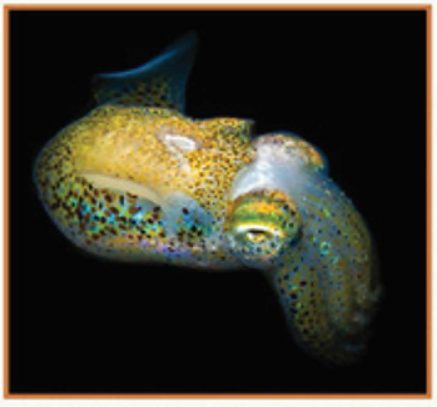
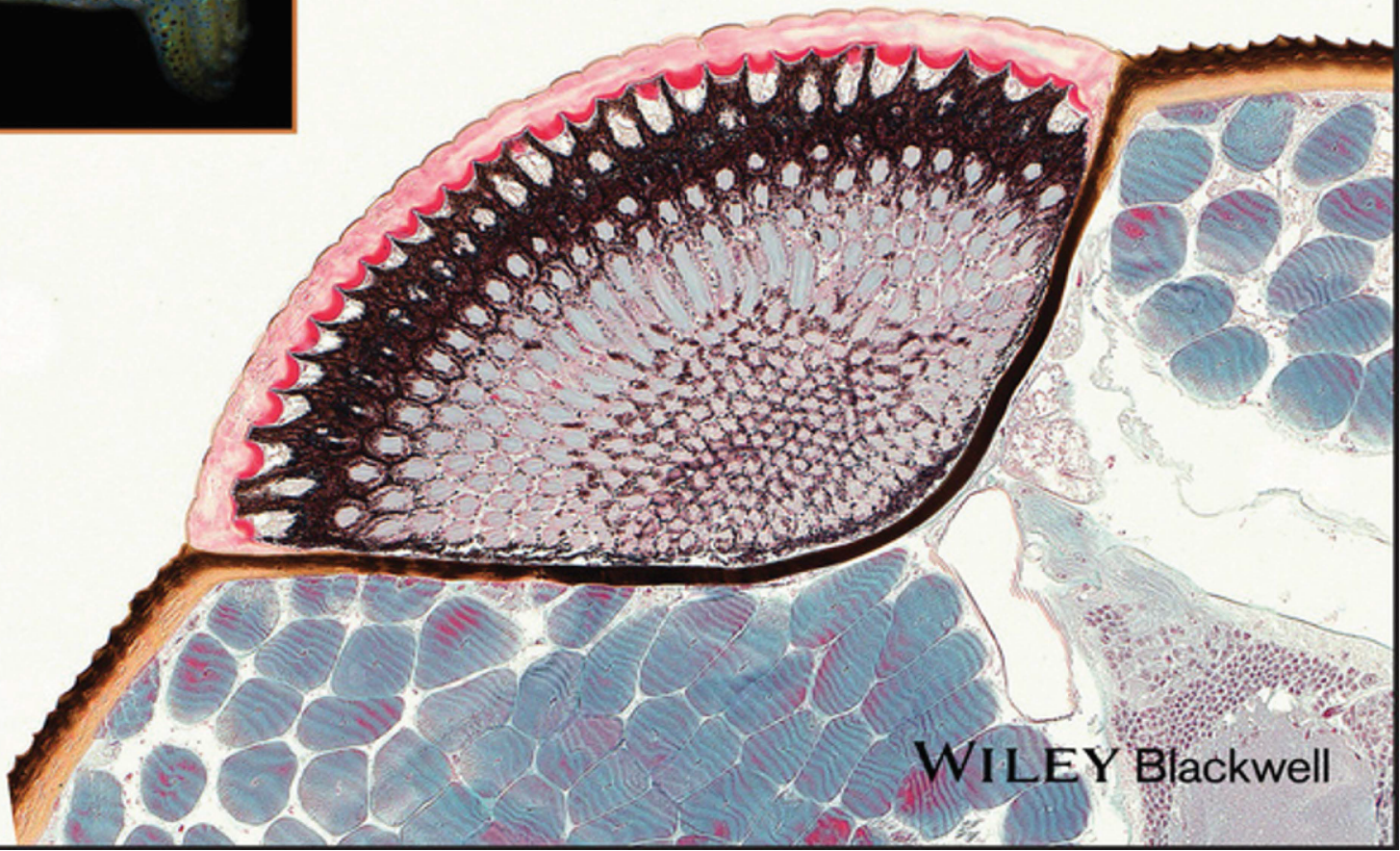
INVERTEBRATE HISTOLOGY

EDITED BY
ELISE E.B. LaDOUCEUR





WILEY Blackwell

Invertebrate Histology

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Foreword

Veterinary medicine is a dynamic profession that began over 250 years ago to heal and protect working and warring equids along with livestock for food and other human-use products. The profession has come a long way since the 1700s, most notably in the breadth of species embraced, and the information that exists and is being explored related to this taxonomic diversity. Increasing human population growth, commerce, technology, and animal welfare are all contributing to this expansion. Our profession is more diverse than ever, and a growing part of that diversity is the inclusion of over 97% of the animal kingdom: the invertebrates.

Dr LaDouceur and her internationally recognized contributors have assembled an organized, easy to navigate, comprehensive, and richly illustrated work focused on the microanatomy and histology of the invertebrates. It is certainly the only book of its kind on the market and one that is long overdue. The text is richly illustrated with beautiful images, drawings, and micrographs, detailing the normal gross and microscopic anatomy of the species covered. Chapters also describe how to properly and efficiently process invertebrate tissues for histology. This is critically important as standard vertebrate tissue-processing methods frequently do not apply to invertebrates. Anatomic features like chitinous shells, glass spicules, calcium carbonate skeletons, and mesoglea, to name a few, may require specialized fixatives, processing, and staining techniques.

One of the biggest challenges for a clinician or pathologist is being able to recognize and become familiar with what is normal about an animal. This challenge is especially pertinent when dealing with nondomestic species. There is no greater or more diverse animal classification than the invertebrates, estimated to include over 1.3 million described species (and it's likely that the global total could be 10 times this number), representing at least 40 phyla. The editor and authors have wisely focused on the taxa that are the most economically important and/or in need of conservation, protection, and veterinary support. This includes species commonly displayed in zoos and aquaria, taxa that are utilized in the laboratory for research, and animals that are kept as pets.

This detailed and thorough text is a windfall for our profession and anyone working on the health and welfare of these animals. Pathologists, veterinary clinicians, histology technicians, invertebrate zoologists, and students studying in these areas will all find this book highly useful and important for their work. The timing for this book could not be better. I'm sure you, the reader, will agree with me, and find this one of the most important references on your bookshelf, in your laboratory, or digitally on your computer.

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1

Echinodermata

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1.1 Introduction

Phylum Echinodermata consists of three subphyla (Asterozoa, Echinozoa, and Crinozoa) and five main classes. Subphylum Asterozoa contains two extant classes: Asteroidea (sea stars, sea daisies) and Ophiuroidea (brittle and basket stars). Echinozoa contains two extant classes: Echinoidea (sea urchins, sand dollars) and Holothuroidea (sea cucumbers). Subphylum Crinozoa contains only one extant class: Crinoidea (feather stars, sea lilies). There are 7000 living species of echinoderms (Mulcrone 2005). All are marine and almost exclusively benthic. Some subphyla are mobile (Asterozoa, Echinozoa) and others are sessile (Crinozoa), though some sea lilies have been documented to swim significant distances. Echinoderms do not appear to have near relatives among other invertebrate phyla.

Most members of Echinodermata are dioecious and undergo sexual reproduction, with a few species reproducing asexually. Holothuroids are gonochoric (Leake 1975). Asexual reproduction through fragmentation may occur in some Asteroidea and Holothuroidea due to trauma or predation. The diet varies widely by class, with Asterozoa being carnivorous, Echinozoa and Crinozoa being vegetarian browsers and filter feeders, and Holothuroidea being detritivores. Significant conservation concerns and anthropogenic stressors include commercial fisheries, which impact diet availability, particularly clams, mussels, and oysters, and the pet trade through individual animal collection and the collection of coral and live rock causing habitat loss. Environmental concerns include habitat destruction and direct animal impacts due to ocean acidification. Population declines due to disease such as the Caribbean *Diadema antillarum* mortality event in 1983–1984 (Carpenter 1990; Lessios 2016) and "wasting disease" events across multiple species of asteroid (Hewson et al. 2014; Menge et al. 2016) have more recently received significant focus. Certain Asteroidea are keystone species in their ecosystems, critical for controlling prey populations and diversity. Echinoidea and Holothuroidea are of paramount importance to marine ecosystems because of respective roles in counteracting macroalgal competition with corals, and recycling nutrients from decaying organic matter.

1.2 Gross Anatomy

Uniting features of all echinoderms include radial symmetry (pentamerous symmetry), a tricoelomate body cavity, and a body wall composed of calcite endoskeletal plates (dermal ossicles) connected by "mutable collagenous tissue." Most internal features, including the alimentary system, reproductive system, nervous system, respiratory system, and a unique water vascular system, share similar basic plans between the subphyla. The basic echinoderm body plan has 10 divisions: five radii (rays or arms) which alternate with five interradii (interrays). Typically, there is an oral surface with a central mouth and an aboral surface that contains the anus. Despite these commonalities, morphology does vary widely and thus representative examples of each subphylum are discussed separately.

The asteroid (sea star) body plan consists of a central disc with typically five but in some species (sun stars) up to 40 or more individual rays. Rays are broad based and arise from the lateral margins of the disc. They taper distally and each ray terminates in one or more tentacle-like sensory tube feet and a red eyespot. The aboral surface is dorsal and contains the anus at the center of the central disc, which may not be grossly apparent. The madreporite, bearing

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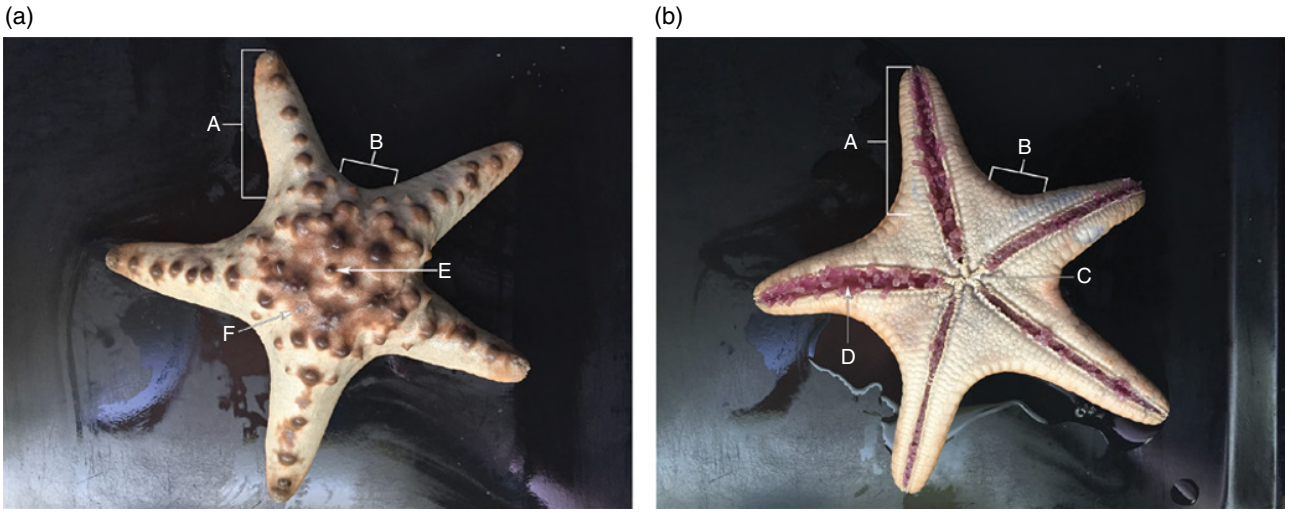


Figure 1.1 Representative image of the aboral (a) and oral (b) surface of a chocolate chip sea star (*Protoreaster nodosus*) demonstrating pentamerous symmetry. Labels include (A) radius, (B) interradius, (C) mouth, (D) ambulacral groove, (E) anus, and (F) madreporite.

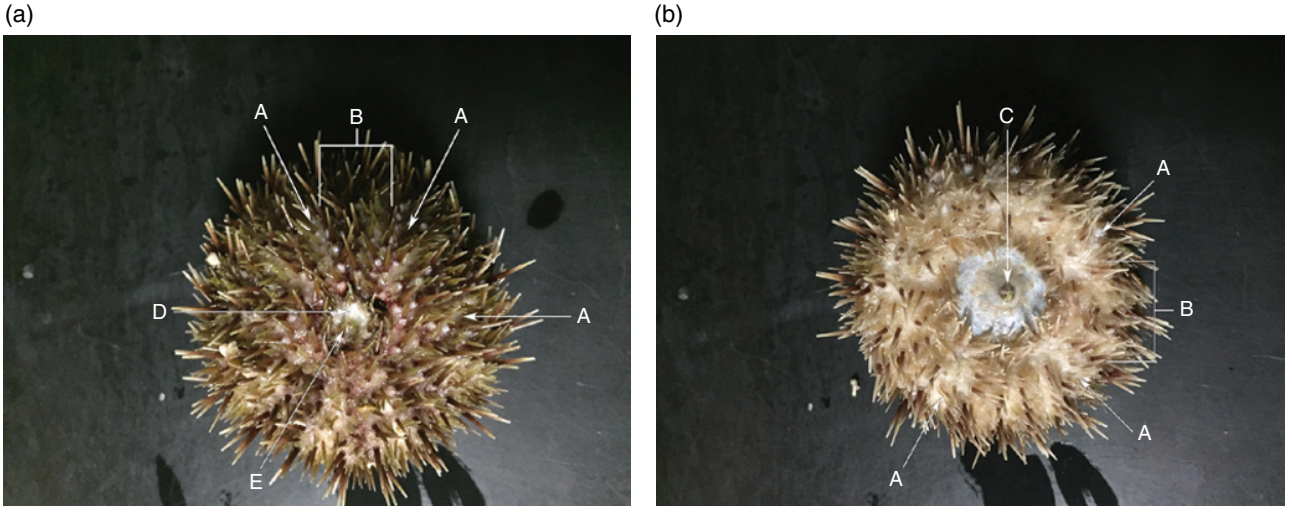


Figure 1.2 Representative image of the aboral (a) and oral (b) surface of a purple urchin (*Arabacia punctulata*) demonstrating pentamerous symmetry. Labels include (A) ambulacral plates, (B) interambulacral plates, (C) mouth, (D) anus, and (E) madreporite.

openings of the water vascular system, is on one side of the disc near the interradius of the first and second rays (Figure 1.1a). The oral surface is ventral and in contact with the substrate. Originating at the mouth and extending the length of each ray is a prominent groove, the ambulacrum (ambulacral groove). Two to four rows of tube feet (podia) lie within the ambulacral groove (Figure 1.1b). The margins are lined by moveable spines that can close over the top of the groove. Ophiurids (brittle and basket stars) demonstrate similar morphology. They typically have five rays, but these are distinctly offset from a round to pentagonal central disc. The rays are typically very long, slender, and very flexible. In basket stars the rays are highly branched. The disc has a proportionally smaller diameter compared to most sea stars. Ophiurid rays lack an ambulacral groove and the tube feet lack distal suckers as they are not typically used for movement.

Echinoidea lack rays and have either a slightly compressed globoid body plan (urchin) or a flattened body plan (sea biscuits, sand dollars). Similar to asteroids, they have a dorsal aboral surface with central anus (Figure 1.2a) and ventral oral surface with a central mouth (Figure 1.2b). Urchins have 10 radial sections, consisting of five pairs of ambulacral plates alternating with five pairs of interambulacral plates, which converge at the oral and aboral poles to form the test (i.e. outer shell). The ambulacral plates bear tube feet and are penetrated by pores that communicate internally with ampullae of the water vascular system,

whereas the larger interambulacral plates lack tube feet (Figure 1.3). On the oral surface, the plates meet, forming a large aperture centrally that contains the mouth and peripheral peristomial membrane. Surrounding the peristomial membrane are five specialized podia (buccal podia) and five pairs of gills. At the aboral pole, the anus is surrounded by a circular membrane, the periproct. There is a ring of five specialized plates (genital plates), surrounding the periproct, one of which is modified to form the madreporite. An additional five smaller plates, ocular plates, are interdigitated with the genital plates. Together, these 10 plates form the apical system.

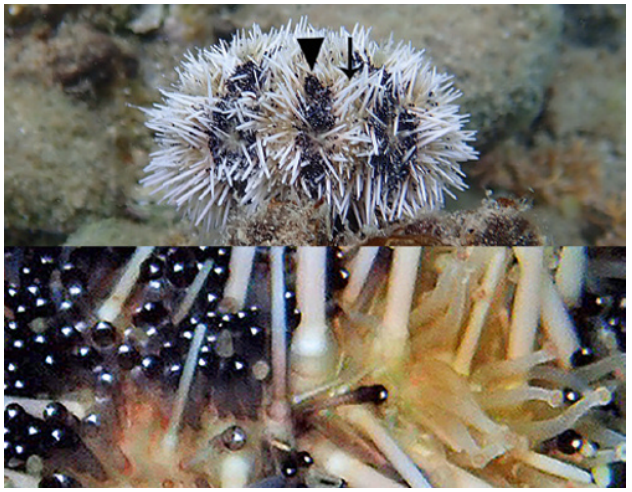


Figure 1.3 Image of a white sea urchin (*Tripneustes ventricosus*) demonstrating the distinction between ambulacral (arrow, inset right) and interambulacral (arrowhead, inset left) plates; tube feet are lacking in the latter where black-pigmented pedicellariae predominate.

Spines are arranged symmetrically in meridional rows along both ambulacral and interambulacral areas with the longest spines near the equator and shortest near the poles. Most urchins have long primary spines and shorter secondary spines equally distributed over the surface. Some species only have primary spines. Spines are cylindrical, taper to a point, and attach to the plates by a tubercle, resembling a ball and socket joint. Sand dollars and sea biscuits have a dorsoventrally compressed body plan compared to urchins, but similar anatomic features. The ventral ambulacral areas are called phyllodes and have tube feet modified for feeding and adhesion. The dorsal ambulacral areas are called petaloids (or petals) and tube feet are broad, flat, and specialized for respiration (gills).

In sea cucumbers, the main body axis is long and the oral surface including the mouth is at the anterior end of the animal and the body axis is parallel to the substrate (Figure 1.4a,b). The mouth is often surround by specialized tube feet (buccal podia) that are large and highly branched. The side of the body that lies on the substrate (ventral surface) contains three ambulacra that are referred to as

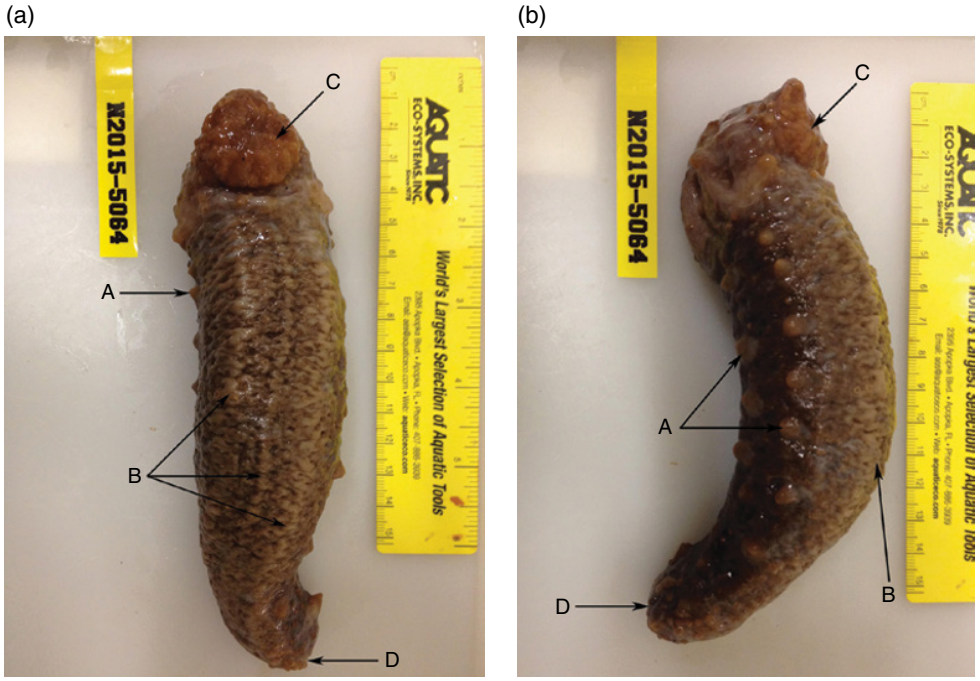


Figure 1.4 Representative image of the ventral (a) and lateral (b) aspects of a California giant sea cucumber (*Parastichopus californicus*). Labels include (A) dorsal ambulacra, (B) ventral ambulacra, (C) buccal podia, and (D) anus.

the sole. The dorsal side contains two ambulacra. Some burrowing species lack this differentiation. Tube feet can be arranged in prominent rows, be spread uniformly over the surface, or may be absent. When present, those on the ventral surface typically have suckers. Those on the dorsal surface are greatly reduced and often lack suckers.

The crinoids (sea lilies) have a different body plan from previously discussed subphyla. They have a long stalk extending from the aboral surface, which attaches the animal to the adjacent substrate. The oral surface is positioned along the uppermost portion of the body (crown). The crown demonstrates similar morphology to the body of other echinoderms. It consists of a central disc with an aboral calyx that is heavily calcified and an oral (dorsal) membranous wall called the tegumen. The mouth is often central or near the center. Ambulacral grooves radiate from the mouth, across the tegumen and into the rays. The anus opens on the oral surface in the interambulacrum and is often at the tip of a prominent anal cone. Rays radiate from the margin of the crown and typically range from 5 to 10. Additional branching is present in some species. In feather stars, each arm has a series of pinnately arranged jointed appendages called pinules creating the gross appearance of a feather. Ambulacral grooves are present and arranged similarly to sea stars. Along the margins there are moveable flaps (lappets) that alternately expose or cover the groove. Three tube feet, which are fused at their base, are present on the inner side of each lappet.

1.2.1 Keys for Dissection/Processing for Histology

In large Asteroidea, gross necropsy is approached from the oral surface. Morphometrics (weight, disc diameter, ray length) can be collected and the animal is placed in dorsal recumbancy. The disc can be opened circumferentially along the junction of the radius/interradius with the body wall, exposing the intestinal tract and gonads (Figure 1.5a). Each ray can be opened along both lateral aspects, removing the ambulacral groove to expose the pyloric cecae, gonads, and internal aspects of the tube feet (ampullae) (Figure 1.5b). Dissection with the animal immersed in sea water (natural or artificial) can help maintain organs in a more natural position and make them easier to assess grossly and dissect. Individual organ samples can be collected into 10% formalin for histology including sections of the body wall. In echinoids, two approaches are possible. The body can be opened circumferentially along the equator of the specimen (Figure. 1.6a) or can be opened dorsal to ventral through the anus and mouth (Figure 1.6b). Holothuroids can be opened with two incisions beginning at the mouth and following along the lateral aspects of the two dorsal ambulacra to the level of the anus. Upon removal of the dorsal aspect of the body wall, the coelomic cavity is readily viewed (Figure 1.7a). It is important to recognize upon opening the animal whether the full complement of viscera is still present as these animals, when stressed, can self-eviscerate (Figure 1.7b).

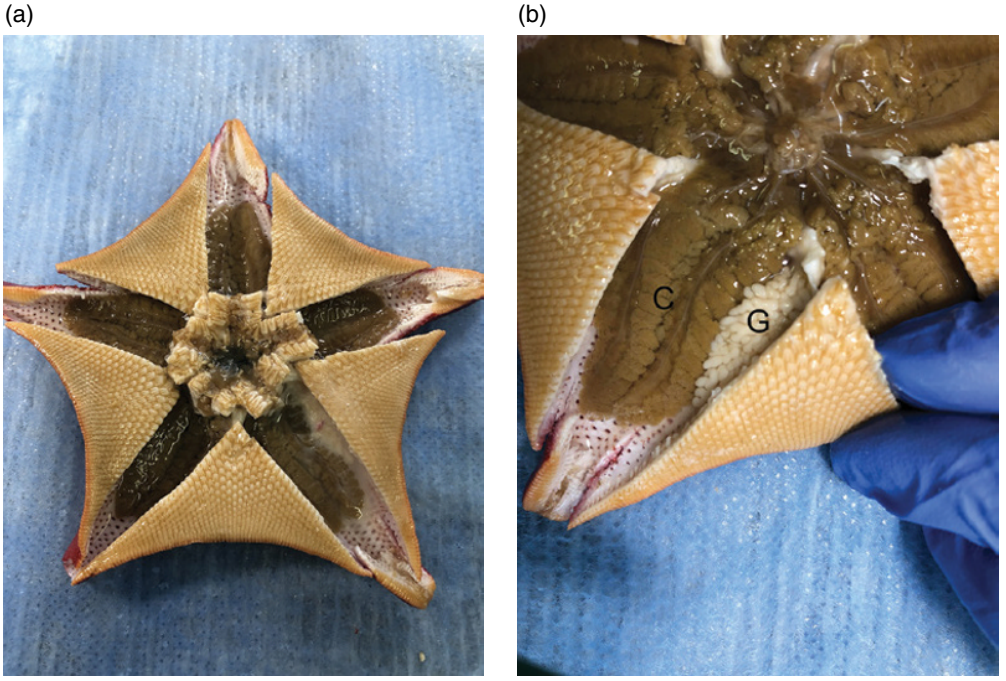


Figure 1.5 Gross necropsy image (a) of a bat star (*Patiria miniata*) open at necropsy. Higher magnification of one of the arms is provided (b) and shows the pyloric cecae (C) and the gonads (G). *Source:* Image courtesy of L. Abbo, Marine Biological Laboratory.

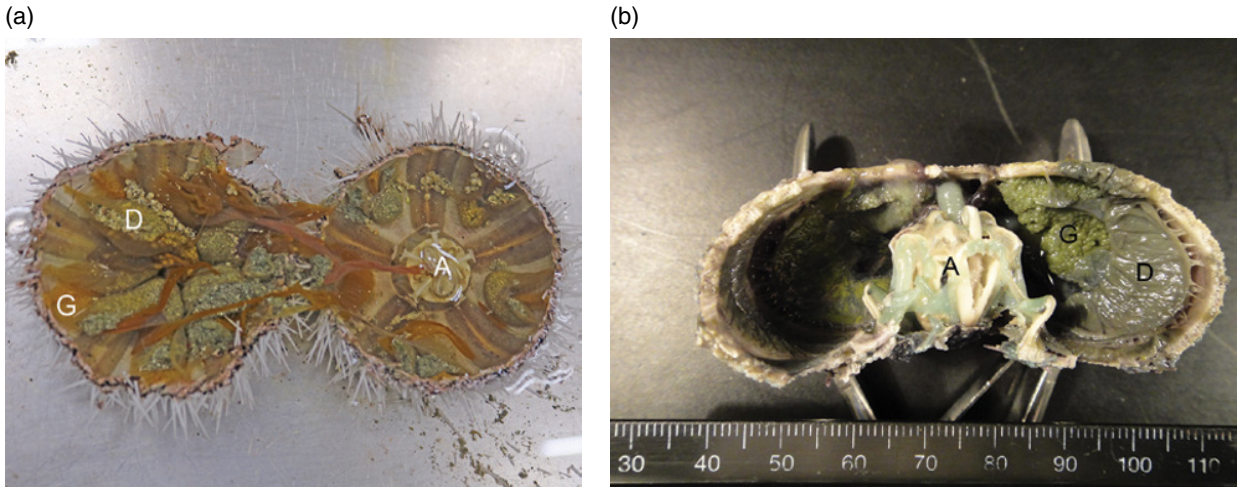


Figure 1.6 Gross necropsy images of urchin open at necropsy. Images include a white sea urchin opened at the equator and submerged in sea water (a) and a purple urchin (*Arabacia punctulata*) opened dorsoventrally (b), showing the gonads (G), digestive tract (D), Aristotle's latern (A), and ampullae (Am).

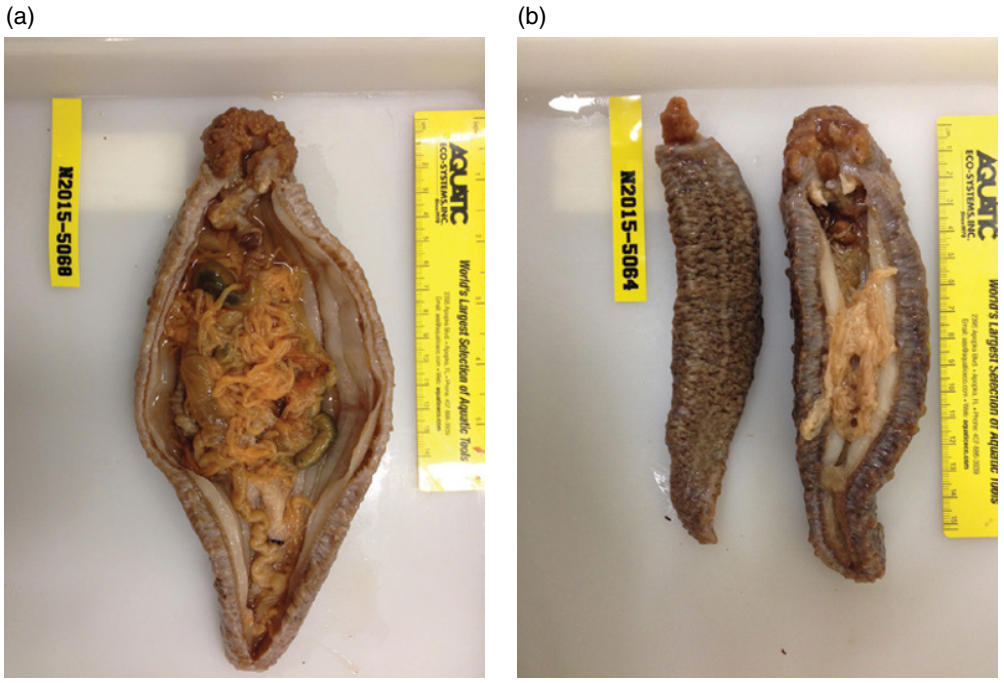


Figure 1.7 Gross necropsy images of a California giant sea cucumber opened along the dorsum removing the dorsal ambulacrum. Images include an animal that has not spontaneously eviscerated prior to death (a) and one that has spontaneously eviscerated (b).

Whenever possible, specimens should be fixed and processed for histopathology entire or as cross-sections as this permits evaluation of the different relationships of various organ systems to one another. Fixation in 10% formalin is adequate for soft tissues but postfixation decalcification of the body wall is required for routine histopathology. Methods of decalcification include fixation in Davidson's solution, postfixation decalcification in EDTA or with formic (DeltaFORM®; CalEx™ II), hydrochloric acid or formic/hydrochloric acid mixes (XL-Cal®). Decalcification can introduce histologic artifacts into tissues, specifically spaces/clearing in the cellular matrix of the endoskeleton due to gas accumulation. The more aggressive/rapid the decalcification process, the greater the disruption produced. Use of a fixative which has some decalcifying properties, such as Davidson's solution, can reduce the need for postfixation decalcification. In cases where body wall histopathology is the most critical system to be evaluated, plastination at a laboratory that specializes in bone histopathology is recommended to permit processing of fully

mineralized tissues. The cuticle is destroyed by fixation in phosphate-buffered glutaraldehyde but preserved by fixation in sea water-osmium, seawater-permanganate, and sea water-glutaraldehyde, modified Dalton's fixative or in a glutaraldehyde-osmium sequence with ruthenium red (Holland and Nealson 1978). The latter fixative is most successful in preserving the cuticle in most echinoderms due to the high acid mucopolysaccharide content in most species and the ruthenium red complex.

1.3 Histology

Histologic features of echinoderm organ systems are described in the following sections. A summary of each organ system and organs is provided in Table 1.1 and provides standardized nomenclature for histologic studies.

1.3.1 Body Wall/Musculoskeletal System

The body wall of echinoderms consists of three major layers: (i) an outer monolayered epidermis, (ii) a middle connective tissue dermis containing an endoskeleton and muscle, and (iii) an internal monolayered coelomic epithelial lining (Figure 1.8a–c). There is a sensory nerve net (ectoneural nerve net or subepidermal nerve plexus) associated with the epidermis. A similar sensory and motor nerve net is associated with the coelomic epithelium (hyponeural nerve net). Nerve nets can be difficult to appreciate on hematoxylin & eosin (HE) stained histologic sections. A multilayered cuticle composed of proteoglycans and mucopolysaccharides covers the epidermal surface, but is frequently lost during fixation and processing (Holland and Nealson 1978; McKenzie and Grigolava 1996). Cuticular layers can be discerned by TEM and are summarized in Table 1.2. In Echindoidea, Asteroidea, and Ophiuroidea, there are essentially three described layers: (i) fibrous outer layer; (ii) granular middle layer; (iii) fibrous inner layer. Crinoidea lack an inner fibrous layer. Holothuroidea have a unique outer rodlet layer and fibrogranular inner layer. In some species, symbiotic bacteria occupy the space between the cuticle and the epidermis. The microvilli and cilia of the epidermal cells project into the lower two layers of the cuticle but do not extend into the outer coat (Ameye et al. 2000).

The epidermis is composed of simple cuboidal to columnar epithelium of several cell types, best differentiated by electron microscopy. These include supporting cells, secretory cells, pigmented cells (chromatophores and iridophores), sensory cells, nerve cells, and coelomocytes. Supporting cells have microvilli along their apex and may have cilia. They have basally located oval nuclei and a prominent nucleolus. Secretory cells are nonciliated with microvilli present only at the apex. Although five types of secretory cells are recognized by electron microscopy, the features discernible by light microscopy are variations in vacuolar size, shape, and staining characteristics. This discerns essentially two cell types: mucous gland cells, with finely granular contents, and muriform cells filled with coarse spherules (Hyman 1955) (Figure 1.9). In some

Table 1.1 Organs for histologic evaluation in Echinodermata.[a]

Organ system		Organs
Body wall/musculoskeletal		Cuticle, epidermis, dermis/mutable collagenous tissue, dermal ossicles, skeletal muscle, paxillae[b], pedicellariae[b]
Water vascular system		Madreporite, stone canal, circumoral ring canal, radial canal, tube feet
Digestive	Alimentary canal	Mouth, esophagus, stomach, intestine[b], rectum[b]
	Pyloric and rectal cecae	Digestive tubules, pyloric duct, rectal duct
Excretory		Heart, axial canal, axial hemal vessel, tube feet, papulae
Circulatory		Heart, axial organ, axial hemal vessel, hyponeural (*oral*) hemal ring, gastric hemal ring, genital hemal ring
Immune		Coelomocytes
Respiratory		Papulae (*gills*), tube feet
Nervous		Circumoral nerve ring, radial nerve, superficial and deep nerve nets
Reproductive	Male	Testis, sperm ducts
	Female	Ovary, oviduct
	Ovotestis[b]	Ovary, testis
Special senses/organs		Eyespots, sensory tube feet

[a] Alternative names for organs are provided parenthetically, in italics.
[b] If present in a given species.

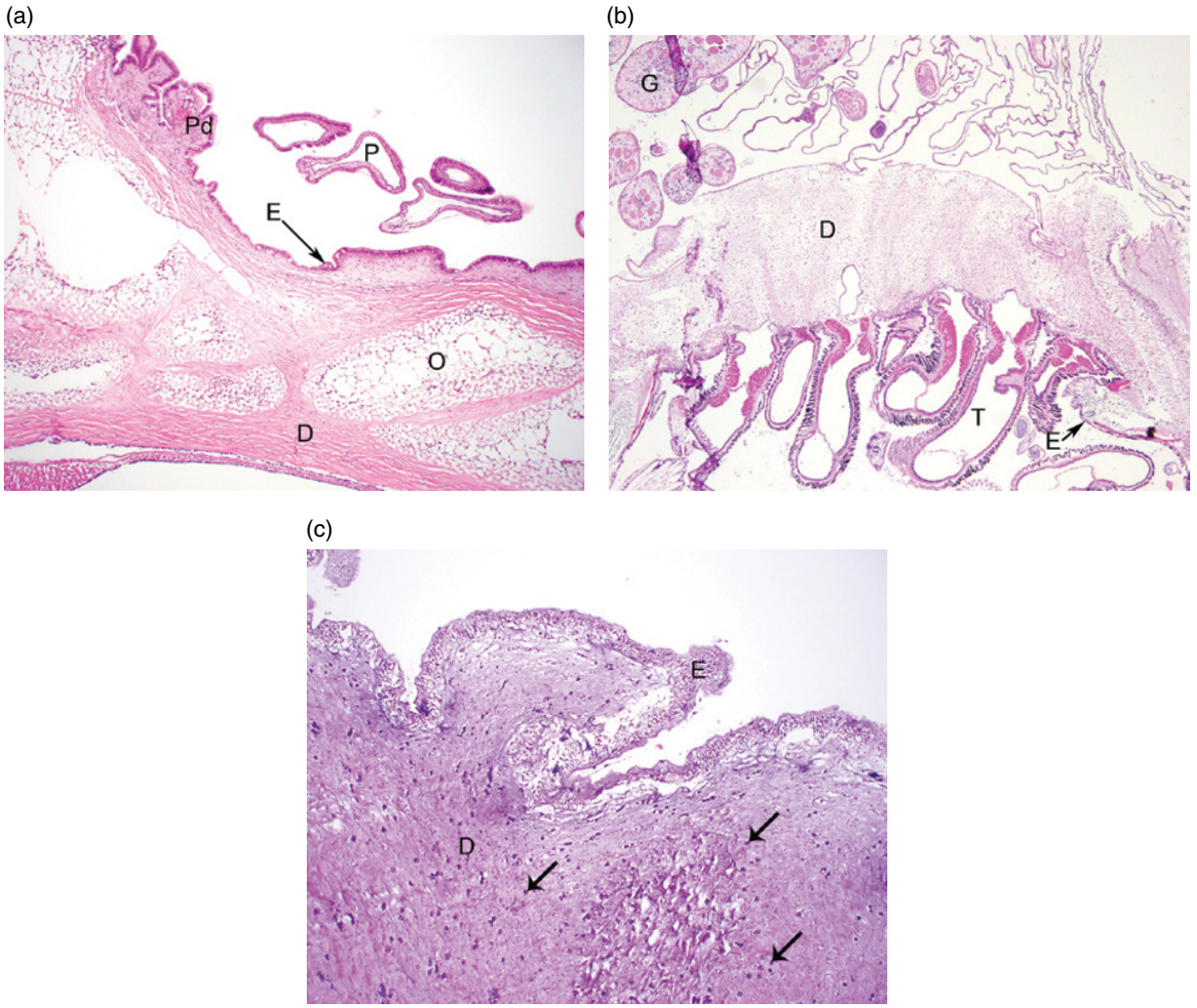


Figure 1.8 Low-magnification image of the histology of the body wall of an (a) ochre sea star (*Pisaster ochraceus*), (b) white sea urchin, and (c) California giant sea cucumber. Hematoxylin & eosin (HE), 100×, 40×, 100×, respectively. D, dermis; E, epidermis; G, gonads; O or arrows, ossicles; P, papulae; Pd, pedicellaria; T, tube feet.

Table 1.2 Cuticular layers in echinoderms (Holland).

Class	Layers present
Crinoidea	Fibrous outer layer ("fuzzy layer") Granular inner layer
Echinoidea	Fibrous outer layer Granular middle layer Fibrous inner layer
Asteroidea	Fibrous outer layer Granular middle layer Fibrous inner layer
Ophiuroidea	Fibrous outer layer Granular middle layer Fibrous inner layer
Holothuroidea	Outer, rodlet layer Granular middle layer Fibrogranular inner layer

echinoderms, especially echinoids, epithelial cell types may be difficult to differentiate histologically. In areas surrounding papulae (eversions of the coelomic cavity used for respiration in Asteroidea), the epidermis may contain multicellular glands with specialized secretions. Sensory nerve cell bodies and their axons may be visible basally within the epidermis, often referred to as the subepidermal plexus (or the ectoneural nerve net). The sensory layer is thinnest near the papulae and thickest in the oral region where it forms a circumoral nerve ring. The sensory layer often forms a ring around the base of ossified appendages. Coelomocytes may be present in the epidermis due to their role in phagocytosis and excretion of waste products to the environment. Their features are described later. The inner body wall consists of a simple layer of squamous sparsely ciliated epithelial cells that line the coelomic cavity.

The dermis is composed of mutable collagenous tissue and an endoskeleton composed of interconnected plates,

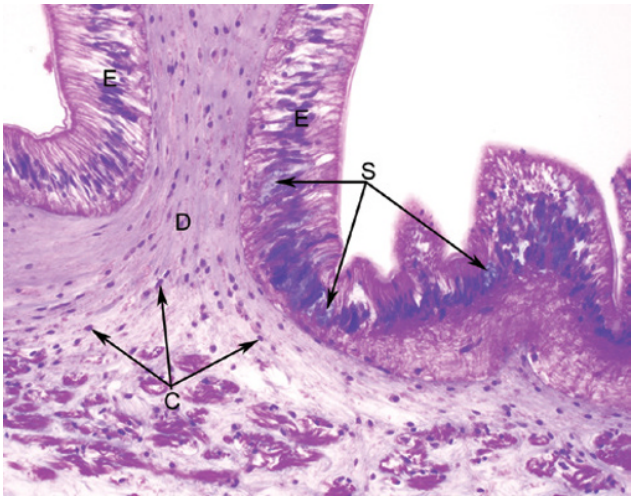


Figure 1.9 Histology of the epidermis of a sunflower sea star (*Pycnopodia helianthoides*). Individual cell types are difficult to discern with light microscopy. The columnar epidermis (E) has occasional secretory cells (S). The subjacent dermis (D) contains many coelomocytes (C). 400×, Lee's methylene blue (LMB).

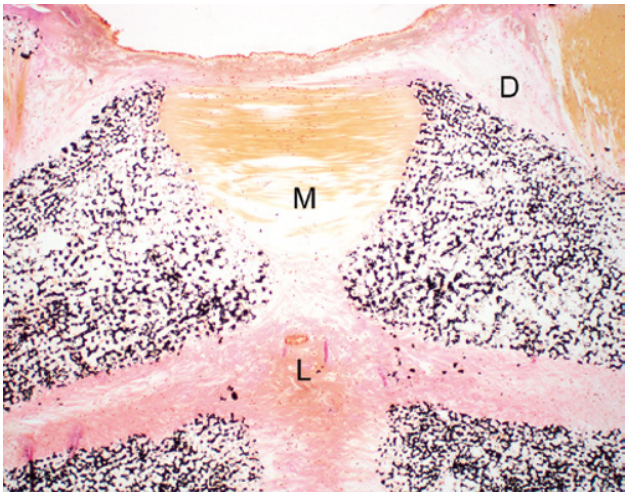


Figure 1.10 Low-magnification image of the histology of a sunflower sea star ossicle demonstrating dermal, ligamentous, and muscular attachments. 200×, von Kossa.

which may be articulated to form a rigid structure. The endoskeleton is composed of magnesium-rich calcium carbonate, as magnesian calcite, devoid of an organic matrix (Cavey and Märkel 1994). Magnesium, substituting for calcium, is a unique feature of the echinoderm skeleton relative to other invertebrates (Raup 1966). Endoskeletal plates are of various shapes and are often called ossicles. Ossicles are separated into small interdigitating sections that are adjoined by collagenous ligaments and skeletal muscle (Figure 1.10). They are typically adorned by tubercles that articulate with movable ossified appendages, such as spines or calcareous protuberances, pedicellariae, and

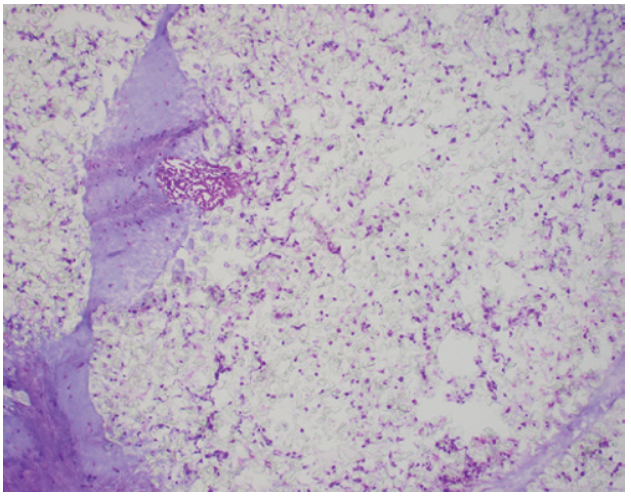


Figure 1.11 Higher magnification image of the histology of an ochre sea star ossicle demonstrating the sclerocyte lattice (plastinated section). 400×, LMB.

sphaeridia. Specialized ossicles called paxillae are present on the aboral surface of certain sea star species and facilitate burrowing. In ophiuroids, ossicles form larger plates called shields and each arm segment (article) is composed of four shields, two lateral, one aboral and one oral, with the lateral shields having large spines. Echinoids lack a muscle layer in the body wall because skeletal plates are fused and immobile, although muscle tissue is still present at the sites of articulation of the spines. In holothurorids, the ossicles are present but microscopic and are randomly distributed throughout the dermis. Some have paired specialized ossicles, the anchor and anchor plate, which assist in attaching species that lack tube feet to the substrate. A ring of well-developed ossicles is present around the mouth and esophagus providing attachment sites for the buccal podia. Well-developed longitudinal bands of smooth muscle are present along each ambulacrum.

