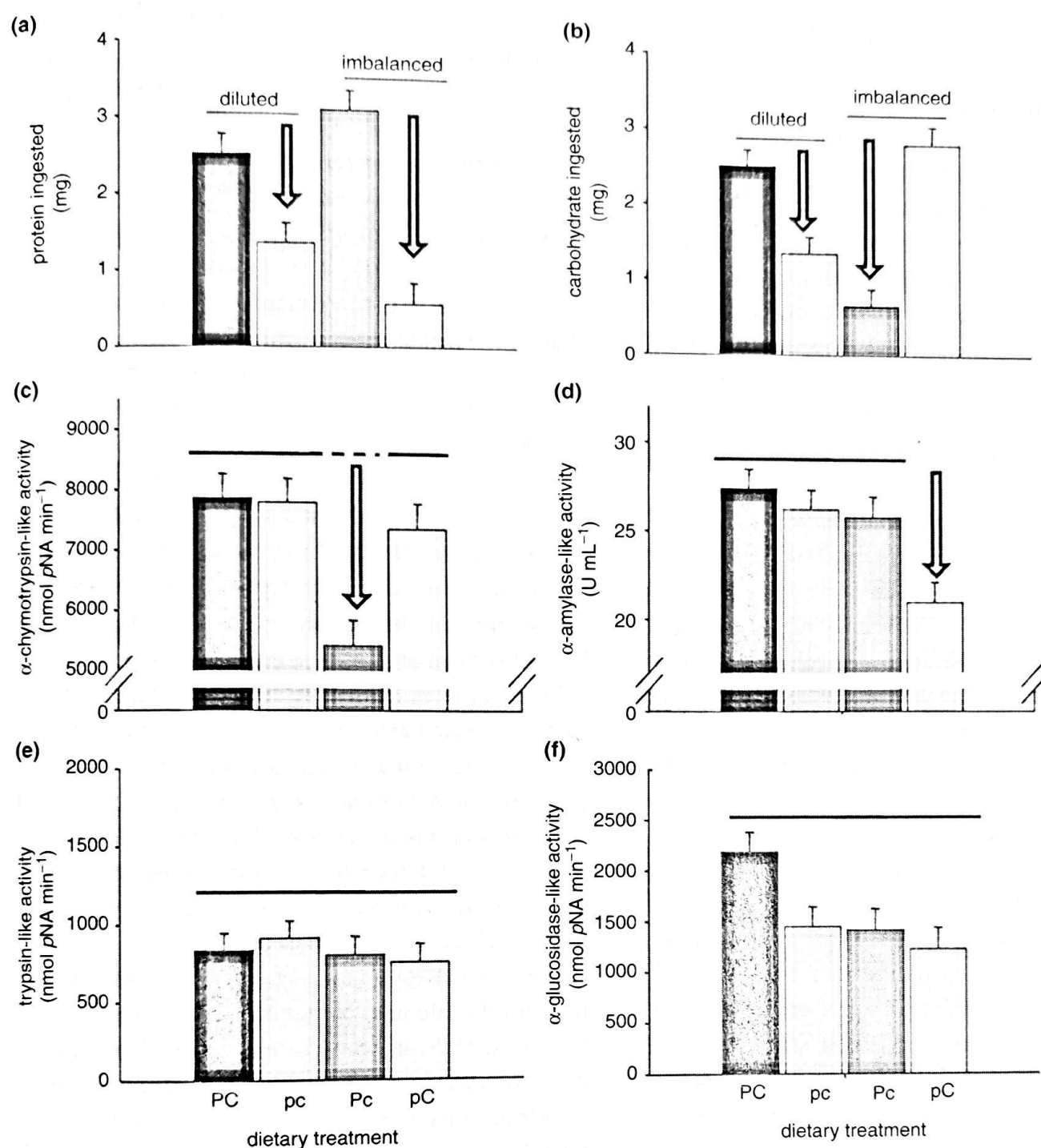


Figure 3.21 The amount of (a) protein and (b) carbohydrate consumed in the meal prior to determining enzyme activity. The four treatment diets contained combinations of high or low concentrations of protein and carbohydrate (both high, PC; both low, pc; high protein, low carbohydrate, Pc; low protein, high carbohydrate, pC). (c)–(f) Total enzyme-like activity of four digestive enzymes (α -chymotrypsin, trypsin, α -amylase, α -glucosidase) is shown in the foregut contents, where activity levels for all enzymes were greatest and where the majority of digestion occurs in locusts. (c) α -chymotrypsin-like activity and (d) α -amylase-like activity were strongly down-regulated only when their nutrient substrate was present in the diet in excess relative to the other macronutrient (i.e., on Pc and pC for α -chymotrypsin and α -amylase, respectively). Treatment did not affect the activity of (e) trypsin-like activity or (f) α -glucosidase-like activity. The down arrows indicate a significant difference ($p < 0.05$) and bars with a line across the top are not statistically different. (c–f) Values are ANCOVA-adjusted (GIT weight) means $\pm$ s.e. (c) $p = 0.010$; (d) $p = 0.039$; (e) $p = 0.989$; (f) $p = 0.308$. Reproduced from figure 1 of Clissold *et al.* (2010).

other midgut trypsin, called early trypsin, is synthesized constitutively.

The regulation of digestive enzyme production has been investigated in an isolated midgut ceca preparation developed for the cricket *Gryllus bimaculatus*, revealing a contribution of multiple regulatory processes. Secretagogue regulation was evident for amylase production, which was stimulated by the presence of maltose and glucose, but not for production of trypsin, carboxypeptidase or aminopeptidase, as tested with protein, peptides and amino acids. The neuropeptide type-A allatostatin also promoted the secretion of amylase, but not trypsin.

3.3 Absorption

Absorption refers to the uptake of compounds in the gut lumen across the gut wall to the hemocoel. At the cellular level, most absorption is transcellular, meaning that the compound is transported across the apical cell membrane of gut epithelial cells, transits the cell and is exported at the basal membrane. Paracellular transport, i.e., between cells of the gut epithelium, is generally uncommon.

This section considers the absorption of organic compounds, particularly products of digestion (sugars, amino acids, lipid products, etc.), inorganic ions and water. The products of extra-oral and midgut digestion are absorbed principally across enterocytes of the midgut, while the hindgut is the main site of absorption of water and ions.