Histologically, the endoskeleton consists of a three-dimensional crystalline latticework, the stereom. Post decalcification, the calcite trabeculae are evident as clear spaces that may be artifactually collapsed. The fluid-rich stroma that marginates trabeculae forms a honeycomb structure and contains sclerocytes that produce, modify, and envelop the skeleton (Figure 1.11). Sclerocytes are stellate mesenchymal cells that are typically in contact with trabeculae, and may be sparse within fully developed ossicles (Märkel and Roser 1983). In growing ossicles, sclerocytes form syncytia. Coelomocytes (discussed later) are common among the stroma, but may not necessarily be evenly distributed and can lead to a false impression of inflammation. Specialized phagocytes are capable of reabsorbing calcite from the ossicles (Ruppert et al. 2004). In echinoids, these are termed skeletoclastic cells and they

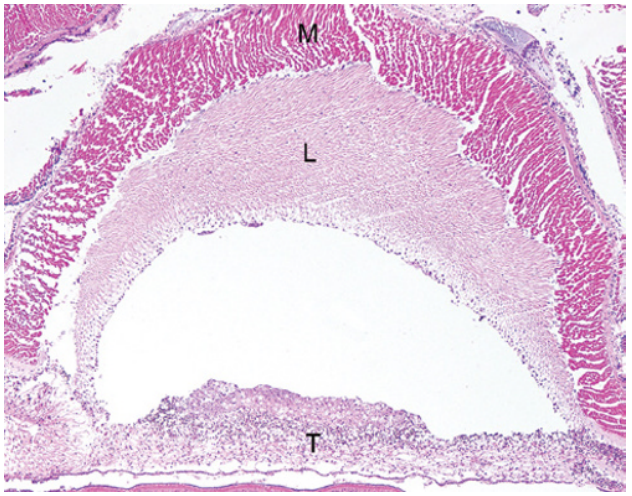


Figure 1.12 Histology of the base of a white sea urchin spine at the ball and socket joint. 400×, HE. M, muscle; L, ligament; T, test.

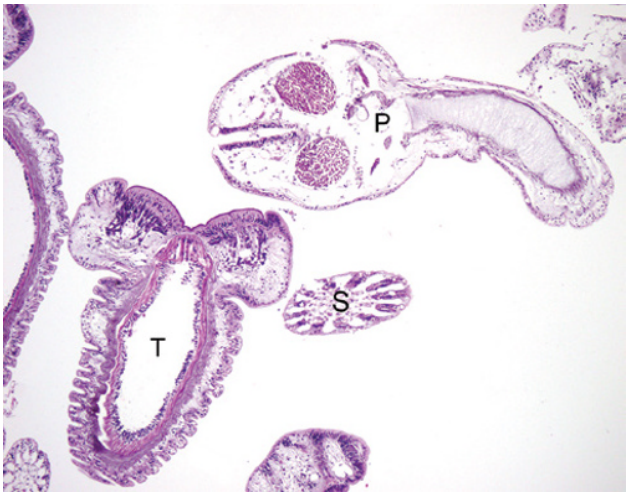


Figure 1.13 Histology of white sea urchin appendages including pedicellaria (P), spine (S), and tube foot (T). 100x. HE.

are syncytial phagocytes that resemble osteoclasts (Cavey and Märkel 1994).

The osseous appendages have components that are similar to the body wall. All are covered in epidermis and contain an assemblage of dermal tissues described above. The echinoid spine consists of similar latticed endoskeleton with a central meshwork or hollow area surrounded by radiating longitudinal septae. The base of a spine adjoins to a tubercle of the test with ligaments of mutable collagenous tissue (i.e., the catch apparatus) encircled by bundles of smooth muscle cells (Figure 1.12). Distal spines of some urchins may be surrounded by a poison sac that has a collagenous connective tissue wall, and a lumen containing dissociated cells and debris (Cavey and Märkel 1994). Pedicellariae, present in Echinoidea and Asteroidea, clean the body surface and protect against sediment and small organisms. Microscopically, they consist of a stalk bearing a moveable head (Figure 1.13). Pedicellariae can be classified into a variety of types based on the size and shape of the head, and the number of jaws (i.e., tridentate, trifoliate, ophiocephalous, and globiferous). Most often, they have three elongate and distally narrowed jaws, each supported by a valve-type ossicle, and supplied by adductor, abductor, and flexor muscles. The latter may be composed of smooth or striated myocytes. The stalk is supported by a rod-shaped ossicle that may distally transition to a cavity filled with mucosubstances (Ghyoot et al. 1987). The epidermis is similar to that covering the test, but may be heavily ciliated along the stalk and inner jaws. Globiferous pedicellariae may carry venom sacs or epidermal glands on the inner jaws and these may be composed of more than one type of secretory epithelial cell (Ghyoot et al. 1994).

Dermal spaces between the endoskeleton are composed of fibrous connective tissue populated by stellate cells (Hyman 1955). A unique connective tissue termed mutable collagenous tissue is present in the body wall of all classes of echinoderms. Mutable collagenous tissue is controlled through a nonmuscular nervous system and can change its mechanical properties within one second to a few minutes from flaccid to rigid (Motokawa 1984, 2011; Wilkie 2002). The histologic features of mutable collagenous tissue (also called catch connective tissue) are not unlike dense irregular and regular connective tissues present in vertebrates. It is composed of individual collagen fibers with intervening ground substance that are arranged in perpendicular or parallel arrays depending on the species (Motokawa 1984). Interspersed among the fibers and ground substances are small numbers of immune cells (morula cells, coelomocytes). The function of this tissue varies by species and body wall structure. In holothuroids and asteroids, this tissue plays a significant role in overall body tone. In asteroids and echinoids, it plays a role in spine posture and prevents spine disarticulation. In crinoids, it controls the flexibility of the stalk (cirral) ligaments. In all species, it plays a significant role in autotomy (Motokawa 1984).

1.3.2 Water Vascular System

The water vascular system is a hydraulic system used for substrate adhesion, locomotion, and in some echinoderms prey manipulation. In many species tube feet also play an important role in respiration and excretion. It is composed of the madreporite, stone canal, circumoral ring canal, radial canal, ampullae, and tube feet (also called podia). The madreporite is a porous ossicle on the aboral surface of

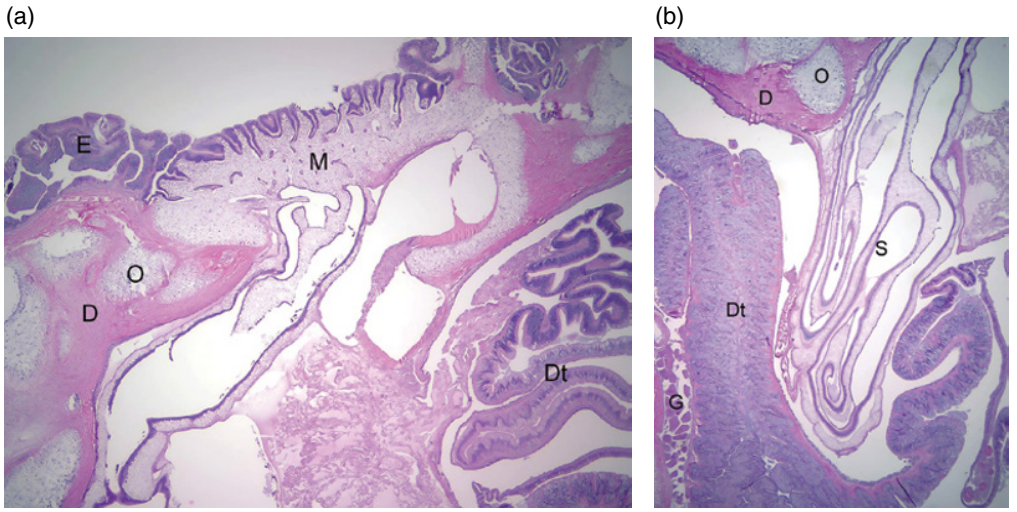


Figure 1.14 Histology of the madreporite (a) and stone canal (b) in a mottled star (*Evasterias troschelii*). 25×, 50×, HE. D, dermis; Dt, digestive tract; E, epidermis; G, gonad; M, madreporite; O, ossicles; S, stone canal.

sea stars, sand dollars, and sea urchins and the oral surface of brittle stars. In sea cucumbers the madreporite is internal. Also known as the sieve plate, the madreporite functions as a valve which communicates with surrounding sea water. The madreporite and stone canal maintain fluid volume in the water vascular system (Ferguson 1990; Ferguson and Walker 1991). Coelomic fluid fills the water vascular system and is osmotically and ionically similar to sea water (Freire et al. 2011).

The madreporite, when present externally on the disc or test, has a surface epithelium similar to the epidermis (Figure 1.14a). It is connected to the stone canal, which consists of scroll-shaped calcareous rings or spicules (Figure 1.14b). The stone canal connects to the circumoral ring canal that gives rise to five radial canals. In Echinoidea, the ring canal may form a small outpocketing at the top end of each tooth, termed polian vesicles. The radial canals extend into the rays through the ambulacral ossicles, or in Echinoidea to the inner ambulacrum surface (Figure 1.15). These terminate in the tube feet, which consist of an interior bulb (an ampulla) and an external foot (a podium). Ciliated myoepithelium, a combination of muscle cells and support cells that histologically resemble cuboidal epithelial cells, lines the entire interior of the water vascular system (Cavey and Märkel 1994). Cilia create flow in the internal canals to help with fluid transport while muscle contraction generates hydraulic pressure to move the tube feet. Exterior to the myoepithelial lining is a connective tissue layer and an external layer of coelomic epithelial cells.

The ampullae are elongate sacs that may be divided from the radial canal by a valve and have circular and longitudinal layers of muscle fibers. The podia consist of a stalk and terminal disc. They have layers similar to the body wall – an outer epidermis, middle connective tissue layer, and interior coelomic epithelial lining (Hyman 1955). The epidermis of the podia contains larger numbers of secretory cells than the rest of the body. The epidermis of the disc becomes thickened and is composed of ciliated columnar cells, larger numbers of secretory cells and neurosensory cells with a more prominent subepidermal nerve plexus, and is supplied by many subepidermal glands that may include mucous cells and granular secretory cells (Nichols 1961). Subjacent to the glands, the disc may be supported by latticed endoskeletal fragments. In addition to the subepidermal nerve plexus, a podial nerve may be evident coursing longitudinally on one side of the stalk. The stalk consists mainly of a cylinder of collagenous

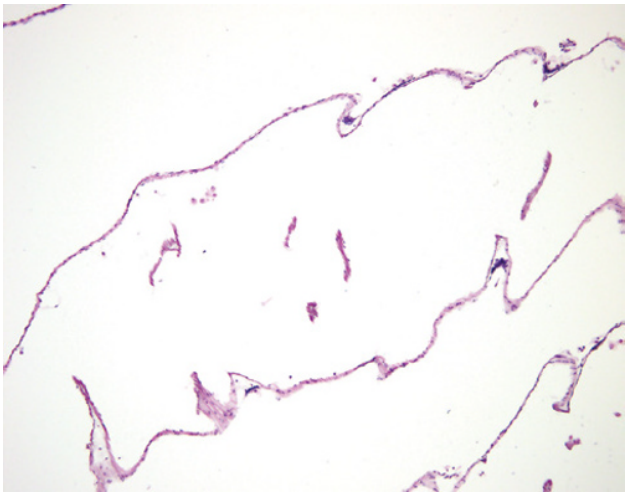


Figure 1.15 Histology of the water vascular (radial) canal in a white urchin. 200×, HE.

connective tissue (potentially divided into outer thicker longitudinal and inner thinner circular layers), supported by calcareous spicules (Figure 1.16). There is a central lumen (or hydrocoel) lined by a similar myoepithelium as observed throughout the water vascular system. Podia also have thick longitudinal retractor muscles which can contract the podia and push coelomic fluid back into the ampullae.



Figure 1.16 Histology of a tube foot in a mottled star. 25×, HE. C, connective tissue; D, disc; E, epidermis; H, hydrocoel; M, muscle; O, ossicle; S, stalk.

1.3.3 Digestive System

Echinoderms are a diverse group of animals with different nutritional strategies reflected in their digestive tracts. All consist of a simple tubular structure extending from the mouth to the anus with varying modifications that aid in digestion. In asteroids, the alimentary canal consists of a mouth, esophagus, stomach (cardiac, pyloric), intestine, and rectum. The mouth is at the center of the peristomial membrane and is separated by a muscular sphincter from the short esophagus and a more complex stomach. The cardiac portion of the stomach is large and has 10 distinct pouch-like structures (radial pouches). Five of the pouches extend into the lumen of the arm from the disc and are attached to the ambulacral ossicles by muscle and dense connective tissue. A pair of gastric ligaments anchors the esophagus and permits retraction of the cardiac stomach in species that evert it during feeding. Above the radial pouches are five interradial pouches that eventually transition into the pyloric portion of the stomach. The pyloric stomach is smaller, flattened, and "star shaped" with five ducts that each extend into the central coelomic cavity of each ray and connect with the heavily branched pyloric cecae. The upper portion of the stomach tapers to form a short intestine that can have its own series of short blind sacs (intestinal cecae). The intestine connects to the short rectum and anus (Leake 1975; Ruppert et al. 2004).

The gastrodermis of the asteroid cardiac stomach is a pseudostratified columnar epithelium (Figure 1.17a). These cells lie on a basal lamina and basiepithelial nerve plexus with a connective tissue wall and outer coelomic epithelial liming. Circular and longitudinal muscle layers are interwoven into the coelomic lining. The gastrodermis is composed of supporting cells, secretory cells, and two

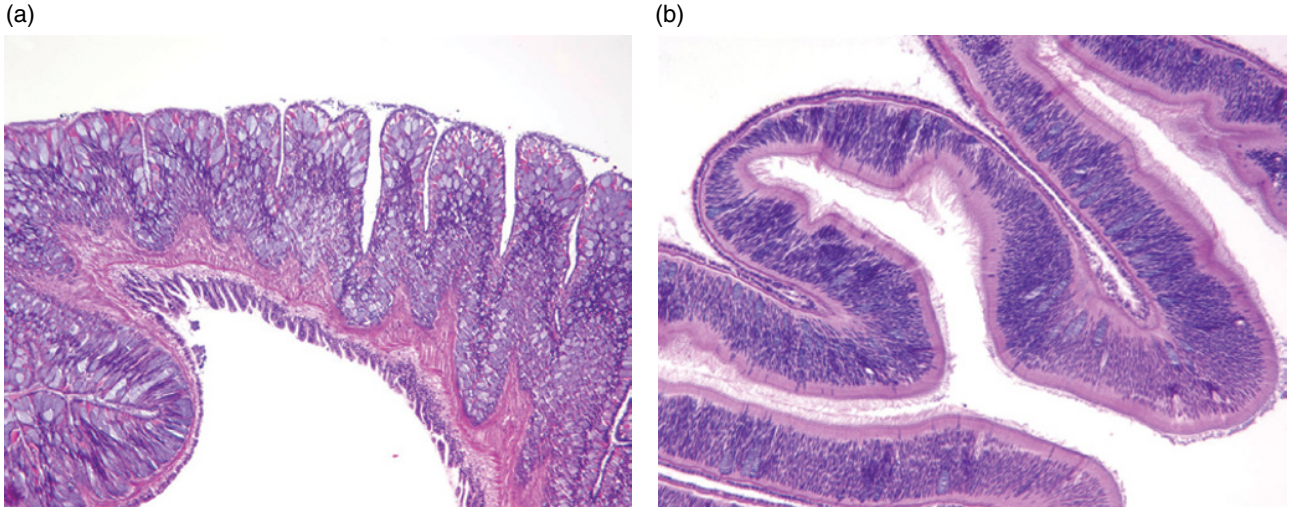


Figure 1.17 Histology of the cardiac (a) stomach in a mottled star (100×, HE) and pyloric stomach (b) of a mottled star (200×, HE).

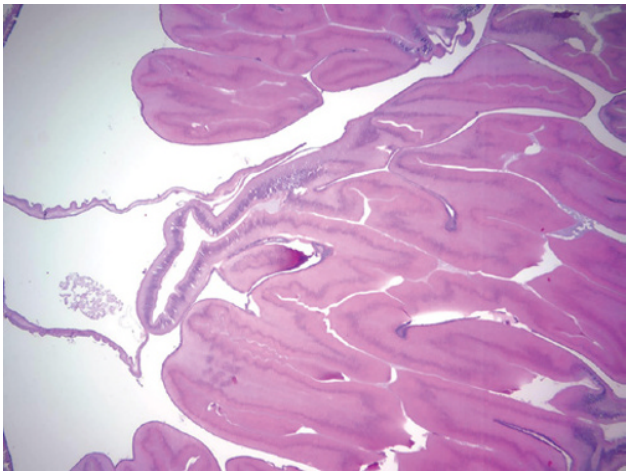


Figure 1.18 Histology of the pyloric cecae of a mottled star. 25×, HE.

types of coelomocytes. Supporting cells have a single cilium and numerous long microvilli. Secretory cells have no cilia and are either mucous or glandular in type. Two types of coelomocytes are normally seen in the gastrodermis and are found at all levels of the gut wall. The gastrodermis of the pyloric stomach is similar to the cardiac. Both the gastrodermal lining and the entire wall are thinner in the pyloric stomach due to reduced presence/thickness of the basiepithelial nerve layers, connective and muscle tissue layers (Figure 1.17b).

The pyloric and intestinal cecae are only present in asteroids. They are foliate structures created by extensive diverticula, which extend laterally from a medial duct. The diverticula are further divided into secondary chambers that are arranged parallel to the median duct. The lining of the pyloric cecae consists of very tall ciliated supporting cells and glandular secretory cells (mucous and zymogen cells) which are most abundant in the distal chambers of the pyloric cecae. Storage cells, cells containing large lipid vacuoles and polysaccharide and glycogen laden vacuoles, are more abundant distally (Hyman 1955; Leake 1975) (Figure 1.18).

The gastrodermis of the intestine and intestinal cecae is a ciliated pseudostratified columnar epithelium that in some areas may be compressed into a simple columnar epithelium and appear similar to the lining of the stomach. The epithelium is composed of supporting cells and two types of mucous secretory cells. The muscle, connective tissue, and nervous components are poorly developed in the intestinal cecal wall. The gastrodermis of the rectum and anus are identical and consist of a pseudostratified columnar epithelium composed predominantly of monociliated supporting cells attached to a basal lamina. The basiepithelial nerve plexus is reduced to absent. The connective tissue layer is thicker than in the intestine and pyloric cecae (5–10 μm thick) and is composed of thin elastic fibers (Hyman 1955). In Ophiurioidea, the digestive system is composed of a mouth, esophagus, stomach, rectum, and anus but lacks an intestinal tract and all components have histologic features similar to those described in asteroids.

In Echinoidea there is a mouth and a unique masticatory apparatus, Aristotle's lantern, followed by the esophagus, intestine, rectum, and anus. Aristotle's lantern is a pentamerous cone made of 40 ossicles including five teeth, adjoined by muscles and confined by coelomic membranes. At the ventrum of the lantern, the mouth is surrounded by a peristomial membrane, composed of mutable collagenous tissue covered in epidermis. Food passes through the mouth into a short pentagonal pharynx suspended in the center of the lantern. The pharynx transitions to esophagus at the top of the lantern. The esophagus ascends and then loops back as intestine. A blind pouch, variably referred to as stomach or cecum, may be present at the junction of esophagus and intestine. The intestine coils along the inside of the test, suspended by peritoneal membranes (i.e., mesenteries). The first nearly complete coil courses counterclockwise (when viewed from a dorsal or aboral aspect), and this segment is sometimes referred to as the stomach, or small or inferior intestine. Most echinoids have a slender extension of the intestine that accompanies this first coil at its inner border, termed the siphon, and it is believed to facilitate extraction of water from food. Then, the intestine turns back on itself and courses dorsally and clockwise to form a second coil, and this segment is sometimes referred to as the large or superior intestine. Finally, the terminal intestine forms the rectum that ascends to the interior of the periproct and forms the anus.

Histologically, the echinoid digestive tract has layers similar to other echinoderms. The epithelial lining is composed of tall columnar ciliated epithelial cells termed enterocytes, some of which bear microvilli, and others that may be distinguished as mucous cells (Figure 1.19). Similar to the epidermis, there is a subtle nervous layer at the base of enterocytes. Subjacent to this is a thin layer of connective tissue, followed by a thinner layer of muscle cells, typically arranged in a circular pattern relative to the lumen. The outer layer consists of a simple layer of flagellated cuboidal epithelial cells, as found on coelomic surfaces of other viscera. Glandular crypts may form where shortened enterocytes segmentally invaginate. Oral (small) and aboral (large) intestine may be histologically distinguished by differential presence of glands, villi, thickness, or prominence of microvilli (Work n.d.; Francis-Floyd et al. 2020). The siphon is histologically similar to the small intestine, only of smaller diameter. Histologic sections through the lantern typically feature major ossicles (i.e., the pyramids, compass, and rotula), teeth, interpyramidal (or comminator)

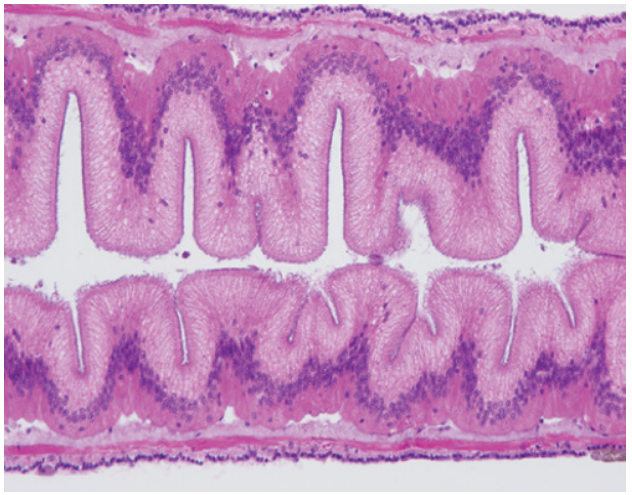


Figure 1.19 Histology of the large intestine of a white urchin. 200×, HE.

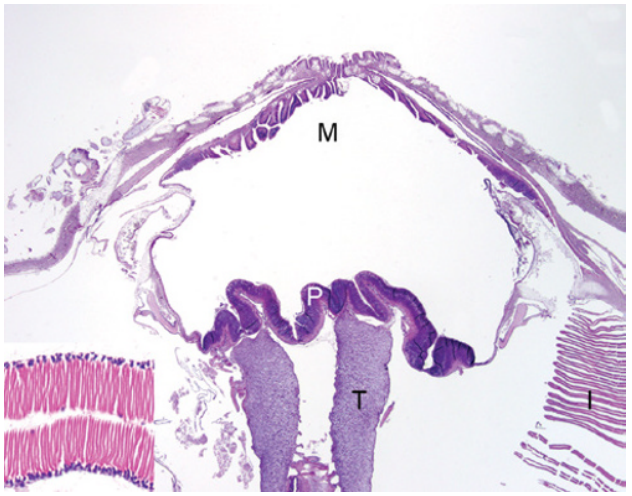


Figure 1.20 Low-magnification histology of anatomy of Aristotle's lantern in a white urchin. Inset shows closer view of interpyramidal muscle. 20×, HE. I, interpyramidal muscle; M, mouth; P, pharynx; T, teeth.

muscles, the pharynx, peristomial membrane, the circumoral nerve ring, and sometimes gill at the lateral margin of the lantern (Figure 1.20). The central cavity of the lantern coelom that surrounds the pharynx reflects between folds of interpyramidal muscle. Its myocytes are arranged into rows along a thin connective tissue septum and are covered by a layer of squamous and ciliated adluminal cells (Märkel et al. 1990). The protractor and retractor muscles exterior to the base of the lantern are instead arranged into fascicles within connective tissue matrix (Ziegler et al. 2012).

In Holothuroidea, there is a mouth, pharynx (calcareous ring), esophagus, stomach, anterior and posterior intestine, and cloaca. The mouth is at the center of a buccal membrane and is surrounded by a muscular sphincter. This leads to a short pharynx enclosed in a ring of ossicles. The stomach may not be present in some species and is generally not as well defined as in Asteroidea. The pharynx and stomach have a tall columnar epithelial lining composed of supporting and glandular cells showing mucous cell differentiation. Both have an internal cuticular lining unlike other species. The intestinal tract in holothuroids is extensive and is the primary site of digestion. The anterior portion (small intestine) has an extensive associated vascular system. It is lined by tall ciliated epithelial cells with prominent glandular differentiation and has a thin muscular wall. The posterior portion (large intestine) has a thinner epithelium with more prominent mucous cell differentiation. The digestive system of Crinoidea is confined to the disc and consists of a mouth, esophagus, intestine, rectum, and anus (anal cone) (Ruppert et al. 2004). Histology is similar to previously described echinoderm species.

1.3.4 Excretory System

In most echinoderms nitrogen excretion is primarily in the form of ammonia, which can diffuse across thin portions of the body wall at the papulae and tube feet. Coelomocytes facilitate excretion of other nitrogen-containing metabolites (urates) and particulates through pinocytosis. Coelomocytes accumulate waste material internally and carry these accumulations to the gills, tube feet, and axial organ for either disposal or storage. Crinoids have no specialized excretory organs but are believed to be ammonotelic.

1.3.5 Circulatory System (Hemal System or Axial Complex)

In Asteroidea, hemal sinuses at the margins of the gut drain to the hemal ring that surrounds the base of the esophagus. The axial duct arises from the hemal ring, courses with the stone canal to the dorsal/aboral body, and enters the axial complex beneath the madreporite. The axial organ is adjoined by the axial duct, forming a junction between the coloemic cavity, water vascular system, and hemal system. The exact role of the axial complex is currently undetermined. Hypotheses include roles in respiration, excretion, and waste disposal, an immune organ, a gland of unknown purpose, a coelomocyte-producing organ, a site of cell degradation, or a heart (Ziegler et al. 2009).

Histologically, hemal sinuses (or lacunae) have a wall of connective tissue that is lined exteriorly by coelomic epithelium. Muscle fibers in circular or longitudinal profile

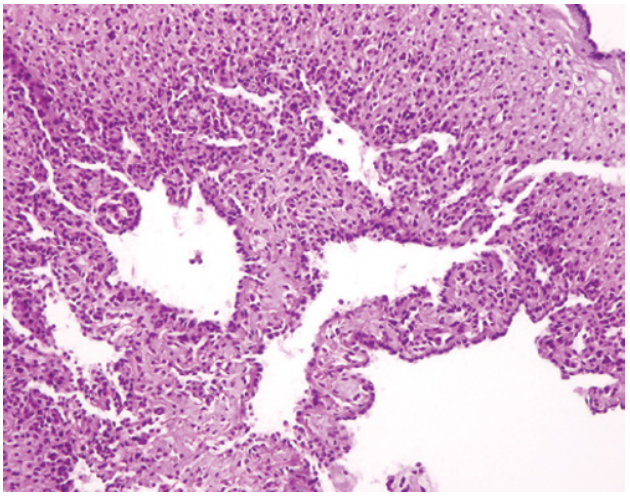


Figure 1.21 Axial gland in a white urchin. 400×, HE.

are scant throughout the wall. There is no inner lining or endothelium. Pigmented cells presumed to be phagocytes laden with melanin are often within vessels of the hemal system, and these may increase with age. The axial gland (or axial organ) is associated with the stone canal and consists of meshwork of connective tissue populated by coelomocytes (Figure 1.21) (Ziegler et al. 2009). Invaginations of the coelomic lining and lacunae penetrate the hemal sinuses. Cells containing melanin pigment are often within the stroma (Bachmann and Goldschmid 1978). The external surface of the axial gland is lined by coelomic epithelium.

Five pairs of Tiedemann's bodies adorn the hemal ring at the interradial areas in Asteroidea and the dorsal lantern in Echinoidea. In echinoidea they are formed where the coelomic lining of the dorsal lantern engages with evaginations from the radial canals (Cavey and Märkel 1994). Histologically, these are similar to the axial organ (Figure 1.22). A meshwork of connective tissue is permeated by canaliculi lined by coelomic epithelium. Coelomocytes and pigmented cells are also similarly frequent.

1.3.6 Immune System

Coelomocytes exist within the fluid of the coelomic cavity, water vascular system, and hemal system, and are seen throughout all tissues of the body (Holland et al. 1965). They play diverse roles including nutrient delivery, waste excretion, phagocytosis, immune response, clotting, and wound healing. Nine different coelomocyte types have been described in sea stars (Kanungo 1984) but by light microscopy these cell types are not discernible. Some discerning features are evident using electron microscopy. Coelomocytes in echinoids include phagocytes (amoebocytes), spherule cells, and vibratile cells (Cavey and Märkel 1994), best distinguished by cytology. Phagocytes are the most abundant and may have cytoplasmic foreign material. Vibratile cells are small, round, and flagellated. Coelomocytes with eccentric nuclei and cytoplasmic inclusions are nonphagocytic and often referred to as granular or spherule cells, which are further named according to the color of their inclusions (i.e., red or colorless). Red spherule cells contain echinochrome, a red naphthaquinone pigment. In holothuroids there are six different types of coelomocytes recognized, including morula cells, amoebocytes, crystal cells, fusiform cells, vibrate cells, and lymphocytes. By light microscopy, however, only two coelomocyte types, hyalinocytes (agranulocytes) and granulocytes, are discernible (Xing et al. 2008). Hyalinocytes are characterized by a central nucleus and scant cytoplasm lacking granules. Granulocytes share similar features but have fine granular cytoplasm.

1.3.7 Respiratory System

Echinoderms have limited anaerobic capacity and are very sensitive to oxygen availability. Gas exchange with the water vascular system occurs through the tube feet in all

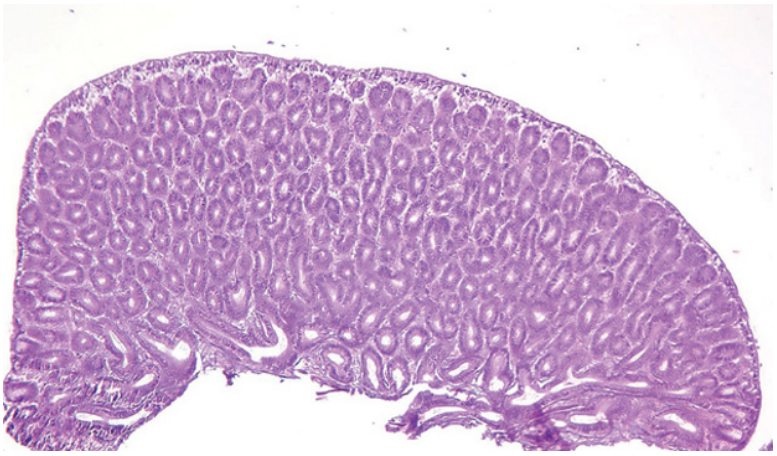


Figure 1.22 Tiedmann's body in a mottled star. 100×, HE.

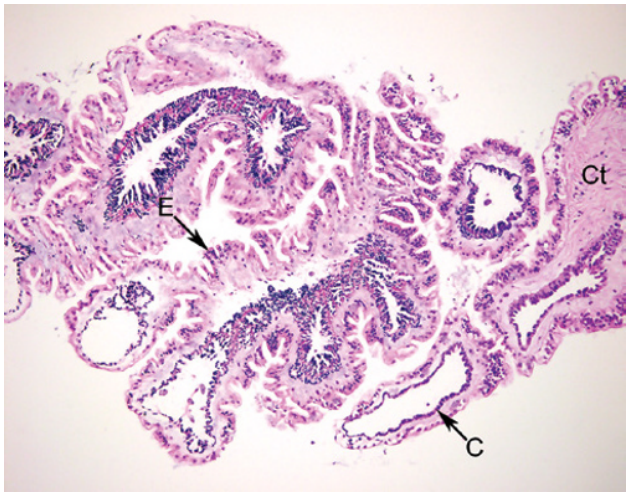


Figure 1.23 Histology of gills (papulae) in a white urchin showing epidermal surface (E), supported by connective tissue (Ct), and a central lumen lined by coelomic epithelium (C). 200×, HE.

echinoderms. To enhance gas exchange to the coelomic viscera and muscles of the disc and rays, all echinoderms except crinoids also have specialized evaginations of the coelomic epithelium, which extend through or between the endoskeletal plates of the body wall to the external body surface and function as "gills." Gas exchange via diffusion occurs between the external sea water and internal coelomic fluid across the extremely thin body wall.

In asteroids these evaginations of the body wall are called papulae. They can be branched and in species with paxillae, the papulae typically sit in the water-filled branchial space beneath this umbrella-shaped specialized surface structure. In regular echinoids there are five pairs of peristomial gills on the peristomial membrane, at the margin of each interambulacral plate, that likely provide gas exchange for the muscular apparatus of the lantern. These originate as evaginations from the peripharyngeal (lantern) coelom and have similar histologic features to asteroid papulae. Coelomic fluid is pumped to and from the peristomial gill lumen by the muscles and ossicles of Aristotle's lantern. In irregular echinoids, modified tube feet of the petaloids act as gills.

Histologically, peristomial gills, papulae, and petaloids are similar (Figure 1.23). They consist of a simple ciliated epidermis composed of supporting cells, a thin connective tissue dermis and a single layered coelomic epithelium lining a central canal. In echinoids, the coelomic epithelium forms small papillary invaginations into the central sinus when contracted. Pigmented cells and coelomocytes are often present, and their extrusion across the epidermis has given rise to the theory that gills have an excretory function (Cavey and Märkel 1994).

Holothuroids have specialized podia near the oral cavity (buccal podia) and tube feet which, similar to other species, function as gills. The primary respiratory organ, which provides gas exchange to the coelomic viscera, is paired internal respiratory trees, which arise as diverticula from the wall of the cloaca. These diverticula form a highly branched system of blind-ended tubes that contain sea water. Histologically, the structure of the respiratory tree is similar to papulae and peristomial gills. The internal surface is covered by a simple low cuboidal epithelium separated from the external coelomic epithelium by a very thin connective tissue dermis. Gas exchange occurs across the surface from sea water that is actively pumped into the respiratory tree from the cloaca.

1.3.8 Nervous System

The nervous system in echinoderms lacks ganglia, which are present in most other invertebrate species. The central nervous system in asteroids consists of a central circumoral ring and five radial nerves that extend within the center of the ambulacral groove to the tip of each ray. Each have a sensory and a motor component. The peripheral nervous system consists of the intraepithelial nerve nets previously described in the body wall. The sensory ectoneural nerve net extends along the epidermis and the motor hyponeural nerve net extends along the coelomic epithelial lining. These nerve nets are connected by neurons that cross the dermis. In Echinoidea, the ectoneural nerve system is the main component and consists of a circumoral nerve ring, radial nerves, podial nerves, and subepidermal nerve plexus. The radial nerves arise from the circumoral nerve ring and extend through the lantern and along the ambulacral plates, coursing between the radial canal and test. Radial nerves give rise to podial nerves that supply the tube feet. The hyponeural nerve system is a series of five radially positioned plaques of nervous tissue below the circumoral nerve ring. Some regular sea urchins have an entoneural nerve system consisting of a nerve ring around the periproct which gives rise to innervation of the gonads.

Histologically, the nerve ring and radial nerves of Asteroidea and Echinoidea have a distinct outer sensory layer, which communicates with the ectoneural system, and an inner hyponeural layer, which communicates with the motor components. The motor portions of the radial nerve innervate the ampullae, tube feet, and body wall musculature. In all other echinoderm classes other than Asteroidea, the circumoral nerve ring and radial nerve cords have been internalized. The ectoneural portions of the circumoral nerve ring and radial nerves are further

isolated in a specialized epineural canal, which is lined by ciliated epithelium (Figure 1.24).

1.3.9 Reproductive System

Asteroids and echinoids are dioecious and each has gonads suspended by mesenteries either as five paired structures within the ray or as five individual gonads suspended from the interradius. The gonad is connected by a short gonoduct to a gonopore opening at the base of the arms in asteroids or in the genital plates on the aboral surface of echinoids. Gonads have similar structures, whether ovary or testicle. They consist of an outer genital sac which has a thin connective tissue wall, an outer coelomic epithelial lining and an internal lining of germinal epithelium. Muscle fibers may be sparsely present within the connective tissue. Germ cells develop peripherally and mature centrally. Ovaries contain oogonia progressing to large well-developed vitellogenic oocytes centrally. Testicles contain spermatogonia progressing to small round spermatozoa centrally (Figure 1.25). Sex may be histologically indiscernible in reproductively inactive or immature individuals. Somatic cells (nutritive phagocytes) are present in both sexes of echinoids and dominate during periods between and leading up to gonadogenesis (Figure 1.26) (Walker et al. 2007).

Holothuroids are dioecious but gonochoric and have an ovotestis rather than a separate ovary and testicle.

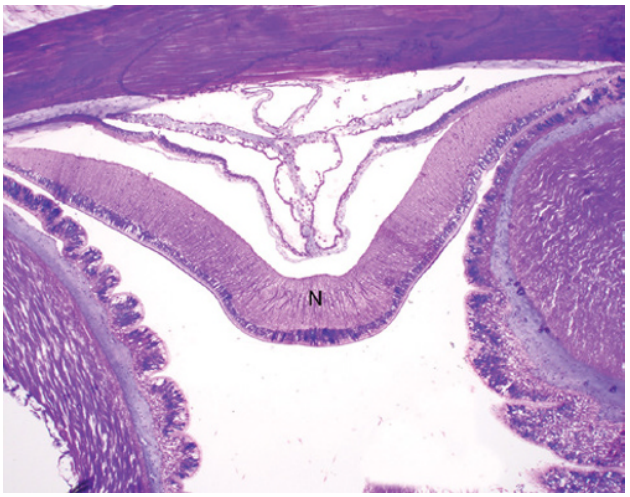


Figure 1.24 Histology of the ventral nerve cord (N) in a mottled star. 100×, LMB.

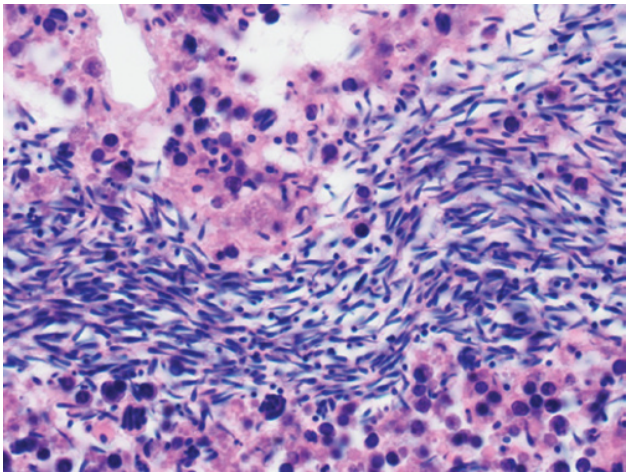
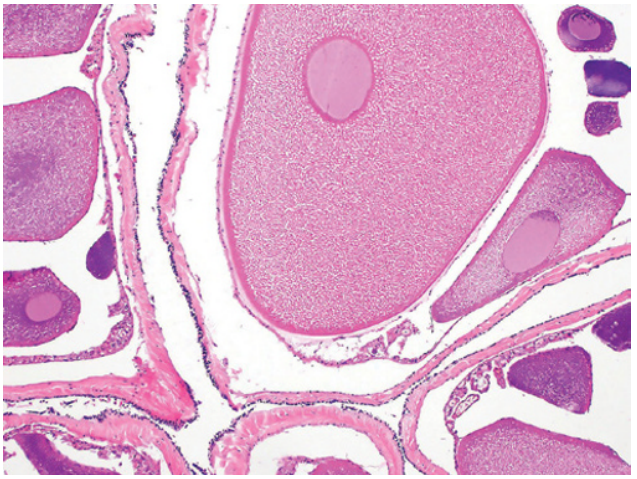


Figure 1.26 Nutritive support cells in the gonad of a sand dollar. 100×, HE.

(a)



(b)

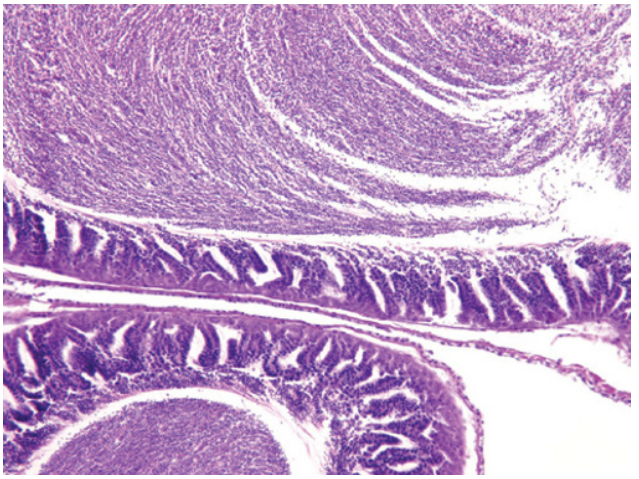


Figure 1.25 Histology of the ovary (a) in a Caribbean thorny star, and testicle (b) in a mottled star. 200×, HE. *Source:* (a) Image courtesy of Elise LaDouceur.

The gonad is composed of a large tuft of finely branched tubules covered by thin layers of coelomic epithelium and muscle. It is lined by germinal epithelium that shows differentiation toward both ova and sperm. It is connected by a gonadal duct to a gonopore located immediately behind the mouth at the base of the buccal podia. This gonopore is lined by a simple columnar epithelium.

1.3.10 Special Senses

In echinoderms, the integument including all appendages could be considered a sensory organ due to the presence of neurosensory cells throughout the epidermis. These cells are particularly concentrated on the surface of discs of the podia, at the bases of the spines and pedicellariae, along the margins of the ambulacral grooves and at the tips of the terminal tentacles and likely provide light, tactile, and chemical reception (Ruppert et al. 2004). All of these receptors connect with the subepidermal superficial nerve net mentioned previously. The primary defined sensory organs in asteroids are eyespots and sensory tube feet (previously described). Eyespots consist of ocelli, each of which is formed by a cup of epidermal cells containing red pigment filled with receptor cells. The receptor cells are connected to the radial nerve cord at the base of the sensory terminal tentacle on the oral side of each arm. The cuticle is thickened in these areas, ultimately focusing light onto the receptors like a lens (Leake 1975). Specialized sense organs are absent in Ophiuroidea. Sphaeridia are minute appendages in the ambulacral regions of non-cidaroid echinoids that are thought to be equilibratory organs. Histologically, sphaeridia consist of a spherical solid (nonmeshed) ossicle covered in ciliated epidermis, and attached to a tubercle by a muscle sheath and thin band of connective tissue (Cavey and Märkel 1994). Within Holothuroidea, burrowing members of Apodida have a single statocyst adjacent to each radial nerve at the junction of the nerve with the calcareous ring. Some Apodida also have an eyespot at the base of each tentacle (Ruppert et al. 2004).

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2

Porifera

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2.1 Introduction

Sponges (Porifera) belong to an ancient metazoan lineage that represents one of the earliest branches of the animal tree (Simion et al. 2017). The Porifera represent one of the most diverse taxa of sessile invertebrates with over 9000 extant species. Phylum Porifera comprises classes Demospongiae, Calcarea, Homoscleromorpha, and Hexactinellida. Sponges form a monophyletic group with two clades: Demospongiae + Hexactinellida and Calcarea + Homoscleromorpha.

Sponges are aquatic, mostly marine, sedentary multicellular animals, with filtration feeding and respiration. The body shape of sponges is very diverse; they may be film-like, encrusting, lumpy or spherical, tubular, branching, flabellate, etc. The body size of sponges varies as much as their body shapes, from 3–10 mm to 1.5–2 m. Their organization is particular; they have no distinct gut, muscles, gonads, nervous system, or respiratory system; however, sponges have a complex system of canals and chambers for water pumping – the aquiferous system (Table 2.1).

Age approximations of sponge species range from several months in freshwater sponges to 100 years in some marine sponges. However, research on the Caribbean giant barrel sponge *Xestospongia muta* suggests that this species might be capable of living more than 2000 years (McMurray et al. 2008).

For sponges, both asexual and sexual reproductions are characteristic. Sexual reproduction is fundamentally no different from similar processes in other multicellular animals. Sponges can be oviparous and viviparous. In the first case, sponges are usually dioecious, while in the second, often hermaphrodites. In many viviparous sponges embryonic development is accompanied by deep destruction of aquiferous system (see section 2.4.4.3). Asexual reproduction occurs in all poriferan clades. It may proceed by fragmentation, gemmulogenesis, and budding (for review see Fell 1974, 1993; Simpson 1984; Ereskovsky 2010). With few exceptions, sponges have a biphasic pelagobenthic life cycle with a tiny, planktonic ciliated larva that metamorphoses and grows into a large, benthic adult that is sexually reproductive (Ereskovsky 2010).

Sponges are mostly filter-feeding animals. They are the only type of Metazoa, with the exception of Placozoa, that lack phagocytoblasts in the form of intestinal epithelium. Practically all of the covering cells and many cells of the internal space participate in the capture of food particles (microbes, microalgae, organic particles, dissolved organic matter) in sponges (Hahn-Keser and Stockem 1997). However, sponges can be "carnivorous." These sponges feed almost exclusively on small crustaceans, which are entangled in a kind of "trapping network" formed by long thread-like outgrowths covered by a pinacoderm with microscleres in the form of anchors on the surface (Vacelet & Boury-Esnault 1995). Digestion, lasting for several days, is carried out both extracellularly and intracellularly in the mesohyl (Vacelet and Duport 2004).

Presently sponges are gaining increased scientific attention because of their secondary metabolites and biotechnological applications. Unique and innovative structural leads have been discovered with cytotoxic, antifouling, antitumoral, antibiotic, antiviral or cytoprotective, enzyme-inhibitory, antiinflammatory and anti-Alzheimer activities. Sponges could also be promising, highly biocompatible biomaterial for stem cell-based tissue engineering applications.

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Table 2.1 Organs for histologic evaluation in Porifera.[a]

Organ system		Organs
Body wall – ectosome		Glycocalyx, cuticle, exopinacoderm, dermal membrane, cortex
Digestive		No special system
	Alimentary canal	No special organs; aquiferous canals perform these functions
	Digestive organs	No special organs; choanocyte chambers perform these functions
Excretory		No special organs or structures; these functions are realized at the cellular level
Circulatory		No special system; aquiferous system perform these functions
Aquiferous system		Ostia, subdermal (*vestibular*) cavities, inhalant canals, prosodus, choanocyte chambers/tubes, aphodus, exhalant canals, atrium, oscula
Immune		No special organs or structures; functions are realized at the cellular level
Respiratory		No special organs or structures; functions are realized at the cellular level
Nervous		No special system; some functions are realized at the cellular level
Reproductive		Only temporary structures
	Male	Temporary spermatocysts
	Female	Temporary incubate chambers and follicles
Special senses/ organs		No special system or organs; this function is realized at the cellular level

[a] Alternative names for organs are provided parenthetically, in italics.

2.2 Gross Anatomy

The superficial region of the sponge body is devoid of choanocyte chambers (which are a component of the aquiferous system) and referred to as the ectosome (Figure 2.1a). The main component of the body, which occupies the middle part of the body wall and includes choanocyte chambers, is referred as the endosome or choanosome (Figure 2.1a). The hypophare is located in the basal part of the sponge and delimited by the endopinacoderm (an internal epithelial layer) from the endosome and by the basopinacoderm (an external epithelial layer) from the external milieu. The hypophare consists of the mesohyl (which is the mesenchyme) devoid of any elements of the aquiferous system (Figure 2.1a). The aquiferous system is a continuous water-conducting system of variably branching tubes between the ostia and the oscules, which comprises the inhalant system, choanocyte chambers or tubes and the exhalant system.

The rigidity of the sponge body is ensured by the collagen and spongin fibrils (in some Demospongiae orders) of the mesohyl and by the inorganic skeleton, consisting of either calcium carbonate ($CaCO_3$) (Calcarea, some Demospongiae) or silica (SiO_2) (Hexactinellida, Demospongiae, Homoscleromorpha). Inorganic skeleton may be represented by separate small elements (spicules), connected or fused spicules, or monolithic mineral skeleton (see section 2.4.3.1). All skeleton structures are secreted or assembled by special cells.

The classes of sponges differ by the type of their organization. Sponges from classes Calcarea, Demospongiae, and Homoscleromorpha have a cellular level of organization and are combined into the nonsystematic group Cellularia. In contrast, the body of sponges from class Hexactinellida is mainly built by a voluminous network of syncytial formations. In this chapter we describe mostly the histology of Cellularia; for Hexactinellida see Leys et al. (2007).

Representatives of the class Calcarea Bowerbank, 1864, the calcareous sponges (Figure 2.1b), are characterized by a calcium carbonate mineral skeleton in the form of free diactines (i.e., spicules with one axis), triactines (i.e., spicules with three rays), tetractines (i.e., spicules with four rays), and/or multiradiate spicules. This class includes approximately 770 species. A dense basal skeleton, with the main spicules cemented together, is sometimes present. The aquiferous system may have various organizational shapes, termed asconoid, solenoid, syconoid, sylleibid, or leuconoid (see section 2.4.2.1 for definitions of these shapes). Calcareous sponges are viviparous, with hollow larvae (calciblastula and amphiblastula). All Calcarea are marine sponges.

The class Homoscleromorpha Bergquist, 1978 includes 120 species (Figure 2.1c). The inorganic skeleton, if present, consists of small siliceous calthrops (equiangular tetraxon with equal rays) and/or their derivatives. The aquiferous system is sylleibid or leuconoid, often with vast basal exhalant cavities. Choanocyte chambers are large.

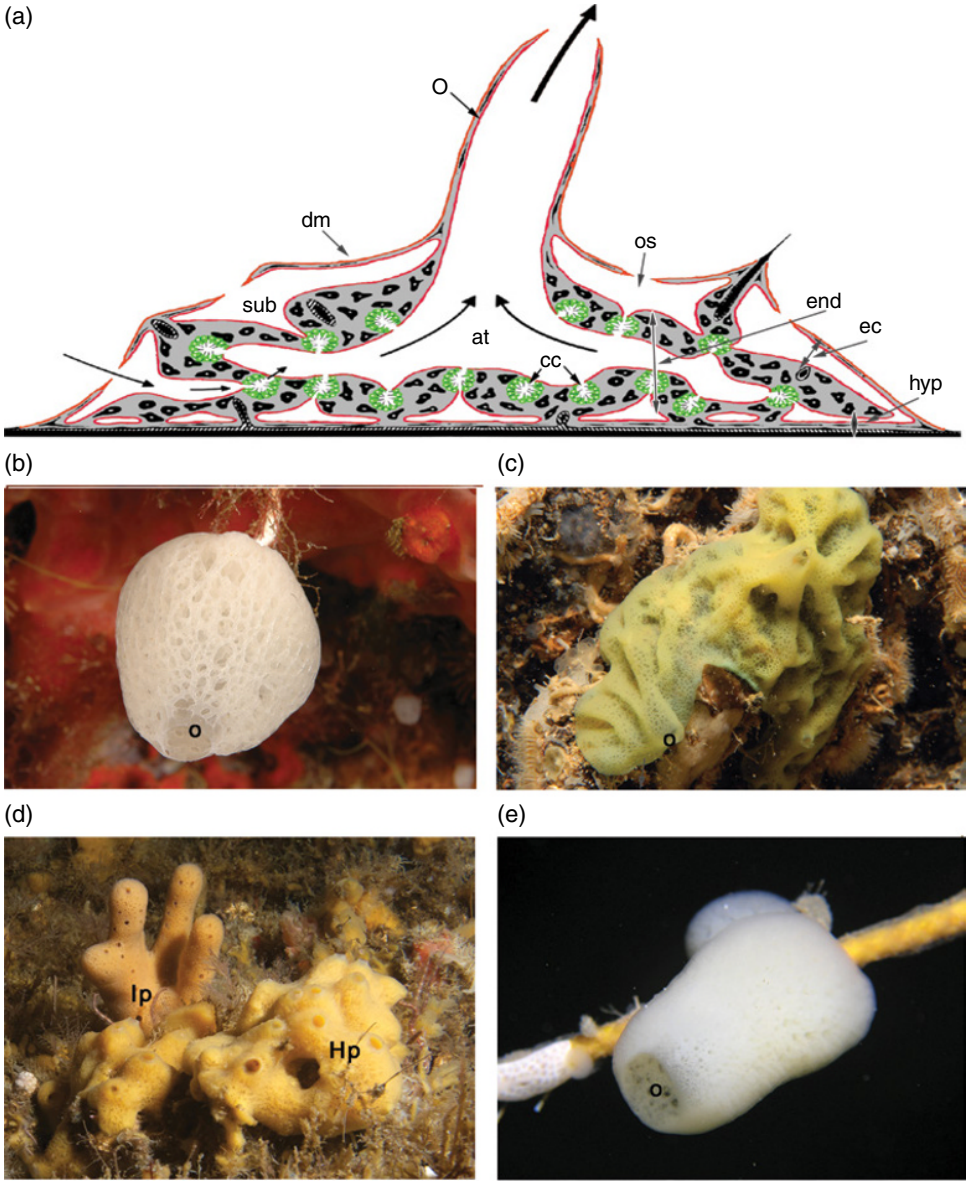


Figure 2.1 Gross anatomy. (a) Scheme of sponge organization; black arrows: water currents. (b) *Clathrina arnesenae* (Calcarea, Calcinea) *in vivo*. (c) *Oscarella viridis* (Homoscleromorpha) *in vivo*. (d) *Isodictya palmata* (Ip) and *Halichondria panicea* (Hp) (Demospongiae) *in vivo*. (e) *Oopsacas minuta* (Hexactinellida) *in vivo*.

True basement membrane underlies the choanoderm (which is the epithelium lining choanocyte chambers) and the pinacoderm (which is the epithelium lining the body cavities, external surfaces, and all portions of the aquiferous system except the choanocyte chambers); pinacocytes are flagellated. All the Homoscleromorpha are viviparous with hollow cinctoblastula larvae (entirely hollow flagellated larva, with a belt of cells with intranuclear paracrystalline bodies in the region of the posterior pole). They can be both gonochoric and simultaneous hermaphrodites. All homoscleromorphs are marine sponges.

The class Demospongiae Sollas, 1885 (about 8850 species) comprises sponges whose skeleton consists either of spongin fibers only or of spongin fibers in combination with siliceous spicules (usually, mega- and microscleres) (Figure 2.1d). Megascleres are larger than microscleres, and are mostly monoaxial and tetraxial. In some groups, the reduced spicular skeleton is compensated by a complex organic one (see section 2.4.3.2); in some other groups, there are no special skeletal elements at all. In several groups, a hypercalcified basal skeleton develops in addition to other skeletal elements. The aquiferous system is

leuconoid. Some sponges from the order Poecilosclerida lost the aquiferous system and became carnivorous. Some species are boring sponges and live in the midst of various calcareous substrate, contributing to its bioerosion. The larvae are mostly parenchymellae or, in some groups, single-layer larvae. Reproductive strategies within the class are oviparity and viviparity. Demosponges inhabit marine and fresh waters.

Representatives of the class Hexactinellida Schmidt, 1870 (about 670 species), commonly called glass sponges, are very variable in shape (Figure 2.1e). Typically, spicules are represented by hexactins (six rays), with three axes. Spicules are divided into micro- and megascleres, the latter, often fused together, forming rigid skeletal lattices. Dense spongin or nonspicular skeletons are absent. Tissues of glass sponges are syncytial and consist of the dermal and atrial membranes, the internal trabecular reticulum enclosing cellular components of the sponge and flagellated chambers. Separate nucleated cells are located in syncytial pockets. Large flagellated chambers are organized according to leuconoid type. All glass sponges are viviparous, with the trichimella larva (larva with median zone of multiciliated mononucleate cells, with syncytial structures and special larval stauractin skeleton). Hexactinellida are marine, mainly deep-sea, sponges.

2.2.1 Keys for Dissection/Processing for Histology

In general, standard protocols of tissue processing for histology are suitable for sponges. However, sponge tissues are highly sensitive to fixation procedure and subsequent manipulations. Sponges should be fixed and processed as soon as possible after collection. Contact with air should be avoided at any stage of processing, especially during manipulations with live sponges, when air could cause severe damage to fine structures in aquiferous systems. The majority of sponges possess porous tissues, highly permeable for solutions, thus requiring a shorter time for each step of the protocol. The fixation for histology should be done at 6 °C within 2–12 hours preferably in Bouin fixative or in 4% formaldehyde on sea water for marine sponges. For sponges with a mineral skeleton, its elements (spicules) should be removed after the fixation procedure by applying 5% hydrofluoric acid (for silica spicules) or 5% solution of ethylenediaminetetraacetic acid, disodium salt (EDTA) (for calcareous spicules) for two hours at room temperature. Then fixed tissues should be dehydrated through an ethanol series, placed in toluene or xylene and, finally, embedded in paraffin. Sections, 5–7 µm in thick, are mounted on glass slides and stained, with hematoxylin and/or eosin.

2.3 Histology

2.3.1 Particularity of Sponge Tissues

A characteristic feature of the Porifera, distinguishing them from the other Metazoa, is a high plasticity of cellular differentiation, anatomic and tissue structures throughout the life cycle. Various differentiated cells of the sponge can move, transdifferentiate, and switch functions. The direction of the differentiation depends on the current needs of the organism. Thus, the sponge is constantly in the state of rearrangement of all its structures (Gaino and Burlando 1990; Bond 1992; Gaino et al. 1995; Maldonado and Uriz 1999; Galera et al. 2000). This “chronic morphogenesis” contributes to the growth of the animal, reconstructing of somatic tissue after degradation during sexual and asexual reproduction, and during movements of the animal (Pavans de Ceccatty 1979; Bond 1992; Gaino et al. 1995; Bonasoro et al. 2001; Lavrov and Kosevich 2018).

In many Demospongiae, some stages of ontogenesis are accompanied by profound reconstructions of all the anatomic and histologic systems (Ereskovsky 2000), which can result in the destruction of all or most of the aquiferous system. These reconstructions may be caused by adaptation to adverse conditions (Simpson 1968; van de Vyver and Willenz 1975), regeneration processes (Borisenko et al. 2015; Ereskovsky et al. 2015), formation of reduction bodies (as delimited by a pinacoderm multicellular mass, consisting primarily of archaeocytes and presumably capable of reorganizing into a new functional sponge; reduction bodies result from a tissue disorganization of freshwater and estuarine demosponges) (Simpson 1984), gemmulogenesis (formation of gemmules, resistant asexual reproductive bodies) or sexual reproduction (Simpson 1984; Ereskovsky 2000; Ereskovsky et al. 2013). In general, these massive rearrangements do not interfere with the normal transport of water through the sponge and occur continuously along with the active pumping of water.

Histologically, the sponge body is divided into three parts: the outer epithelial layer (exopinacoderm and basopinacoderm), the inner epithelial layers (choanoderm and endopinacoderm), and mesohyl, the inner space of the sponge body that is enclosed by the epithelial layers.

2.3.2 Bordering Tissues – Epithelia

2.3.2.1 Pinacoderm

The pinacoderm is represented by the exo-, baso-, and endopinacoderm. Exopinacoderm forms the external cover of the sponge. Basopinacoderm develops at the sponge base, attaching it to the substrate. Endopinacoderm forms the walls of the subdermal cavities and the aquiferous

system canals, excluding regions of choanocyte chambers (which are lined by choanoderm; see below). There are, correspondingly, several types of pinacocytes.

The **exopinacoderm** consists of the exopinacocytes – the covering cells of the sponge, that may be T-shaped or spindle shaped in cross-section (Figures 2.1a, 2.2a,b, 2.3c,d,f). The spindle-shaped exopinacocytes are described in many Demospongiae from the orders Spongillidae and Poecilosclerida (Bagby 1970; Weissenfels 1989), in all the Homoscleromorpha (Muricy et al. 1996, 1999; Ereskovsky et al. 2014) and in some Calcarea (Borojevic 1969; Eerkes-Medrano and Leys 2006). The T-shaped exopinacocytes are described in part of Demospongiae and Calcarea (see section 2.4.1) (Boury-Esnault 1973; Willenz and Hartman 1989; Ereskovsky et al. 2011; Lavrov et al. 2018).

In most sponges, exopinacocytes lack specialized cell junctions, but are united with a well-developed adhesive system (Blumbach et al. 1998; Schütze et al. 2001); for example, in *Hippospongia communis*, *Ephydatia fluviatilis*, *Sycon coactum*, *S. ciliatum*, and *Leucosolenia variabilis*, the sites of exopinacocyte contacts have electron-dense thickenings of the membranes resembling *zonula adhaerens* (Pavans de Ceccatty et al. 1970; Pottu-Boumendil 1975; Eerkes-Medrano and Leys 2006; Lavrov et al. 2018). In Homoscleromorpha exopinacocytes have specialized cell junctions, and the exopinacoderm in general has all the structural features of eumetazoan epithelium (Ereskovsky and Tokina 2007). Unique characteristics of the homoscleromorph exopinacocytes are the flagellum (Muricy et al. 1996, 1999; Ereskovsky et al. 2014), and the ability to synthesize spicules (Maldonado and Riesgo 2007). Exopinacoderm contains ostia – numerous microscopic structures, 4–100 μm in diameter, through which water is drawn into the aquiferous system of the sponge.

Exopinacoderm exhibits many functions characteristic of the typical eumetazoan epithelia, such as absorption, secretion, transport, excretion and protection (Harrison and de Vos 1991; Meyer et al. 2006; Leys and Hill 2012). Moreover, exopinacocytes are capable of contractile responses (Pavans de Ceccatty 1986; Wachtmann and Stockem 1992a, b; Adams et al. 2010), amoeboid movement (Ereskovsky et al. 2015), incorporation of exogenous silica particles (Bavestrello et al. 1998), and phagocytizing of food particles (Willenz and van de Vyver 1982).

The **basopinacoderm** consists of the basopinacocytes – flattened cells, which are located at the basal surface of the sponge and function in attachment of the sponge to the substrate. Synthesizing basal spongin and fibronectin, basopinacocytes function as spongocytes (Garrone and Rozenfeld 1981; Labat-Robert et al. 1981). During sponge growth, marginal basopinacocytes actively secrete proteins that make up spongin (Garrone 1978).

In the demosponges with a massive calcareous skeleton, such as *Acanthochaetetes wellsi* (order Clionaida), *Ceratoporella nicholsoni* and *Stromatospongia norae* (order Agelasida), basopinacocytes participate in the formation of this skeleton (Willenz and Hartman 1989; Reitner and Gautret 1996). In calcareous sponge *Petrobiona massiliana* basopinacocytes participate in formation of the basal massive skeleton by producing an extracellular organic framework that might guide the assemblage of submicronic amorphous Ca- and Mg-bearing grains into higher structural units (Gilis et al. 2012).

Basopinacocytes of the freshwater demosponges have a well-organized cytoskeleton (Wachtmann et al. 1990; Wachtmann and Stockem 1992a, 1992b; Kirfel and Stockem 1997; Adams et al. 2005). Actin is located in the cortical layer and in the fibrils in the cytoplasmic matrix. Microtubules radiate from the perinuclear zone, finishing at the cell periphery. At the same time, intermediate filaments have not been described. In *Ephydatia muelleri*, basopinacocytes were shown to have desmosome-like junctions (Pavans de Ceccatty 1986).

The **endopinacoderm** consists of the endopinacocytes – flattened, polygonal cells, spindle shaped on cross-section (Figures 2.1a, 2.2b, 2.3c; see also Figure 2.7a,b,f). The endopinacocytes are divided into prosopinacocytes, lining the inhalant canals, and apopinacocytes, lining the exhalant canals of the aquiferous system. The external surface of the endopinacocytes is covered with a glycocalyx layer (Boury-Esnault et al. 1984; Vacelet et al. 1989; Harrison and de Vos 1991). The basal surface often forms numerous projections (pseudopodia) for anchoring in the extracellular matrix. In all Homoscleromorpha and in some Demospongiae, endopinacocytes bear flagella (Boury-Esnault et al. 1984; Vacelet et al. 1989; Ereskovsky et al. 2014). In particular, this is the case in most studied representatives of the orders Dictyoceratida and Dendroceratida (Thiney 1972; Donadey 1982; Vacelet et al. 1989; Boury-Esnault et al. 1990). The presence of flagella appears to be associated with the involvement of endopinacocytes in the generation of water currents through the aquiferous system. However, in some demosponges, the endopinacocytes lining the oscular tube (large exhalant opening) have short nonmotile cilia, presumably with sensory function, which may be involved in the coordination of simple sponge behavior (Ludeman et al. 2014).

Generally, endopinacocytes contact each other by simple overlap. However, in the oscular tubes of freshwater sponges they are united by desmosome-like junctions (Masuda et al. 1998). Endopinacocytes of Homoscleromorpha are joined by *zonula adhaerens* junctions and underlined with basement membrane (Ereskovsky and Tokina 2007; Ereskovsky et al. 2009).

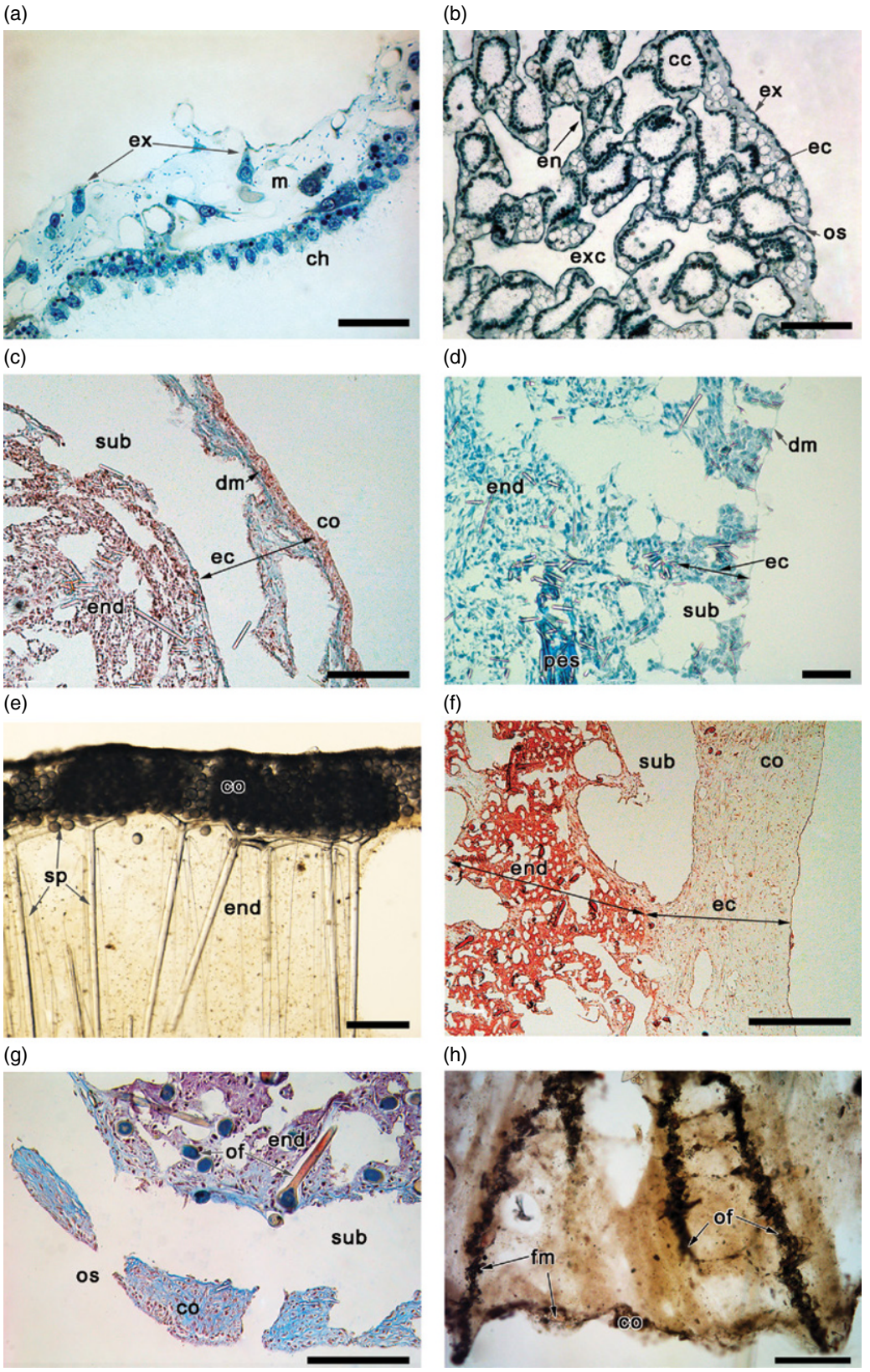


Figure 2.2 The ectosome, dermal membrane, and cortex. (a) Semithin section of a body wall of asconoid *Leucosolenia variabilis* (Calcarea, Calcaronea, Leucosolenida), showing a very thin ectosome, T-shaped exopinacocyte, mesohyl, and choanoderm. (b) Semithin section of leuconoid *Oscarella tuberculata* (Homoscleromorpha, Oscarellidae), showing a very thin ectosome, flat exopinacocytes, and endosome. (c) Histologic section of the ectosome of *Crambe crambe* (Demospongiae, Poecilosclerida). (d) Histologic section of the ectosome of *Petrosia ficiformis* (Demospongiae, Haplosclerida). (e) Histologic section of the ectosome of *Geodia atlantica* (Demospongiae, Tetractinellida) showing a cortex, reinforced with special skeletal elements – microscleres (astroscleres), and the endosome with the megascleres. *Source:* Image courtesy of Paco Cardenas. (f) Histologic section of the ectosome of *Pleraplysilla spinifera* (Demospongiae, Dictyoceratida). (g) Histologic section of the ectosome of *Spongia officinalis* (Demospongiae, Dictyoceratida). (h) Histologic section of upper part of *Dysidea incrustans* (Demospongiae, Dictyoceratida), showing the cortex with foreign material embedded into the organic skeleton. Scale bars: (a) 20 μm; (b,c,d) 100 μm; (e) 1 mm; (f,g,h) 500 μm.

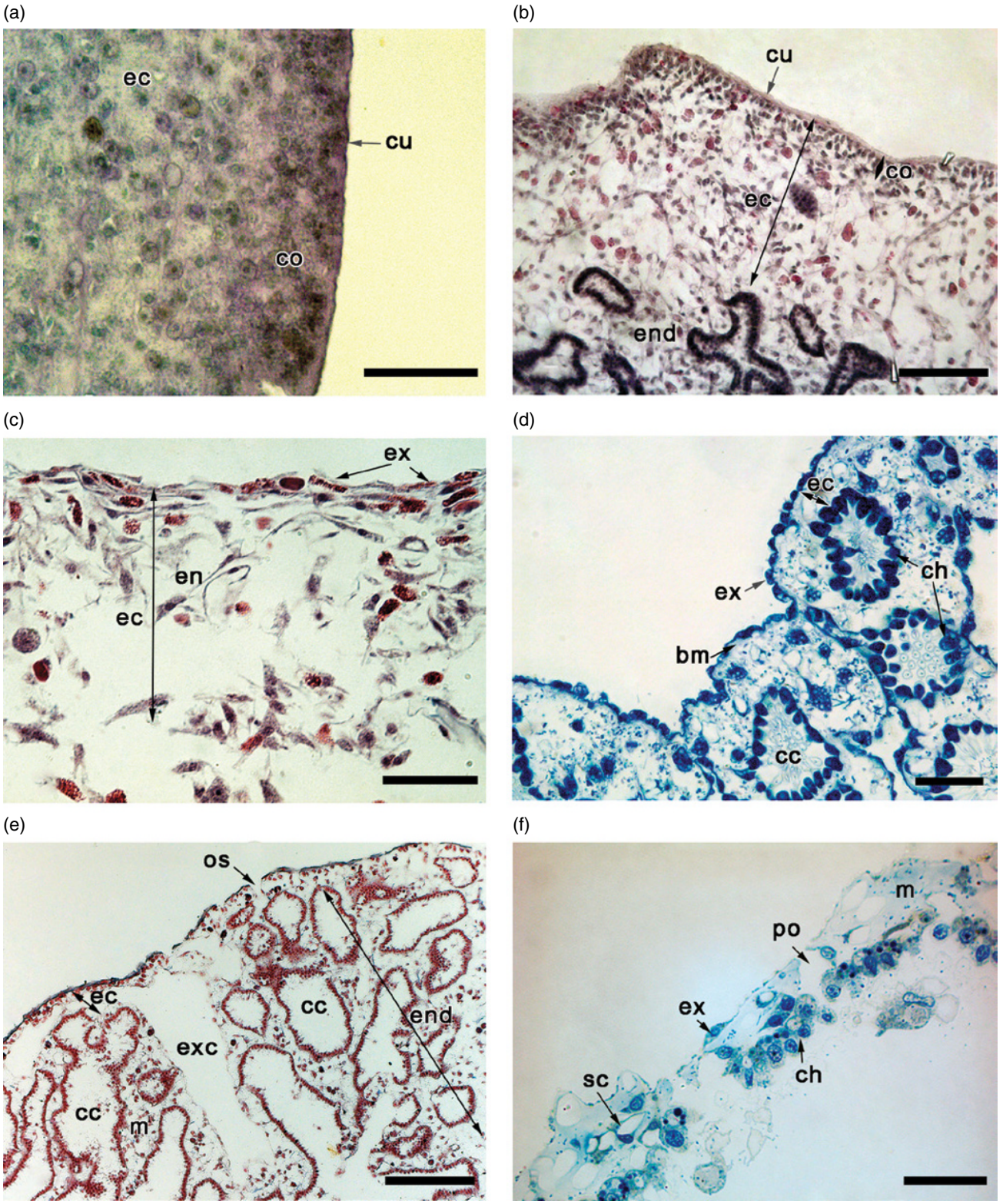


Figure 2.3 Cuticle, exopinacoderm, and pores. (a) Histologic section of *Aplysina cavernicola* (Demospongiae, Verondiida): cortex and cuticule. (b) Histologic section of *Halisarca dujardinii* (Demospongiae, Chondrillida): ectosome, noncellular, amorphous cuticule. (c) Histologic section of ectosome *of Lubomirskia baicalensis* (Demospongiae, Spongillida), showing flat exopinacocytes. (d) Semithin section of ectosome and choanosome of *Oscarella lobularis* (Homoscleromorpha, Oscarellidae), showing a basal membrane lining pinacoderm and choanocyte chambers. (e) Histologic section of upper part of *Halisarca dujardinii* (Demospongiae, Chondrillida): ectosome, choanosome, and multicellular pores. (f) Semithin section of a body wall of asconoid *Leucosolenia variabilis* (Calcarea, Calcaronea, Leucosolenida), showing porocytes. Scale bars: (a,c,d) 50 µm; (b) 100 µm; (e) 200 µm; (f) 20 µm.

Endopinacocytes and choanocytes of some demosponges, homoscleromorphs, and calcareans are thought to be functionally and ontogenetically interrelated. For example, during reparative regeneration the choanocytes of different species from these taxa can differentiate into endopinacocytes by reduction of the flagellum and the microvilli and the subsequent flattening of the cell (Diaz 1974; Borisenko et al. 2015; Ereskovsky et al. 2015; Lavrov et al. 2018).

The apopylar cell is a particular cell type forming the boundary between apopinacoderm (epithelium lining the exhalant canal) and choanoderm (see section 2.3.2.2) and linking choanocytes and apopinacocytes (de Vos et al. 1990). Apopylar cells display morphologic characteristics intermediate between those of choanocytes and pinacocytes. In Homoscleromorpha, these cells possess a flagellum and an unfolded collar of microvilli (Boury-Esnault et al. 1984). In demosponges, the apopylar cells were observed in all investigated species of Dictyoceratida and Dendroceratida, in some Chondrosida (*Halisarca dujardinii*, *Thymosia guernei*) and Haplosclerida, in the freshwater sponge *E. fluviatilis* and in *Tethya wilhelma* (Tethyida) (Langenbruch et al. 1985; de Vos et al. 1990; Hammel and Nickel 2014). Earlier, these cells were referenced as "cone cells" and "cell-ring" (de Vos et al. 1990). In *T. wilhelma*, the apopyle (the exit from the choanocyte chamber) has also a reticuloapopylocyte, a modified apopylar cell, which has numerous small intracellular pores, which give them a mesh or grid-like morphology (Hammel and Nickel 2014).

2.3.2.2 Choanoderm

The choanoderm consists only of choanocytes, which form the choanocyte chambers (or tubes in asconoid and solenoid sponges) (see section 2.4.2) (Figure 2.2b; see also Figure 2.6a–e). Contrary to the pinacoderm, the choanoderm has a cubic or palisade epithelium. Choanocytes can be cylindrical, cubic, trapezoid, or slightly flattened. These cells bear a flagellum surrounded with a collar of cytoplasmic microvilli interconnected by glycocalyx bridges. In some demosponges choanocytes have a periflagellar sleeve, such as in Suberitidae, Polymastiidae, Acanthochaetetidae, and Halisarcidae (Connes et al. 1971; Boury-Esnault et al. 1990, 1994; Ereskovsky et al. 2011).

Another cell type associated with the choanocyte chambers of some demosponges is the central cell (Reiswig and Brown 1977; Diaz 1979; Langenbruch and Scalera-Liaci 1986; Langenbruch and Jones 1989; Sciscioli et al. 1997; Ereskovsky et al. 2017a). Central cells have an irregular, branched shape with numerous projections and holes. The cell is perforated with a vast canal, into which flagella of the choanocytes enter. The central cells participate in the regulation of beating of the choanocyte flagella within a chamber and thus in the regulation of water currents through the aquiferous system.

2.3.3 Tissues of the Internal Environment

Diverse cells, which compose the tissue of the internal environment of sponges, are located in the mesohyl – a highly complex system occupying the internal parts of the animal's body, between its surface and elements of the aquiferous system. Besides cells, the mesohyl includes a skeleton (both organic and mineral) (see section 2.4.3), organic ground substance, which encompasses all cellular and skeletal elements, dissolved macromolecules and often bacteria, archaea and cyanobacteria. The mesohyl has a parenchymal structure with various cell types intermixed with each other and with noncellular elements. No structural compartments can be defined in the mesohyl. Moreover, the majority of cells demonstrate apparent motility and the ability to transdifferentiate, making the structure of the mesohyl highly unstable.

However, the sponge cell populations of the internal environment can be subdivided according to their functions: supportive-connecting tissue, protective-secretory tissue, and contractile cells, which occur in some demosponges (Ereskovsky 2010). Although these tissues are not structurally delimited from each other, they comprise groups of cells with a specific function.

In addition, occasionally during various stages of sexual and asexual reproduction the gametes, embryos, and specific somatic cells participating in gamete formation (trophoblasts, nurse cells, histoblasts, thesocytes, etc.) develop in the mesohyl, significantly changing its overall structure (see section 2.4.4).

2.3.3.1 Supportive-Connective Tissue

This tissue comprises a variety of cells participating in the formation of organic and mineral skeleton and ground substance of the mesohyl.

Collencytes (lophocytes) are mobile cells participating in the secretion of collagen and formation of its fibrils (Figure 2.4a). These cells are frequently characterized by a nucleus without nucleolus, a well-developed rough endoplasmic reticulum (RER), few nonspecific inclusions, and specific vacuoles containing collagen, which can be dense and homogenous or contain clear fibrillar material. Collencytes are devoid of phagosomes. These cells can be found all over the mesohyl. The secretory activity of these cells is evident from the deposition of oriented collagen fibrils near the cells, sometimes attached to the cell membrane (Borojevic 1966; Bonasoro et al. 2001).

Two names are used for this type of cell (Lévi 1970; Simpson 1984; Boury-Esnault and Rützler 1997): *collencyte*

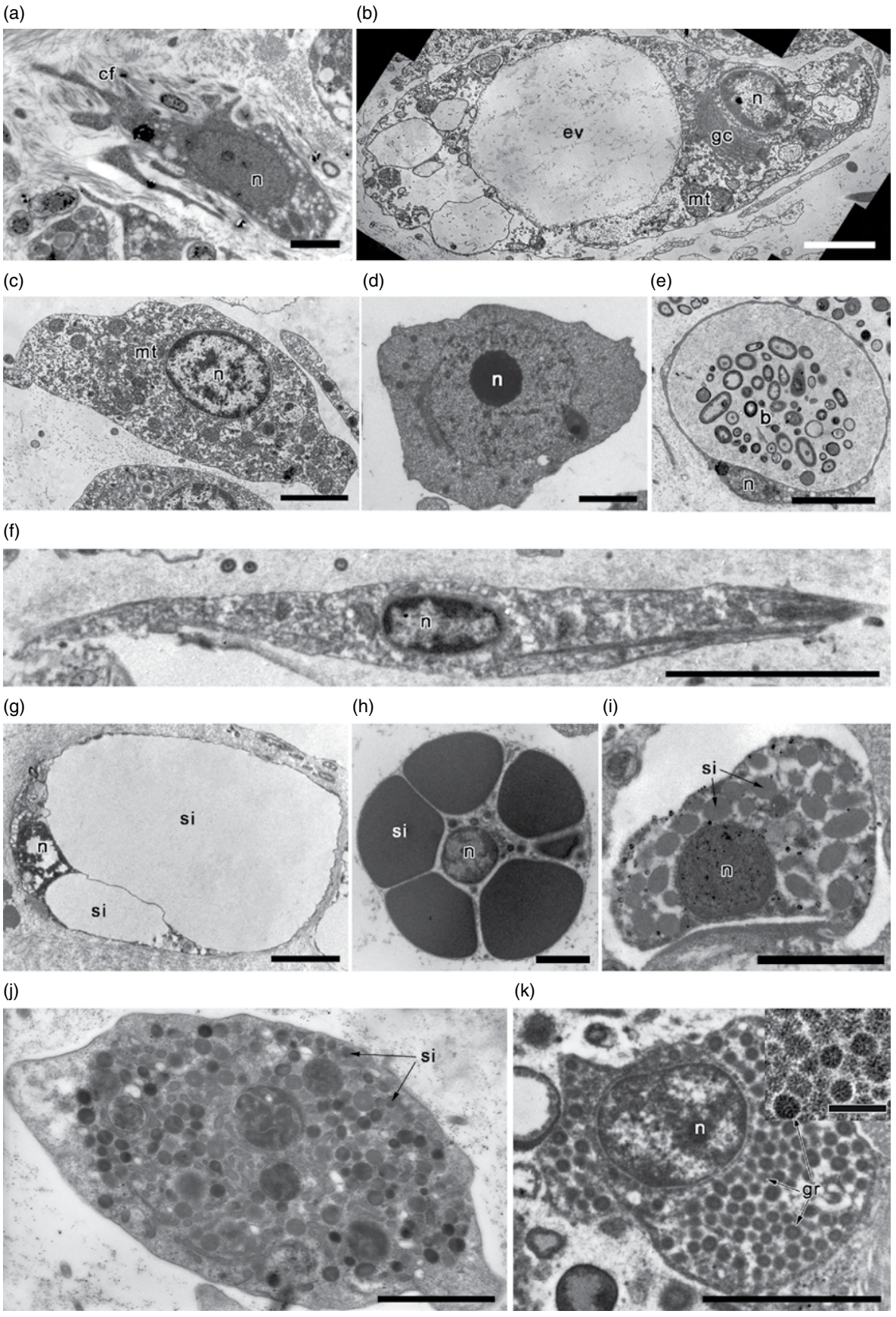


Figure 2.4 Cells of the internal environment. (a) Lophocyte of *Chondrilla* sp. (b) Sclerocyte of *Leucosolenia variabilis*. (c) Amoebocyte of *Leucosolenia variabilis*. (d) Archaeocyte of *Crellomima imparidens*. (e) Bacteriocyte of *Aplysina cavernicola*. (f) Myocyte of *Leucosolenia* sp. (g) Vacuolar cell of *Oscarella tuberculata*. (h) Spherulous cell of *Halisarca caerulea*. (i) Granular cell of *Chondrilla* sp. (j) Microgranular cell of *Halisarca dujardinii*. (k) Gray cell of *Chondrilla* sp. Inset – glycogen rosettes. Scale bars: (a,c,d,g,h,i,j,k) 2 µm; (b) 1 µm; (e,f) 5 µm; (inset) 0.25 µm.

usually refers to a stellar-like or spindle-like cell, while *lophocyte* refers to an obviously motile cell with anterior–posterior polarity and often forming a collagen bundle, which is associated with its posterior pole (Garrone 1978; Bonasoro et al. 2001).

Spongocytes are amoeboid cells responsible for the secretion of spongin in different forms (see section 2.4.3.2). Spongocytes always form groups of several cells during spongin secretion. They are characterized by a nucleus with nucleolus, well-developed RER, perinuclear cisterns of Golgi complex and vesicular cytoplasm, containing numerous homogenous dense inclusions with spongin precursor (Garrone 1978).

Special types of spongocytes participate in the development of gemmules (a resistant asexual reproductive body, composed of internal mass of archaeocytes [thesocytes] charged with reserves and enclosed in a noncellular protective envelope) in freshwater sponges from the family Spongillidae. These spongocytes form a palisading epithelium around the developing gemmule and secrete collagenous shell and chitin for its coat (de Vos 1971, 1977; Langenbruch 1981, 1982; Ehrlich et al. 2013).

Sclerocytes are mobile cells, secreting elements of the mineral skeleton – spicules. Depending on the size and hence type of spicule produced (megasclere or microsclere) (see section 2.4.3.1), the sclerocytes are divided into megasclerocytes and microsclerocytes. Megasclerocytes have a nucleus with nucleolus, prominent Golgi complex, free ribosomes, mitochondria, few phagosomes and cisterns of RER (Figure 2.4b). Microsclerocytes are characterized by smaller size and a nucleus without nucleolus (Wilkinson and Garrone 1980; Garrone et al. 1981; Custodio et al. 2002).

Microscleres and microsclerocytes are characteristic only for Demospongiae and Hexactinellida. The mineral skeleton of Homoscleromorpha and Calcarea consists of megascleres of different size, so representatives of these classes have only megasclerocytes.

Silica spicules of Demospongiae, Hexactinellida, and Homoscleromorpha are synthesized intracellularly in vacuoles around an organic axial filament (Uriz et al. 2003; Uriz 2006; Leys et al. 2007; Maldonado and Riesgo 2007). During synthesis of large spicules, which are bigger than sclerocytes, several cells join (Uriz et al. 2003). In Demospongiae and Hexactinellida the membrane forming the spicule vacuole has a specific structure and is called a silicalemma (Uriz et al. 2003; Uriz 2006; Leys et al. 2007). It pumps silica inside the vacuole, producing a higher concentration inside for effective deposition around the organic axial filament (Uriz 2006). No information exists about the structure of analogous membranes in Homoscleromorpha.

In contrast, in calcareous sponges spicules are always synthesized extracellularly by the group (2–6 cells) of sclerocytes, which are joined by septate junctions and form an extracellular vacuole, where increased concentration of calcium ions is produced (Jones 1970; Ledger and Jones 1977; Uriz 2006).

Transport cells are peculiar amoeboid cells of the mesohyl described from the freshwater sponge *E. fluviatilis* (Nakayama et al. 2015). Transport cells are attached to the newly synthesized megascleres and transport a spicule from its place of synthesis to the final position in skeletal framework. No specific structural features of the transport cells are yet known. This cell type is defined only by its location on the newly synthesized megascleres, motile behavior, and specific expression of the gene EflSoxB1 (Nakayama et al. 2015).

2.3.3.2 Protective-Secretory Tissue

Various amoeboid cells and cells with specific inclusions compose this tissue. The functions of protective-secretory tissue include transfer and distribution of nutrition and oxygen, excretion, immune protection, and secretion of specific substances.

Amoebocytes *sensu lato* are common motile cells of the mesohyl. There is no clear definition of these cells and at various times they were called thesocytes (Sollas 1888), spherulous cells (Topsent 1892), polyblast or hyaline cells (Tuzet and Pavans de Ceccatty 1958), amoebocytes (Müller 1911), or nucleolated amoebocytes (Wilson and Penney 1930; Faure-Fremiet 1931; Efremova 1972). They occur in all regions of the mesohyl and often are the main cell type in it. The amoebocytes have a large nucleus with nucleolus associated with Golgi complex, numerous RER cisterns and unspecific inclusions, especially phagosomes, and symbiotic zoochlorellae in the cytoplasm of freshwater sponges (Figure 2.4c) (Gilbert and Allen 1973; Williamson 1979). Amoebocytes are traditionally considered to execute several functions including digestion and distribution of nutrition, immune response in the form of phagocytes, elimination of refractory leftovers, and functioning as stem cells. Considering the broad variety of executed functions and absence of obvious structural features, amoebocytes could represent a highly heterogenous cell group and should be further researched. Currently, one subpopulation of amoebocytes can be distinguished – archaeocytes.

Archaeocytes are amoebocytes with a high nuclear/cytoplasmic ratio, with cytoplasm reach in RER and ribosomes and devoid of special cytoplasm inclusions (Figure 2.4d) (Smith and Hildemann 1990; Harrison and de Vos 1991). They occur in Demospongiae and Hexactinellida (in which they are the only cellular elements independent from the main syncytial tissues) and represent one of the stem lines in sponges of these classes (Lévi 1970; Korotkova, 1981, 1997; Simpson 1984; Harrison and de Vos 1991; Funayama, 2008, 2018).

In addition, participation of some amoebocytes in various immune reactions is well known (Smith and Hildemann 1986, 1990), and some attempts to distinguish this subpopulation have been made, using molecular markers (Funayama et al. 2005).

Bacteriocytes represent mobile cells with specific vacuoles, containing various symbiotic prokaryotes. This cell type is known only in demosponges. Bacteriocytes can contain single large or several small vacuoles with symbionts (Figure 2.4e) (Vacelet 1970; Vacelet and Donadey 1977; Bigliardi et al. 1993; Vacelet and Boury-Esnault 1996; Maldonado 2007). Bacteriocytes participate in food digestion in carnivorous sponges (Vacelet and Duport 2004) and are responsible for vertical transmission of symbionts in some demosponges (Ereskovsky et al. 2005; Maldonado 2007), as they penetrate the embryos during their development and remain intact until the larval settlement and metamorphosis (Lévi and Lévi 1976).

Cells with specific inclusions are an important element in sponge mesohyl. This heterogenous cell group includes various cells types, which are united by the presence of specific inclusions in the cytoplasm but differ by structure of these inclusions. Currently, the function of most cells with specific inclusions is unknown. Some evidence indicates that these cells can realize the content of their vacuoles in the mesohyl, participate in metabolism of glycogen, excrete metabolic by-products, and produce metabolites with antibiotic functions, which may be involved in the regulation of symbiotic bacteria or defense against foreign bacteria. Cells with inclusions can be found in most demosponges and homoscleromorphs but are rare in calcareous sponges and hexactinellids. According to Simpson (1984), cells with specific inclusions are subdivided into two major categories: cells with larger inclusions and cells with smaller inclusions.

Cells with larger inclusions. Vacuolar cells (cystencytes) are characterized by the presence of one or several large transparent vacuoles, occupying almost all cytoplasm of these cells (Figure 2.4g; see also Figure 2.7b,f). The free cytoplasm is reduced to a thin film around inclusions and nucleus. The nucleus may be displaced to the cell periphery by the inclusions. In addition, cytoplasm may contain Golgi complex, some RER and smooth endoplasmic reticulum, few mitochondria, and small phagosomes. Cystencytes are vacuolar cells of freshwater sponges, having one large inclusion with amorphous material of a polysaccharide nature (Tessenow 1969; Pottu-Boumendil 1975; Ereskovsky et al. 2016). Vacuolar cells can be used as a diagnostic characteristic in closely related species of sponges without a skeleton, for example *Oscarella* and *Halisarca* (Muricy et al. 1996; Ereskovsky 2006, 2007).

Spherulous cells have several large membrane-bounded inclusions (0.8–8 μm diameter). Free cytoplasm is reduced to narrow strands between the inclusions and on the cell periphery. The cytoplasm contains few mitochondria and rare cisterns of RER (Figure 2.4h; see also Figure 2.8f). The nucleus is usually small, anucleolated, and deformed by the inclusions. Inclusion content is usually homogenous but can be paracrystalline, fibrillar, or lamellar (Diaz 1979; Thompson et al. 1983; Bonasoro et al. 2001; Ereskovsky et al. 2017b). In some species, spherulous cells are localized near the sponge surface or aquiferous system canals, although they can lie diffusely in the mesohyl (Uriz et al. 1996; Bonasoro et al. 2001; Ereskovsky 2007; Maldonado 2016). The presumable function of the spherulous cells varies in different species, indicating possible heterogeneity of this cell type. The spherulous cells were reported to participate in storage of various metabolites (including toxic ones) (Thompson et al. 1983; Uriz et al. 1996; Becerro et al. 1997), immune response against nonsymbiotic bacteria, defense against fouling, predation by release of metabolites (Thompson et al. 1983; Ternon et al. 2016), excretion (Vacelet 1967; Donadey 1978; Maldonado 2016; Ereskovsky et al. 2020), and mesohyl extracellular matrix synthesis and maintenance (Donadey and Vacelet 1977; Donadey 1982; Bretting et al. 1983; Smith and Hildemann 1990).