3.3.1 Transport of amino acids and protein

Most research on the absorption of sugars and amino acids has focused on their passage across the apical membrane of enterocytes. Multiple sugar and amino acid transporters have been identified in various insects. Some transporters mediate active transport, i.e., transport dependent on energy to move a substance against its concentration

gradient; others mediate passive transport, i.e., transport not requiring energy and dependent on a higher concentration in the gut lumen than in the enterocyte cell. Passive transport can be promoted by the metabolism of the transported compound in the enterocyte, thereby maintaining a steep concentration gradient across the apical membrane.

The midgut amino acid transporters in most insects that have been studied belong to the Na^+ -coupled neurotransmitter and amino acid symporter family, known as family SLC6. Multiple transporters are expressed, with overlapping specificities for amino acids. Some are very specific – e.g., NAT6 and NAT8 in the distal midgut of the mosquito *Anopheles gambiae* transport just aromatic amino acids. Other SLC6 transporters have a very broad range. Notably, the neutral amino acid transporter in *Drosophila* (DmNAT6) can mediate the transport of most amino acids apart from lysine, arginine, aspartate and glutamate; and, remarkably, it can also take up D-isomers of several amino acids. This capability can be linked to the abundance of D-amino acids in the cell walls of bacteria, which are an important component of the natural diet of *Drosophila* species. DmNAT6 is an active transporter, capable of mediating uptake against the concentration gradient.

Exceptionally, amino acid transport in the midgut of larval Lepidoptera is coupled to K^+ ions, not Na^+ ions. Multiple transporters are involved, with a range of specificities, including two neutral amino acid transporters in *Manduca sexta* (KAAT1 and CAATCH1), both of which are members of the SLC6 family. The cotransport of the K^+ ions and amino acid into enterocyte cells is coupled to the ATPase-dependent extrusion of K^+ ions from adjacent goblet cells, which play a crucial role in alkalinization of the gut lumen (Fig. 3.7). The coupled functions of electrogenic K^+ transport and K^+ /amino acid uptake are mediated by different cells, presumably because the high EMF generated by the goblet cells could compromise the function of the SLC6 and other transporters.