Granular cells have numerous membrane-bound inclusions (0.5–2 μm diameter) in their cytoplasm. The inclusions of the granular cells are smaller, and their number is higher in comparison with spherulous cells (Figure 2.4i). The shape of inclusions varies from round to irregularly ovoid. Using electron microscopy, the inclusions are usually homogenous, but they also can be fine-grained or have a fine-grained periphery with a homogenous central region. The content of the inclusions is often separated from the surrounding membrane by the transparent space. The nucleus is round with or without a nucleolus. A few phagosomes, unspecific inclusions and vacuoles can appear in the cell cytoplasm (Pomponi 1976; Ereskovsky et al. 2011, 2017a, 2017b; Willenz et al. 2016). The exact functions of granular cells are unknown, but they may be involved in the immune response, as the inclusion contents show antimicrobial activity (Krylova et al. 2003). In addition, in some demosponges maternal granular cells penetrate into the forming larvae (Ereskovsky and Gonobobleva 2000; Rützler et al. 2003) and can be retained there until the beginning of metamorphosis (Gonobobleva and Ereskovsky 2004).

Granular and spherulous cells can be used as a diagnostic characteristic in closely related species (Pomponi 1976; Boury-Esnault et al. 1994; Bergquist 1996; Muricy et al. 1996; Reveillaud et al. 2012; Gazave et al. 2013; Willenz et al. 2016).

Microgranular cells are characterized by cytoplasm filled with minute dense granules (~0.09–0.3 μm diameter). The nucleus is often anucleolated and cytoplasm contains few mitochondria and RER cisterns (Figure 2.4j) (Sciscioli et al. 2000; Pinheiro et al. 2004). These cells could contribute to the synthesis of glycoprotein components of the extracellular matrix. Others functions of the microgranular cells remain unknown.

Cells with smaller inclusions. Gray cells (glycocytes) contain numerous small ovoid membrane-bounded inclusions (~0.2–0.8 μm diameter). The inclusions are acidophilic and osmiophilic. Another characteristic feature of these cells is glycogen rosettes in the cytoplasm (Figure 2.4k). Besides the glycogen rosettes, the cytoplasm of gray cells contains well-developed RER and Golgi complex (Boury-Esnault 1977). The nucleus usually contains a small nucleolus. Presumably these cells participate in glycogen metabolism (Boury-Esnault 1977) and also have been considered as immunocytes, responsible for the allogeneic response (Humphreys 1994; Yin and Humphreys 1996; Sabella et al. 2007).

Rare type of cells with inclusions. The types of cells with inclusions described above are widespread and found in many sponge species. In addition to these, several rarer types of cells with inclusions occur in some sponges: rhabdiferous cells (Simpson 1968; Smith 1968; Smith and Lauritis 1969; Ereskovsky et al. 2011), sacculiferous cells (Smith 1968; Smith and Lauritis 1969), spumeuse cells (Donadey and Vacelet 1977; Donadey 1982), globoferous cells (Borojevic and Lévi 1964; Simpson 1968) and styllocytes (Harrison et al. 1974). These rare cell types have been found in one or several sponge species, consequently additional studies of their structure and functions are required. Moreover, these rare cells with inclusions could possibly be species-specific modifications of common types of cells with inclusions.

Contractile cells of the mesohyl, myocytes, are found in the mesohyl of many demosponges and calcareous sponges. In some sponges, the myocytes can occur in large concentric multilayered structures (sphincters) around large exhalant canals and oscula, while in other species they lie sparsely in the mesohyl near the aquiferous system canals and/or dermal membrane (Bagby 1966). The myocytes are fusiform cells with an ovoid nucleus, lying in the central part of the cell (Figure 2.4f). Other organelles (Golgi complex, mitochondria, unspecific inclusions) are usually located near the poles of the nucleus. The bundles of myofilaments are concentrated at the cell periphery. In some sponges, the myocytes have two types of filaments, which are spatially organized, forming regular patterns (e.g., *Tedania ignis*) (Bagby 1966; Thiney 1972). Considering their position and ultrastructural features, the myocytes are thought to be contractile cells, which regulate the diameter of large canals of the aquiferous system, thus participating in the regulation of water flow.

2.3.4 Loose Connective Tissues (Mesohyl)

The mesohyl occupies the internal spaces of the sponge body and is delimited by the pinacoderm and choanoderm. The degree of mesohyl development varies greatly according to the type of sponge body organization: in asconoid sponges the mesohyl has a thickness of only dozens of micrometers (see Figure 2.6a), while in leuconoid sponges it comprises the main volume of a sponge (see Figure 2.6e).

The mesohyl is a complex compartment, comprising numerous cells of different types, organic and inorganic skeletal components, collagen fibers, unstructured extracellular ground substance, and symbiotic organisms. It does not have a permanent structure or structural units, appearing as a highly dynamic and variable system, although the mesohyl of the specialized parts of the sponge body (e.g., cortex, dermal membrane, etc.) can have permanent structural features like extracellular matrix arrangement and/or cell type composition (see section 2.4.1).

All mesohyl cells are in constant movement (Bond 1992; Gaino et al. 1995). However, semipermanent structures can appear in the mesohyl. Mesohyl cells have a tendency to form small transient groups of 2–10 cells. Sometimes such groups are united in a single accumulation of cells, moving in the same direction, so-called cell tracts. Cell tracts can be formed in various regions of the sponge mesohyl, but usually are characteristic for regions of growth and leading edge of moving sponge (Bond and Harris 1988; Bond 1992). The only known permanent structures in sponge mesohyl are peculiar cellular strands, described in the genus *Aplysina* (Leys and Reiswig 1998). These strands run through the endosome of the sponge and are composed of elongate cells tightly aligned along bundles of collagen (Figure 2.5). The cells have a permanent position in the strands and do not actively move. According to experiments, the strands are involved in nutrition transport and thus may represent a primitive nutrient transport pathway (Leys and Reiswig 1998).

The mesohyl always comprises well-developed extracellular matrix. A common fibrillar component in mesohyl of all sponges is collagen. The collagen fibrils may be dispersed through the mesohyl or form bundles and tracts. In some species collagen fibrils are the only skeletal elements and are greatly elaborated (see section 2.4.3.2). The mesohyl ground substance is rich in glycoproteins, but also contains fibronectin, various glycosaminoglycans, mucopolysaccharides, proteoglycans, sugars (including the unusual arabinose), amino acids, etc. (Gross et al. 1956; Katzman

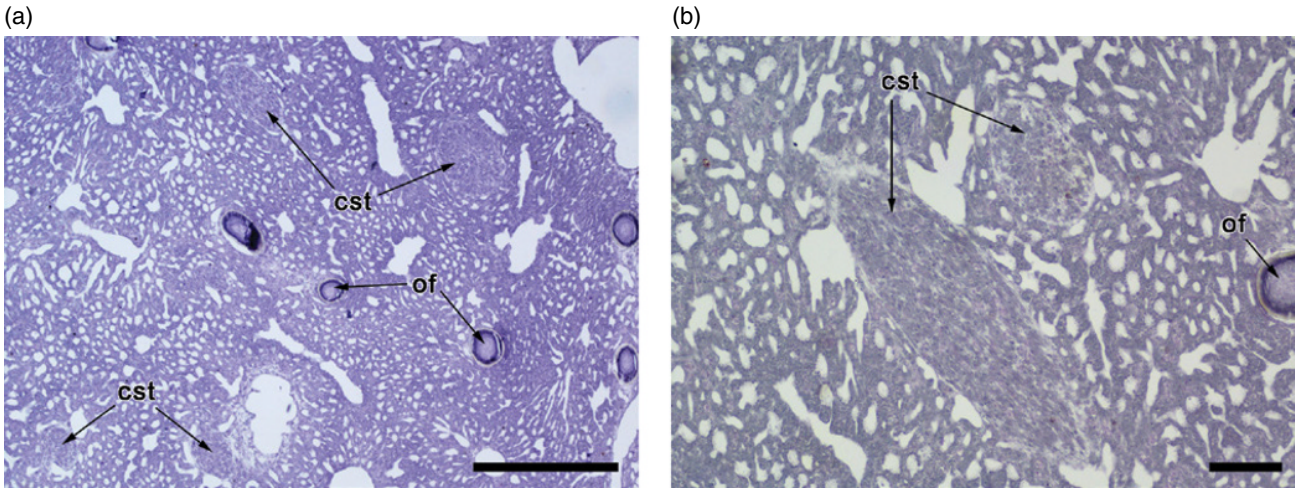


Figure 2.5 Mesohyl cellular strands of *Aplysina cavernicola*. (a) General view of endosome with several cellular strands. (b) Structure of cellular strands. Scale bars: (a) 500 µm; (b) 100 µm.

et al. 1970; Evans 1975; Junqua et al. 1975; Garrone 1978). The composition of ground substance varies by species and even from individual to individual within a single species. It appears likely that a considerable proportion of the components of the mesohyl ground substance is synthesized and released by various cells with specific inclusions (see section 2.3.3.2). The mesohyl ground substance and collagen fibrils represent a basic scaffold for mesohyl cells and may play a crucial role in cell–cell or cell–matrix interactions, immune reaction, self/nonself recognition, cytodifferentiation, cell aggregation (e.g., during formation of gemmules), and possibly other physiologic processes.

The mesohyl varies in cell type and composition, both between and within species. For instance, the mesohyl of calcareous sponges contains few cells, most of which are sclerocytes, while amebocytes and cells with specific inclusions are rare (Eerkes-Medrano and Leys 2006; Lavrov et al. 2018). In homoscleromorphs, the mesohyl contains numerous cells with inclusions, especially vacuolar cells, which can be represented by several types (see Figure 2.7b) (Gazave et al. 2013). Both calcareous and homoscleromorph sponges could lack archaeocytes in their mesohyl. Demosponges usually have highly cellular mesohyl amoebocytes, archaeocytes, skeleton-secreting cells and several types of cells with inclusions, which vary from species to species (Simpson 1984). The intraspecies variations in mesohyl cell composition occur due to different physiologic states of sponge tissues, mainly during the reproduction and life cycles (see section 2.4.4.3).

All sponges are associated with microbial communities, with representatives of 41 different prokaryotic phyla (Thomas et al. 2016; Moitinho-Silva et al. 2017), which are located in the mesohyl, extracellularly, in the ground substance, or in the special cells, bacteriocytes (Lee et al. 2001). Sponge species were observed to harbor dense communities of symbiotic microorganisms in their tissues, while others were almost devoid of microorganisms. The former were termed "high microbial abundance" (HMA) and the latter "low microbial abundance" (LMA) sponges (Hentschel 2003). In HMA sponges, microbial biomass can comprise up to one-third of the total biomass (Vacelet 1975), and bacterial densities are 2–4 orders of magnitude higher than in LMA sponges. Moreover, HMA microbiomes are highly complex, while LMA microbiomes are mainly represented by Proteobacteria and Cyanobacteria (Moitinho-Silva et al. 2017). The sponge-associated microorganisms participate in nutrient cycling, vitamin and secondary metabolism, and chemical defense (Taylor et al. 2007; Webster and Taylor 2012).

2.4 Organ Systems

2.4.1 Body Wall – Ectosome

Sponges lack a body wall, homologous to eumetazoans (because sponges lack embryonic anlages homologous of these animals) (Ereskovsky and Dondua 2006). External surfaces of sponges have an important role in the exchange of particles and gases between the animal and the environment, and may help maintain the constancy of the sponge's internal milieu and separate it from the surrounding water. The exopinacocytes are the main cells of the dermal structures of sponges.

The ectosome is the peripheral zone of a sponge, devoid of choanocyte chambers. This region is directly in contact with the external environment. The internal surface of the ectosome is often separated from the endosome by aquiferous system cavities or vestibules, coated with endopinacocytes.

The ectosome has a very variable thickness. It is reduced to a single exopinacoderm layer with external glycocalyx and thin layer of extracellular matrix in *Oscarella* and asconoid Calcinea (Figure 2.2a,b); it can reach 2–5 mm in some Demospongiae where it is reinforced by a very important spicular or collagenic skeleton (Figure 2.2e,f).

The number of symbiotic microbes may be different in the ectosome and endosome. For example, the remarkable scarcity of bacteria in the ectosome of *Ceratoporella nicholsoni* and *Stromatospongia norae* (Willenz and Hartman 1989), relative to the choanosome, can be compared to the great reduction of bacterial density observed in superficial regions of *Aplysina aerophoba* (Vacelet 1975). In contrast, in sponges with photosynthetic symbionts (unicellular algae or cyanobacteria), the number of symbionts in the superficial regions of the sponge body is much higher in comparison to deeper parts (Sarà and Liaci 1964; Oren et al. 2005).

The ectosome is the general term for describing the peripheral zone of the sponge body. In different sponges, the ectosome shows various modifications, having specific names – for example, in the cortex, the ectosome is reinforced with specific skeletal elements.

The ectosome could include the following structures:

- glycocalyx layer
- cuticle (if present)
- exopinacoderm
- pores or ostia
- dermal membrane
- cortex (if present)
- inhalant canals
- lacunae and subdermal cavity.

The exopinacocytes of all sponges produce an external layer of mucopolysaccharides – the **glycocalyx**, which is continuous along the surface of the pinacoderm. The glycocalyx can have a variable thickness in different seasons and in different species and be modified into a cuticle. It plays a role in the adhesion of external particles to cell surfaces, prior to their phagocytosis (Willenz 1982).

The **cuticle** is a noncellular, amorphous, sometimes fibrillar covering present in some sponges (Figure 2.3a,b). An external cuticle has been recorded in Dictyoceratida (Garrone 1975; Donadey 1982; Teragawa 1986), Verongida (Vacelet 1971), Chondrosiida (Vacelet and Perez 1998), Chondrillida (Ereskovsky et al. 2011; Willenz et al. 2016), Poecilosclerida (Bagby 1970; Turon et al. 1999), and Tetractinellida (Simpson et al. 1985). The cuticle has been recognized as a structure allowing isolation of the sponge tissues from the environment for cell repair, reorganization, or survival during adverse environmental conditions (Vacelet 1971; Diaz 1979). Another possible function of the cuticle is defense against harmful epibionts since it is periodically shed (Connes et al. 1971; Donadey 1982).

The **exopinacoderm** forms the external cover of the sponge (Figure 2.3c,d,f). The surface part of an exopinacocyte is polygonal in shape and covered with a self-secreted glycocalyx. The exopinacocytes can secrete components of the extracellular matrix and synthesize collagen (Garrone 1978; Simpson 1984; Gaino et al. 1986). The exopinacocytes of Homoscleromorpha are closely associated with the underlining dense fibrillar layer, the basal membrane, comprising collagen IV, laminin, and tenascin (Boute et al. 1996). This basal membrane is identical to the lamina reticulata in the basal lamina of the vertebrate epithelia (Figure 2.3d) (Humbert-David and Garrone 1993; Boute et al. 1996).

The exopinacoderm contains **ostia** or pores – numerous microscopic structures 4–100 µm in diameter, through which the water is drawn into the aquiferous system of the sponge. In most Demospongiae and in all Homoscleromorpha, ostia are intercellular (Figures 2.1a, 2.2b,g, 2.3e; see also Figure 2.7a,b,e). In the Calcarea and many Demospongiae, the ostia are formed inside special cylindrical tubular cells, the porocytes (Figure 2.3f) (Jones 1966; Eerkes-Medrano and Leys 2006; Lavrov et al. 2018). The porocytes contact both exopinacocytes and choanocytes or endopinacocytes by their lateral surfaces. In *Sycon coactum*, porocytes can contract in response to mechanical stimulation and treatment with anesthetics (Eerkes-Medrano and Leys 2006). The porocytes of some Demospongiae from order Haplosclerida are flattened cells with a central or peripheral opening, which can open and close like a sphincter (Harrison 1972a; Weissenfels 1980; Willenz and van de Vyver 1982; Langenbruch and Scalera-Liaci 1986; Harrison et al. 1990). Thus, they may constrict or dilate the pore (Harrison 1972b) and influence the rate of flow of environmental water into the sponge.

The **dermal membrane** is a part of the ectosome, which comprises the exopinacoderm and endopinacoderm, lining subdermal spaces (subdermal cavities) and forming the inner surface of the dermal membrane, and thin mesohyl in between these pinacoderms (Figures 2.1a, 2.2c,d) (Bagby 1970; Willenz and Hartman 1989). The mesohyl layer includes a dense fibrillar component, consisting of collagen fibrils scattered between pinacocyte layers (Garrone and Pottu 1973; Garrone and Rozenfeld 1981; Willenz and van de Vyver 1982; Teragawa 1986). The dermal membrane can have a special "dermal skeleton" that differs from the ectosomal skeleton and serves as the diagnostic characteristic in taxonomy. This skeleton is often formed by special categories of spicules (e.g. in Poecilosclerida).

The **cortex** is the superficial specialized reinforced part of the ectosome. It is characteristic for syconoid and leuconoid sponges. The cortex is not an obligatory structure for all

Porifera; if present, it occupies the area immediately below the exopinacoderm and consists of a specialized portion of the mesohyl. The cortex can be highly structured and contains (i) layers or tracts of cells (Figure 2.2c), (ii) special skeletal elements (could include special spicules that are absent in other body parts) (Figure 2.2e), (iii) spongin fibers or exceptionally dense fibrils (Figure 2.2f,g), (iv) a combination of these, and (v) occasionally foreign material embedded into the tissue (Figure 2.2h) (Teragawa 1986). In some species the cortex has variable thickness (10–100 µm).

The cortex can comprise particular cell types, including lophocytes (Paris 1961), degenerating spongocytes (Connes et al. 1972), spherulous cells, and granular cells (Simpson 1968). The particular skeletal elements of the cortex may be represented by specific types of inorganic microscleres (e.g. Tetractinellida, Poecilosclerida) or organic fibrils (e.g. Ircinia, Dysidea – Dictyoceratoda) in a special arrangement.

The cortex presumably acts as a special supportive device for openings of the canal system and as a protective layer (Vacelet 1971) with the result that superficial injury does not involve the choanocytes.

2.4.2 Aquiferous System

2.4.2.1 Types of Aquiferous System

The circulatory aquiferous system is the most characteristic feature of the poriferan organization (Figure 2.1a). Water drawn into the inhalant canals via ostia moves through inhalant canals to choanocyte chambers and then, via the system of exhalant canals, to the large exhalant opening – the osculum. The aquiferous system brings water through the sponge to the cells responsible for food gathering and gas exchange. At the same time, excretory and digestive wastes are expelled by way of the water currents. The unidirectional flow of water is ensured by the coordinated beating of the choanocytes' flagella. The aquiferous system is a modular, easily rearranged system (Gaino et al. 1995; Plotkin et al. 1999; Ereskovsky 2003).

The aquiferous system is situated, mainly, in the endosome (choanosome) – the internal region of a sponge, comprising the choanocyte chambers. The aquiferous system consists of three main parts: (i) inhalant system – canal system between ostia and prosopyle (entrance of choanocyte chamber); (ii) choanocyte chambers; and (iii) exhalant system – canal system between the apopyle (exit from choanocyte chamber) and osculum.

However, some representatives of the families Cladorhizidae and Esperiopsidae (Poecilosclerida, Demospongiae) lack all the elements of the aquiferous system (Vacelet 2006, 2007; Ereskovsky and Willenz 2007) due to the change of their nutrition strategy from water pumping to predation.

Five types of aquiferous system occur in sponges:

1) asconoid (Greek, skin bag) – ostia lead directly to the internal cavities completely lined with choanoderm, which open via an osculum (Figure 2.6a)
2) solenoid (Greek, tube) – ostia open to a network of anastomosed tubes completely lined by choanoderm (i.e., choanocyte tubes), which lead into an atrium lined with endopinacoderm, opening via an osculum (Figure 2.6b)
3) syconoid (Greek, fig) – ostia open directly to radially elongated choanocyte chambers or to short inhalant canals, which are connected to elongated choanocyte chambers via prosopyles; the choanocyte chambers are connected to a single atrium via apopyles; the atrium, lined with endopinacoderm, opens via an osculum (Figure 2.6c)
4) sylleibid (Greek, collect + ibi) – ostia lead to short inhalant canals which connect to prosopyles of choanocyte chambers, arranged radially around large exhalant canals; chambers are connected to exhalant canals via apopyles; the exhalant canals lead to the atrium, opened via oscula (Figure 2.6d)
5) leuconoid (Greek, a disease like elephantiasis) – ostia open to short inhalant canals or to large subdermal cavities, leading to inhalant canals; inhalant canals are connected via prosopyles to numerous small choanocyte chambers, scattered in the mesohyl; choanocyte chambers lead to exhalant canals via apopyles; exhalant canals open to atria which lead to oscula (Figure 2.6e).

2.4.2.2 Histology, Cell Types, Arrangement, Extracellular Structures

2.4.2.2.1 Canals of Aquiferous System: Inhalant System

Incurrent openings – ostia, pores. These structures could be intracellular, formed by special cells, porocytes (Figure 2.3f), or intercellular, formed as an opening between adjacent pinacocyte margins (Figure 2.7a,b,e). However, in some demosponges, such as *Eunapius fragilis* (Spongillida), both types of ostia occur (Harrison and de Vos 1991). The ostia have the ability to open and close within a relatively short period of time for flow-regulating purposes (Harrison 1972a; Weissenfels 1980). Hence number and diameter of ostia within specimens appear highly variable at any given time.

In some demosponges (e.g., order Poecilosclerida), the ectosome forms specialized inhalant structures: (i) pore sieve (fr. crible) – a contractile cluster of ostia, located on the sponge surface; (ii) pore groove – a furrow on the sponge surface, where the ostia are located (Boury-Esnault 1972); (iii) poral face – a specific surface on the sponge body, where all ostia are located (e.g., family

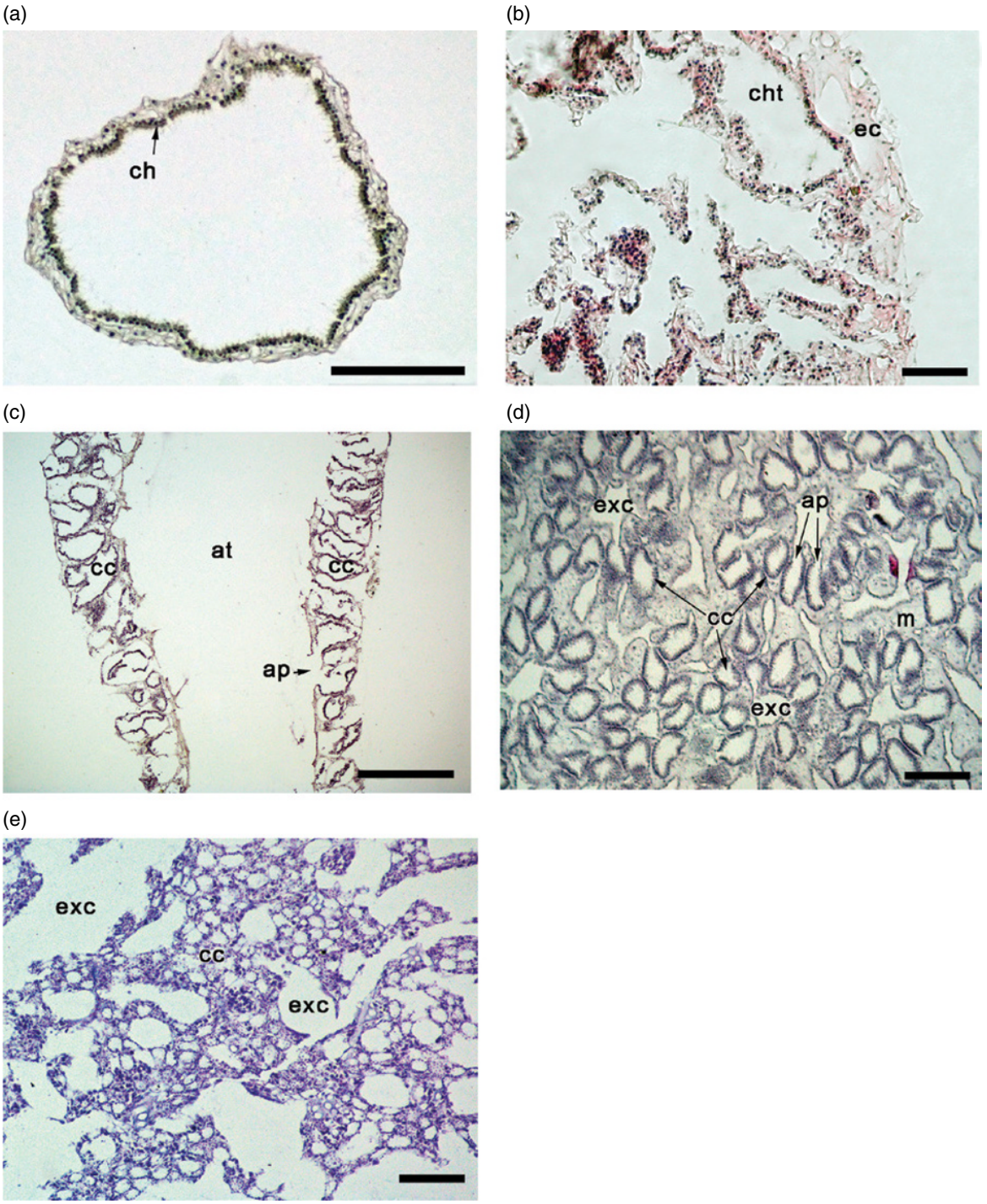


Figure 2.6 Types of aquiferous systems in sponges. (a) Asconoid aquiferous system in *Clathrina clathrus*. (b) Solenoid aquiferous system in *Leucascus* sp. *Source:* Image courtesy of M. Klautau. (c) Syconoid aquiferous system in *Sycon ciliatum*. (d) Sylleibid aquiferous system in *Oscarella tuberculata*. (e) Leuconoid aquiferous system in *Myxilla incrustans*. Scale bars: (a,d,e) 100 μm; (b) 500 μm; (c) 50 μm;

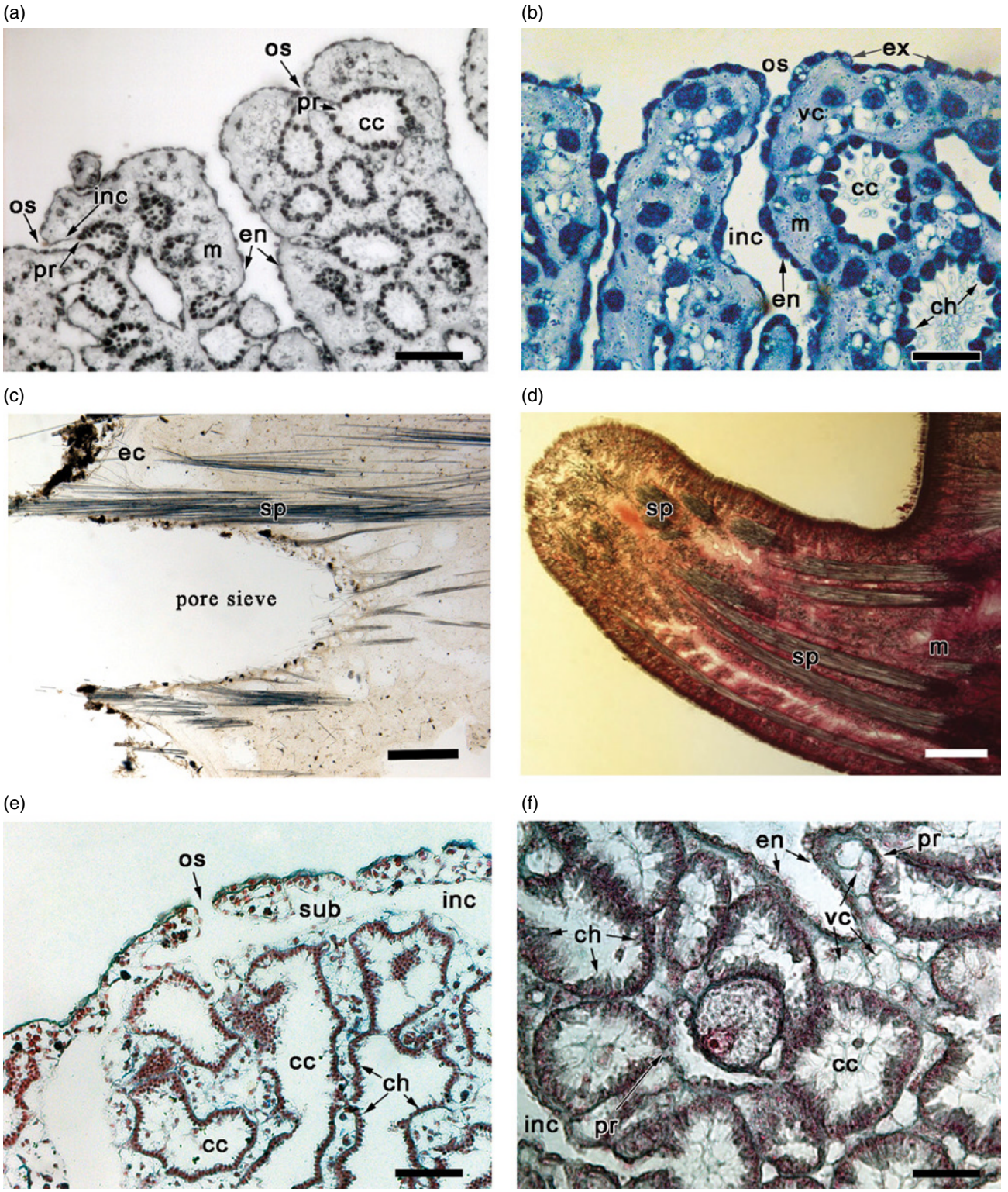


Figure 2.7 Inhalant aquiferous system. (a,b) Ostia and inhalant canals lined with prosendopinacocytes in *Oscarella lobularis*. (c) Porocalyx in *Cinachyrella apion*. *Source:* Image courtesy of Paco Cardenas. (d) Papilla of *Proteleia sollasi*. *Source:* Reproduced with permission from Plotkin et al. (2016). (e) Pores, inhalant canals and subdermal (vestibular) cavities in *Halisarca dujardinii*. (f) Prosopyles in *Halisarca dujardinii*. Scale bars: (a) 100 μm; (b,f) 50 μm; (c) 20 μm; (d) 1 mm; (e) 200 μm.

Thorectidae, order Dictyoceratida) (Cook and Bergquist 2002). All mentioned inhalant structures are underlaid by a large inhalant cavity, the vestibule (Boury-Esnault 1972).

Another type of specialized inhalant structure is the porocalyx which is a circular, poriferous depression in the ectosome, disturbing the cortex structure and appearing as a distinctive oval or flask-shaped pit. The porocalyx bottom contains the inhalant and, occasionally, also exhalant orifices. Porocalices may be contractile. These structures are typical for some demosponges from family Tetillidae (order Tetractinellida) (Figure 2.7c) (Rutzler 1987).

In some demosponges there are special structures associated with the aquiferous system – papillae which are nipple-like protuberances projecting from the sponge surface and bearing either ostia, oscula, or both, discussed in detail by Simpson (1984) (Figure 2.7d).

Inhalant canals lead from ostia to choanocyte chambers (Figures 2.7a,b, 2.8b,f). In most sponges, ostia open directly into the inhalant canals. However, in many cases smaller canals, called canalicules, connect ostia with the larger inhalant canal. The inhalant canals and canalicules are lined by special endopinacocytes called prosopinacocytes. The prosopinacoderm often has intercellular gaps, allowing water flow between the canal lumen and mesohyl.

Subdermal (vestibular) cavities. Most demosponges possess a leuconoid canal system, in which inhalant water, passing through ostia, enters a large subepithelial space, the incurrent vestibule or subdermal cavity. These structures are located just below the exopinacoderm or dermal membrane and are lined with prosopinacocytes (Figures 2.2c,d,f,g, 2.7e).

The **prosodus** (Greek *prosodos*, procession) is a tiny canal connecting the larger inhalant canal with the entrance to the choanocyte chamber, a prosopyle. Prosodus occurs only in sponges with leuconoid aquiferous systems.

The **prosopyle** (Greek *prósō*, forward + *pýlē*, gate) is the opening through which water enters a choanocyte chamber (Figures 2.7a,f, 2.8b). Prosopyles can be formed by prosopinacocytes, that contact the choanocytes and form a structure like a pore (pinacocytic prosopyle), as in *Tethya wilhelmia* (Hammel and Nickel 2014), or by pseudopodial extensions between adjacent choanocytes, forming small gaps (choanocytic prosopyles), as in *Petrosia ficiformis* (Langenbruch and Scalera-Liaci 1990).

2.4.2.2.2 Choanocyte Chambers According to Boury-Esnault and Rützler (1997), a choanocyte (= flagellated) chamber is any cavity lined by choanocytes and located between the inhalant and exhalant systems. The structure of choanocyte chambers and the way they are attached to each canal are characteristic of sponge families and genera (Boury-Esnault et al. 1984, 1990).

In sponges with asconoid and solenoid aquiferous systems, it is possible to consider all the internal cavities, lined by choanocytes as one branching choanocyte tube (Figure 2.6a,b).

In sponges with syconoid aquiferous systems, the large choanocyte chambers are ovoid and lie perpendicular (radially) to the central atrial cavity (Figures 2.6c, 2.8a).

The choanocyte chambers of the sponges with sylleibid and leuconoid aquiferous systems are categorized according to the type of their connection to the inhalant and exhalant canal systems (Sollas 1888). There are three main types of chamber organization.

1) In the aphodal choanocyte chamber, water enters directly from inhalant canals through prosopyles and leaves through a narrow canal, the aphodus, which lies between the chamber and exhalant canal (*Plakina trilopha*) (Figure 2.8d).
2) In the diplodal chamber, water enters by a prosodus and leaves through the aphodus, connecting the chamber with the exhalant canal (Figure 2.8e). The aphodal and diplodal choanocyte chambers are delimited externally by the mesohyl (*Pseudocotricium jarrei*, *Chondrosia reniformis*, *Tethya wilhelma*).
3) In the eurypylous choanocyte chamber, water enters from the inhalant canal directly through prosopyles (typically, these are spaces between choanocytes and termed choanocytic prosopyles) and leaves through a large apopyle, that opens directly to the large exhalant canal (*Spongilla lacustris*, *Oscarella* spp., *Aplysina*) (Figure 2.8b,c,f). Two types of eurypylous choanocyte chambers are known.
   - The first type, which we propose to call a mesohylar eurypylous choanocyte chamber, is characterized by direct contact of choanocytes with the mesohyl (e.g., Homoscleromorpha, Verongida) (Figure 2.8b,c).
   - In the second type, which we propose to call a canal eurypylous choanocyte chamber, described in many Haplosclerida, the choanocyte chambers lie free in the lumen of inhalant canals and are separated from the mesohyl by endopinacocytes of these canals (Langenbruch 1988, 1991; Langenbruch and Jones 1990) (Figure 2.8f). In these chambers choanocytes are arranged in a regular hexagonal pattern with uniform interstitial spaces around each cell – choanocytic prosopyles. In this case two modes of organization are observed: (i) the choanocyte chamber is completely enveloped by pinacocytes (e.g., *Haliclona elegans*, *Haliclona mediterranea*, *P. ficiformis*), and (ii) choanocyte epithelia partially enveloped by pinacocytes (e.g., *Haliclona fulva*, *Niphates digitalis*) (Langenbruch 1991). In the Homoscleromorpha,

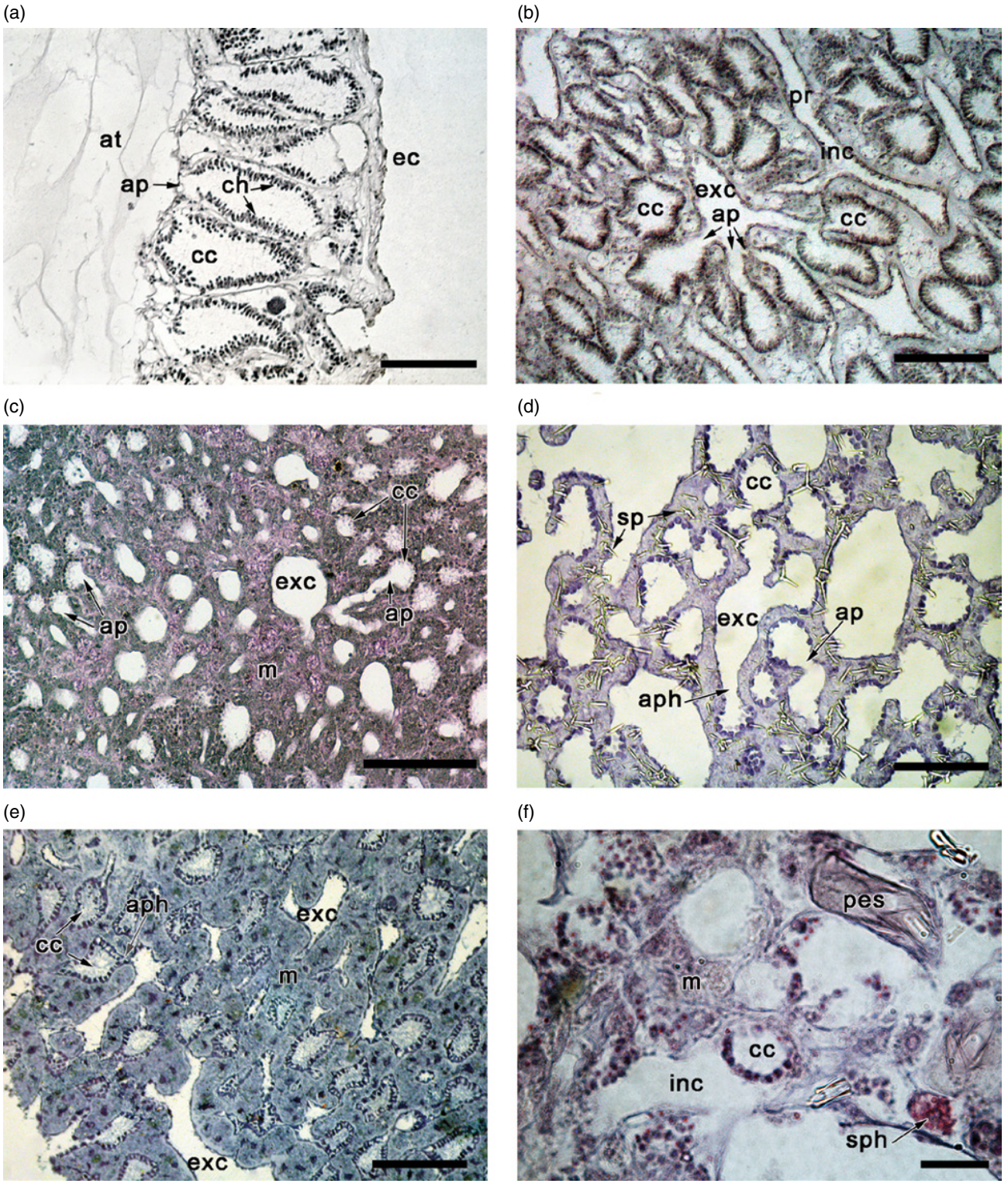


Figure 2.8 Choanocyte chambers. (a) Syconoid choanocyte chambers in *Sycettusa murmanensis*. (b) Mesohylar eurypylous choanocyte chambers in *Oscarella tuberculata*. (c) Mesohylar eurypylous choanocyte chambers in *Aplysina cavernicola*. (d) Aphodal choanocyte chambers in *Plakina trilopha* with the spicules dispersed in the mesohyl. (e) Diplodal choanocyte chambers in *Pseudocorticium jarrei*. (f) Canal eurypylous choanocyte chambers in *Haliclona fulva*. Scale bars: (a,b,c,d) 100 µm; (e) 50 µm; (f) 20 µm.

choanocytes are connected to each other by close junctions along their lateral surfaces, and there is a basement membrane and collagenous mat covering the chamber (Figure 2.7b).

Choanocyte chambers of leuconoid sponges can have different shapes and sizes, from small and spherical or ovoid (Figures 2.6e, 2.8f) to long and tubular (Figure 2.7e). The volume of chambers within different species varies between 350 (Agelasida) and 480 000 μm^3 (Halisarcidae) (Boury-Esnault et al. 1990). Halisarcidae have tubular branched choanocyte chambers, which are the largest known chambers in Demospongiae (Figure 2.7e; see also Figure 2.12a) (Ereskovsky et al. 2011). Larger chambers are found in the sponges with a less extensive mesohyl. Small chambers occur mostly in sponges with a dense mesohyl. Differences also exist in choanocyte size and shape, collar and flagellar length and ornamentation, and anchorage of the choanocytes in the mesohyl (Boury-Esnault et al. 1984, 1990). The number of choanocytes per chamber varies from 5 (Agelasida) to 2800 (Halisarcidae) (Boury-Esnault et al. 1990).

2.4.2.2.3 Canals of Aquiferous System: Exhalant System The **apopyle** (Greek *apo*, from + *pylē*, gate) is an opening in a choanocyte chamber, connecting it with an exhalant canal. The apopyles are larger than prosopyles. De Vos et al. (1990) described three types of apopyle organization. The first is characterized by the absence of apopylar cells. In this case the choanocytes and apopinacocytes directly contact each other. This type of apopyle is found in demosponges from the orders Suberitida, Clionaida, Tetractinellida, and Axinellida. In the second type, a structure of apopylar cells connects choanocytes and apopinacocytes and each has a flagellum directed toward the exhalant canal. This type was described in Homoscleromorpha, Dysideidae, Aplysillidae, and Halisarcidae (Figure 2.8b–e). The third type of apopyle is found in *Ephydatia fluviatilis* (Spongillida) and *Petrosia ficiformis* (Haplosclerida). In this case, the apopylar cells are completely immersed inward, so that they are invisible from the outside and give a specific cone shape to the apopylar opening from the inside (Langenbruch et al. 1985). The localization of these cells suggests that they play a role in controlling water current. In addition, in *Tethya wilhelma* the apopyle also has a reticuloapopylocyte (see above) (Hammel and Nickel 2014).

The **aphodus** (plural **aphodi**; Greek *aphodos*, departure) is the short canal leading from an apopyle of a choanocyte chamber to an exhalant canal (Figure 2.8d,e).

Exhalant canals gather water from the choanocyte chambers and lead it to larger canals, which finally merge in a large atrial cavity, underlying an osculum (Figure 2.8b–e). Exhalant canals are lined by apopinacocytes, which in some sponges may bear a flagellum (Donadey 1979; Boury-Esnault et al. 1984; Hammel and Nickel 2014). The lining of the exhalant canals structurally appears more homogenous than the lining of inhalant canals and may contain porocytes, connecting the canal lumina with the mesohyl.

The **atrium** is the central exhalant, preoscular cavity. It is not characteristic of sponges with asconoid aquiferous systems. The atrium is lined with apopinacocytes and is roofed by the exhalant dermal membrane (Figures 2.1a, 2.6c, 2.9c). In some cases, mostly in encrusting sponges, the large exhalant canals merging at the atrium run parallel to the sponge surface, thus forming radiating "astrorhizae."

The **osculum** is a large opening through which the water leaves a sponge. Oscula are bounded externally by exopinacocytes, while their inner surface is formed by endopinacocytes (Figure 2.1a–c,e). The oscula of some sponges are lined internally by ciliated cells as in Hexactinellida (Leys et al. 2007), Homosclermorpha (Boury-Esnault et al. 1984), and freshwater sponges (Saller 1990; Ludeman et al. 2014). It is supposed that these ciliated cells have sensory functions (Ludeman et al. 2014).

In the oscular rim of *Microciona prolifera*, *Tedania ignis*, and some other sponges, contractile cells – myocytes – are present (Bagby 1966) (see section 2.3.3). These cells can change the diameter of the oscular opening, thus controlling water currents through the aquiferous system. The freshwater sponge *E. fluviatilis* has no myocytes but does have contractile pinacocytes with actin bundles in its oscular diaphragm (Masuda et al. 1998).

2.4.3 Skeleton

The typical sponge skeleton is a composite of organic and inorganic materials that form a scaffold-like framework supporting the sponge body. The organic material is composed of various types of fibrillar collagen, which can form fibers up to several millimeters in thickness. This proteinaceous material provides a flexible but sturdy matrix for the sponge skeleton. The inorganic material is composed of either silica (SiO_2) or calcium carbonate ($CaCO_3$) in the form of calcite or aragonite. Sponges are the only animals that use hydrated silica as a skeletal material.

The distinction of inorganic material has important taxonomic and phylogenetic value. About 92% of all living sponge species are siliceous. Sponges belonging to the classes Demospongiae, Hexactinellida, and Homoscleromorpha produce siliceous spicules although some demosponges (*Chondrosia, Halisarca, Hexadella, Myxospongia*) and homoscleromorphs (*Oscarella, Pseudocorticium, Aspiculophora*) have lost the inorganic component of the skeleton. All representatives of the class Calcarea

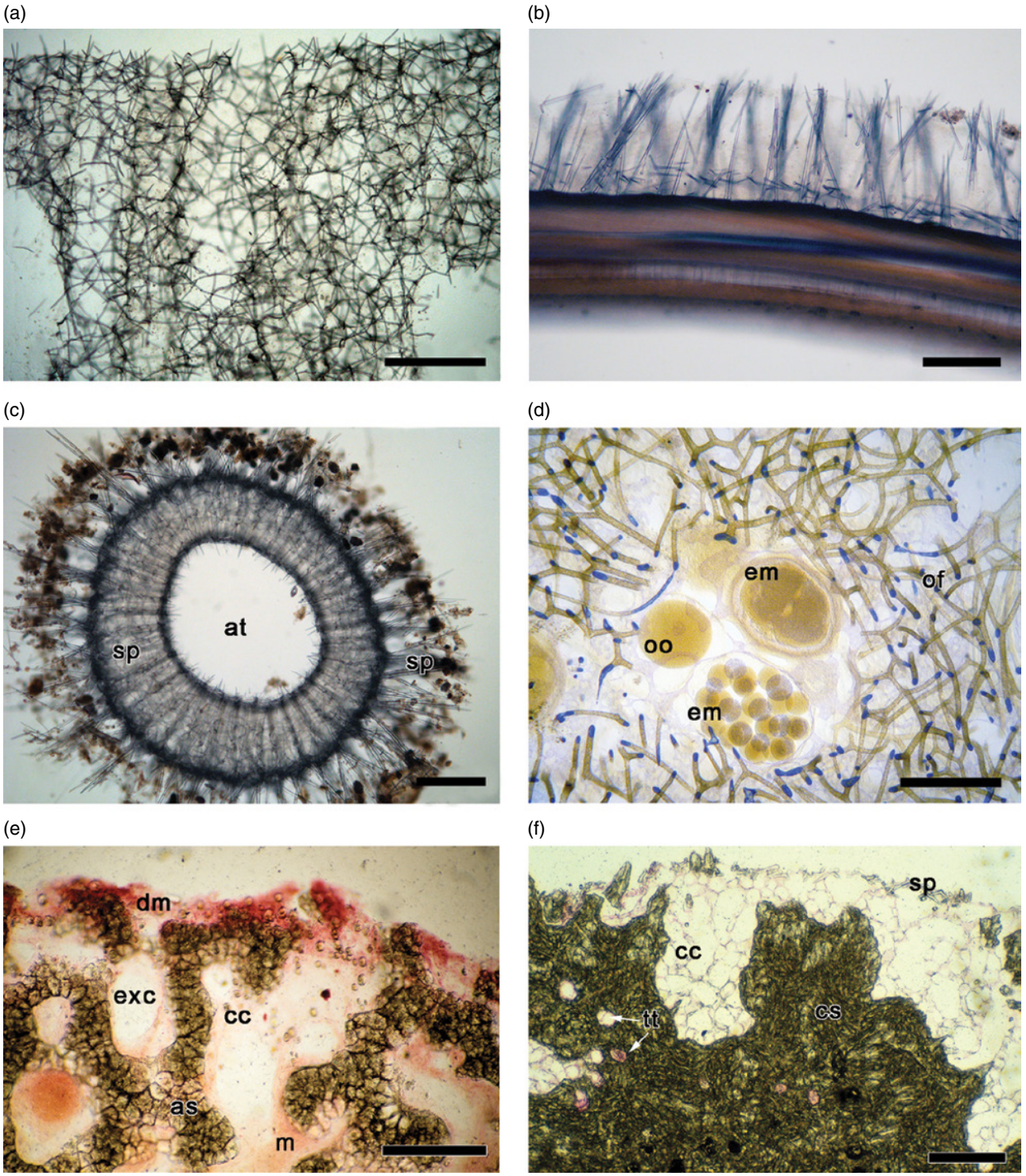


Figure 2.9 Skeleton. (a) Inorganic skeleton (SiO_2) of *Haliclona* sp. with regular reticulation of multispicular tracts of megascleres. (b) Inorganic skeleton (SiO_2) of *Protosuberites mereui* with choanosomal skeleton of brushes of tylostyles, erected from the substrate. (c) Inorganic skeleton ($CaCO_2$) of *Sycon vigilans* with radial organization around a central atrium (at). (d) Organic skeleton of *Spongia officinalis* with homogeneous skeletal fibers. (e) Hypercalcified *Ceratoporella nicholsoni* (Demospongiae) with aragonitic skeleton. *Source:* Image courtesy of J. Vacelet. (f) Hypercalcified *Petrobiona massiliana* (Calcarea). *Source:* Image courtesy of J. Vacelet. Scale bars: (a) 500 µm; (b,c) 200 µm; (d,e,f) 300 µm.

produce calcitic spicules. Spicules may be either dispersed in the mesohyl (Figure 2.8d) or assembled into a defined three-dimensional framework structuring the soft tissue (Boury-Esnault and Rützler 1997; Uriz 2006). Spicules are divided into megascleres and microscleres, according to their size, morphology, and role in the skeletal framework. Megascleres are usually assembled into tracts (Figure 2.9a,b) and maintain the gross form of the sponge, while microscleres are dispersed throughout the sponge body and support various microanatomical structures (Figure 2.2e). In addition to a spicular skeleton, some demosponges and calcareous sponges develop a massive basal skeleton, composed of calcite or aragonite.

2.4.3.1 Inorganic Skeleton

The inorganic skeleton in the form of siliceous spicules in Demospongiae includes about 12 basic types of megascleres and 28 types of microscleres; in Homoscleromorpha, about four megascleres; in Hexactinellida, 20 basic types of megasclere and 24 types of microscleres (Boury-Esnault and Rützler 1997; Tabachnick and Reiswig 2002). Megascleres in both demosponges and hexactinellids usually form the main sponge skeleton (Figure 2.9a,b). Spicules can be joined by spongin (demosponges), fuse (some hexactinellids), or articulate with each other (Lithistidsa). Microscleres may be widespread in the sponge body or are concentrated in the ectosome-forming crusts of the cortex (Figure 2.2e) or spread in the choanosome. In demosponges there are six elemental types of spicule frameworks, with intermediate forms, that can be differentiated: hymedesmoid, plumose, axial, radiate, reticulated, and disarranged (Boury-Esnault and Rützler 1997; Uriz 2006). In some demosponges (e.g., Lithistida) and many Hexactinellida, the spicules may be linked or fused into such a rigid framework that it is capable of fossilizing.

Skeletons made up of calcium carbonate usually appear in the form of networks of spicules or rarely can be massive, occurring in combination with spicular elements. Calcareous spicules are made of calcium carbonate, mainly crystallized as magnesium-rich calcite (Jones and Jenkins 1970). The majority of Calcarea have a skeleton composed of free spicules, without calcified nonspicular reinforcements. Frameworks in most calcareous sponges are simple and delicate but well organized, with several spicule types localized in particular regions of the sponge body. The skeleton in general has radial organization around a central atrium (Figure 2.9c).

In addition to a spicular skeleton, some representatives of the Demospongiae and Calcarea secrete a massive basal skeleton, composed of calcite or aragonite. All such species are referred as hypercalcified sponges. Living hypercalcified sponges are restricted to deep or cryptic habitats like bathyal cliffs, sublittoral dark caves, and coral reef tunnels (Vacelet et al. 2010).

In living hypercalcified Demospongiae, several morphologic types or grades of organization are represented (Vacelet et al. 2010). The chaetetid type corresponds to laminar or domical sponges in which the superficial parts of the skeleton display a honeycomb structure, with more or less hexagonal tubes, somewhat resembling the coallites of scleractinian corals but smaller. The living tissue occurs as a thin veneer at the surface and within the outer parts of the tubes (Figure 2.9e). This type is known in the Ceratoporellidae, Merliidae, and Acanthochaetetidae.

The stromatoporoid type is found in domical to flattened, laminar sponges with a calcified skeleton consisting of a meshwork of tubes, pillars, and laminae. This type is known in Calcifibrospongia and Astrosclera.

In the sphinctozoid type found in Vaceletia (Dictyoceratida), the skeleton is external, resulting in a discontinuous growth, with separate chambers linked by a central siphon.

Only a few living calcareous sponges of the orders Murrayonida (Calcinea), Lithonida, and Baerida (Calcaronea) secrete massive or reinforced calcareous skeletons (Vacelet et al. 2002a, b). In Murrayonida, the basal skeleton reticulates with a meandering structure made up of fused, irregularly shaped calcitic sclerodermites, generally without entrapped spicules. In Baerida, the basal skeleton is composed of a solid mass of calcite, consisting of spiny elongated or irregular sclerodermites that form a series of crests between which lies the living tissue (Figure 2.9f).

2.4.3.2 Organic Skeleton

In addition to an inorganic skeleton, all sponges have a collagenous organic skeleton. This collagenous skeleton may include two types of the fibrils: (i) "classic" collagen fibrils of 20–25 nm in diameter, which are cross-striated ultrastructurally (striations are not visible by light microscopy) and (ii) thin (10 nm) spongin fibrils.

The fibrillar collagen is located throughout the mesohyl of the sponge and is the only form of organic skeleton characteristic for all sponge classes (Garrone 1985). Such fibrils are always presented in the sponge mesohyl, while the extent to which fibrillar collagen reinforces the sponge varies in different classes and species. In Calcarea, the fibrillar collagen is always lightly dispersed through the mesohyl, never occurring in dense concentration, except for the formation of sheaths around spicules (Jones 1967; Ledger 1974). In contrast, in some demosponges (e.g., *Halisarca*) and homoscleromorphs (e.g., *Oscarella*), which are devoid of inorganic and spongin skeletons, the fibrillar collagen plays a central role as a skeleton component and is greatly elaborated (Bergquist 1996). In such cases, collagen

fibrils are usually organized into bundles, which are interlaced and form complex three-dimensional supporting structures (Garrone et al. 1975).

In contrast to fibrillar collagen, which shows more or less the same structure in all sponges, the spongin skeletal structures are diverse, but occur only in demosponges. Spongin is a collagenous protein (Exposito et al. 2002), which was called "spongin B" by Gross et al. (1956). According to Garrone (1978), the spongin microfibers can appear in demosponges in five different states.

1) Spongin microfibrils form an adhesive layer, which attaches sponges to their substrate. In this form, spongin appears in all demosponges (Borojevic and Lévi 1967; Garrone 1985). However, the participation of chitin in the formation of a sponge holdfast has been proposed in Lubomirskiidae freshwater sponges (Ehrlich et al. 2013).
2) Another widespread form of spongin is perispicular spongin, characteristic of demosponges with inorganic skeletons, composed of spicules (Figure 2.8f). Usually, the perispicular spongin is deposited at the points of intersection of spicules, incorporating their ends, thus uniting single spicules to integrate the skeleton (Weissenfels 1978; Willenz and Hartman 1989; Galera et al. 2000). In some cases, perispicular spongin can be highly developed and form spiculated fibers. The spiculated fibers are macroscopic spongin structures, which envelop whole spicule tracts. Occasionally, the spiculated fibers can be very wide with only a thin row of spicules in the center (Garrone 1969, 1978; Garrone and Pottu 1973).
3) In some demosponges (e.g., orders Verongiida, Dendroceratida, Dictyoceratida) spongin appears as macroscopic fibers, reaching a thickness of several millimeters. These fibers begin from the basal adhesive layer of spongin but are submersed into the sponge body (Figures 2.2g, 2.9d). In some species (e.g., freshwater sponges), all spongin fibers inside the body are covered with a continuous epithelium, connected with the basopinacoderm. Thus, in these species the whole spongin skeleton is an exoskeleton (Weissenfels 1978; Garrone 1985). The spongin fibers form a complex skeleton with hierarchic structure and of either a dendritic or an anastomosing pattern. The internal structure of the fibers varies: they can have fine fibrillar pith (central area of a fiber, made up of more or less diffuse wisps of collagen or of a coarsely granular collagenous material), surrounded by the laminar bark (the dense area of compacted spongin, in which concentric layers are visible) (e.g., Dendroceratida, Verongiida) (Figure 2.2g), or be homogenous (e.g., Dictyoceratida) (Garrone 1978). In Verongiida, the outermost layer of the fiber is composed of the chitin, making them more rigid and chemically resistant (Ehrlich et al. 2007). The content of chitin in the fibers varies between 10% and 60% depending on the species (Ehrlich et al. 2018). Occasionally, cellular (degenerate spongocytes) elements or exogenous particles (sand grains) (Cerrano et al. 2007) are incorporated into the fibers.
4) In contrast to fibers, which are organized into a continuous skeleton, spongin can appear in the form of individual macroscopic skeletal elements. In the genus Ircinia, in addition to fibers, spongin appears as macroscopic filaments, which are up to several millimeters in length and dozens of micrometers thick and terminate with a knob at each end (Garrone et al. 1973; Junqua et al. 1974). Another form of individual spongin macrostructure is spiculoids of the genus Darwinella, which are regular diactinal, triactinal, and tetractinal structures, resembling the siliceous spicules (Bergquist 1996).
5) The last spongin formation is a deposition of its fibrils in the gemmular coat. In this case spongin microfibers are densely packed and form thick layers around the forming gemmule (see section 2.3.3.1).

2.4.4 Reproductive System

For sponges, both asexual and sexual reproductions are characteristic. Sexual reproduction is fundamentally the same as similar processes in other multicellular animals. No sexual dimorphism exists in sponges. Sponges can be oviparous (Figure 2.10a) and viviparous (brooding) (Figures 2.10 c,d, 2.11c,d, 2.12b). In the first case, the sponges are usually gonochoric (Figure 2.10a), while in the second, they are often hermaphrodites (Figure 2.10b) (Ereskovsky 2010, 2018). Viviparous sponges release larvae and oviparous sponges release zygotes or unfertilized eggs. Embryonic development in the oviparous sponges is always external, leading to free-swimming larvae. Viviparous or ovoviviparous sponges are characterized by brooding of embryos in the mesohyl or inside a special temporary structure – follicles (Ostrovsky et al. 2016). The resulting free-swimming larvae exit through exhalant canals of the aquiferous system. Direct development without a larval stage exists in some demosponges (Sarà et al. 2002).

The only common feature for all sponge species is the absence of organized gonads; gametogenesis is usually diffuse. A characteristic feature of this process in sponges is the origin of gametes by direct transformation from the somatic cells – choanocytes or, rarely, archaeocytes. Otherwise, the stages and cytologic features of gamete development in sponges are similar to those in other animals (Boury-Esnault and Jamieson 1999; Maldonado and Riesgo 2008; Ereskovsky 2010).

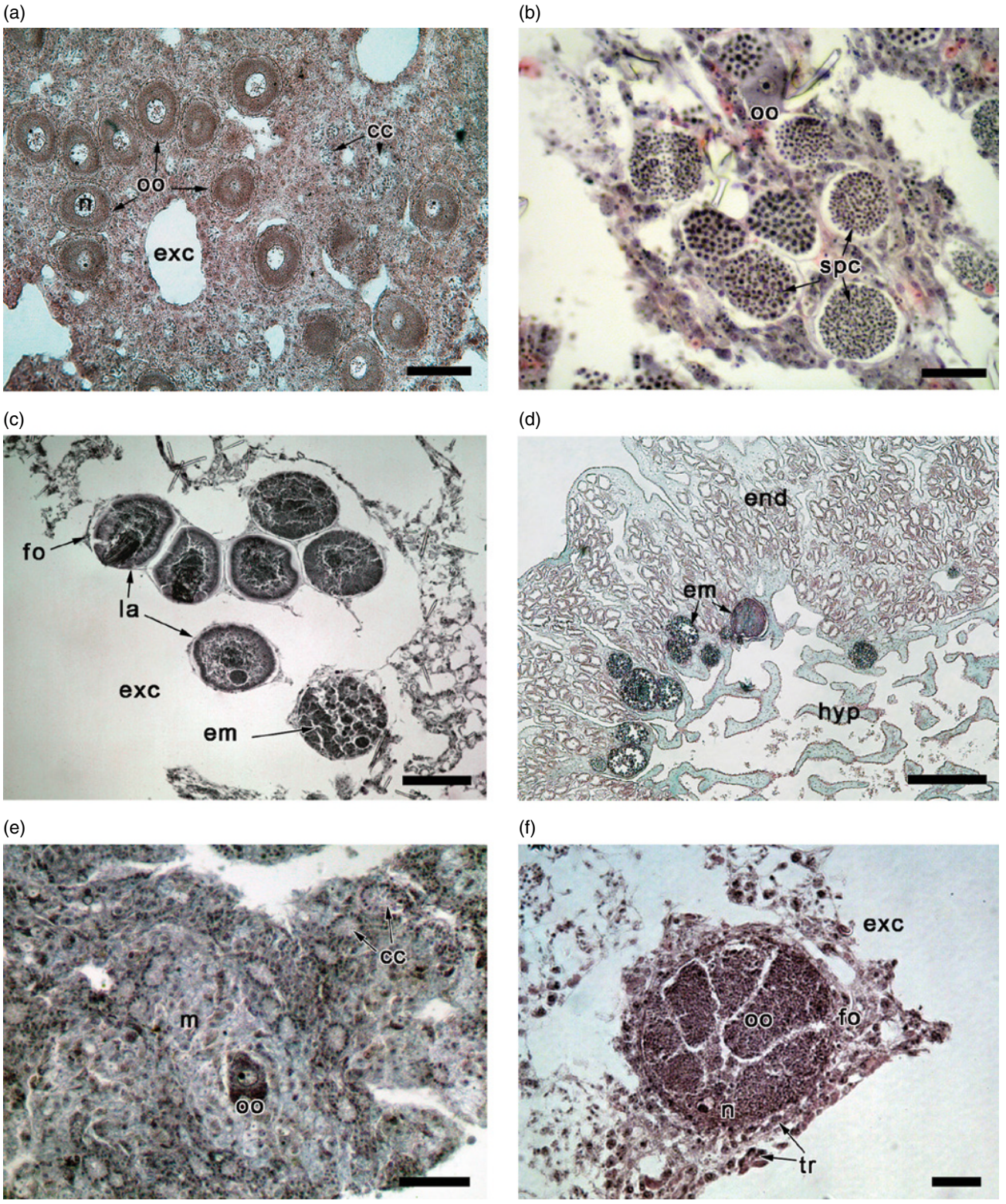


Figure 2.10 Reproduction, female. (a) Oviparous gonochoric sponge *Aplysina cavernicola* with oocytes diffusely distributed in the endosome. Note the aquiferous system degradation. (b) Viviparous hermaphrodite sponge *Esperiopsis koltuni* with oocyte and spermatocysts diffusely distributed in the endosome. (c) Viviparous *Haliclona aquaeductus* with the clusters of embryos and larvae in brooding chamber. (d) Basal position of embryos in the endosome of *Oscarella tuberculata*. (e) Amoeboid-like oocytes in *Crellomima imparidens*. (f) Amoeboid cells and trophocytes, concentrating around the egg of *Haliclona aquaeductus*. Scale bars: (a) 100 μm; (b,e,f) 50 μm; (c) 250 μm; (d) 300 μm.

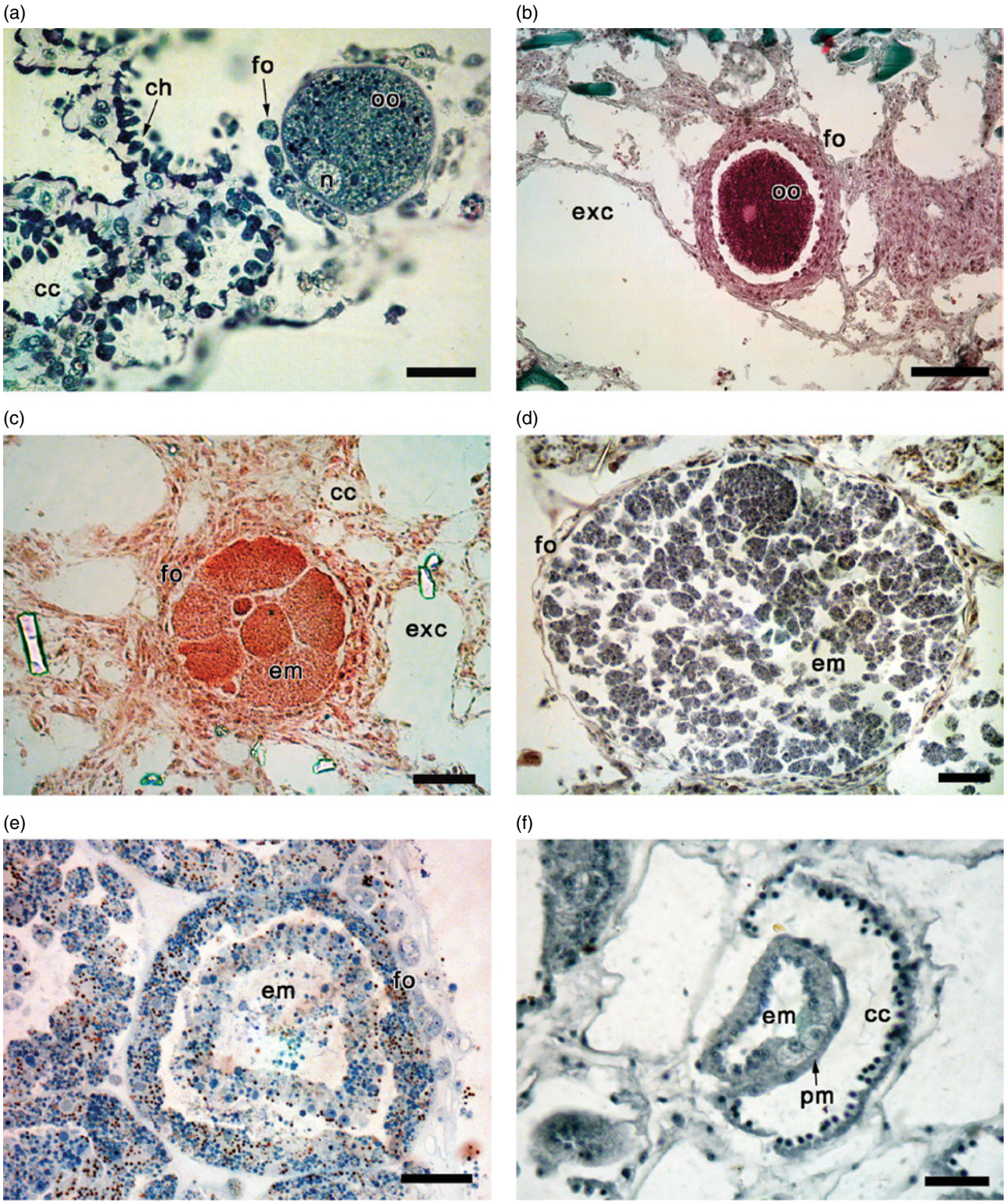


Figure 2.11 Follicle. (a) Beginning of follicle development in *Oscarella nicolai*. (b) Multilayer follicle in *Spongia officinalis*. (c) Multilayered follicle around cleaving embryo in *Iophon piceum*. (d) Monolayered follicle around a morula in *Haliclona aquaeductus*. (e) Complex two-layered follicle in *Clathrina arnesenae*. (f) "Placental membrane" in the embryo of *Sycon raphanus*. Scale bars: (a,e,f) 25 μm; (b) 200 μm; (c) 100 μm; (d) 50 μm.

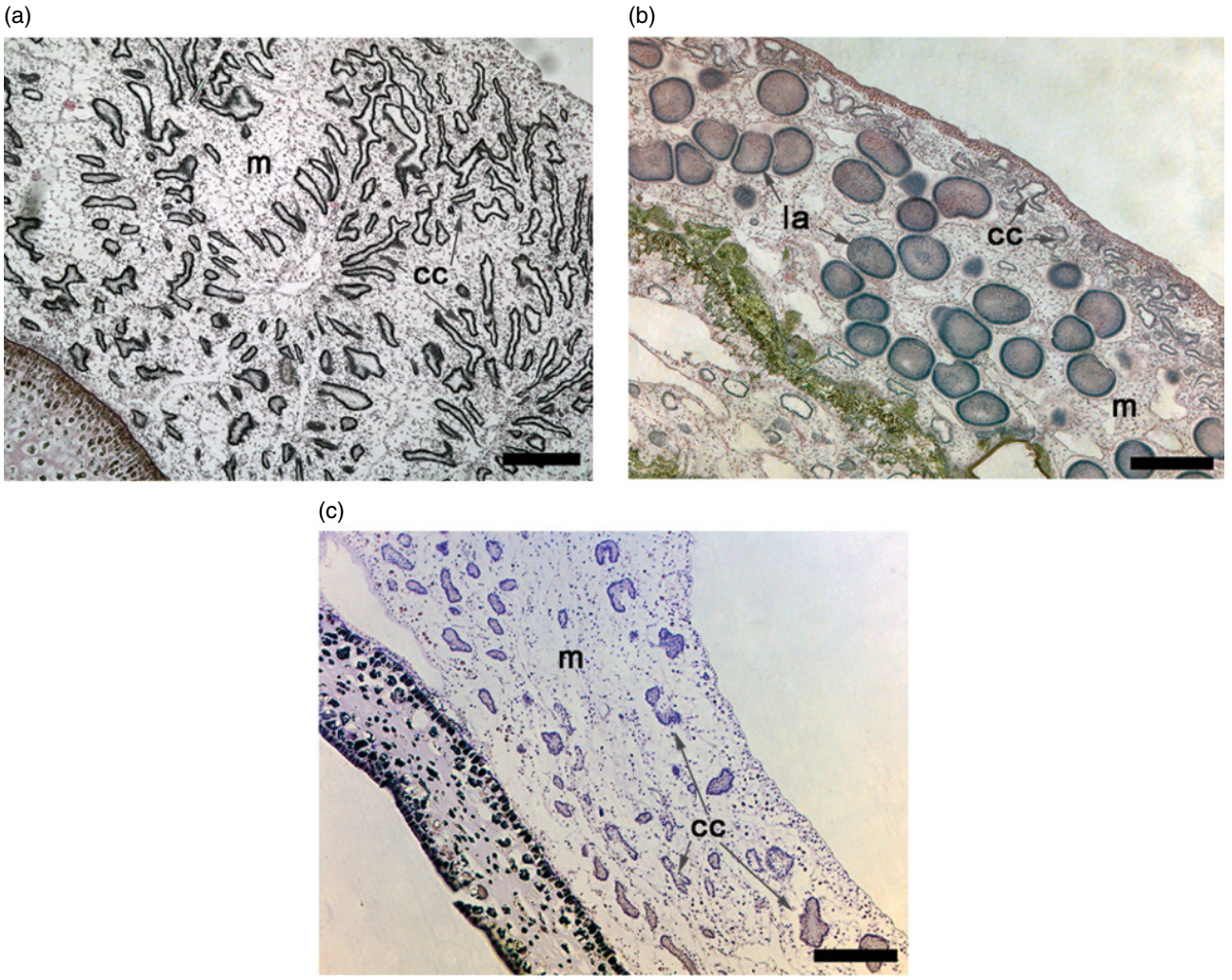


Figure 2.12 Tissue modification during sexual reproduction in *Halisarca dujardinii*. (a) Tissues in nonbreeding sponge. (b) Tissues of sponge with prelarvae, showing reduction of aquiferous system in the endosome. (c) Postreproduction rehabilitation of parental sponge tissue. Scale bars: 250 μm.

2.4.4.1 Female

In both oviparous and viviparous sponges, female gametes develop in small clusters (Figure 2.10c), located diffusely in the endosome (Figure 2.10a,b), or in the basal part of the body (Figure 2.10d) in encrusting sponges.

At the early stages of oogenesis, oocytes have an amoeboid-like shape and migrate through the mesohyl (Figure 2.10e). As the oocyte grows, it takes an oval form and proceeds to vitellogenesis, which passes via autosynthesis, heterosynthesis or both processes simultaneously. Heterosynthesis involves participation of different somatic cells and is typical for most viviparous sponges. These somatic cells (choanocytes, different mesohyl cells) are often referred to as trophocytes or "nurse cells." However, strictly speaking, sponges do not possess true trophocytes (Ereskovsky 2010). In some demosponges (orders Haplosclerida, Spongillida, and Suberitida), vitellogenesis is accompanied by the emergence of a population of specialized phagosome-rich amoebocytes that migrate toward the oocyte and are phagocytosed by it (Figure 2.10f).

In most sponges, at the end of vitellogenesis the oocyte stops near the exhalant canal and is surrounded by a temporary follicle, where embryos develop (Figure 2.11a–e). Embryonic development of ovoviviparous and viviparous sponges proceeds in these temporary follicles. In many viviparous demosponges during vitellogenesis, amoeboid cells, concentrating around the growing oocyte, form a well-developed multilayered capsule that makes up the follicle (Figures 2.10f, 2.11b,c). The phagocytosed cells of this capsule become yolk granules in the oocyte (Diaz 1973; Fell and Jacob 1979; Witte and Barthel 1994; Gerasimova and Ereskovsky 2007). As its cells are phagocytosed, the capsule gets thinner, and all that is left around the mature

egg is a single-layer follicle (Figures 2.10f, 2.11d). The follicle consists of flat pinacocyte-like cells, originating from the choanocytes, amoebocytes, or endopinacocytes, and an external layer of collagen fibers parallel to the cell surface that are synthesized by mesohyl cells (Figure 2.11d) (Fell 1983; Ereskovsky 2010).

In some calcareous sponges from subclass Calcinea, a two-layered follicle forms (Figure 2.11e). The external layer consists of dense extracellular matrix, the internal layer is made up of large cells, which may be cubic, prismatic, or flattened. Follicular cells are close to the embryo; they produce projections on the side, opposite to the embryo. These projections anchor the follicle cells in the extracellular matrix (Ereskovsky and Willenz 2008).

In many viviparous demosponges from orders Haplosclerida, Dendroceratida, Dictyoceratida, and Suberitida, groups of 6–20 oocytes of different stages concentrate into a common collagenous brood chamber (Figure 2.10c) (Ereskovsky 2010; Degnan et al. 2015). Inside the brood chambers, embryos are enveloped by follicles.

In some calcareous sponges from subclass Calcaronea, a special structure made up of flattened cells, derived from choanocytes, is formed during embryonic development. This structure is referred to as the placental membrane (Figure 2.11f). The placental membrane ensures the embryo's nutrition and participates in inversion (Duboscq and Tuzet 1937; Lufty 1957; Lanna and Klautau 2012). This structure is formed from parent choanocytes that gradually spread around the embryo (Gallissian 1983; Gallissian and Vacelet 1992; Lanna and Klautau 2012).

2.4.4.2 Male

In Hexactinellida, Demospongiae, and Homoscleromorpha, spermatogenesis proceeds in spermatocysts – temporary spherical structures bounded by flattened somatic cells diffusely distributed in the endosome (Figure 2.10b). Calcaronea have no spermatocysts (Boury-Esnault and Jamieson 1999; Maldonado and Riesgo 2008; Ereskovsky 2010, 2018). The spermatocysts are surrounded by the follicle cells derived from the transformation of pinacocytes or archaeocytes. In demosponges, cell junctions between the follicle cells are simple (apposition) with no detectable membrane specialization (Riesgo et al. 2008). The follicular cells of the spermatocysts of Homoscleromorpha possess specialized cell junctions and basement membrane (Ereskovsky 2010; Riesgo et al. 2007). Development of male gametes within a cyst is usually synchronous. In Homoscleromorpha, there is a gradient of male gamete maturation within a cyst, a feature they have in common with the Eumetazoa (Gaino et al. 1986; Riesgo et al. 2007; Ereskovsky 2010).

2.4.4.3 Reproduction and Tissue

During sexual reproduction, sponge tissue and the elements of the aquiferous system may be completely or partially destroyed, depending on the intensity of gametogenesis and embryogenesis. It occurs in both oviparous and viviparous sponges, gonochoric and hermaphroditic species. This period is marked by the complete disorder of central and basal parts of the choanosome. Normal tissue organization persists only in the narrow marginal zone of the sponge. For example, in *Halichondria panicea* and *H. dujardinii* after intensive gametogenesis and embryogenesis, the endosome transforms almost completely into a "gonad" filled with brood chambers containing larvae (Figure 2.12a,b) (Barthel 1986; Witte and Barthel 1994; Ereskovsky 2000; Gerasimova and Ereskovsky 2007). Slow postreproduction rehabilitation of parental sponge tissue continues after the end of reproduction (Figure 2.12c).

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Abbreviations for Figures

ap apopyle
aph aphodus
as aragonitic skeleton
at atrium
b bacteria
bm basal membrane
cc choanocyte chamber
cf collagen fibrils
ch choanocytes
cht choanocyte tube
co cortex
cs calcareous skeleton

cst cellular strands
cu cuticle
dm dermal membrane
ec ectosome
em embryos
en endopinacocytes
end endosome
ev extracellular vacuole for spicule synthesis
ex exopinacocytes
exc exhalant canal
fm foreign material
fo follicle
gc Golgi complex
gr glycogen rosettes
hyp hypophare
inc inhalant canal
la larva
m mesohyl
mt mitochondria
n nucleus
o osculum
of organic fibers
oo oocyte
os ostia
pes perispicular spongin
pm placental membrane
po porocyte
pr prosopyle
poc porocalyx
sc sclerocyte
si special inclusions
sp spicules
sph spherulous cell
spc spermatocyst
sub subdermal cavity
tr trophocytes
tt trabecular tract
vc vacuolar cells

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3

Cnidaria

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3.1 Introduction

The phylum Cnidaria contains an estimated 11 000+ living species (World Register of Marine Species 2018) and is almost exclusively an aquatic group, primarily marine but with some freshwater species and a few terrestrial parasitic species (Atkinson et al. 2018). There are two distinct body forms: the polyp, which is often sessile, and the medusa, which is normally free-floating (Figure 3.1). Familiar groups include corals, sea fans, jellyfish (sea jellies), sea anemones, and the common laboratory hydra. The name cnidarian means "stinging creatures." Cells called cnidocytes – stinging cells – are unique to this phylum.

The phylum is divided into multiple classes. The four main classes are the Anthozoa (sea anemones, hard or stony corals, and soft corals such as sea fans or sea whips), Scyphozoa (jellyfish or sea jellies), Hydrozoa (hydra and fire corals), and Cubozoa (box jellies). Newer classes have been acknowledged including Staurozoa (stalked jellyfish) and, within the intracellular parasitic subphylum Myxozoa, the classes Myxosporea and Malacosporea (Fiala et al. 2015). Older classifications combined cnidarians with ctenophores (comb jellies) in the phylum Coelenterata, but currently they are recognized as distinct phyla.

Life expectancy in cnidarians is often difficult to ascertain. Some colonial corals have been estimated to exceed 4200 years (Devlin-Durante et al. 2016; Roark et al. 2009). Medusae of many species are fast growing, mature within a few months, and die after breeding. They are prone to damage from wave action, currents, and predators. There are anecdotal reports of individual sea anemones and jellies living up to 50 years.

Cnidarians reproduce both sexually and asexually, most species incorporating both methods. They produce gametes (eggs and sperm), can be monoecious, producing both eggs and sperm, or dioecious, with individuals of separate sexes (gonochoric). Monoecious species are also referred to as hermaphroditic, although they are usually not self-fertilizing even if they are simultaneous hermaphrodites; other species can be sequential hermaphrodites, switching the type of gametes produced. Gametes are usually released into the water column, known as spawning, though some species brood fertilized gametes within the body cavity or within external structures on the body wall. Spawning depends on various environmental conditions such as water temperature and light, and often occurs at specific times of the year. Fertilized eggs develop into planulae, larvae that can swim or crawl using cilia and eventually attach to a substrate. The attached end becomes the aboral end of a polyp.

In many Scyphozoan species, the polyp absorbs the tentacles and begins to split horizontally into a series of discs that become juvenile medusae (ephyrae). This process is known as strobilation. Cubozoan polyps undergo complete metamorphosis (do not undergo strobilation), and each polyp transforms into a single medusa. Hydrozoa have a variety of life cycles, some with no polyps and some with no medusae. Anthozoans have no medusa stage, and polyps are responsible for sexual reproduction. Cnidarians can also reproduce asexually by various means. New polyps can bud off from parent polyps or split in half to expand or begin new colonies. These polyps are genetically identical to each other, or clones. Some hydrozoan medusae can also divide in half. Many cnidarians (in particular the stony corals) can be fragmented, where a portion of a colony is broken or separated off from the main colony (parent colony),

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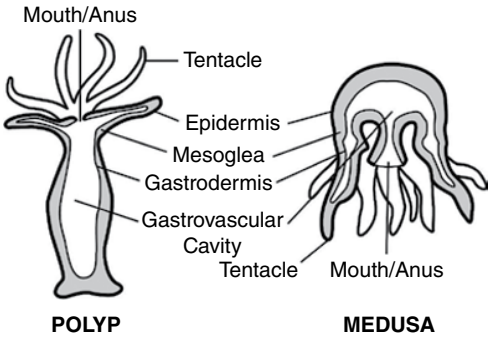


Figure 3.1 Cnidarian body forms: polyp and medusa. *Source:* Kevin Seline, artist.

to form a new colony. This technique is being used in many restoration efforts (Berzins et al. 2007, 2011).

Cnidarians are considered carnivorous animals. Smaller species feed on plankton, whereas larger forms can capture and ingest larger prey items, such as fish, worms, and crustaceans (Meredith et al. 2016; Milisenda et al. 2018; Sullivan et al. 1994). Certain groups, in particular most of the reef-building or stony corals, have a symbiotic relationship with single-celled dinoflagellates known as zooxanthellae (mostly in the genus *Symbiodinium*) (LaJeunesse et al. 2018). The algal cells undergo photosynthesis and exchange nutrients and waste molecules with the cnidarian host cells. They also often provide color to the host and can be expelled from the host cell or die within the host cell in reaction to various environmental stressors (e.g., temperature, light, pH, and salinity changes), resulting in a loss of color, often referred to as coral bleaching. The coral can be recolonized by other subtypes or clades of dinoflagellates that may be better adapted to the new environment but the coral may die before that can happen.

From a human point of view, the most noticeable aspect of cnidarians involves coral reefs. Reefs are located around the world in shallow, deep, warm, and cold waters. They are often referred to as the "rainforests" of the ocean. They contain some of the most biodiverse and oldest ecosystems on earth, providing home to more than 25% of all marine life, including one-third of all marine species, yet comprise less than 1% of the earth's surface (www.coralreef.gov). Millions of people and thousands of communities in more than 100 countries depend on reefs for food, protection (barrier reefs are natural storm barriers), and jobs, which generate billions of dollars of resources and services (for food, tourism, shelter). Cnidarians are also an important part of food webs, being both predators as well as prey. For example, as predators, they can have a significant impact on zooplankton and larval populations (Crum et al. 2014; Meredith et al. 2016; Purcell and Arai 2001); flamingo tongue snails graze on sea fans; parrot fish scrape up stony coral polyps including some of the supporting exoskeleton (calcium carbonate particles later excreted contribute to sandy beaches); and medusoid forms are often ingested by other jellies, sea turtles, and fishes.

New discoveries in biochemical and biomechanical properties of cnidarians are leading to new tools for our medicine cabinets. Bone graft materials from stony coralline species, due to chemical and structural characteristics similar to those of human cancellous bone, have been investigated (Pountos and Giannoudis 2016). Some cnidarians possess bioluminescence or fluorescent proteins that are used as gene markers (Prasher 1995) and their mucus contains antibiotic compounds (Ritchie 2006). From an adverse perspective, while most nematocysts cannot penetrate human skin, there are some species of cnidarians whose nematocysts can be large with long skin-penetrating tubules and whose toxins/venoms can pose serious threats (Bentlage et al. 2010). However, scientists are looking into puncture mechanics of nematocysts that can be utilized to deliver therapeutics in a wearable drug delivery patch (Oppegard et al. 2009).

Threats to cnidarians include climate change, infectious diseases, development of shorelines, increase in surface runoff, land-based sources of pollution, ship groundings, and damaging fishing techniques (dynamite, nets, etc.). As a possible indicator of some of these detrimental changes, there has been an increase in the frequency of jelly blooms (Purcell 2012). Changes in currents, nutrients, light, and temperature from agricultural or urban runoff can increase the growth of the plankton on which sea jellies feed. Jellies can also tolerate the low oxygen levels caused by eutrophication, itself a result of pollution by runoff; however, their predators may not survive these conditions. As another example, the underlying support system of a coral reef is a calcium carbonate structure that accretes slowly over many years. Ocean acidification poses a threat by altering the concentration of carbonate ions required to maintain this process (Albright et al. 2018).

3.2 Gross Anatomy

3.2.1 General Characteristics

Cnidarians are soft-bodied, diploblastic (two cell layers) metazoans (animal kingdom), with primary radial symmetry (although with some variations). The two adult cellular layers, the epidermis and gastrodermis, are separated by a nonliving gelatinous layer, the mesoglea, which ranges from a thin sheet to a thick, mucoid ("jelly-like") material. They are basically sac-like organisms, with a single opening leading into a body cavity, the gastrovascular cavity, and can have one of two basic body types, polypoid or medusoid (Figure 3.1).

The single orifice functions as both a mouth and an anus. The polyp is normally a sessile form and the main body is a tubular or cylindrical column with the oral end, or oral disc, directed upward. The oral opening, or mouth, is surrounded by tentacles, which are extensions of the body wall. The opposite side, the aboral end, is usually attached to solid surfaces (although some species can burrow into soft sediment and in other species, polyps are surrounded by connective tissue or exoskeleton) and is often referred to as the base or basal plate. The medusa is usually a free-swimming "umbrella" or bell-shaped form, with the convex side (exumbrella) directed upward. The mouth is located in the center of the concave undersurface (subumbrella) and the tentacles hang down from the margin of the bell. Some cnidarians pass through both forms in their life cycle, while others exhibit only the polyp or the medusa form. Cnidarians can be found in colonies or exist as individuals and exhibit a wide range of sizes – microscopic to massive reef formations to medusae measuring several meters in diameter and with tentacles 20+ meters long (Brusca et al. 2016).

The gastrovascular cavity is often subdivided by mesenteries, infoldings of the mesoglea and gastrodermal layers. Mesenteries provide structural support and increase the surface area of the gastrodermis. In many species, mesenterial or gastric filaments are located on free margins of mesenteries, and the cnidoglandular band epithelium of the filament is armed with abundant numbers of nematocysts and cells producing enzymes for digesting prey. Gonadal tissue is usually located within the mesenteric mesogleal regions of polyps or in the gastric space of medusae.

In medusae, the mesoglea is the main supporting structure, whereas in polyps, water in the gastrovascular space can act as a hydrostatic skeleton. Epitheliomuscular cells are cuboidal to columnar to flattened cells (depending on the species) that span the thickness of the epithelium, with their bases concentrated into attachment sites (myonemes) along the mesogleal pleats (Leclère and Röttinger 2017). The musculature is mostly diffuse but there are distinct tracts of muscles such as the circular bands in mouths of anemones (acting as a sphincter muscle) or along bell margins in medusae (facilitating water movement for locomotion).

There are no discrete respiratory, excretory, or circulatory structures. Nutrient uptake in cnidarians can occur by endocytosis of particulate food, absorption of dissolved organic compounds or utilization of organic compounds leaked from the symbiotic dinoflagellates. Respiration and excretion occur by direct exchange through cell membranes using passive and active transport processes. There is no centralized nervous system, only a diffuse network of nerve cells, the nerve net. Some species have ocelli that are light-sensitive structures (Martin 2002), statocysts for detection of gravity and orientation, and primitive chemosensory pits. The cnidarians' immune system is also very basic. They do not have an acquired immune system but several innate mechanisms provide protection. The mucus contains bactericidal and other antimicrobial activity, colonial organisms can recognize self from nonself, and most (if not all) possess amoebocytes, pleomorphic cells with phagocytic and biochemical properties that function as the principal cell of the innate immune system in cnidarians (Bosch and Rosenstiel 2016; Ocampo and Cadavid 2014; Ritchie 2006).

3.2.1.1 Anthozoan Specifics

Anthozoans are exclusively marine, do not have a medusa stage, and can be solitary or colonial. The subclass Hexacorallia contains the anemones and hard or stony corals (order Scleractinia) and the subclass Octocorallia contains the octocorals, soft corals and gorgonians such as sea fans and sea pens. In the Hexacorallia, the mesenteries are usually paired and in multiples of six, whereas in octocorals there are just eight pinnate tentacles (with side-branches or lateral outfoldings) and eight complete mesenteries.

In colonial stony corals (scleractinians) and the octocorals (all octocorals are colonial), the polyps are connected and the organisms secrete several different types of support structures. The stony corals secrete an aragonite (calcium carbonate) exoskeleton. The entire skeleton is known as the corallum and develops into many different growth forms depending on species of coral – including massive, laminar, branching, and encrusting (Peters 2016). These large exoskeletons form the familiar coral reefs. Figures 3.2 and 3.3 help provide a visual guide to the following anatomic descriptions of a scleractinian coral.

The polyps occupy only the surface of the corallum. The size of the corallum expands in size as the polyps increase in number by budding. Each polyp sits in a calcium carbonate cup known as a corallite. The wall of the corallite is the theca and the floor is the basal plate. As the overall complex increases in size, the bottoms of the corallites are sealed off by transverse calcareous partitions called dissepiments, each becoming the new basal plate for the polyp. Spacing between dissepiments varies. Some species have very narrow spaces with dense partitions, often in the slower growing corals. In faster growing, often branching species, the spaces are large with thinner partitions, and can fragment very easily. The spaces are often colonized by other organisms (primarily fungi and protozoa) (Marcelino et al. 2018). Little is known about these communities and whether they affect the overlying coral tissues. Calcareous partitions, septae, radiate from the theca and basal plate toward the center and provide support to the mesenteries. The size and shape of these calcareous septae are distinctive for each species and are often used in species identification.