Amino acid transporters are also expressed in the apical membrane of the insect hindgut, where they mediate the uptake of amino acids in the primary urine produced in the Malpighian tubules (see Chapter 18). For example, glycine, serine, alanine and threonine are actively reabsorbed into the cells of the rectal pads of the locust by an Na^+ -cotransporter of the SLC6 family. Proline is also taken up, and is a major respiratory substrate of rectal cells.

Amino acid transport across the basolateral membrane of gut epithelial cells is not well understood, but active transport is not generally involved. Both amino acid uniporters and amino acid exchangers have been described.

There is also evidence for the movement of proteins across the gut wall to the hemolymph. For example, the hemolymph of blood-feeding insects, including tsetse fly, mosquitoes and fleas, routinely contain antibodies derived from the vertebrate blood on which they feed; and the hemolymph of the silkworm, *Bombyx mori*, has active urease proteins derived from their diet of mulberry leaves. It is unclear whether proteins are transported by the transcellular or paracellular route; it may vary with protein and insect species. There is unambiguous evidence that absorbed proteins are cleared from the hemocoel over a period of hours, mediated by degradation and transfer to other organs – e.g., fat body, ovaries. It is uncertain whether these proteins have any nutritional value to the insect.

3.3.2 Transport of sugars

Carbohydrates are absorbed mainly as monosaccharide sugars across the midgut epithelium, but the molecular basis of sugar transport has been little-studied. There is a general expectation of parallels with glucose absorption in the mammalian intestine, in which transport is transcellular and mediated by a Na^+ /glucose cotransporter SGLT1 (a member of the Na^+ /solute symporter family) and two facilitative glucose transporters, GLUT2 and GLUT5 (both members of the major facilitator superfamily,

MFS). Consistent with this expectation, glucose transport across the midgut of the hymenopteran parasite *Aphidius ervi* is mediated by a SGLT1-like transporter on the apical membrane, together with a GLUT2-like transporter on both the apical and basolateral membranes of the enterocytes; and a second passive transporter similar to GLUT5 is implicated in fructose uptake. This condition is not, however, universal among insects. For example, genome annotation of the pea aphid, *Acyrtosiphon pisum*, revealed no Na^+ /solute symporter with plausible specificity for sugars, but 29 candidate sugar transporters in the MFS family, equivalent to GLUT. These included an abundantly expressed gene *ApSt3*, a hexose uniporter with specificity for glucose and fructose in the distal midgut. Aphids presumably do not need to take up sugars against the concentration gradient because their diet of plant phloem sap is sugar-rich, and a concentration gradient from gut lumen to epithelial cell and hemocoel is maintained by the excess sugar in the gut lumen. The metabolism of the absorbed sugars, both as a respiratory fuel and in trehalose and glycogen synthesis, may also promote sugar absorption.

3.3.3 Transport of lipids and related compounds

The products of lipid digestion include free fatty acids, glycerol, mono- and diacylglycerols and lysophospholipids. These compounds can move relatively freely across membranes, and they are absorbed principally across the midgut epithelium, although absorption in the foregut, e.g., the crop of the cockroach, *Periplaneta americana*, can also occur.

Two factors promote lipid absorption. The first is emulsification, which assists their diffusion across the aqueous boundary layer to contact the apical membrane of the gut epithelial cells. Insects lack the bile salts that are crucial for emulsification in the vertebrate gut (this, incidentally, is why the

insect-neutral lipases do not have a colipase cofactor, which is required by the pancreatic lipase of vertebrates to hydrolyze the bile salt-covered lipid in the vertebrate gut). Instead, emulsification in the insect gut is promoted by the formation of fatty acid-amino acid complexes and glycolipid complexes, and the formation of micelles of fatty acids and lysophospholipids.