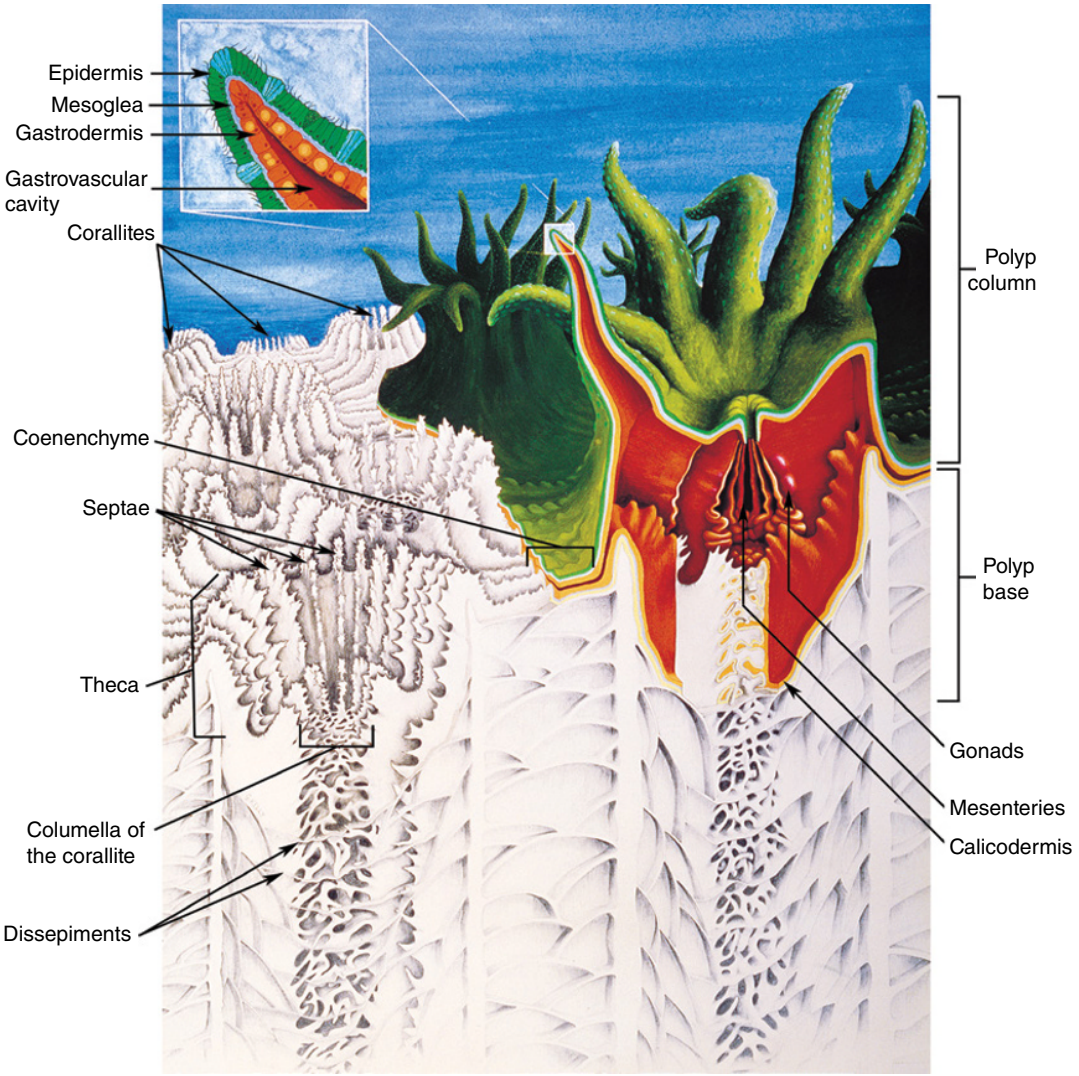
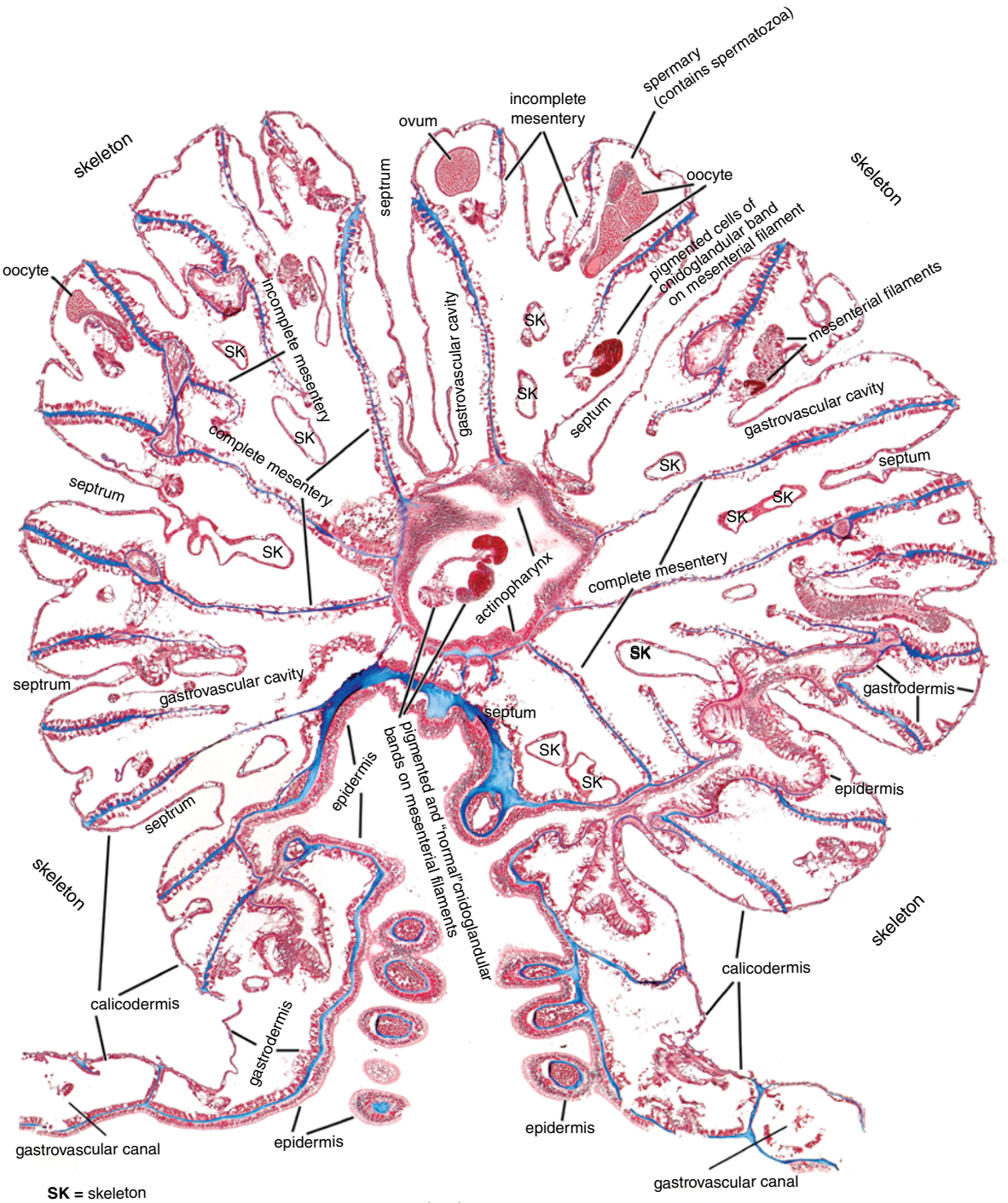


Figure 3.2 Gross anatomy of a scleractinian polyp from an imperforate coral. *Source:* Reprinted with permission from *Corals of the World* (Veron 2000). Painting by Geoff Kelly.

The part of the polyp that can extend above the theca is the column, and portion below the surface, sitting in the cup, is the polyp base. The skeleton between the polyps is known as the coenosteum. The polyp epithelium lining the cup assists in the building of the calcified exoskeleton and is known as the calicodermis. The tissue connecting the polyps is known as the coenenchyme and consists of two full sets of tissue layers, separated by gastrovascular canals. In imperforate corals (usually the dense, slower growing corals such as *Orbicella* spp.), this network extends over the surface of the skeleton. In perforate corals, the connecting tissue is both on the surface and embedded within the skeleton, with canals piercing through the walls of the corallites and surrounding skeleton (e.g., *Acropora* spp., *Porites* spp.). The lumens of the gastrovascular cavities are connected via the canals to adjacent polyps and fluid, food, molecules, and disease agents can be spread to adjacent polyps through these tubes.

Octocorals do not secrete the large exoskeleton of the stony corals but most contain small, often microscopic calcium carbonate pieces called spicules or sclerites embedded in the coenenchyme (Figures 3.4 and 3.5). Scleroblasts secrete these minute structures into various shapes, sizes, and colors giving the octocorals their texture, and at times color, and protect the tissue from predators. They often are used in species identification. Corticocytes, also in the coenenchyme, form an axis epithelium to secrete a flexible or stiff internal axial rod as a supportive base. These rods are composed of an organic material, a protein-mucopolysaccharide complex, called gorgonin. Desmocytes, interspersed with corticocytes (Figure 3.29), anchor the tissue to the gorgonin. Around the rod is a layer of coenenchyme of variable thickness



SK = skeleton
complete mesentery = mesentery attaches to the actinopharynx
incomplete mesentery = mesentery does not attach to the actinopharynx, free edge has mesenterial filament along its margin.
The mesenterial filaments of this species have red-pigmented regions (which show up bright red here) in addition to the "normal" cnidoglandular bands (contain nematocysts and granular gland cells).

Annotation by Esther Peters Histological preparation by the students of the MUSC Histotechnology Program

Figure 3.3 Overview of key structures in a polyp from the scleractinian coral *Orbicella faveolata*. SK, skeleton. *Source:* Reprinted with permission from NOAA Coral Disease and Health Consortium (Galloway et al. 2007). Section prepared and stained by the Medical University of South Carolina's Histotechnology Program 2007 students. Photomicrograph prepared by James H. Nicholson.

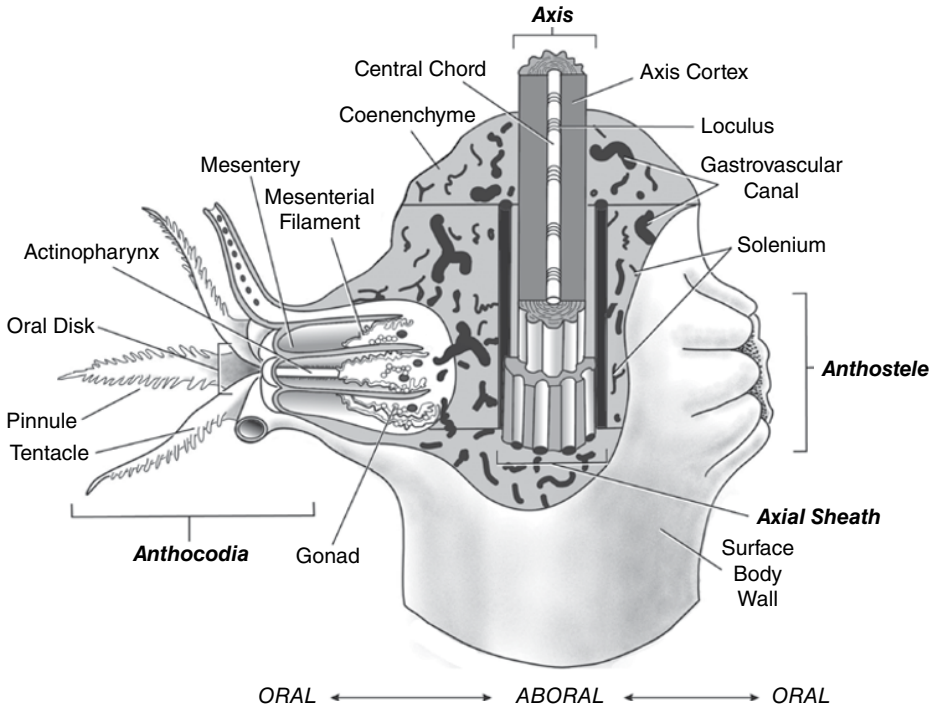


Figure 3.4 Gross anatomy of an octocoral (gorgonian polyp). *Source:* Reprinted with permission from NOAA Coral Disease and Health Consortium (Galloway et al. 2007). Adapted from an illustration in Bayer et al. (1983). Jennifer Clark, artist.

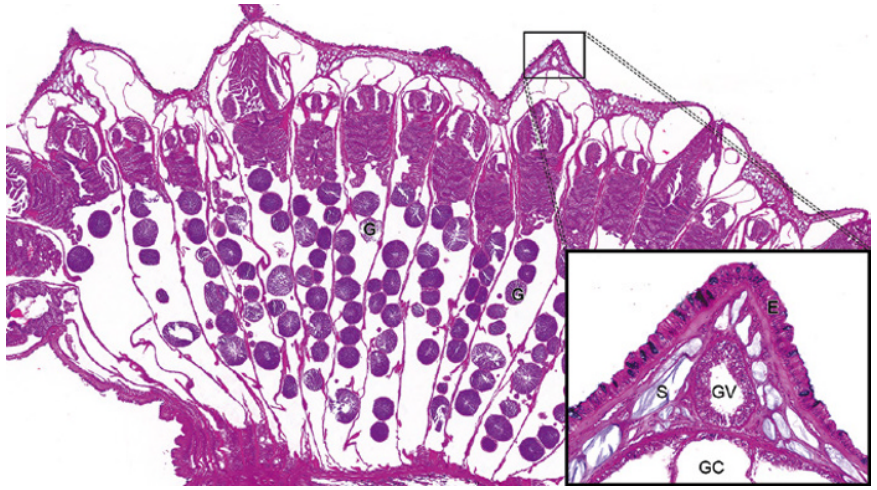


Figure 3.5 Subgross photomicrograph of a sea pen (order Pennatulacea). The epidermis (inset, E) is subtended by coenenchyme-containing amoebocytes (not visible from this magnification) and sclerites (inset, S). Vertically oriented mesenteries extend down from the superficial coenenchyme mesoglea and contain mesenteric filaments and gonads (G; sperm). A gastrovascular canal (inset, GV) is lined by gastrodermis. Larger gastrovascular spaces are gastrovascular cavity (inset, GC). 200× (inset 1000×). HE.

connecting polyps and perforated by gastrodermal epithelium-lined tubes (gastrovascular canals and smaller tubes, solenia.) which are continuous with the gastrovacular cavities of other polyps. The portion of the polyp wall adjacent to the coenenchyme is often thickened and reinforced with sclerites and is known as the anthostele.

3.2.1.2 Scyphozoan Specifics

The predominant body form of scyphozoans is the medusa and species in this group are commonly referred to as jellyfish or sea jellies. The bell of the medusa is typically a thick mesogleal layer and differences in the bell margin often differentiate species. Tentacles may be present or absent and

the margin is often scalloped or lobed. Sensory centers are situated on club-shaped structures called rhopalia found along the bell margin in the notches between lobes, and can contain chemosensory pits, statocysts, and ocelli (photosensitive structures of differing complexity). The mouth is in the center of the undersurface of the bell and may be suspended on a tubular extension called the manubrium. Portions of the mouth edge may be drawn into four or eight elongate structures known as oral arms, which aid in the capture of prey. The gastrovascular cavity is divided by four mesenteries (referred to as "septa" in some sources) into four gastric pouches or canals, resulting in a tetramerous or quadriradial symmetry. Most cnidae are located in tentacles and oral arms but can also occur in all types of epithelial tissues, including epidermis and gastrodermis (Ruppert et al. 2003). Gametes arise along the mesenteries or on folds of the gastrodermis. Gametes can sometimes be observed grossly as four horseshoe-shaped organs suspended in the bell, with the "open" side of the horseshoe facing the oral cavity; this is most obvious in *Aurelia aurita*.

3.2.1.3 Cubozoan Specifics

The Cubozoa have cube-shaped medusae and include sea wasps and box jellies. The venom from their nematocysts is very toxic and, in some cases, can be fatal to humans. From each corner, short stalks called pedalia bear one or more tentacles. The margin of the bell is folded inward to form the velum (velarium). This structure restricts the aperture of the bell, helping to create a more powerful flow of water when the bell contracts. On the underside of the bell, in the center, is the mouth on an elongate structure, the manubrium. The mouth leads into the gastrovascular cavity which is partitioned by mesenteries into four gastric pockets (again with tetramerous or quadriradial symmetry) extending to the tentacles through canals. Along the bell margin, between the pedalia, are sensory structures, rhopalia. Cubozoans are unique because of the possession of complex eyes (Coates 2003). In each rhopalium are two complex eyes with a lens, retina, and cornea. Statocysts and ocelli are also present.

3.2.1.4 Hydrozoan Specifics

In most hydrozoan species, the polyp is the predominant body form. The medusa stage is often small, and a few species never go through a medusoid stage. The medusa is the sexually reproducing stage in most hydrozoans. Medusae form by budding from polyps and have a velum inside the bell of the medusa. Around the margin is a ring of tentacles. Inside the umbrella, the mouth can be supported by an elongate tubular manubrium. Gametes form on the sides of the manubrium. Some forms, such as *Hydra* spp., exist as solitary polyps but most hydrozoans are colonial. The initial polyp produces new polyps by budding and develops a network of interconnected tubes, called stolons. The lumen of the stolon is connected with that of the gastrovacular cavities of the polyps (Buss et al. 2013). In some species, the polyps secrete calcareous coatings, such as in the fire coral, *Millepora* spp. This exoskeleton, called a coenosteum, is perforated by pores for the polyps. Some hydrozoans have developed pelagic colonies (siphonophores) made up of interconnected polyps and medusoids forming polymorphic structures with different functions called zooids. These pelagic forms are often confused with the medusae of scyphozoans. The "Portuguese man-of-war" is an example of a pelagic colonial hydrozoan.

3.2.2 Keys for Dissection/Processing for Histology

Collecting specimens from the field should include gathering as much information as possible such as environmental conditions, condition of tissues, and gross photographs. Thorough protocols have been developed for obtaining stony coral samples (Price and Peters 2018). Consult papers that have used histology in studies on other cnidarians to develop the best procedure to use.

Multiple options for fixation have been used with cnidarians; most commonly these include: (i) a 10% formalin-seawater fixative, (ii) Helly's Modified Fixative, (iii) Bouin's, and (iv) Z-Fix Concentrate®, a zinc formaldehyde solution that must be diluted with ambient sea water (see Appendix 3.1 at the end of this chapter). Routine sea water-formalin is an easy fixative but provides less cellular detail because it does not fix the membranes well. Helly's is very good for preserving cellular structure and intracytoplasmic granules but may cause some shrinkage. Bouin's is excellent for preserving cellular structure, but the sample should remain in the Bouin's for only a few hours or the tissue will become hardened; the excess fixative must be removed by multiple changes of 70% ethanol. Z-Fix Concentrate diluted with sea water provides better detail than regular formalin and specimens can remain in this fixative indefinitely. The diluting sea water should be of the same or nearly same salinity as the water from which the cnidarians were collected, because they do not osmoregulate.

The size or thickness of the specimen must be thin so the fixative will penetrate it easily, and 10–20 times the volume of fixative per volume of tissue is required. Jellyfish, anemones, and large fleshy corals need to be trimmed into slices no thicker than 5 mm. Most specimens will retract their polyps or tentacles when handled. They can be anesthetized with a magnesium sulfate solution (see Appendix 3.1) to help relax tissues. Using the technique of agarose preembedding (termed "enrobing") prior to decalcification of the exoskeleton from

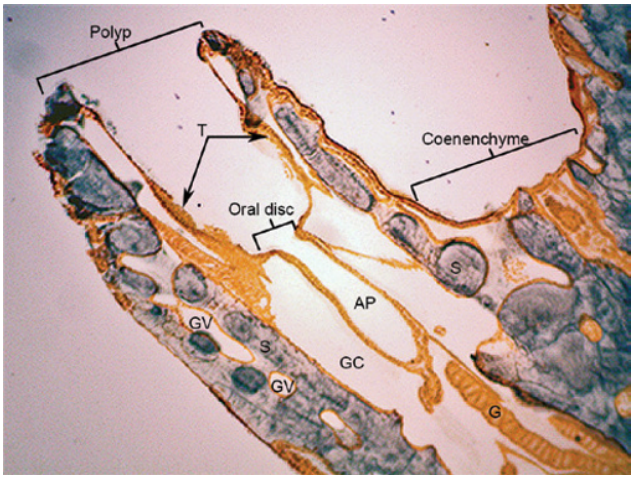


Figure 3.6 Undecalcified *Acropora cervicornis* section prepared using resin embedding and petrographic thin section techniques (Price and Peters 2018) with the blue-gray aragonite crystals of the skeleton (S) still present. The yellow-orange staining areas are the coenenchyme tissue and one coral polyp is contracted into its corallite, showing tentacles (T), actinopharynx (AP), gonad (G), gastrovascular cavity (GC), and gastrovascular canal (GV). 100×. *Source:* Photo credit: Kathy L. Price.

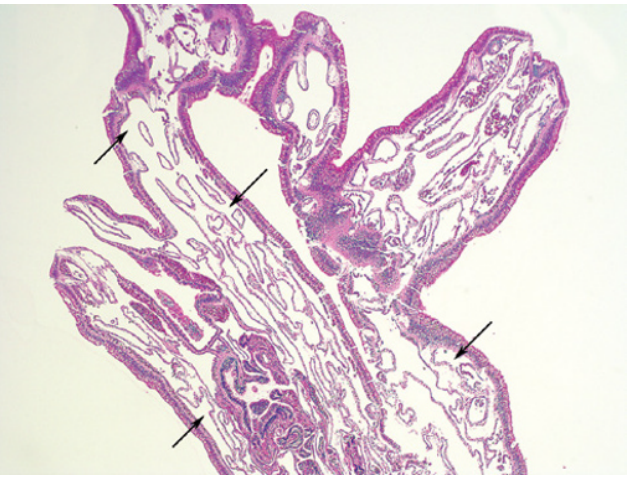


Figure 3.7 *Acropora cervicornis* tissue section with aragonite skeleton dissolved (arrows pointing to some of the spaces where the skeleton used to be present). 40×. HE.

scleractinian corals and sclerites from octocorals is recommended for preserving spatial orientation and overall ease of sectioning (Bythell et al. 2002) (see Appendix 3.2). Sections can be cut without decalcification with a lapidary trim saw (Figure 3.6). After fixation and decalcification (if needed), tissue samples must be rinsed to remove excess solutions, trimmed to fit into tissue-processing cassettes (no thicker than 3 mm), and processed to embed the sections in paraffin or plastic (e.g., JB-4 methylmethacrylate, epoxy, other resins), depending on the microtomy equipment available for sectioning the resulting tissue blocks. The sections are then mounted on glass microscope slides, dried well, and stained (Figure 3.7).

Hematoxylin and eosin (HE) stain is generally used for routine identification of tissues. Nuclei will stain blue with hematoxylin, mucus in mucocytes will stain pale blue/purple, and proteins, especially those in the mesoglea, granular gland cells, and myonemes, stain pink with eosin. Special stains such as Masson's trichrome can distinguish collagen in the mesoglea (blue) and the contractile myonemes (red). Pentachrome stains are useful for observing changes in the mucopolysaccharides of the mucocytes. Alcian blue (AB) and periodic acid–Schiff reagent (PAS) will stain carbohydrates and using them together can distinguish between neutral mucins and acidic mucins. A thorough discussion of staining techniques can be found in Price and Peters (2018).

3.3 Histology

Although similarities and differences among the four major groups of the phylum Cnidaria were introduced, most images in this section are from members of the class Anthozoa, subclass Hexacorallia, order Scleractinia (stony corals). This reflects their recent use in restoration efforts (Berzins et al. 2007, 2011) and in long-term monitoring programs, as indicators of environmental stress and in disease evaluation (Bythell et al. 2002; Peters 2016). Several excellent references on anatomy and histology are available including Fautin and Mariscal (1991), Galloway et al. (2007), Lesh-Laurie and Suchy (1991), Peters (2016), and Thomas and Edwards (1991). Other papers have been published on particular cnidarian taxa, including octocorallia (Bayer et al. 1983; Fabricius and Alderslade 2001; Goldberg 1976; Moore et al. 2016), corallimorpharia (Fautin 2016), and cerianthid anemones (Peters and Yevich 1989). Table 3.1 provides a list of the tissue types evaluated histologically in cnidarians.

3.3.1 Epithelium

The outer cell layer in adult cnidarians has been referred to as ectoderm or ectodermis and the inner layer as endoderm or endodermis. However, in recent discussions (Peters 2016) the terms *epidermis* and *gastrodermis* refer to adult epithelia that are derived from embryonic ectoderm and endoderm, respectively. The simple embryonic ectoderm and endoderm layers differentiate into different cell types whose exact origins often are still being investigated. The epidermis (ectoderm) and gastrodermis (endoderm) are separated by a nonliving gelatinous layer, the mesoglea (derived primarily

Table 3.1 Tissues, structures, and cells for histologic evaluation in cnidarians.

Tissue type/system	Tissue name	Structure/cell name, type
Epithelium/ Integument	Epidermis (embryonic ectoderm)	Simple columnar epithelium/pseudostratified columnar
		Ciliated or flagellated columnar cell (also known as supporting cell or collar cell; can be difficult to appreciate under light microscopy)
		Mucocyte
		Cnidocyte (nematocyst, spirocyst, ptychocyst)
		Granular cell (pigment, glandular)
Epithelium/ Exoskeleton	Calicodermis (Scleractinia)	Calicoblast (squamous to columnar, pleomorphic; secretes organic matrix which assists in the deposition of aragonite crystals)
		Desmocyte
		Mucocyte
	Axis (Alcyonacea)	Corticocyte (squamous to columnar; secrete gorgonin or antipathin)
		Desmocyte
Epithelium/Digestive	Gastrodermis	Body wall
		Mesentery
		Mesenterial filament/cnidoglandular band
		Cuboidal supporting cell (+/- symbiotic dinoflagellates [zooxanthellae] in vacuoles)
		Cuboidal to columnar absorptive/storage cell
		Mucocyte
		Cnidocyte (nematocyst/spirocyst)
		Ciliated columnar supporting cell (difficult to appreciate on light microscopy)
		Collar cell (difficult to appreciate on light microscopy)
		Granular cell (pigment and glandular)
Connective	Mesoglea	Collagen matrix
		Fibroblast (difficult to appreciate on light microscopy)
		Mesogleal pleat
Muscle		Epitheliomuscular cell/myoneme
Nervous	Nerve Net	Neurons (difficult to appreciate on light microscopy), ocelli
Immune		Amoebocyte
		Interstitial cell[a]
Reproductive	Gonad	Ova
		Spermatozoa (in spermaries)

[a] These are considered to be multipotential cells that can migrate through the mesoglea and between epithelial cells and differentiate into other cell types (i.e., epithelial, amoebocyte).
Note: Not all cell types are found in all species and variations occur.

from the ectoderm), which ranges from a thin sheet to a thick, mucoid, gelatinous material (Figures 3.8–3.14).

In anthozoans, at the oral end of the polyp, tentacles surround a relatively flat oral disc. In many scleractinian corals, the tips of the tentacles have a bulbous structure, the acrosphere, which has a concentrated number of cnidocytes (containing nematocysts or spirocysts) used both in prey capture and defense (Figures 3.20–3.22). The tentacle-bearing portion of the octocoral polyp is called the anthocodium. Octocorals typically lack nematocysts and spirocysts on the tentacles. The polyp may possess an operculum made up of eight calcareous scales that can cover withdrawn tentacles. Polyps of some octocoral species can be dimorphic. Larger, normally developed polyps are autozooids, and smaller, less well-developed polyps are siphonozooids (not to be confused with hydrozoan siphonophores, described later).

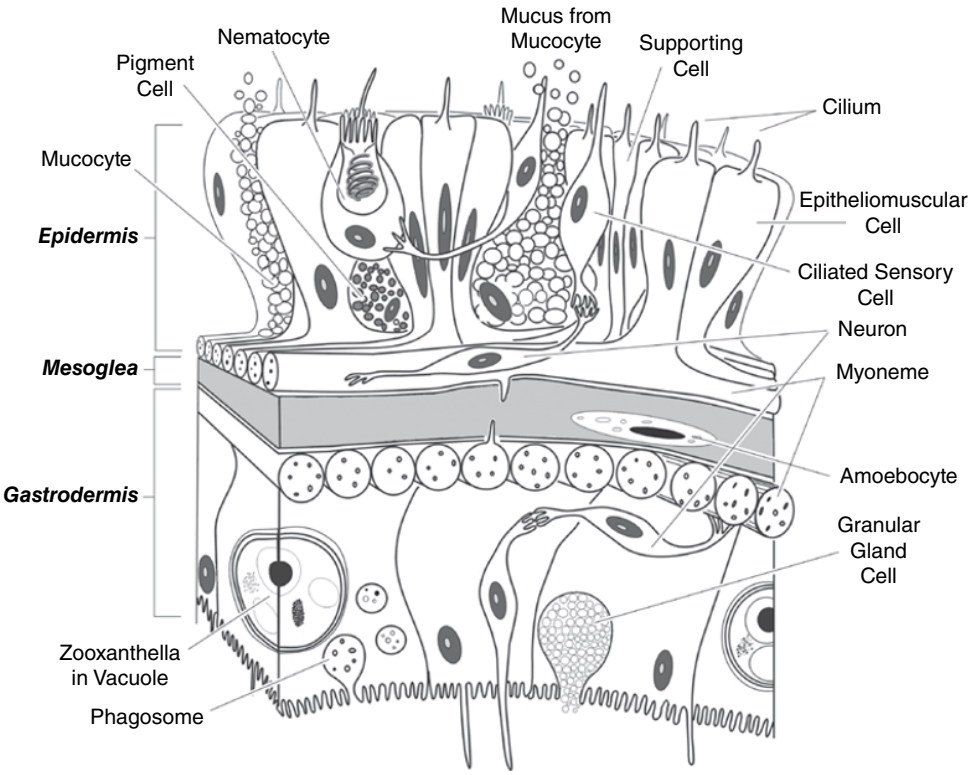


Figure 3.8 Diagram showing the surface body wall of a scleractinian polyp, showing cell types of the epidermis, mesoglea, and gastrodermis. *Source:* Reprinted with permission from NOAA Coral Disease and Health Consortium (Galloway et al. 2007). Jennifer Clark, artist.

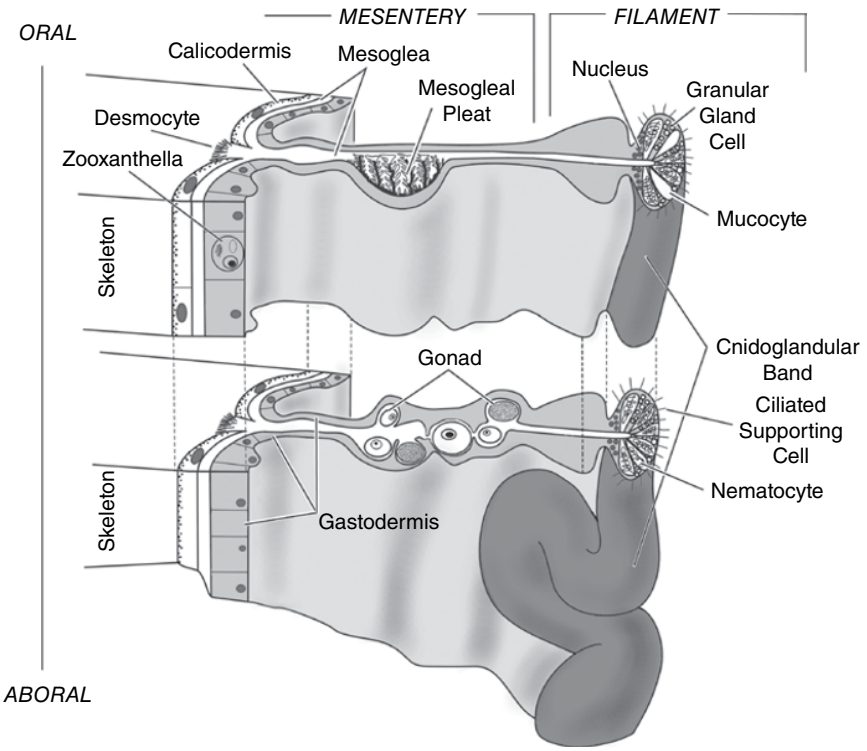


Figure 3.9 Diagram of the key features of a scleractinian polyp mesentery. Features often change, moving from oral to aboral regions. *Source:* Reprinted with permission from NOAA Coral Disease and Health Consortium (Galloway et al. 2007). Adapted Fautin and Mariscal 1991. Jennifer Clark, artist.

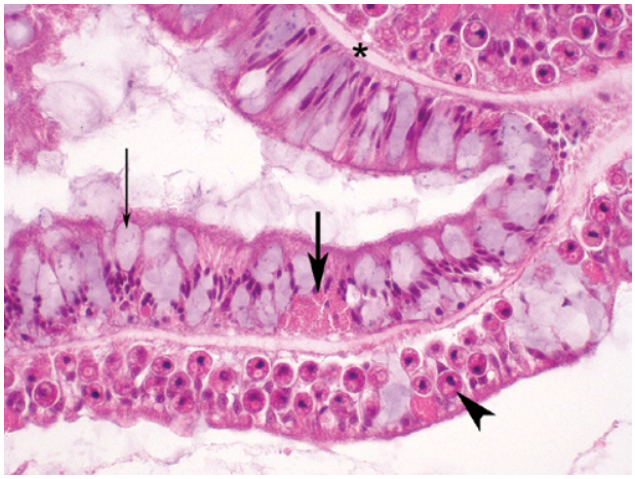


Figure 3.10 Body wall from a scleractinian coral, *Orbicella annularis*. The surface of the coral in contact with sea water is covered by a simple columnar or pseudostratified columnar ciliated epithelium which may contain scattered columnar epitheliomuscular cells (difficult to differentiate with HE stain), and plump mucocytes (*thin arrow*). An eosinophilic granular cell containing fluorescent protein pigment is visible in the midsection of the epithelial band (*thick arrow*). A narrow band of mesoglea (*asterisk*) separates the epidermis from the gastrodermal cells, which have numerous *Symbiodinium* in vacuoles (*arrowhead*). 400×. HE.

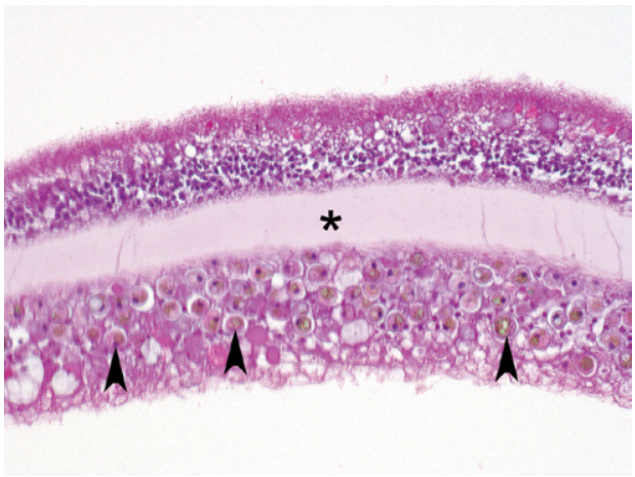


Figure 3.11 Body wall from a scleractinian coral, *Orbicella cavernosa*. The mesogleal layer (*asterisk*) is much thicker in this species. Zooxanthellae in the gastrodermis are highlighted with arrowheads. HE. 400×.



Figure 3.12 Body wall from a branching perforate scleractinian coral, *Acropora cervicornis*, showing the ciliated columnar epidermal cells with cnidocytes containing developing or mature spirocysts (*thick arrow*), a scant mesogleal layer (*thin arrows*) and the gastrodermis containing numerous zooxanthellae (*arrowhead*). 1000×. HE.

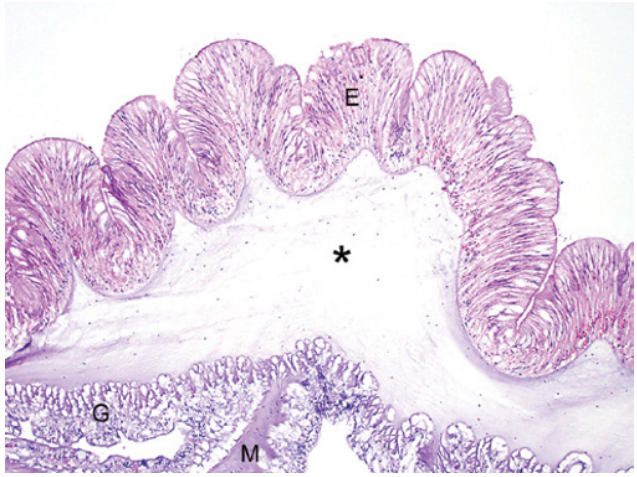


Figure 3.13 Body wall from an aggregating anemone (*Anthopleura* sp.) showing the epidermis (E) that is composed of elongate cells on a basement membrane. Subjacent to the epidermis, the mesoglea (*asterisk*) is a loose collagenous tissue. At the bottom of the image, part of the gastrodermis (G) and a mesentery (M) can be seen. 200×. HE.

In anthozoans, from the mouth, a short, muscular tube, the actinopharynx, leads into the gastrovascular cavity. The mouth opening can be circular, oval, smooth or ridged. The tissue next to the mouth sometimes forms a raised rim around it, known as the peristome. In some species (although scleractinian polyps lack this) there are one to several grooves along the length of the actinopharynx known as siphonoglyphs, which are lined by epithelial cells bearing elongate cilia to assist with moving water and food into the gastrovascular space and waste material out. When siphonoglyphs are present, the animal is classified as having biradial or bilateral symmetry. Tissue partitions, the mesenteries (Figure 3.9 and section 3.3.1.4.3), extend from the body wall into the middle of the sac with gastrodermal tissue on either side of a mesogleal layer. Gastrodermal cells often contain cytoplasmic vacuoles that harbor single-celled symbiotic

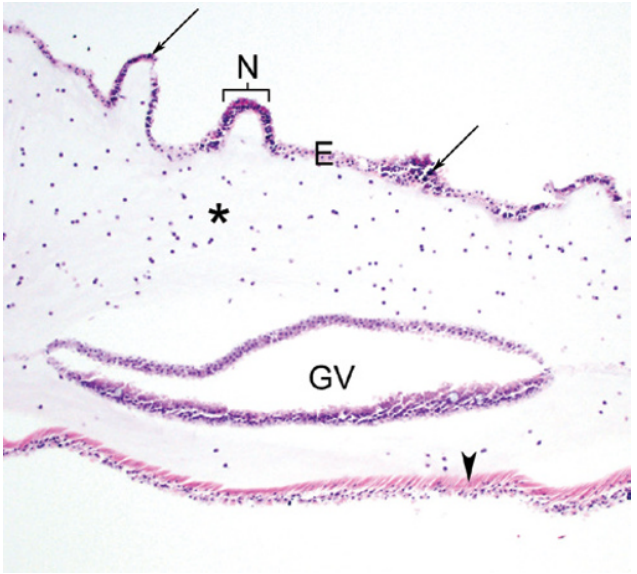


Figure 3.14 Cross-section of a moon jelly (*Aurelia aurita*). The epithelial layer at the top of the image represents the exumbrella, which is composed of a simple epidermis (E). The epidermis contains rosettes of nematocysts (N) and occasional pigment cells (*arrows*). The epithelial layer at the bottom of the image is the subumbrella, which is bilayered with an outer epithelial layer and an inner layer of myocytes with striated myofibers (*arrowhead*). Between the exumbrella and subumbrella is gelatinous ground substance called the mesoglea (*asterisk*) that contains scattered amoebocytes. Embedded in the mesoglea is a cross-section of a gastrovascular canal (GV), which is lined by the gastrodermis. 200×. HE.

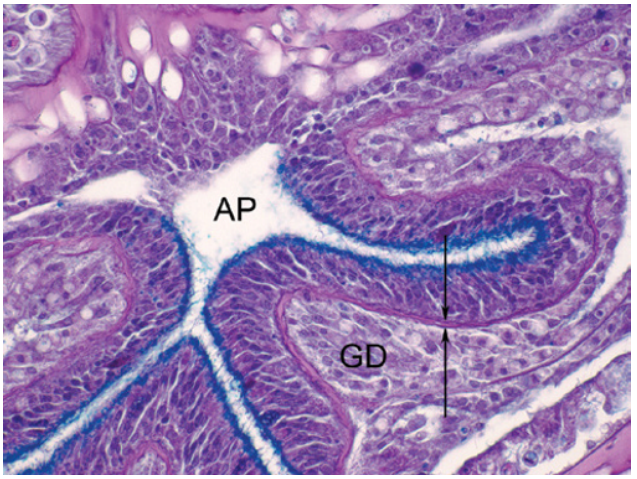


Figure 3.15 Body wall of the gorgonian, *Antillogorgia americana*, sectioned at the level of the mouth. Ciliated columnar epithelial cells line the actinopharnyx (AP), and are separated from the gastrodermis (GD) by an acellular band of mesoglea (*thin arrows*). 600×. PAS/AB.

dinoflagellates, formerly known as zooxanthellae, now referred to by their genus names, for example *Symbiodinium*. Some mesenteries connect with the actinopharynx and are considered "complete" mesenteries, those not connected are "incomplete." The mesenteries increase the surface area of the gastrodermis and also provide some structural support. The free edges of complete mesenteries are modified into thickened coils of white mesenterial filaments (also known as gastric filaments; Figure 3.9). The filament itself is covered by an epithelium of thin columnar ciliated cells for water circulation, acidophilic granular gland cells secreting digestive enzymes, and cnidocytes used in food capture or defense. Adjacent to the band, the gastrodermal cells of the mesenteries are often columnar, phagocytic, and can degrade particulate matter. The mesentery portion near the body wall contains epitheliomuscular (Figure 3.49) cells used to extend or contract the polyp. General organization of these epithelia is displayed in animals from multiple orders in Figures 3.10–3.16.

3.3.1.1 Epidermis

The epidermis is a single layer of cells that can be simple columnar, pseudostratified columnar, cuboidal, and even squamous. Outer surface epithelial cells (those in contact with the sea water) are usually ciliated to help carry mucus or shed sediment. This epidermis contains different cell types, including mucocytes, nematocytes, spirocytes, ptychocytes, neurons (nerve net), sensory cells, acidophilic (eosinophilic) granular gland cells, pigment cells, and epitheliomuscular cells (Figures 3.10–3.16). Numbers and distribution of these cells vary depending on species, and may be least developed in octocorals, but scleroblasts and amoebocytes can form a protective covering layer (Figures 3.17 and 3.18).

3.3.1.1.1 Mucocyte Unicelluar mucocytes in the epidermis secrete mucus, a polysaccharide-protein-lipid complex (Peters 2016), which aids in feeding and sediment removal (Figure 3.19). It provides protection against desiccation and presents a barrier to other stressors, including salinity, temperature changes, or exposure to UV radiation (Krupp 1984; Peters 2016). Mucus can also be involved in self/nonself recognition and in the immune response (Bosch and Rosenstiel 2016). In *Acropora acuminata*, mucous secretion accounts for up to 40% of the net daily fixed carbon (Crossland et al. 1980).

3.3.1.1.2 Cnidae Cnidocytes produce specialized organelles, cnidae or cnidocysts, which are capsules that contain a solid (ptychocysts and spirocysts) or hollow tubule (nematocyst); the latter everts when triggered and assists with prey capture, defense (nematocysts) or adhesion (ptychocysts and spirocysts) (Mariscal et al. 1977). There are over 30 types of cnidae in different cnidarians (Fautin 2009; Östman 2000). Nematocysts are organelles containing hollow, thread-like coils, inverted (like the

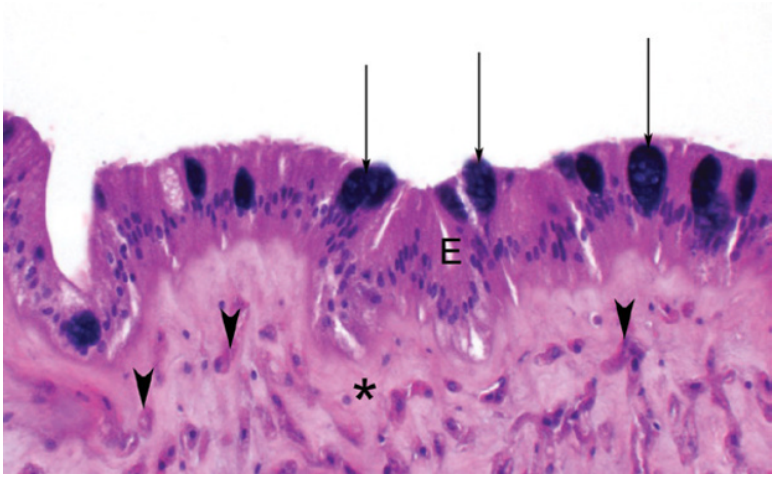


Figure 3.16 Body wall of a sea pen (order Pennatulacea). The epidermis (E) is overlying the mesoglea (*asterisk*), which is studded with amoebocytes (*arrowheads*). The epidermis is predominantly composed of tall, columnar epithelial cells interspersed with occasional mucocytes (*arrows*). 1000×. HE. *Source:* Photo credit: Emily Corbin.

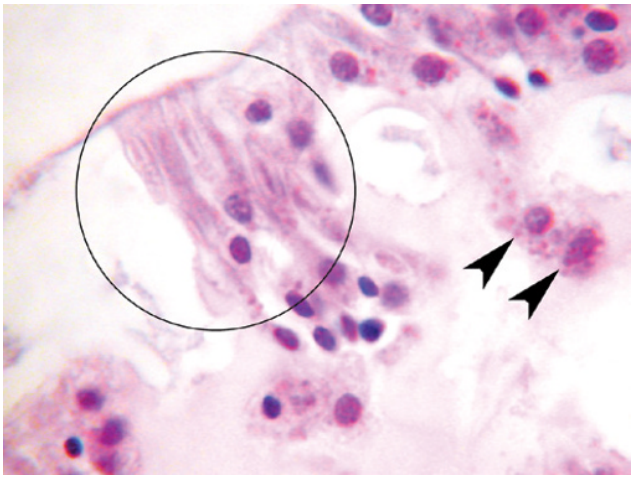


Figure 3.17 High magnification of the surface of a sea fan, *Gorgonia ventalina*, showing cluster of nematocytes (*circle*) and granular amoebocytes (*arrowheads*) among holes left after decalcification of the scleroblasts. 1000×. HE.

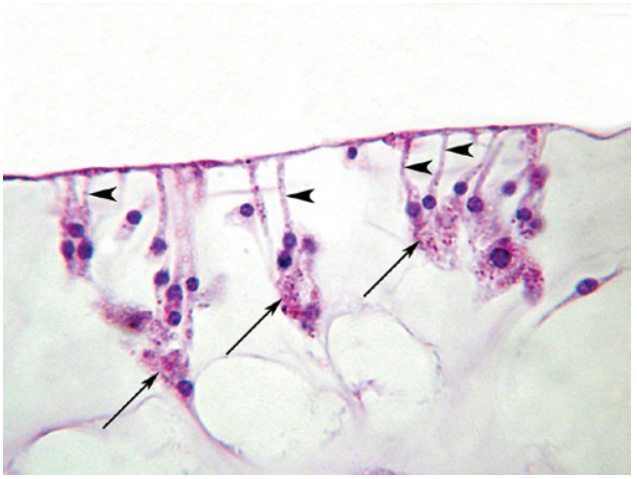


Figure 3.18 High magnification of the surface of another gorgonian, *Plexaurella fusifera*, showing pseudopodia (*arrowheads*) of granular amoebocytes (*arrows*) forming a protective layer of enzyme-containing vesicles to cover the mesoglea, presumably to kill bacteria coming in contact with them. 1000×. HE.



Figure 3.19 Epidermal mucocytes secreting through apical pores (*arrows*) in *Orbicella annularis*. 600×. HE.

finger of a glove turned inward), often with a trigger mechanism, a cnidocil, protruding on the end of the nematocyst capsule. The cnidocil (a modified cilium) extends from the apical surface of the nematocyte and on contact results in eversion of the tubule. The capsule can be covered by a lid or operculum. When triggered by a mechanical or chemical stimulus, the lid opens and the coil evaginates to penetrate and release venomous or acidic compounds from the capsule, used to subdue prey or to repel predators or competitors (Figures 3.20–3.22).