The second factor promoting absorption is the resynthesis of lipids from absorbed compounds in the enterocytes. This metabolic activity maintains a steep concentration gradient, promoting uptake. In all insects studied to date the enterocytes synthesize 1,2-diacylglycerols (DAGs), triacylglycerols (TAGs) and phospholipids. Lipids can also be generated in the enterocytes from absorbed glucose, galactose or other sugars. Lipids are exported from enterocytes to the hemolymph predominantly as DAGs, and are transported to other tissues bound to the protein lipophorin. Lipophorin has also been implicated in the transport of hydrocarbons, carotenoids, sterols and phospholipids. Sterols appear to be absorbed unchanged, but, in some caterpillars, sterols are esterified in the gut cells.

3.3.4 Absorption of inorganic ions

For sodium to move from the gut lumen into the hemolymph of a locust, it must move against the electrochemical gradient (Fig. 3.22). Energy for this active movement is provided by V-ATPase pumps in the apical plasma membranes of the anterior ceca, and by major sodium-potassium exchange pumps in the basal plasma membranes of the rectal cells. Potassium, on the other hand, probably moves passively down its electrochemical gradient into the hemocoel. The electrochemical gradients for chloride and calcium ions are less extreme. In the rectum, chloride ions are actively removed from the lumen by pumps in the apical membranes of the cells and pass passively from the cells to the hemolymph. Calcium is actively moved from the gut lumen to the hemocoel by cells in the midgut.

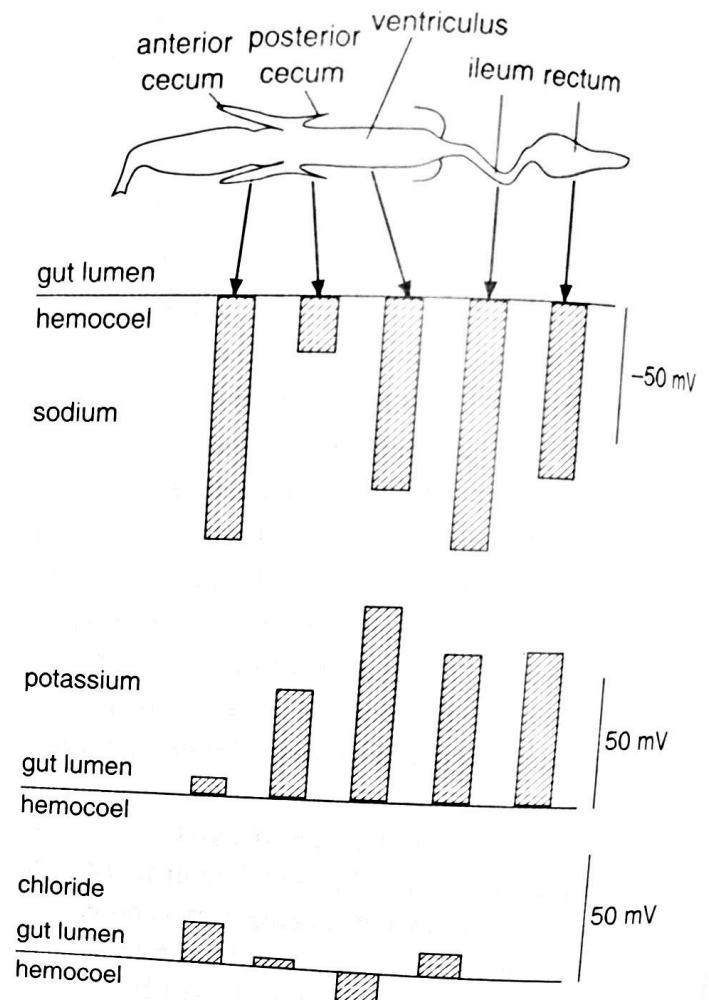


Figure 3.22 Electrochemical gradients across the gut epithelium of a locust. The insects were recently fed. Bars extending into the lumen (upwards) indicate a positive gradient from gut to hemolymph – diffusion into the hemocoel will occur. Bars extending into the hemocoel indicate a negative gradient from gut to hemocoel – passive diffusion into the hemocoel will not occur (after Dow, 1981).