Spirocysts are similar to nematocysts, but instead of spines, the tubule/thread is solid and, when everted, can adhere to any object. Although smaller than nematocysts, spirocysts are often easier to observe on light microscopy as the solid tubule stains brightly with eosin (Figure 3.23). Once activated, the used structure is discarded, sloughed and a new one produced by the cnidocyte. Ptychocysts discharge a long, flattened, sticky tubule to help bind sand grains together to build the tubes in which cerianthid

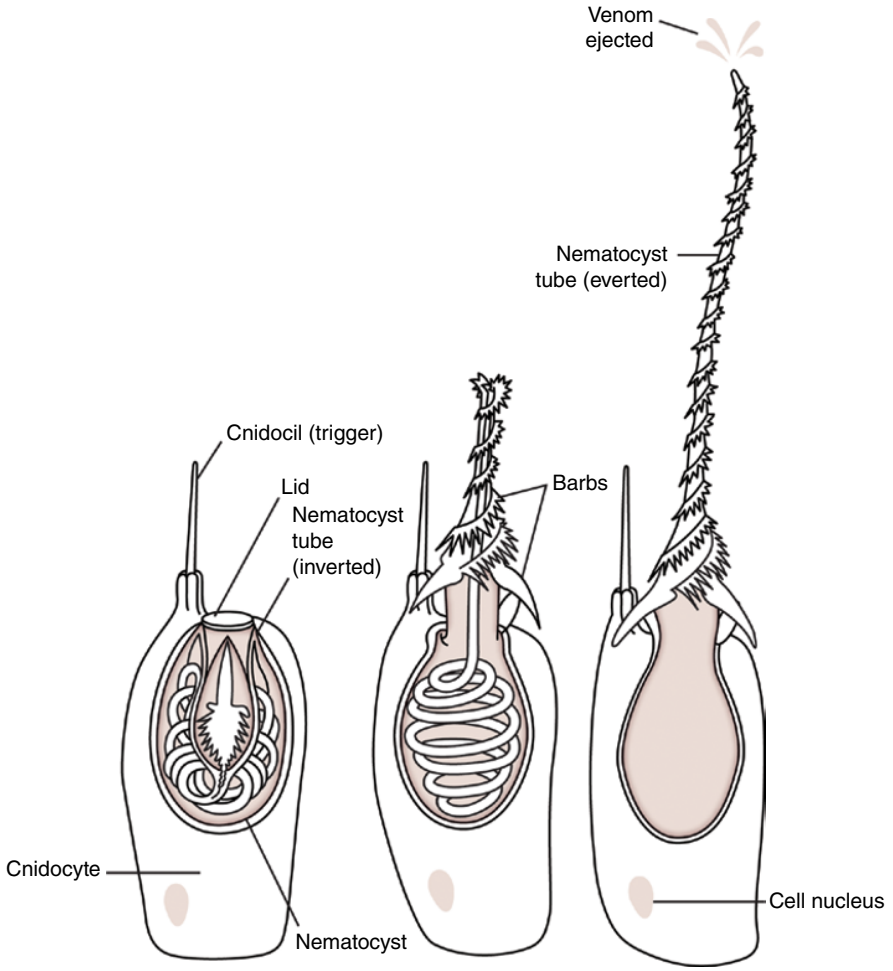


Figure 3.20 Diagram of a cnidocyte (*left*) ejecting venom from the tip of the everted hollow tubule of a nematocyst. *Source:* Image by Byron Inouye. © University of Hawaii. Reprinted with permission from the Curriculum Research & Development Group. Reprinted from the original on the Exploring Our Fluid Earth website at https://manoa.hawaii.edu/exploringourfluidearth.



Figure 3.21 Exumbrella from a moon jelly (*Aurelia auria*). Embedded within the exumbrella are the nematocysts (*arrowheads*). 400×. HE.

anemones burrow (Stampar et al. 2015) (Figure 3.24). Cnidocytes can also occur in dense aggregates called "batteries" or "warts" along tentacles and body walls, or individually. Acontia, which are located in the gastrovascular cavity of anemones, are thread-like tissues containing numerous stinging cells and serve as a unique defense tissue against predators. In some groups of anthozoans, the structure of certain tentacles can be altered often in response to overgrowth pressures. They differ in morphology and aggressive function and include acrorhagi, catch tentacles, and sweeper tentacles (Williams 1991).

3.3.1.1.3 Granular Cell There is some confusion when referencing granular cells. Sometimes they are also referred to as acidophilic or eosinophilic granular cells as a result of the staining characteristics with HE. Granules vary in size, number, uniformity, location, and origin. Special stains are needed to delineate and even then with some difficulty. Three general groupings include granular pigment cells, granular gland cells, and granular amoebocytes. Other granules, such as those in some epidermal supporting cells,

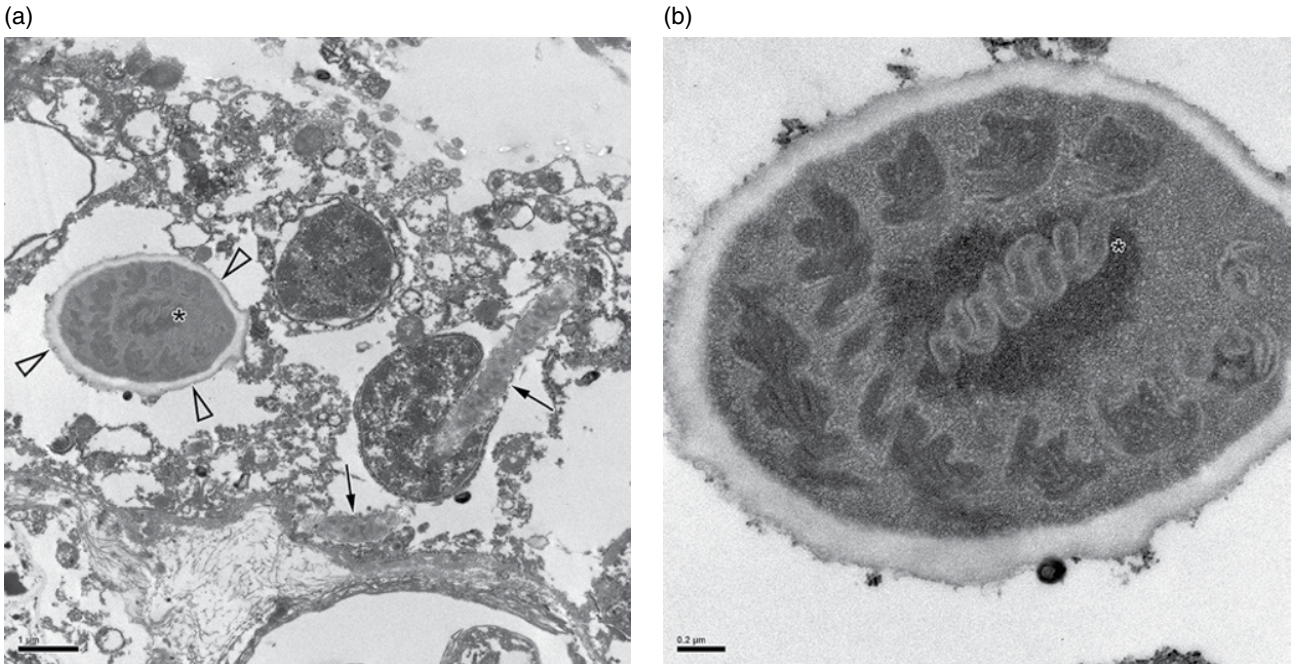


Figure 3.22 Transmission electron microscopy of nematocysts from a spotted jelly (*Mastigias papua*). (a) Arrowheads highlight a nematocyst that is not discharged. Arrows outline discharged hollow threads from nematocysts. (b) Higher magnification of an intact nematocyst highlights the inverted hollow thread with spines (*asterisk*). *Source:* Photo credit: Gary D. Marty, and Electron Microscopy Core Laboratory, UC Davis School of Medicine.

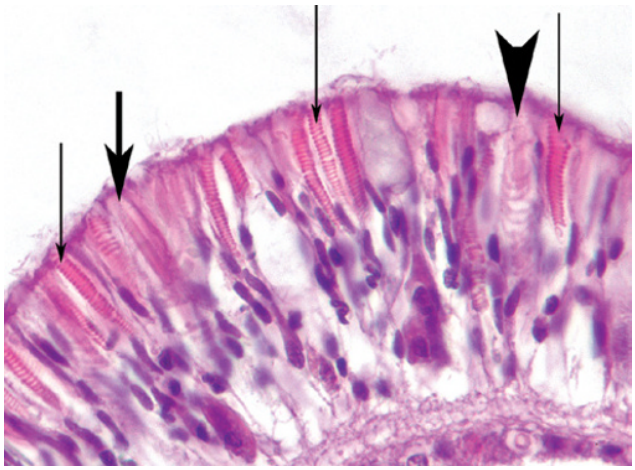


Figure 3.23 The tightly wound tropocollagen-rich tubules of spirocysts are easily seen (*thin arrow*). Two different types of nematocysts are present: the central, elongate rods are the swollen basal portions of the nematocyst type called microbasic mastigophore (*thick arrow*) and the nematocysts with a uniform tubule diameter are called holotrichous isorhizas (*arrowhead*). *Orbicella annularis*. 1000×. HE.

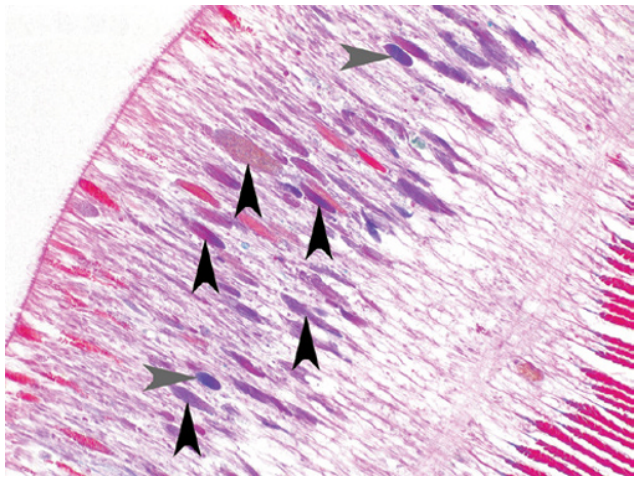


Figure 3.24 Ciliated surface body wall of the burrowing sea anemone, *Ceriantheopsis americanus*, with large, elongated ptychocysts (*black arrowheads*) for tube building; replacements are being synthesized in ptychocytes deep in the epidermis, the smaller blue capsules (*gray arrowheads*). 630×. Heidenhain's aniline blue trichrome.

mucocytes, or within or between gastrodermal or cnidoglandular band cells, have been determined to be bacteria (e.g., Sharp et al. 2010; Wada et al. 2016). Bacteria may stain blue/purple with hematoxylin. Calicoblasts of calcifying anthozoans and hydrozoans might be considered another "granular cell" (Figure 3.28). Cells with granules in the epithelia have also been noted in hydra/hydrozoan tissue sections (Peters, pers. obs.).

Granular pigment cells, in addition to zooxanthellae, provide various colors and color patterns to cnidarians. They are present in the basal epidermis, show variable



Figure 3.25 Epidermal pigment cells (*arrows*), possibly containing fluorescent pigments, in the base of the epidermis of *Orbicella annularis*. 400×. HE.

staining with H&E, and contain smaller granules. Some granules can contain pigments that fluoresce when exposed to different wavelengths and viewed with special filters (e.g., green or red fluorescent proteins) (Peters 2016) (Figure 3.25).

Granular gland cells (also known as zymogen cells, eosinophilic or acidophilic granular gland cells) are secretory cells containing granules or spherical membrane-bound vesicles of enzyme precursors (lysosomes) used for digesting food in the gastrovascular cavity or on the external surface to attack/digest other material or organisms competing for space (Peters 2016). Granules tend to be larger, staining quality of granules may vary, and cells can be found in both epidermal and gastrodermal epithelia (Raz-Bahat et al. 2017). They are present in large numbers in cnidoglandular bands with fewer scattered in the gastrodermis and actinopharynx epidermis. Refer to section 3.3.1.4 for granular gland cell (zymogen) images. (Note: we are not sure yet if these cells found in the oral region mesenteries' gastrodermal cells of *Orbicella faveolata* and *Orbicella annularis* have this role.)

Granular amoebocytes (also known as amoeboid chromophore cells) are present in epidermal and gastrodermal epithelia, and on occasion in mesoglea. They have been noted both in scleractinian corals (for example, in the genus *Porites*) (Palmer et al. 2011) (Figure 3.26) and in

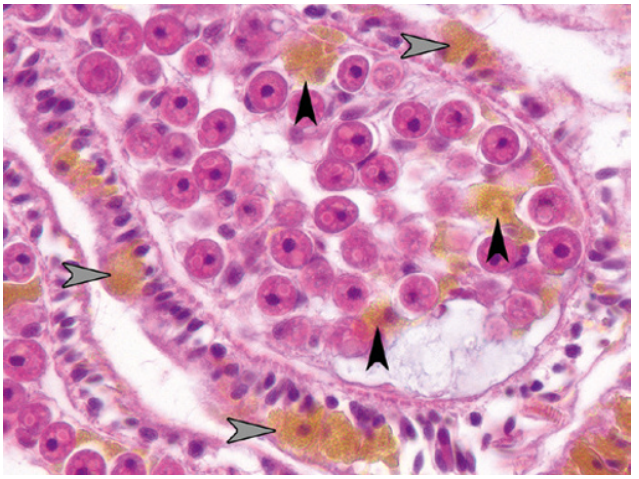


Figure 3.26 Melanin-like pigment-containing granular amoebocytes (aka amoeboid chromophore cells) in both the gastrodermal (*black arrowheads*) and epidermal (*gray arrowheads*) epithelia of the scleractinian coral, *Porites astreoides*. 1000×. HE.

Caribbean sea fans (Mydlarz et al. 2008) and other gorgonians. Granules are small and may stain pink or remain golden to brown when stained with eosin; the latter contain melanin-like pigment, staining black with Fontana–Masson procedure. These cells participate in immune responses for the cnidarians. Melanin-like pigment (brown with HE, green with Giemsa, black with Fontana–Masson) may also develop in epidermal supporting cells or other cells of cnidarians, which requires more study concerning their biochemical nature and roles.

3.3.1.2 Calicodermis

The external basal body wall epithelium is specialized in the stony corals and is known as the calicodermis. It is mainly composed of calicoblasts and desmocytes. Calicoblasts can be columnar, cuboidal or squamous (Figures 3.27 and 3.28). They do not secrete crystals of aragonite, but secrete an organic matrix and coral acid-rich proteins (CARPs) which assist in the deposition of these crystals (Mass et al. 2012, 2017). Recent studies suggest that they become cuboidal to columnar during active matrix secretion. Along the mesogleal/calicodermal border are desmocytes, differentiated epithelial cells that attach to collagen fibers of the mesoglea and produce fibers that extend and attach to the aragonite skeleton (Figure 3.28) (Muscatine et al. 1997). They detach and reattach to the skeleton during episodes of mineral accretion. The calicodermis may have variable numbers of mucocytes but does not contain nematocytes or spirocytes. Cells of the calicodermis (calicoblasts) in corals produce varying amounts of pink (HE) granules, now identified as containing CARPs that are involved in the crystallization of calcium and

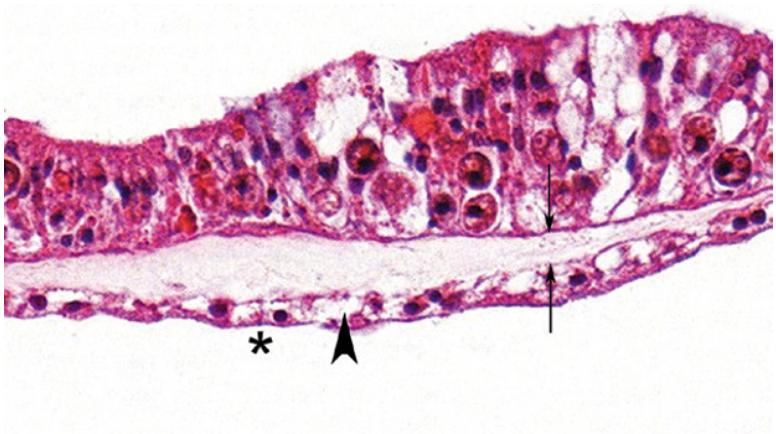


Figure 3.27 Squamous calicoblasts (*arrowhead*) in the calicodermis deep in the corallite, overlying the space where the calcium carbonate skeleton was removed (*asterisk*). A thin mesoglea (*arrows*) separates the calicodermis from the gastrodermis. *Orbicella faveolata*. 400×. HE.

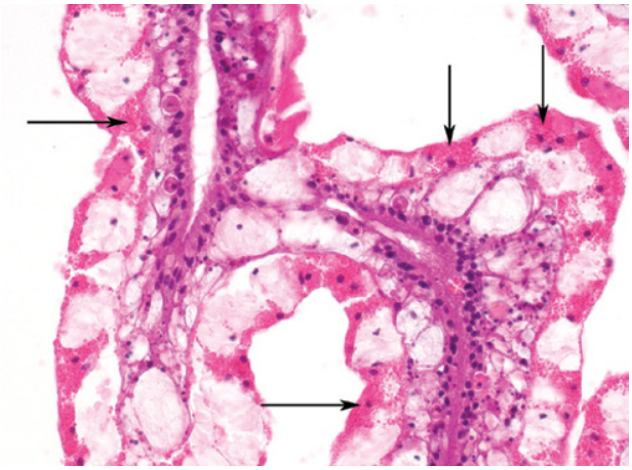


Figure 3.28 Calicoblasts (*arrows*) containing eosinophilic granules in the calicodermis of *Siderastrea siderea*. In this species (and other corals) these cells produce coral acid-rich proteins (CARPs) to form the skeleton. 400×. HE.

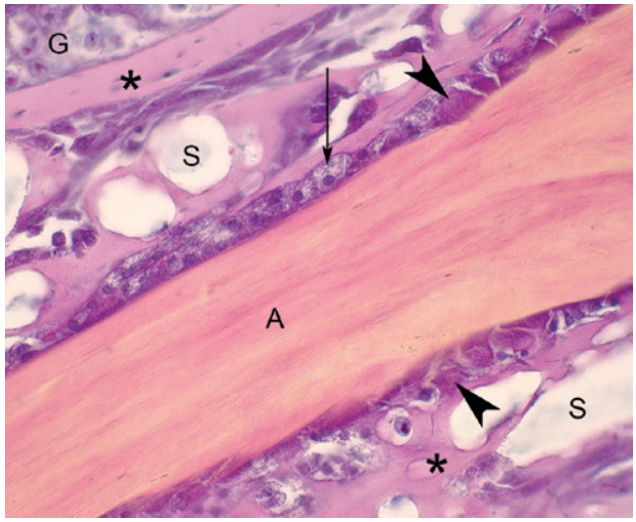


Figure 3.29 Corticocytes (*arrow*) secrete the proteinaceous axis and desmocytes (*arrowheads*) attach the tissue to the axis (A). The axis epithelium is surrounded by mesoglea (*asterisk*), within which are sclerites (S). The gastrodermis (G), with embedded zooxanthellae, borders the other side of the mesoglea. *Gorgonia ventalina*. 600×. PAS/AB/hematoxylin.

carbon dioxide molecules to form the aragonite crystals of their skeletons (Figure 3.28). (Mass et al. 2013).

3.3.1.3 Axis

In gorgonians, the mesoglea covers an axis epithelium, which secretes an internal proteinaceous rod that supports the polyps and tissue of the colony. This epithelium lies between the mesoglea and the axis and is derived from the ectoderm (Figures 3.29 and 3.30) (Peters 2016). Corticocytes secrete the gorgonin matrix and desmocytes attach the tissue to the axis.

3.3.1.4 Gastrodermis

The gastrodermis lines the gastrovascular cavity of all cnidarians, as well as the interconnecting gastrovascular canals and the mesenteries created by the infoldings of the mesoglea in corals. The free margin of a complete mesentery in many anthozoan species has thickened bands or tracts that may be multilobate. These are called mesenterial filaments, the distal cnidoglandular band contains nematocytes, ciliated columnar supporting cells, collar cells, and granular gland cells (zymogen cells containing lysozymes). Digestion is both extracellular (enzymes being released) and intracellular as particles are taken up by absorptive cells in the gastrodermis of the attached mesentery using the processes of phagocytosis, pinocytosis, and/or diffusion (Schlichter 1982). The gastrodermis also contains cnidocytes, amoebocytes, mucocytes, and granular pigment cells. Gastrodermis subjacent to the calicodermis often has different numbers of cell types with fewer mucocytes but more granular gland cells or cells that store lipid droplets more basally in coral polyps (Figure 3.31). Importantly, for many shallow-water species, the gastrodermis can

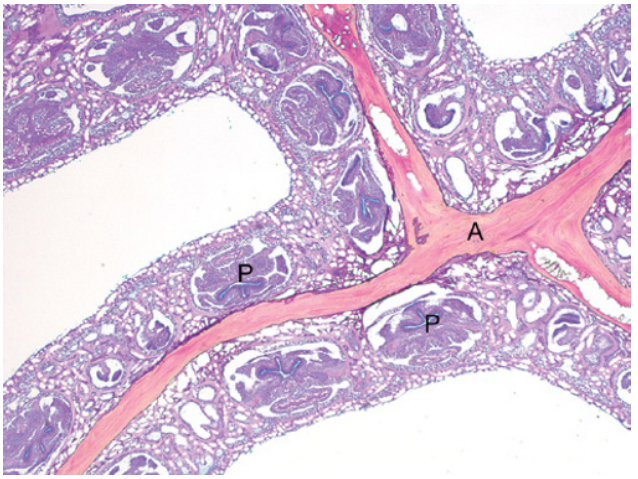


Figure 3.30 Low magnification of a fixed, decalcified sample from the sea fan, *Gorgonia ventalina*. The gorgonin axis (A) is the pink material in the middle of the tissue, which contains numerous polyps (P). The coenenchyme between the polyps consists of mesoglea packed with scleroblasts, gastrovascular canals, and solenia (variably shaped holes). 40×. PAS/AB/hematoxylin.

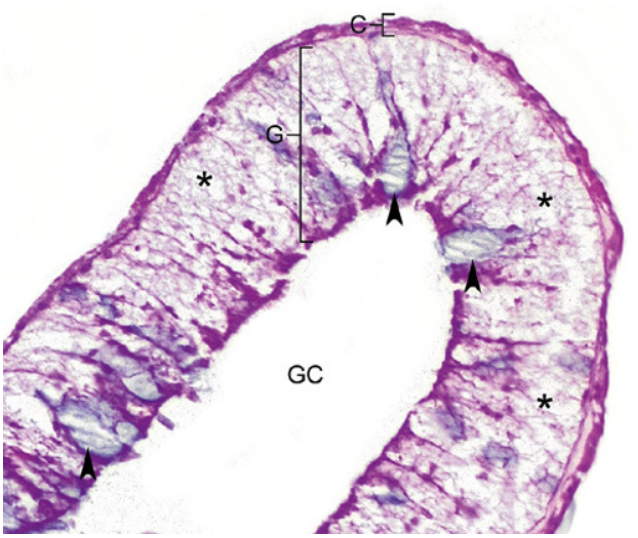


Figure 3.31 Section through the deep basal body wall of the scleractinian coral *Montastraea cavernosa*, showing a thin calicodermis (C) with desmocytes and squamous calicoblasts. The gastrodermis (G) is composed largely of lipid droplet-containing gastrodermal cells (*asterisks*) with scattered mucocytes (*arrowheads*). GC, gastrovascular cavity. 200×. HE.

phagocytose single-cell dinoflagellates, which have been commonly referred to as zooxanthellae, and maintain them in a vacuole without digesting them. These are usually most abundant in the oral surface tissues, especially the gastrodermis of tentacles. The cells can degrade the algal cells in the process of symbiophagy (Downs et al. 2009).

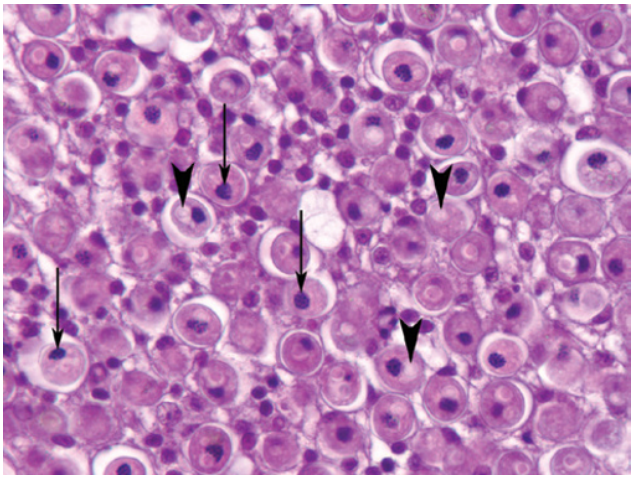


Figure 3.32 Vacuole-bound symbiotic dinoflagellates in the gastrodermis of *Orbicella cavernosa*. Nuclei (*arrows*) and pyrenoids (*arrowheads*) are visible in many zooxanthellae. 1000×. HE.

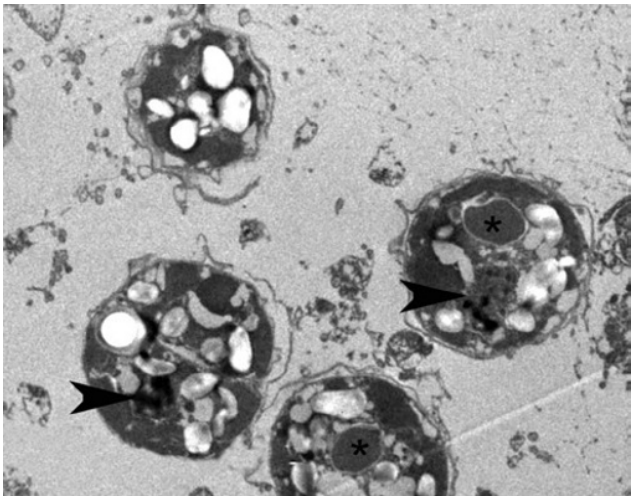


Figure 3.33 Transmission electron microscopy of zooxanthellae from a spotted jelly (*Mastigias papua*); pyrenoids (*asterisks*) and nuclei (*thin arrow*) are occasionally visible. Zooxanthellae range from 5 to 10 µm in diameter. *Source:* Photo credit: Gary D. Marty; and Electron Microscopy Core Laboratory, UC Davis School of Medicine.

3.3.1.4.1 Zooxanthellae Vacuole-bound dinoflagellates, *Symbiodinium* and other genera, undergo photosynthesis and exchange nutrients and waste with coral host cells. They possess photosynthetic pigments, which often provide distinctive colors to their host cnidarians. By light microscopy, a zooxanthella nucleus is visible as a deeply basophilic, oval to square, often eccentrically placed organelle with permanently condensed chromatin (dinokaryon). Pyrenoids, responsible for carbon fixation, are also frequently visible by light microscopy as clear, round cytoplasmic vacuoles containing an approximately 2 µm diameter, eosinophilic organelle. Pigments in the chloroplasts are variably observed in the cytoplasm (Figures 3.32 and 3.33).

3.3.1.4.2 Mucocytes in Gastrodermis The gastrodermis of the surface and basal body walls, actinopharynx wall, and mesenteries can contain variable densities of mucocytes (Figure 3.34). Mucus secreted by these cells is discharged into the gastrovascular cavity and canals; it will stain variably with hematoxylin, alcian blue, or alcoholic saffron, depending on its molecular composition and pH. The role of mucus within the polyps has not been determined, but it may help support the internal bacterial microbiome.

3.3.1.4.3 Mesenteries The mesenteries are partitions of gastrodermis-lined mesoglea that branch from the mesoglea forming the body wall (Figures 3.35 and 3.36). The mesoglea at the free end of each partition is lined by densely packed columnar gastrodermis (called mesenterial filaments; see following section). Complete mesenteries connect to and support the actinopharynx as far as it extends into the gastrovascular cavity of the polyp. The mesenteries frequently contain gonads (but not all of them in some anthozoan species) and scattered amoebocytes, either among epithelial cells or in the mesoglea.

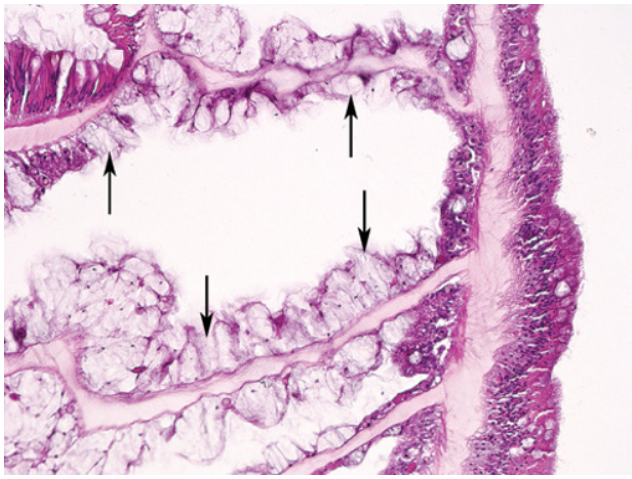


Figure 3.34 Large slightly basophilic mucocytes (*arrows*) line the gastrodermis of the mesenteries in *Orbicella annularis*. 400×. HE.

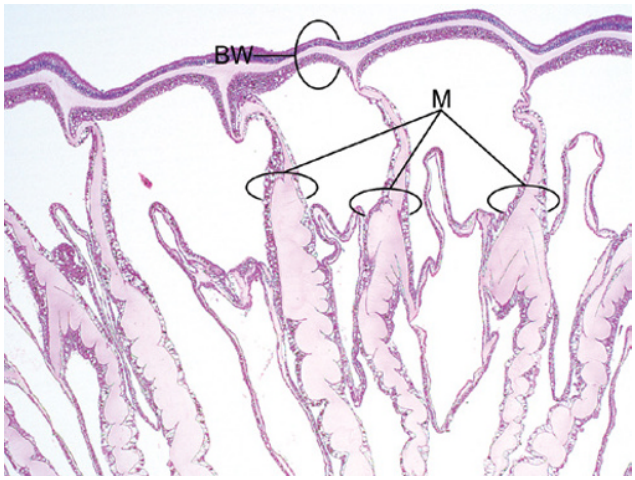


Figure 3.35 Mesenteries of *Montastraea cavernosa*. The thickness of mesenteries (M) and the degree of folding or pleating vary among species, shown here as they attach to the surface body wall (BW). 40×. HE.

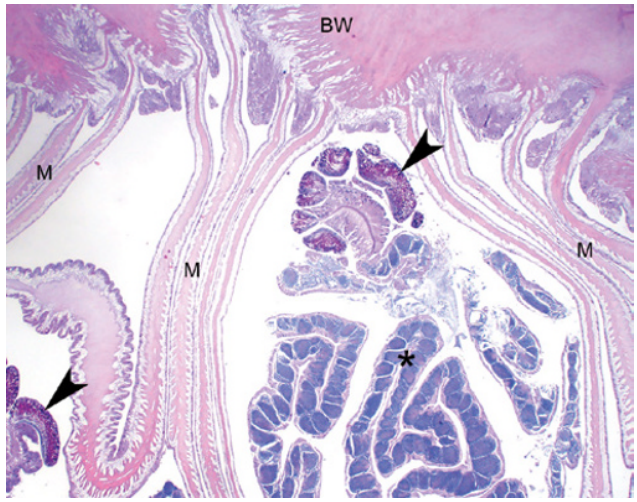


Figure 3.36 Mesenteries of aggregating anemone (*Anthopleura* sp.). The mesenteries (M) are branching membranes from the body wall (BW) that have a thin, central core of mesoglea that is lined on both sides by gastrodermis. The gonads (in this case spermaries) are embedded within the central mesoglea (*asterisk*). Terminal portions of mesenteries are covered by densely packed columnar cells of varied functions called mesenterial filaments (*arrowheads*). 40×. HE.

3.3.1.4.4 Mesenterial Filaments and Cnidoglandular Band The free edge of each complete mesentery below the actinopharynx is thickened and modified into a uni- to multilobate elongated, convoluted ("ruffled or ribbon-like") structure known as a mesenterial filament (Peters 2016). A median band or tract of the mesenterial filament containing nematocytes, ciliated columnar cells, mucocytes, and granular gland cells is called the cnidoglandular band (or lobe) (Figures 3.37–3.44). Granular pigment cells may also be found in mesenterial filaments (Figures 3.41 and 3.42). Filaments can sometimes protrude through the mouth or through pores (cinclides) in the surface body wall to help capture and digest food or act against competitors or invaders.

3.3.2 Connective Tissue System: Mesoglea

The mesoglea supports the epidermis and the gastrodermis. It is an amorphous matrix of a hydrated protein and polysaccharide polymer combined with varying amounts of collagenous fibers and can vary in thickness. It helps maintain the arrangement of cells and cell layers and is important to the

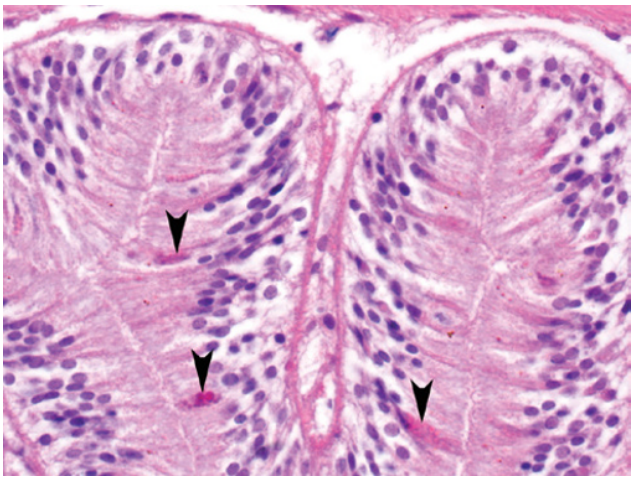


Figure 3.37 Mesenterial filament of a sea pen (order Pennatulacea). The termination of a mesentery has abundant, tall columnar cells on thin extensions of mesoglea. Admixed with columnar cells are occasional granular gland cells (*arrowheads*). 1000×. HE.



Figure 3.38 Mesenterial filaments of aggregating anemone (*Anthopleura* sp.). The sections through the mesenteries appear as branching filaments with a thin central layer of mesoglea (*arrows*) that is lined on both sides by gastrodermis. The free edges of the mesenteries have tightly packed thin columnar gastrodermal cells, including ciliated cells, acidophilic granular gland cells, and nematocytes. 400×. HE.

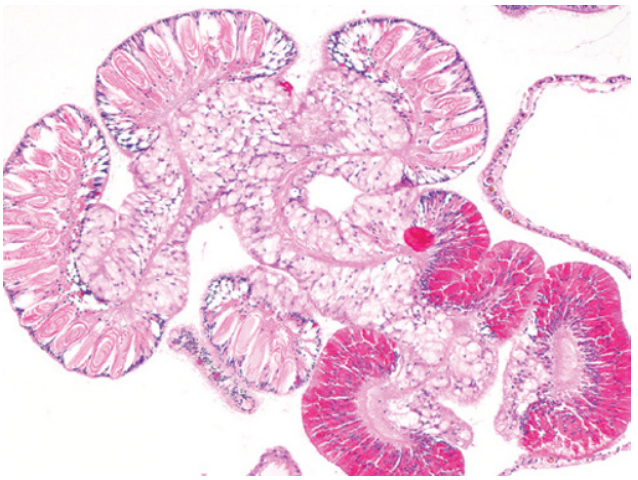


Figure 3.39 Cnidoglandular band and mesentery sections in *Pseudodiploria* sp. 200×. HE.

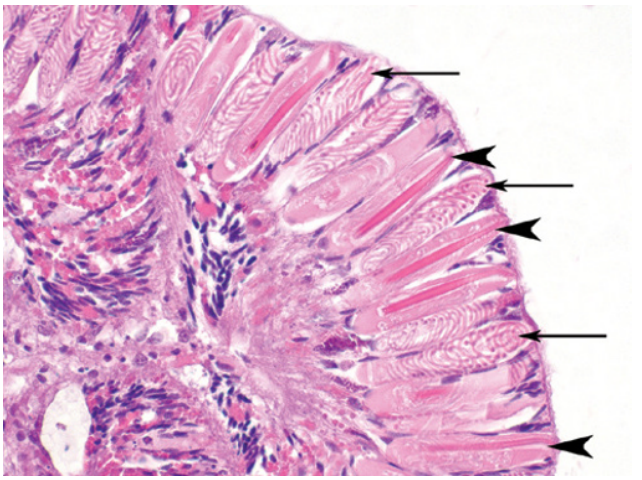


Figure 3.40 Higher magnification of Figure 3.39. Nematocysts in cnidoglandular band, *Pseudodiploria* sp. The central, elongate rods are the swollen basal portions of the nematocyst type called microbasic mastigophore (*arrowheads*); the nematocysts in between them have a uniform tubule diameter and are called holotrichous isorhizas (*arrows*). When discharged, the sharp tip of the tubule penetrates prey, predators, or competitors and allows release of toxins into their tissues. 400×. HE.

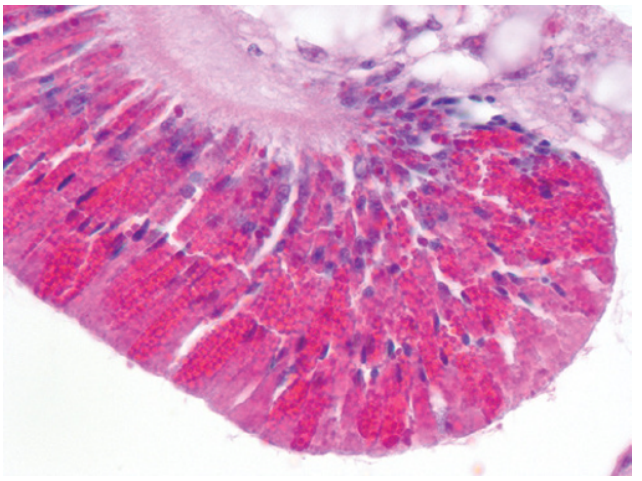


Figure 3.41 Higher magnification of Figure 3.39. Gastrodermal granular pigment cells in *Pseudodiploria* sp. These elongated cells contain uniformly sized granules packed together throughout the cnidoglandular band and are suspected to be pigmented portions of the filament (see Figure 3.42). Enzyme-containing vesicles (granular gland cells) are not so uniform and stain paler pink (see Figure 3.43). 1000×. HE.



Figure 3.42 Pigmented areas on mesenterial filaments of *O. faveolata*. Gross photograph.

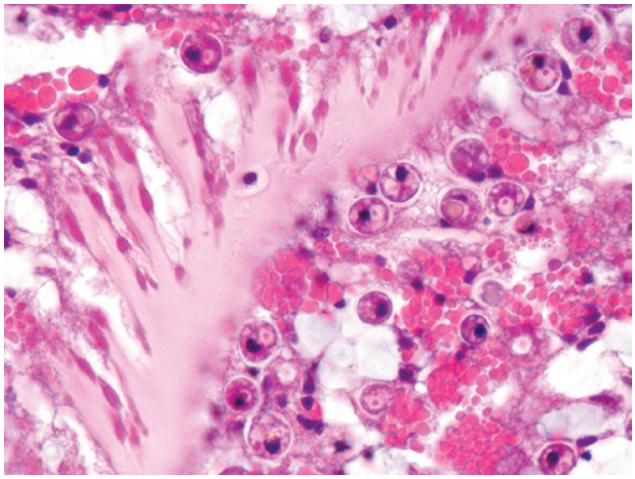


Figure 3.43 Large gastrodermal granular gland cells found in the mesentery of scleractinian corals *Orbicella faveolata* and *O. annularis*. The granules are larger and variably sized, compared to the smaller and more uniform granules in granular pigment cells (see Figure 3.41 for comparison to the latter). Numerous zooxanthellae are also present in the gastrodermis. 1000×. HE.

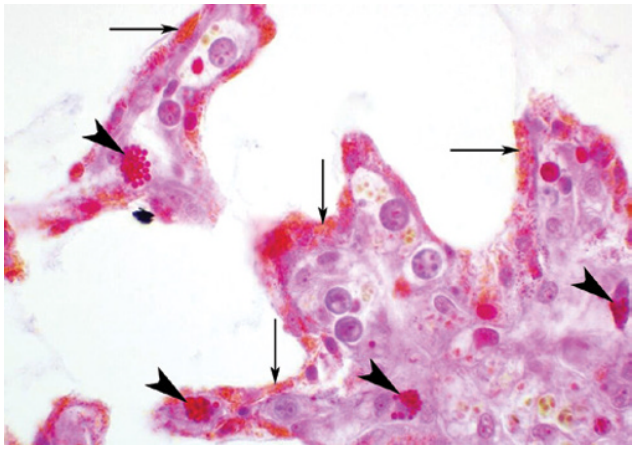


Figure 3.44 Granular cells in the calcifying hydrozoan fire coral, *Millepora* sp. The larger red granules (*arrowheads*) are thought to be granular gland cells producing zymogens in the gastrodermis (digestive enzymes); zooxanthellae are also visible in the gastrodermis. The fine, orange granules (*arrows*) are in calicoblasts, probably containing acid-rich proteins similar to the granules in calicoblasts of stony corals. 400×. Casson's trichrome. *Source:* Tissue section contributed by Alan Davis.

operation of the hydrostatic skeleton, providing stiffness for shape and resilience to reinforce muscle action. It can turn inward to form the supporting structure of mesenteries, which in turn can further fold vertically (like an accordion), creating structures known as mesogleal pleats. Epitheliomuscular cells are anchored into the mesoglea via basal extensions of the plasmalemma (cell membrane) containing actin and myosin filaments; these attachments are known as myonemes. Pleats are thought to allow greater ability to move, bend, and retract, as the additional surface area

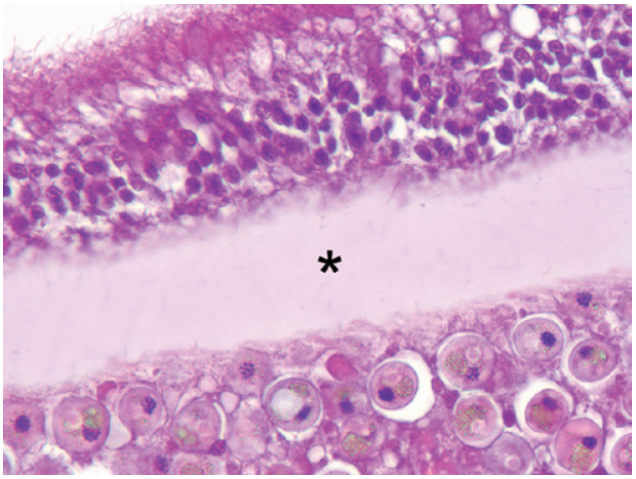


Figure 3.45 Mesoglea (*asterisk*) in coral *Orbicella cavernosa*. Epidermis is on the top of the image and gastrodermis containing zooxanthellae on the bottom of the image. 1000×. HE.

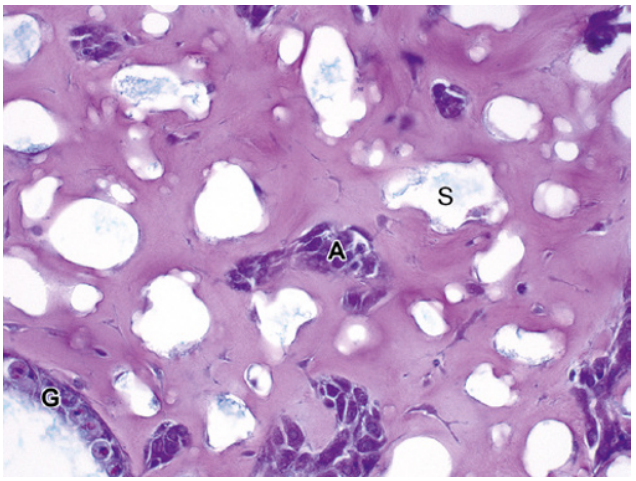


Figure 3.46 Mesoglea in gorgonian *Gorgonia ventalina* showing spaces left by the decalcification of numerous sclerites (S); note blue material in lumens remaining from this process and clusters of amoebocytes (A). Gastrodermis (G) with zooxanthellae is on the bottom left of the image. 100×. PAS/AB/hematoxylin.

supports more myonemes. The mesoglea can contain some cells – fibroblasts that secrete the collagen fibers and ground substance, and migratory ameobocytes (see section 3.3.5).

3.3.2.1 Collagen Matrix

See Figures 3.13 and 3.14 for photomicrographs of mesoglea in an anemone and jelly, respectively. Images of mesoglea in corals are provided in Figures 3.45 and 3.46.

3.3.2.2 Mesogleal Pleat

The gastrodermis of the mesenteries contains packed epitheliomuscular cells along the mesogleal pleats, which form the longitudinal retractor muscles

(Figures 3.47 and 3.48). Here the myonemes extend parallel to the longitudinal axis of the polyp, enabling it to expand and contract lengthwise. Larger polyps have more well-developed pleats, providing more surface area to support more numerous epitheliomuscular cells. They are especially well developed in the mesenteries of large single polyps of mushroom corals, *Fungia* sp., and of the rose coral *Manicina areolata*, a brain coral with multiple mouths (polystomodael) within the ring of tentacles along a meandering valley corallite. These corals live free on the bottom and smaller colonies can adjust their hydrostatic skeletons to turn over or dig through sediment (Uhren et al. 2005). Smaller-polyped genera such as *Acropora* or *Porites* have thin mesoglea and poorly developed mesogleal pleats because they cannot extend far out of their corallites.

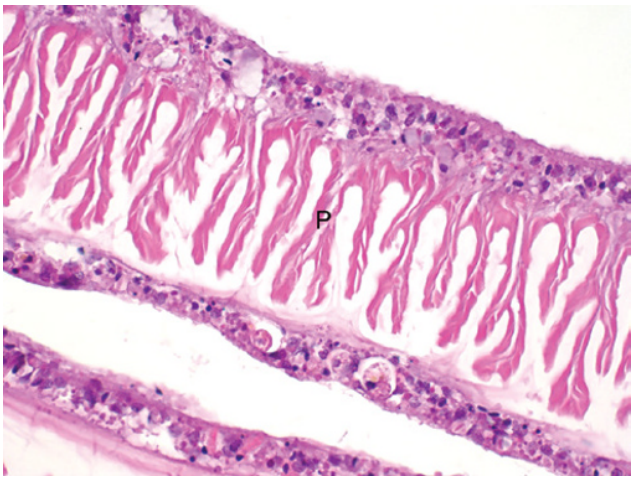


Figure 3.47 Mesogleal pleats (P) in *Fungia* sp. 600×. HE.