3.3.5 Absorption of water

There are two major water-absorbing zones in the insect gut, one in the midgut, where water is absorbed from the food, and the other in the rectum, where water is absorbed from the feces before they exit the body. Absorption in the rectum is a key component of the regulation of body water. Water absorption in the midgut serves to concentrate the food, enhancing both the efficiency of digestion and the maintenance of concentration gradients favorable to the absorption of nutrients across the

all of the gut. It also creates water flows which are important in nutrient absorption and perhaps enzyme conservation. Specifically, absorption in the proximal midgut, especially the midgut ceca (where these structures occur) drives the forward flow of water in the endoperitrophic space, carrying forward the products of digestion. The water may be derived directly from the food, as in well-fed grasshoppers, or drawn forwards as it leaves the Malpighian tubules, as in cockroaches. In larval Diptera, and perhaps in some other insects, the forward movement occurs in the ectoperitrophic space, while fluid in the endoperitrophic space moves backwards. This countercurrent probably improves the efficiency of utilization of the digested materials in the gut.

Water absorption depends on the establishment of an osmotic gradient across the epithelium, such that water passes from a zone of low solute concentration to high solute concentration. In many insects the flux of water from gut lumen to hemolymph is based on

the accumulation of Na^+ or K^+ ions in the extracellular space between the epithelial cells, which has only a few openings to the hemolymph. Water is thus drawn through, or between the cells from the gut lumen into the extracellular space. This creates a hydrostatic pressure which forces water out through the openings into the hemolymph (Fig. 3.23a). This process is energetically very demanding, and is supported by large numbers of mitochondria close to the cell membrane.

Water absorption from the midgut often occurs in localized areas. In cockroaches, grasshoppers and the larvae of some flies (mosquitoes and *Sciariidae*), it occurs in the midgut ceca, while in blood-sucking insects water is removed from the stored meal in the anterior midgut. Deep invaginations of the basal plasma membrane, which may extend more than halfway toward the apex of the cell, are closely associated with large numbers of mitochondria. These provide the energy by which K^+ (or Na^+ in the case of *Rhodnius*) is actively pumped into the

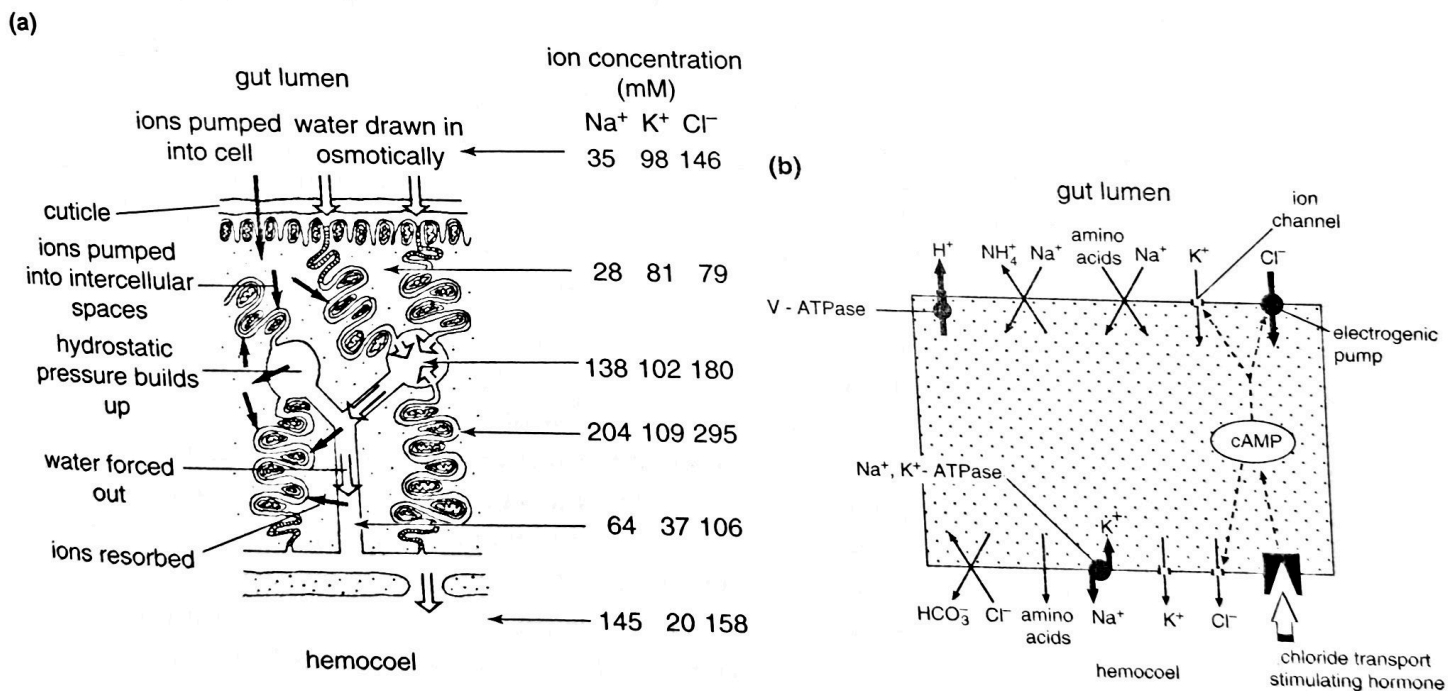


Figure 3.23 Absorption of inorganic ions from the rectum of a grasshopper. (a) Diagram of the principal rectal cells showing the ionic concentrations measured in one experiment (after Phillips *et al.*, 1986). (b) Diagram showing the different pumps and channels involved in the movement of ions from the rectal lumen to the hemocoel (after Phillips and Audsley, 1995).

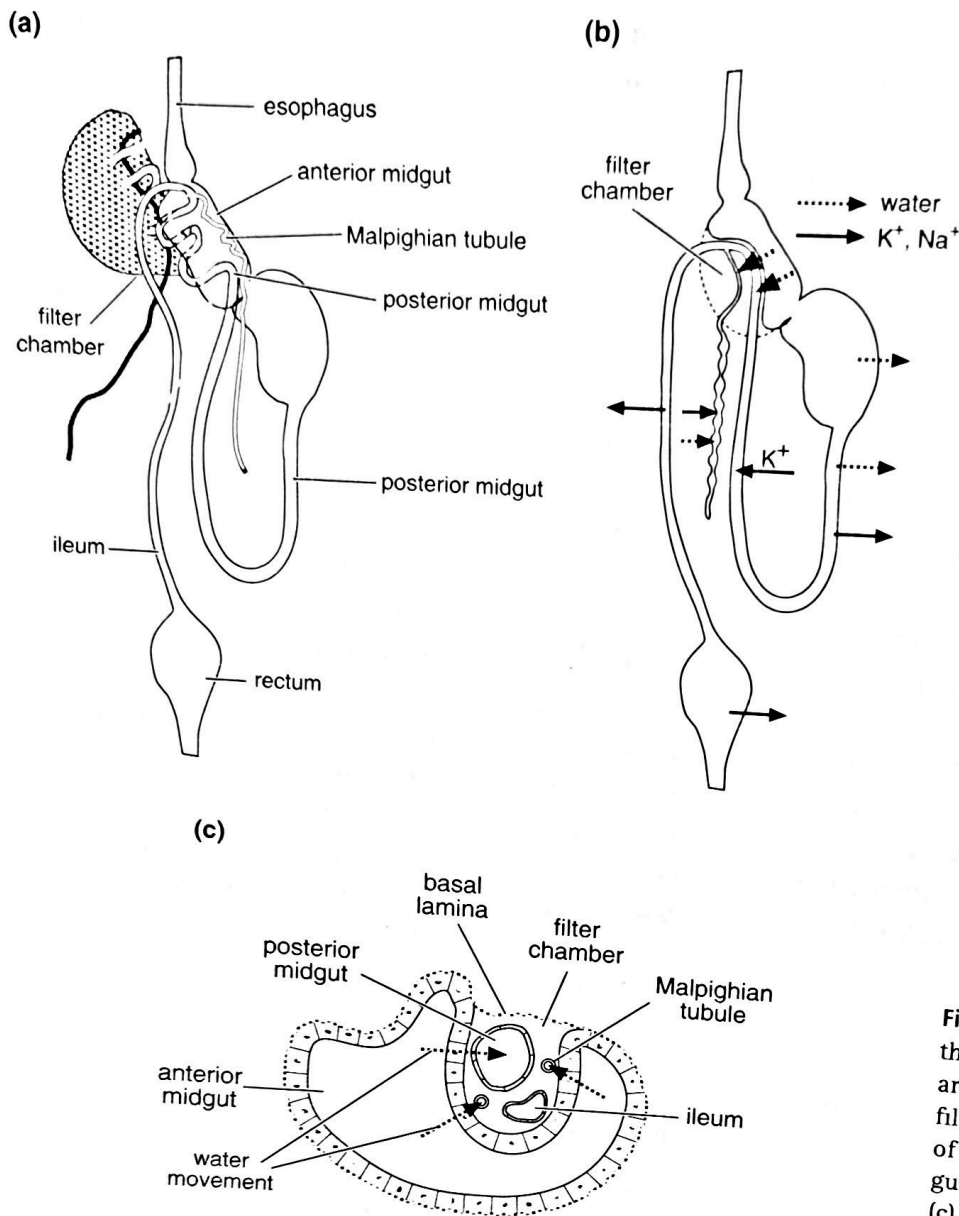


Figure 3.24 Water absorption from the midgut of a cercopid. (a) General arrangement of the gut showing the filter chamber. (b) Postulated movement of salts and water across the wall of the gut (after Cheung and Marshall, 1973). (c) Transverse section of the filter chamber.

intercellular spaces to create a high osmotic gradient between the gut lumen and the intercellular spaces.

The Hemiptera that feed on xylem sap of plants must ingest large volumes because the concentrations of nutrients in these fluids are very low. Modifications of the gut provide for the rapid elimination of the excess water taken in. In cercopids, for example, the anterior midgut forms a thin-walled bladder that wraps around the posterior midgut and the proximal ends of the Malpighian tubules. This arrangement, which is called a filter chamber,

enables water to pass directly from the anterior midgut to the Malpighian tubules (Fig. 3.24). In this way the food is concentrated and dilution of the hemolymph is avoided; and the urine eliminated by the insect is hypo-osmotic to the hemolymph. In this instance, the cells of the midgut are unspecialized, but an osmotic gradient is established from the Malpighian tubules to the midgut. In some bryocorine Miridae the anterior midgut makes contact with a large accessory salivary gland. After feeding, a clear fluid is exuded from the mouthparts,

suggesting that water is withdrawn from the midgut directly to the salivary glands and then eliminated via the mouthparts.

Water absorption from blood stored in the anterior midgut of *Rhodnius* is dependent on the active movement of sodium into the hemocoel. It is believed that, in this case, Na^+ ions are transported out of the cell by Na^+/K^+ exchange pumps in the basal plasma membrane.

In terrestrial and saltwater insects water is absorbed from the rectum. Here, as in the midgut, V-ATPase in the apical plasma membrane probably provides the energy that drives the inward movement of ions and amino acids, principally chloride and proline in the locust (Fig. 3.23b). Water moves across the epithelium due to the increased osmotic pressure. Chloride moves passively into the lateral intercellular spaces and sodium is pumped out by sodium/potassium ATPase so that water is drawn osmotically into the spaces. Hydrostatic pressure forces the solution out to the hemolymph, but ions are reabsorbed as the fluid passes out from between the principal cells. This permits the recycling of the ions and the maintenance of absorption.

3.4 The alimentary tract as an immunological organ

The alimentary tract is one of the most immunologically active organs of the insect, along with the fat body. In most insects, the alimentary tract plays a central role in the response to the many potential pathogens that gain entry to insects via the oral route, and it is also the habitat for a community of resident microorganisms. The immunological response of the alimentary tract is important for both protection against pathogens and management of the resident microbiota.

Midgut immunity has been investigated in detail in *Drosophila melanogaster*. Two complementary inducible immunological responses are important:

the production of reactive oxygen species (ROS) and anti-microbial peptides (AMPs).

ROS are produced by a dual oxidase (DUOX) with two enzymatic activities: NADPH oxidase, which mediates the NADPH-dependent reduction of oxygen to generate superoxide ions, which can dismutate spontaneously to form hydrogen peroxide (H_2O_2); and the peroxidase activity which combines hydrogen peroxide with chloride ions to form hypochlorous acid (HClO). HClO is a very potent microbicide (it is the active component of household bleach). There is genetic evidence that the peroxidase activity of DUOX is absolutely required to protect the insect gut against microbial invasion. ROS and HClO are cytotoxic, damaging DNA, proteins and lipids, and the DUOX system is tightly regulated by complex signaling cascades to minimize autotoxicity. In addition, the ROS are cleared by a catalase (immune-related catalase, IRC). Flies with genetically reduced IRC activity ("knockdown flies") suffer high mortality when infected by pathogens that are not lethal to wild-type flies. It is believed that the high gut ROS levels in IRC-knockdown flies cause redox imbalance and cell damage.

AMPs are amphipathic peptides, 12–50 amino acids in length, that partition readily into membranes and kill microorganisms by membrane permeabilization or via cytoplasmic targets, e.g., inhibition of DNA, RNA or protein synthesis. The *Drosophila* gut cells secrete various AMPs, including drosomycin and diptericin, that are active principally against Gram-negative bacteria. This branch of the local immune response is mediated by the recognition of a major constituent of the bacterial cell wall, peptidoglycan, with signaling via the IMD (immune deficiency) pathway. Expression of AMP genes in the healthy insect is dampened through negative regulation by the transcription factor Caudal, so protecting the resident microbiota from AMP attack. Knockdown of *caudal* gene expression results in altered composition of the microbiota, which becomes dominated by species that are deleterious to the insect. These data illustrate how the

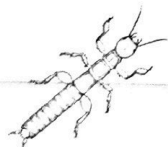
amount and composition of AMPs can function to regulate the composition of the resident microbiota. It appears that the insect gut epithelium does not

produce AMPs regulated by the Toll signaling pathway that are active principally against Gram-positive bacteria or fungi.



Summary

- The alimentary tract comprises three regions: the foregut, midgut and hindgut. The foregut and hindgut are bounded by a chitinous extension of the exoskeleton, and the midgut of most insects bears a peritrophic envelope, comprising proteoglycans on a chitinous framework.
- The foregut of many insects includes a crop, where food is stored. In some insects, the proventriculus of the foregut is modified for mechanical grinding of food particles.
- The midgut is the principal site of digestion and absorption, mediated by the enterocytes, which are the most abundant cells of the midgut epithelium. Digestive enzymes include proteases, carbohydrases (including amylases, and in some insects cellulases) and lipases. Transporters mediating active transport (against the concentration gradient) and passive transport (down the concentration gradient) of sugars and amino acids are known. The uptake of fatty acids, acylglycerols and other products of lipid digestion is aided by emulsification. The midgut is immunologically active, displaying the inducible production of reactive oxygen species and anti-microbial peptides in response to potential pathogens.
- The hindgut includes the ileum (intestinum) and rectum, and mediates the uptake of inorganic ions, water and other solutes derived from the Malpighian tubules. The ileum is also the site of a fermentation chamber bearing cellulolytic microorganisms in some insects, such as termites.



Recommended reading

- Clissold, F. J., Tedder, B. J., Conigrave, A. D. and Simpson, S. J. (2010). The gastrointestinal tract as a nutrient-balancing organ. *Proceedings of the Royal Society of London B* **277**, 1751–1759.
- Hegedus, D., Erlandson, M., Gillott, C. and Toprak, U. (2009). New insights into peritrophic matrix synthesis, architecture, and function. *Annual Review of Entomology* **54**, 285–302.
- Horne, I., Haritos, V. S. and Oakeshott, J. G. (2009). Comparative and functional genomics of lipases in holometabolous insects. *Insect Biochemistry and Molecular Biology* **39**, 547–567.