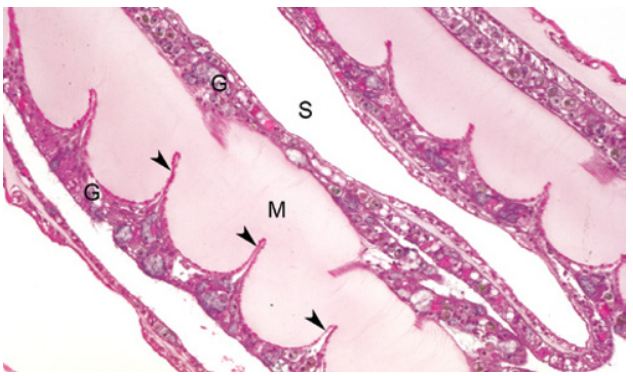


Figure 3.48 Mesogleal pleats (*arrowheads*) in *Orbicella cavernosa* have thick mesoglea (M) between gastrodermis (G). They are present in the gastrovascular cavity between septa (S). 200×. HE.

3.3.3 Muscle

The main muscle cell type of cnidarians is the epitheliomuscular cell, also known as myoepithelial cell. These cells contain contractile myofilaments in basal extensions called myonemes that are often embedded into the mesoglea (Figures 3.49–3.51) (Seipel and Schmid 2006). Epitheliomuscular cells are found in both the epidermis and gastrodermis. In schyphozoans, epitheliomuscular cells are most prominent in the subumbrellar epidermis, where they help to facilitate belling (i.e., contraction of the bell to facilitate swimming/movement in a jelly).

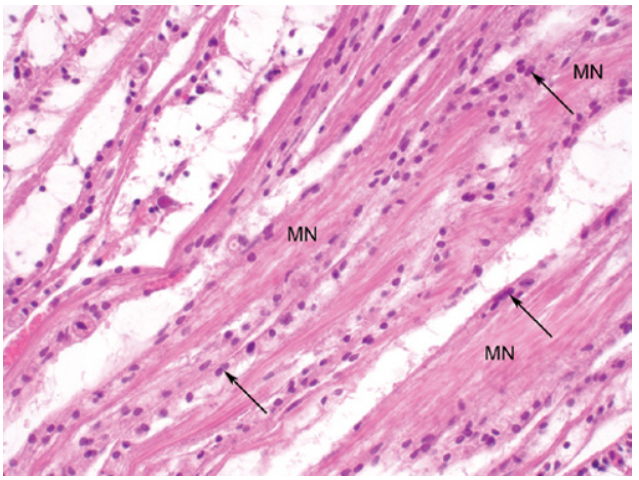


Figure 3.49 Arrays of myonemes (longitudinal section) (MN) from epitheliomuscular cells in the scleractinian coral, *Orbicella franksii*. Myonemes are muscle fibrils. Nuclei of some of the epitheliomuscular cells are highlighted with arrows. 600×. HE.

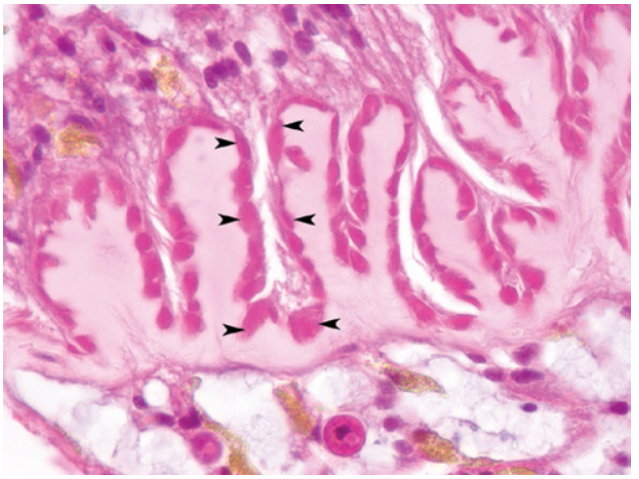


Figure 3.50 Myonemes (*arrowheads*; cross-section), basal extensions of epitheliomuscular cells, embedded in mesoglea of the scleractinian coral, *Porites astreoides* sp. 1000×. HE.

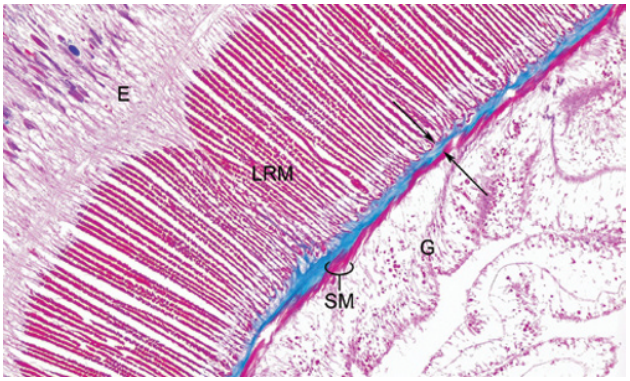


Figure 3.51 Contracted bands of myonemes in cross-section from a burrowing anemone, *Ceriantheopsis americanus*, which is a highly extensible species. Myonemes are present on the gastrodermal (G) and epidermal (E) sides of the mesoglea. The myonemes stain red in contrast to the blue-staining mesoglea (*arrows*). On the epidermal side, the myonemes form a thick layer constituting the longitudinal retractor muscles (LRM). On the gastrodermal side, the myonemes form a thinner layer constituting a sphincter muscle (SM) to reduce the diameter of the anemone. 100×. Heidenhain's aniline blue trichrome.

3.3.4 Nervous System

Neural components in cnidarians are often difficult to visualize under routine light microscopy. With the use of electron microscopy, immunocytochemistry, and other specialized techniques, cnidarians' nervous systems are not as diffuse or undifferentiated as previously thought (Grimmelikhuijzen and Westfall 1995; Kass-Simon and Hufnagel 2016; Westfall 2004; Westfall and Elliot 2002). Cnidarian nerve cells include unipolar, bipolar, and multipolar morphologies. Neurons tend to have a distinctive polygonal shape with a large nucleus and prominent nucleolus, and are organized into nerve nets, nerve tracts, ring nerves, giant nerves and ganglia-like clusters (Figure 3.52). Conducting systems transmit signals from various sensory neurons and across chemical and electrical synapses (Mackie 2004; Westfall et al. 2000) to effector systems such as myonemes, muscle cells, and cnidocytes. Cubozoans have the most complex sensory system of the Cnidaria and possess ocelli, which are analogues of complex eyes that contain lenses and light-sensitive receptor cells.

3.3.5 Immune System

Most (if not all) cnidarians species possess amoebocytes that function as the principal cells of the innate immune system. They are thought to have antimicrobial and antioxidant roles, can phagocytize and digest particulate matter and move food/wastes, and may differentiate into other cell types (Palmer and Traylor-Knowles 2012). Amoebocytes can migrate between epithelial cells and epithelial layers,

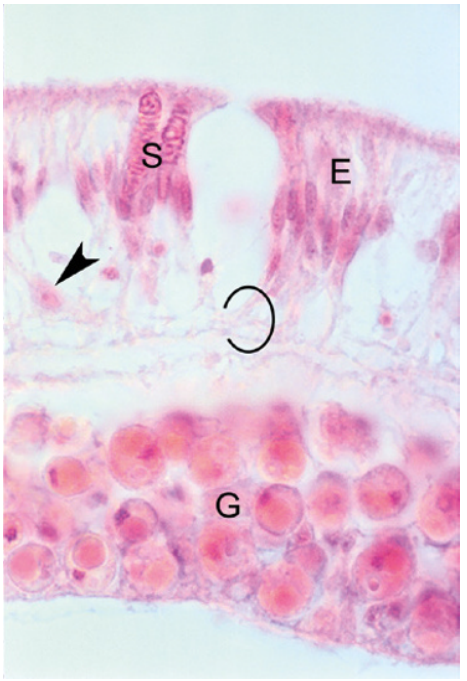


Figure 3.52 Surface body wall of *Manicina areolata*, showing the epidermis (E) with a couple of spirocysts (S) and gastrodermis (G) separated by a thin layer of mesoglea. The subepidermal nerve net (*oval*) is the layer of fine fibrils along the mesoglea, the axons and dendrites of neurons (*arrowhead*). 1000×. HE.

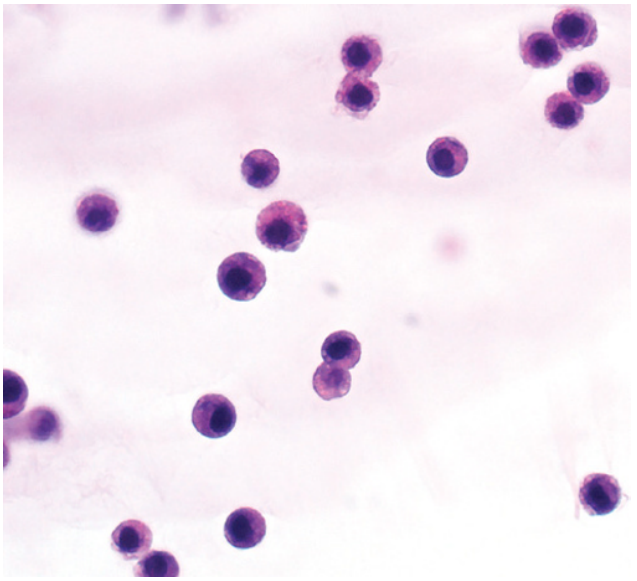


Figure 3.53 Mesoglea of a moon jelly (*Aurelia aurita*). Amoebocytes are scattered in the mesoglea. Amoebocytes are round to polygonal cells with an eosinophilic to slightly vacuolated cytoplasm and a round, dense nucleus. 1000×. HE.

including passing through the mesoglea. They can be difficult to delineate with light microscopy but are typically readily apparent when in the mesoglea (Figures 3.53–3.55).

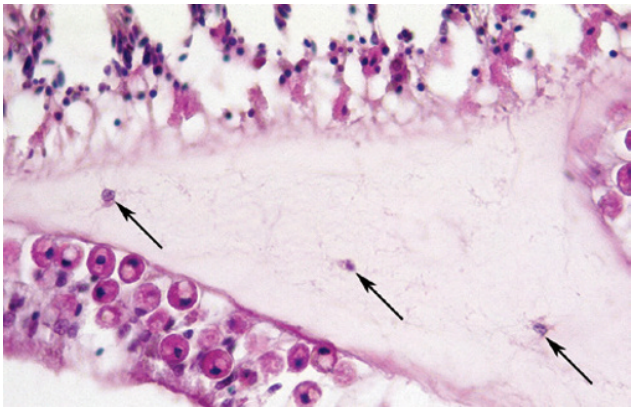


Figure 3.54 Mesoglea of a fleshy scleractinian coral, *Colpophyllia natans*, shows few small amoebocytes (*arrows*) in the mesoglea. 630×. HE.

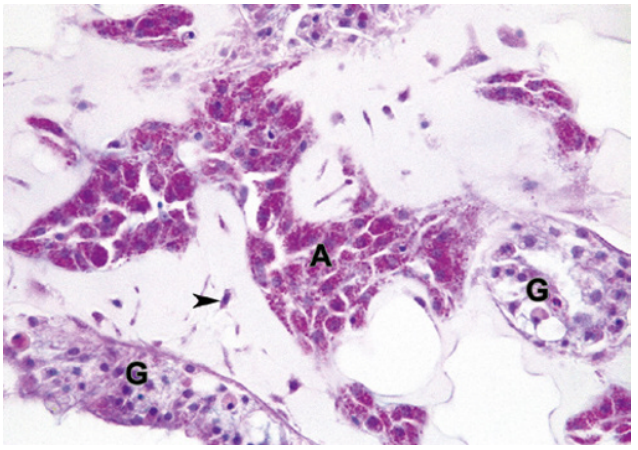


Figure 3.55 Mesoglea of a sea fan (*Gorgonia ventalina*), containing multiple clusters of amoebocytes (A) with abundant acidophilic granules among sections of gastrodermis (G) that line the gastrovascular canals. The smaller cells lacking granules are fibroblasts (*arrowhead*). 630×. HE.

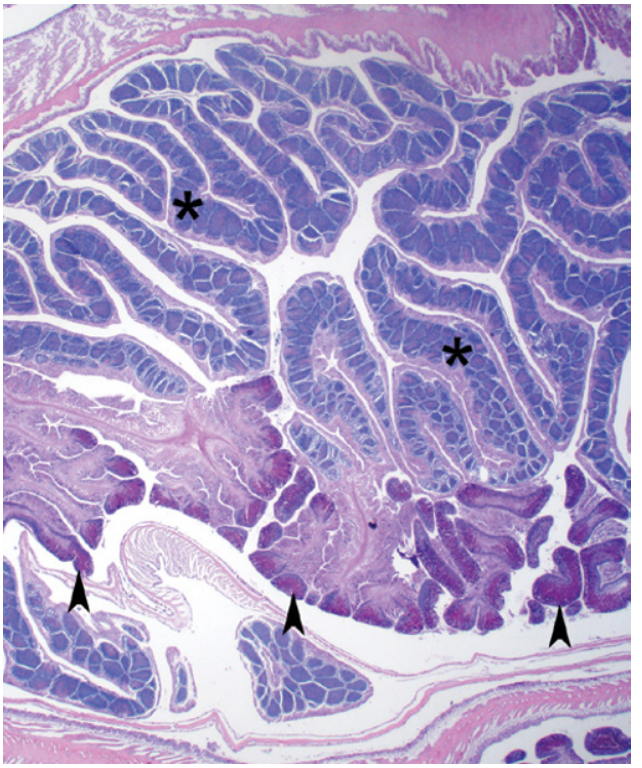


Figure 3.56 Mesenteries in an aggregating anemone (*Anthopleura* sp.). Spermaries (*asterisks*) are embedded in the central mesoglea of mesenteries. The nonreproducing portions of the mesenteries are below the gonads, and the mesenterial filaments (*arrowheads*) are at the free edges of the mesenteries. 40×. HE.

3.3.6 Reproductive Cells

Gonadal development occurs along the mesenteries, surrounded by the mesoglea in the area between the muscles and the mesenterial filaments (Figure 3.56), forming oocytes and spermaries (Figures 3.57 and 3.58). Germ cells originate in the gastrodermis arising from stem cells (sometimes referred to as totipotential, multipotential, or intersititial cells). In scyphozoans, this occurs in the gastrodermal layer on the surface of the four internal septa. In some hydrozoa, the germ cells migrate into the epidermis. In anthozoans, germ cells originate in the gastrodermis of the midportion of select mesenteries and then move into the mesoglea where they mature. All or only some mesenteries may be gametogenic. Some are separate sexes (gonochoric, dioecious), and others are either sequential or simultaneous hermaphrodites. Gametes of both sexes may or may not occur in the same mesentery (Fautin and Mariscal 1991). Eggs may be yolk-filled or not (Figures 3.59–3.62). In spawning species, the mesogleal and gastrodermal layers rupture, releasing gametes into the gastrovascular cavity and then out of the animal through the mouth. In brooder species, developing embryos and planula larvae may be found within the polyp; they are released when they have developed mesenteries and taken up symbiotic dinoflagellates, as seen in the coral *Porites astreoides* (Chornesky and Peters 1987). Ova in a few reef-building tropical scleractinian and soft coral species, a temperate anemone, and a freshwater hydrozoan may incorporate zooxanthellae into the yolk as they develop within the mesentery, but those of other species do not (reviewed in Davy and Turner 2003).

3.3.6.1 Sperm

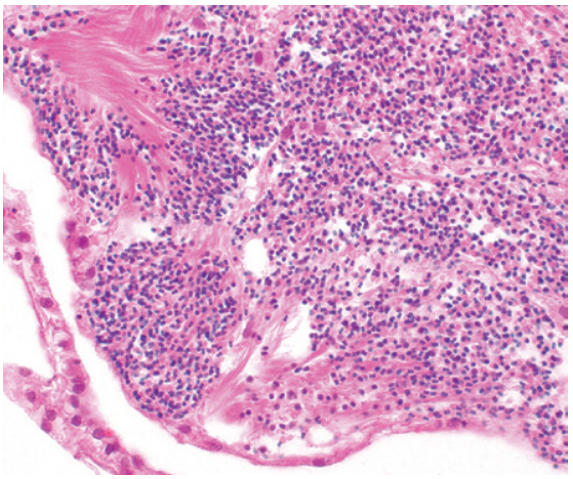


Figure 3.57 Spermatozoa in *Acropora* sp. 600×. HE.

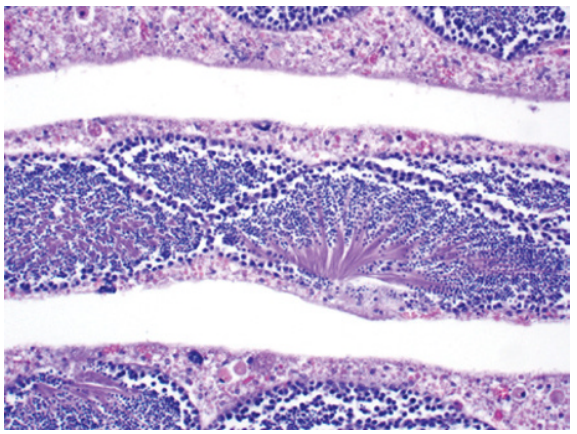


Figure 3.58 Spermatozoa in an aggregating anemone (*Anthopleura* sp.). 200×. HE.

3.3.6.2 Oocytes

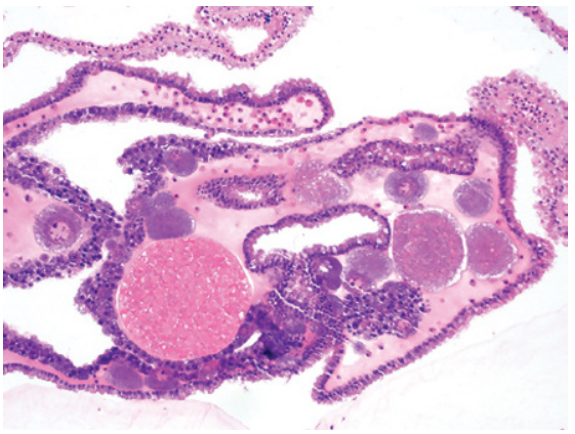


Figure 3.59 Multiple stages of developing oocytes from the jellyfish *Aurelia aurita*. Oocytes are embedded within mesogleal folds of the mesenteries within the gastrovascular cavity. 200×. HE.

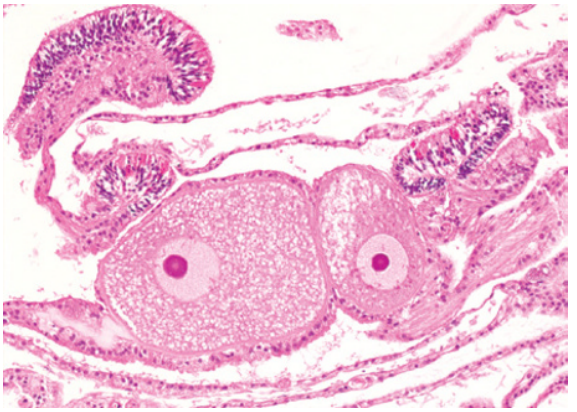


Figure 3.60 Oocytes in *Acropora* sp. 200×. HE.

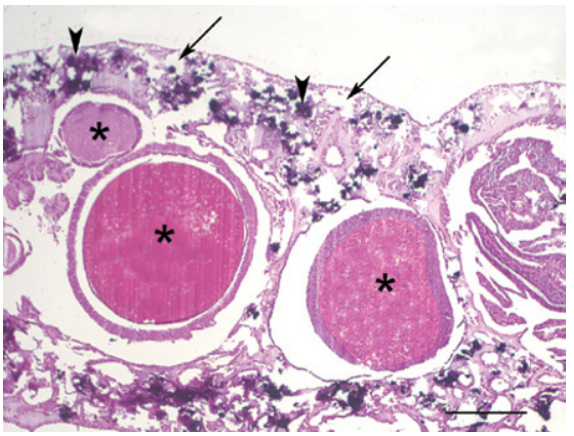


Figure 3.61 Oocytes (*asterisks*) in an unknown species of gorgonian. The tissue has not been completely decalcified, resulting in remnant mineral (*arrowheads*) and artifacts (clear spaces; *arrows*) where the mineralized matrix came out of section. Scale bar, 100 µm. 40×. HE. *Source:* Tissue section contributed by Michael Garner, Northwest ZooPath.

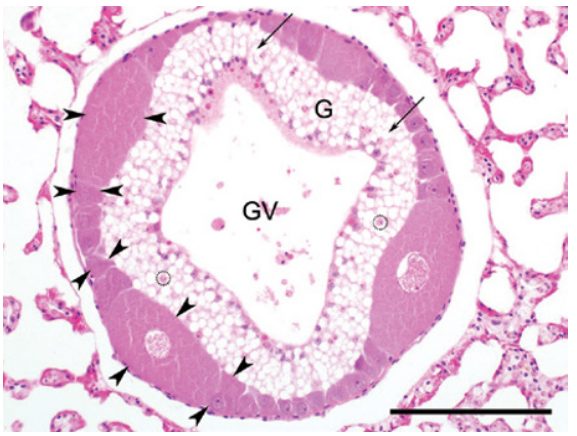


Figure 3.62 Oocytes (*arrowheads*) developing within the medusa stage in the fire coral, *Millepora* sp. Oocytes are in the mesoglea, circumferentially surrounding the gastrodermis (G) that contains a few zooxanthellae (*dashed circles*) and abundant lipid droplets, represented by clear spaces (*arrows*). The central lumen is the gastrovascular cavity (GV). Scale bar, 200 µm. 200×. HE. *Source:* Tissue section contributed by Alan Davis.

3.3.6.3 Asexual Reproduction

Cnidarians often incorporate both sexual and asexual forms of reproduction in their life cycles. Both polyps and medusae can undergo asexual or clonal reproduction, but asexual reproduction more commonly occurs with polyps (Figure 3.63). Forms of asexual reproduction include budding, fission, and pedal laceration. Colonial corals can increase in size by various forms of budding. Extratentacular budding occurs from the lateral polyp body wall outside the tentacles. Intratentacular budding occurs within the circle of tentacles. Fission includes both transverse (uncommon) and longitudinal (common) replication and occurs when the individual organism divides generally into two equal parts. Longitudinal fission occurs along the oral/aboral axis. Strobilation in various scyphozoan species is an example of transverse fission. Pedal laceration occurs at the base of a polyp (often in anemones); fragments of tissue separate from the pedal disc and develop into a new individual. Fragmentation of scleractinians can also be considered a form of asexual reproduction, but it is a passive type of separation when a portion of the stony coral colony breaks off from the parent colony as the result of trauma (e.g., strong wave action, something hitting the colony). Fragmentation is commonly noted in branching scleractinian species such as *Porites* sp., and acroporids. It is frequently used when propagating coral for culture and restoration efforts.

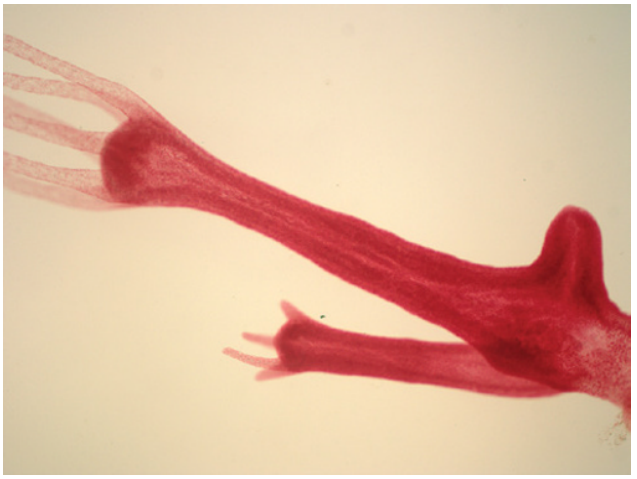


Figure 3.63 *Hydra* spp. exist as solitary polyps and do not have a medusoid form. Some species can produce gametes but more commonly, they reproduce asexually by producing buds in the body wall that break away as they mature. 4×. HE. *Source:* Copyright Carolina Biological Supply Company. Used by permission only.

3.3.7 Parasitic Myxozoa

Myxozoa were originally considered protozoans but are now identified as a subphylum of cnidarians with more than 2000 species. They are small (on average 10–20 μm in diameter) and have undergone an extreme reduction of the cnidarian body plan. The distinctive myxozoan structure, the polar capsule, is now considered to be the equivalent of the nematocyst (or "polar capsule type nematocyst") and the coiled polar filament within the polar capsule is the equivalent of the tubule (Americus et al. 2020). Myxozoa are obligatory endoparasites that use both invertebrate and vertebrate hosts in their life cycle (Chang et al. 2015) and are able to infect freshwater, marine, and terrestrial hosts (Atkinson et al. 2018). Life cycles for many species are still unknown, but generally involve spores with polar capsules containing polar filaments that anchor the spore to the host. Spores penetrating host tissues and developing into multinucleated plasmodia (Figure 3.64) can undergo various transformations to eventually form mature spores containing polar capsules (myxospores or malacospores) (Figure 3.65). They can cause significant economic diseases in cultured fish, including proliferative kidney disease (PKD) and whirling disease in salmonids, and proliferative gill disease (hamburger disease) in catfish.

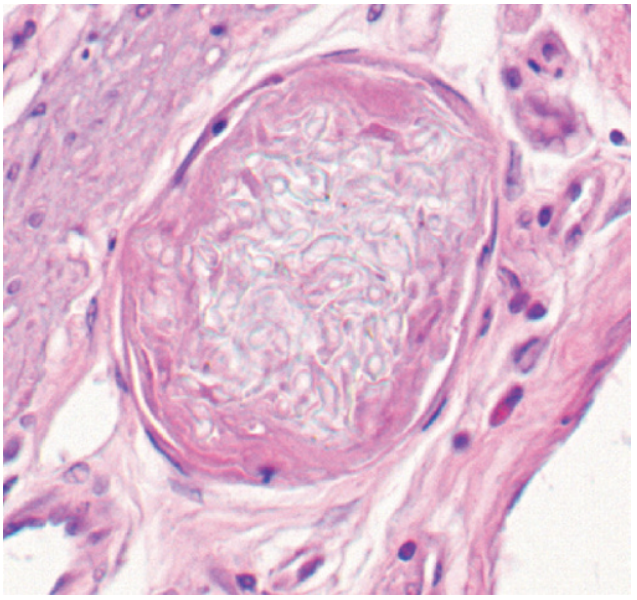


Figure 3.64 A myxozoan plasmodium located in the gill arch of the fish *Lepomis macrochirus*, commonly called bluegill. 600×. HE. *Source:* Photo credit: Alex Primus.

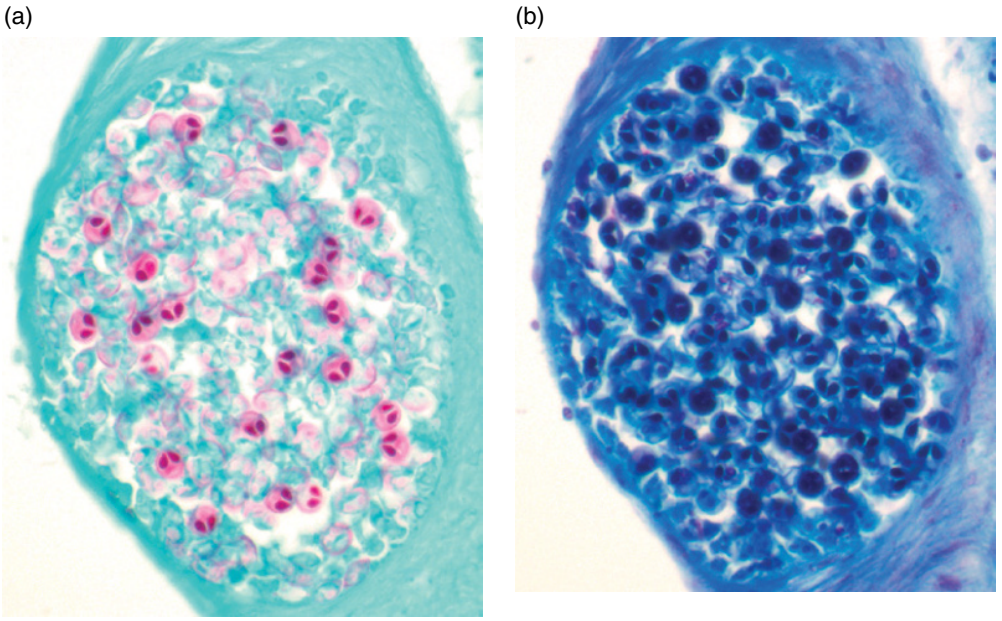


Figure 3.65 Polar capsules of myxozoan spores found in gill arch of *Lepomis macrochirus*. 600×. Acid-fast (a) and Giemsa (b) stains. *Source:* Photo credit: Alex Primus.

3.4 Conclusion

Cnidaria (excluding Myxozoa) have all the basic tissues found in the eumetazoans including epithelia, connective, muscle, and nervous tissues, organized to support and facilitate various physiologic functions such as absorption, reproduction, movement, etc. A complete understanding of the biochemistry and metabolic functions of these structures, however, is still lacking. This basic guide to key structures is intended to provide wider awareness of these animals and to encourage further investigations.

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Disclaimer

The views expressed in this chapter are those of the authors and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or US Government.

Appendix 3.1 Specimen Relaxation and Common Fixative Formulations

A) Magnesium sulfate solution
- 5 grams (g) magnesium sulfate
- 45 milliliters (mL) deionized water

Dissolve in a glass flask. Let the solution reach room temperature. Add the solution in 5 mL increments at 15–20-minute (min) intervals to the specimen maintained in sea water (specimen in a small volume of sea water, just enough to cover it). Continue additions until the specimen is unresponsive to touch. Remove and place into fixative. Patience is necessary, as well as developing innovative methods to keep the organism relaxed and tentacles extended. The amount of time needed to successfully relax the organism will vary with the species and its size.

B) Modified Helly's solution
- 20 g potassium dichromate
- 40 g zinc chloride
- 800 mL sea water

Combine in a glass container and mix well. Can store this solution until ready for use. When ready to use add:

- 12.5 mL 37–40% formaldehyde

Mix. Add specimens and agitate every few hours. Life span of final fixative only 24 hours. Rinse samples in run-

ning water for up to 8 hours or several changes of water over a 24-hour period. May require a few additional changes of 70% ethanol to remove any remaining fixative. Store in 70% ethanol until ready to process further.

C) 10% formalin-seawater fixative
- 10 mL 37–40% formaldehyde
- 90 mL sea water

Mix. Add specimens, cover the container and leave for at least 24 hours. Remove specimen and rinse in fresh water for 5–12 hours prior to processing. May be stored in the sea water-buffered fixative indefinitely.

D) Z-Fixative (Zinc formaldehyde).
- Z-Fix® Concentrate (order from Anatech Ltd. www.anatechltdusa.com)
- Filtered ambient sea water

Mix one part Z-Fix® Concentrate with four parts filtered sea water (should be the same salinity sea water as that in which the organisms have been living); for example, 50 mL Z-Fix® Concentrate and 200 mL sea water will make 250 mL of this fixative solution. Place specimens in solution for at least 48 hours; they can remain in this fixative solution indefinitely.

Appendix 3.2 Basic Histology Protocol for Processing Scleractinian Corals (refer to Price and Peters (2018) for more detailed techniques)

1) Filter sea water through a 0.2 µm filter to be used for the fixative solution.
2) Prepare fixative solution using one part Z-Fix® to four parts filtered sea water.
3) Using a tile saw, cut coral fragments measuring less than 2 cm × 2 cm × 1 cm.
4) Place each coral fragment in a container with fixative so that the fixative-to-coral ratio is at least 10 : 1.
5) Fix for at least 24 hours (may remain in fixative indefinitely), gently agitating (swirl fixative in the container) every few hours.
6) Empty fixative from container, and refill the container with clean sea water or tap water, change water 3–5 times.
7) For samples to be agar enrobed to help maintain tissue orientation (Figure 3.66):
   a) Make 1.5% agar solution (15 g SeaKem® Agar/L fresh water) using hot (90 °C) distilled water with stirring.
   b) Allow agarose to cool to 60 °C.
   c) Drain specimens well, allow to air dry but do not completely dry out.

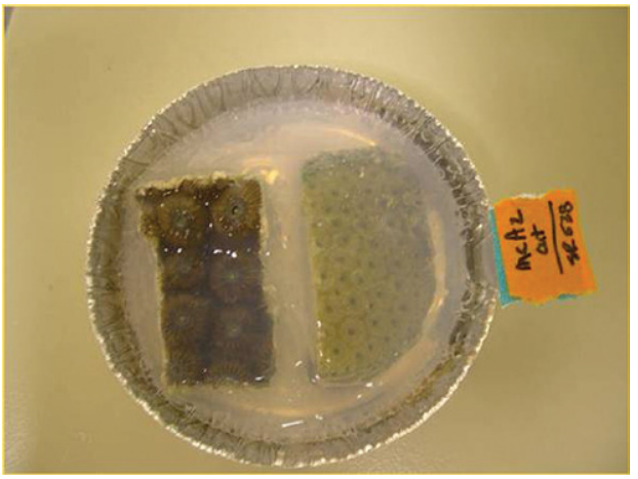


Figure 3.66 Fragments of coral specimens enrobed in agar. *Source:* Photo credit: Kathy Heym.

   d) Place coral fragments in labeled weigh boats and cover the fragments with agar.
   e) Preheat vacuum oven to 40–56 °C (gel temperature).
   f) Place in vacuum oven and pull pressure of 25 mmHg twice. Draws agar into crevices.
   g) Allow to cool before beginning decalcification steps
8) Decalcification (Figure 3.67a–c):
   a) Scrape away the agar from the skeleton side (not the tissue side) until there is ¼" agar remaining on each side.
   b) Cut window in agar to expose the calcium carbonate exoskeleton.
   c) Place sample in container and cover with 10% neutral pH EDTA decalcification solution so that there is an excess of this solution above the sample.
   d) Raise sample above container bottom so that decal solution penetrates all surfaces.
   e) Swirl decal solution in container several times a day to expose tissue to fresh solution.
   f) Change decal solution every 2–3 days; it may take 1–2 weeks to decalcify, depending on the density of the skeleton.
   g) Be careful to remove fragment from decal solution as soon as it is decalcified to avoid overexposure (leads to poor staining).
   h) Rinse fragment well in deionized water.
   i) Orient coral tissue in cassettes for longitudinal sections and oral/aboral cross-sections.
   j) Proceed with routine paraffin processing.
   k) Stain with routine stains (hematoxylin and eosin) or special stains as needed.

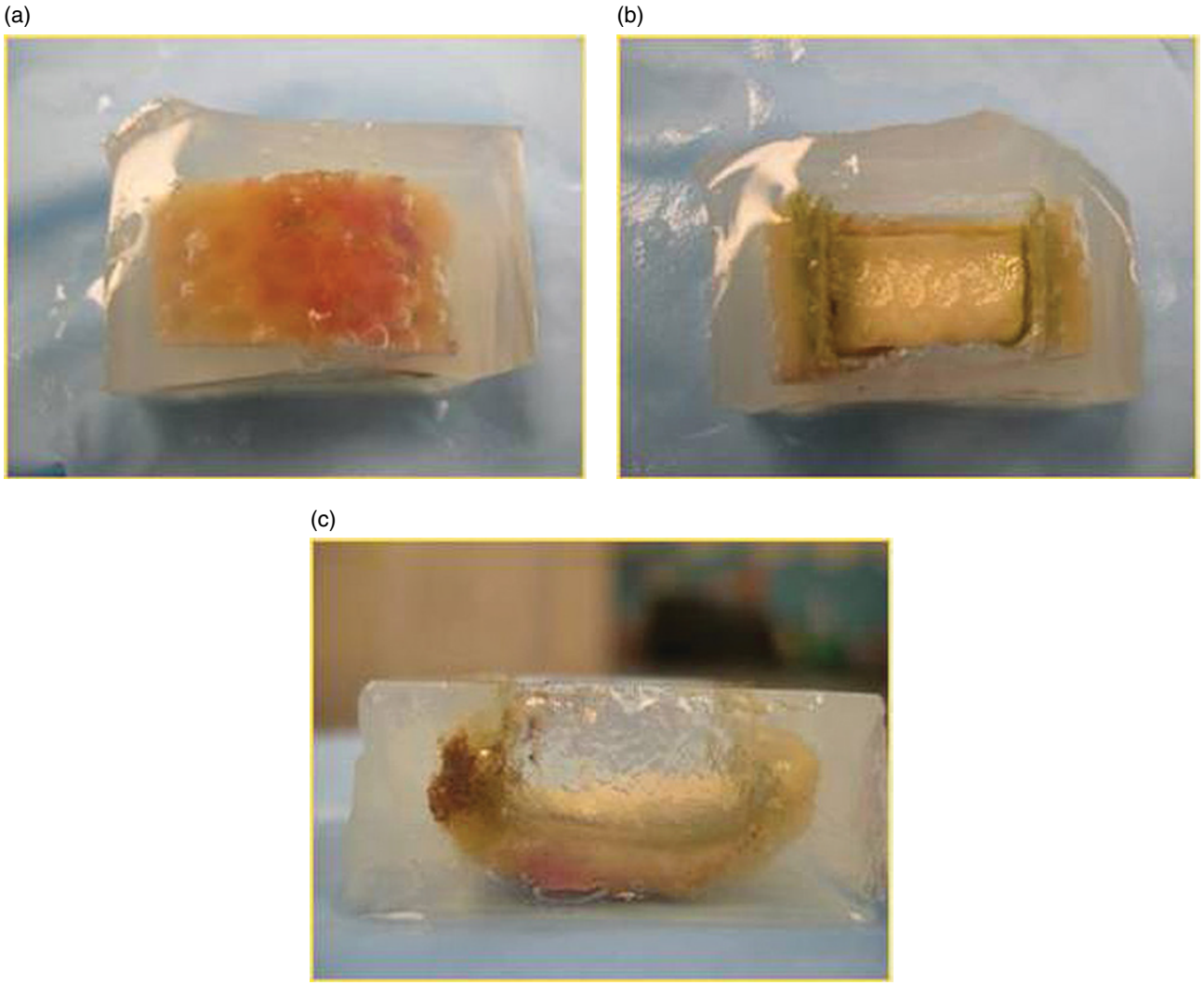


Figure 3.67 Decalcification of coral fragment enrobed in agar. (a) Remove excess agar from fragment. (b) Scrape away agar to expose the calcium carbonate skeleton. (c) Fragment with skeleton dissolved but with coral tissue still present. *Source:* Photo credit: Kathy Heym.

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4

Mollusca

Gastropoda

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4.1 Introduction

4.1.1 Taxonomy

Gastropoda is the largest group in the phylum Mollusca, comprising over 25 000 genus-group names that encompass snails, conch, whelks, cowries, abalone, limpets, slugs, sea hares, and nudibranchs among others (Bouchet et al. 2017; Bouchet and Rocroi 2005). Of the oldest and most evolutionarily successful animals, gastropods are globally distributed and well represented in marine, freshwater, and terrestrial habitats, and their taxonomic diversity is exceeded only by insects (McArthur and Harasewych 2003). The taxonomy, complex and highly debated, is presently under revision, allowing new insights from genetic studies to integrate with the classic phenetics approach. The revised classification approximates evolutionary relation more accurately and is arranged into clades according to common ancestors (Bouchet et al. 2017). Despite these changes, terms based on abandoned subclasses remain in use where they are morphologically informative. For example, opisthobranchs are gastropods with gills to the right and behind the heart, prosobranchs are those with gills in front of the heart, pulmonates are those with lungs instead of gills, and gymnomorphs are those without shells.

4.1.2 Life History

4.1.2.1 Life Expectancy

The life span differs greatly among gastropods. Most of clade Heterobranchia, which includes the majority of terrestrial gastropods, live around one year. In contrast, clade Caenogastropoda often live much longer, even decades. For example, the queen conch (*Lobatus gigas*) is thought to become sexually mature after 3–4 years and have longevity of up to 30–40 years.

4.1.2.2 Reproduction

Similar to other molluscs, the first gastropod life stage consists of free-swimming ciliated trochophore larvae. The next development stage is the veliger, characterized by the growth of lobes, and a torsion process that is unique to gastropods. The body twists 180°, such that the anus and visceral mass become located just behind the head. Over time, currents may disperse these larval phases across long distances. After a period ranging from weeks to months, the veliger metamorphoses into a settled, crawling juvenile. Growth is typically rapid until reaching sexual maturity.

Gastropods reproduce sexually although some parthenogenetic species exist. They are typically dioecious but some are simultaneous hermaphrodites, such as most pulmonates, or sequential hermaphrodites, such as members of superfamily Calyptraeoidea. Some simultaneous hermaphrodites may be capable of uniparental reproduction (Leonard et al. 2007). Sequential hermaphrodites are usually protandric (male-to-female change), but protogyny (female-to-male change) also occurs. Most species have anatomic structures that permit internal fertilization but external fertilization also occurs, particularly in primitive marine species. Gastropods typically copulate during a spawning season, some species showing seasonal movement patterns to meet their mates, and some displaying curious courtship or mating behaviors. Pulmonate snails and slugs may shoot calcareous "love darts" into their mates thought to promote sperm survival (Davison et al. 2005). Apophallation, where the penis is occasionally severed during copulation and then

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eaten by the partner, is poorly understood and best documented in terrestrial slugs (Leonard et al. 2007).

In many gastropods, a storage organ of the female reproductive tract holds sperm prior to fertilization. A mucus- and nutrient-rich secretion ("egg capsule") typically encases fertilized eggs, which may be stored in the uterus until oviposition. Usually, an egg mass is produced at oviposition which may have been fertilized by more than one individual. Females of some species brood developing larvae within anatomic pouches, the mantle cavity, or just inside the shell. Most gastropods are iteroparous, having multiple reproductive cycles over a lifetime. Semelparity occurs in association with annual life span, particularly observed in the clade Heterobranchia (Zając and Kramarz 2017).

4.1.2.3 Diet

The adult gastropod diet varies nearly as wide as their taxonomy. The radula, a tongue-like chitinous structure used for prehension of food, is configured to suit the diet, so much so that its morphology can be used for species identification. Herbivorous and detritivorous species use the radula to scrape food from surfaces. Predatory carnivorous cone snails use a specialized harpoon-like radula supplied by a venom gland to paralyze prey. Other predatory marine snails use the radula to drill through the shells of echinoderms or other molluscs.

4.1.3 Relevance

Gastropods have been an important food source since early human history. Today gastropod agriculture and fisheries are highly valued in international and artisanal markets, including queen conch (*L. gigas*) in the Caribbean, abalone (*Haliotis* spp.) in the Pacific, and periwinkles (*Littorina* spp.) and garden or Roman snails (*Helix* spp.) in Europe. Additionally, many cultures value jewelry or decorations made from the shells of gastropods. Neurobiologists use the nudibranch *Hermissenda crassicornis* and the Californian sea hare (*Aplysia calfornica*) as laboratory animals to study learning and memory. On the other hand, there are numerous problematic invasive gastropods, some representing serious agricultural or ecosystem pests while others are devastating vectors of parasitic diseases. Examples include the Spanish slug (*Arion vulgaris*), the giant African snail (*Lissachatina fulica*), apple snails (family Ampullariidae), the Budapest slug (*Tandonia budapestensis*), the Chinese mystery snail (*Bellamya chinensis*), the white garden snail (*Theba pisana*), and the veined rapa whelk (*Rapana venosa*), among many others. Gastropods are medically significant due to their role as intermediate hosts for flatworm parasites including several species of freshwater snails for schistosomes of birds, humans, and other mammals; pond snails (family Lymnaeidae) for the sheep liver fluke (*Fasciola hepatica*); and several species of terrestrial snails and slugs for the serious zoonoses caused by the rat lungworm (*Angiostrongylus cantonensis*).

4.2 Gross Anatomy

The presence of a well-defined head and foot, and asymmetric organs, a feature resulting from the unique torsion process that occurs during embryonic development, characterize the gastropods. The anus and visceral mass (containing much of the digestive and reproductive tracts, kidney, and heart) lie dorsal to the head and to one side of the median plane of the body. Many organs that are paired in other molluscs, such as gill (or ctenidium), gonad, and kidney (or nephridium), are undeveloped or lacking on one side (usually the left) of the body. Some gastropods, particularly opisthobranchs and pulmonates, detort in later developmental stages such that these features may be less obvious (Gosliner 1994). Many gastropods have a shell, in which case it is usually spiraled with a right-handed aperture (Figure 4.1) (Hyman 1967), but may be absent or reduced, especially in

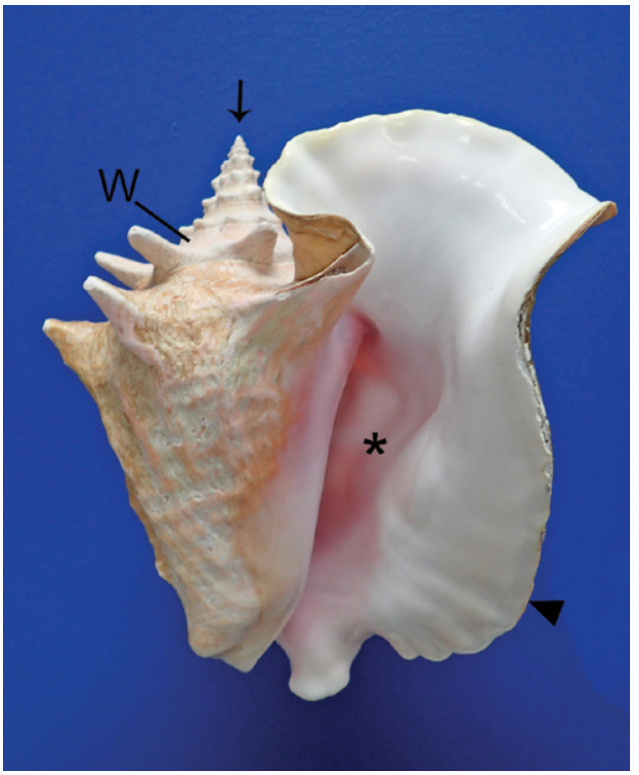


Figure 4.1 Shell of *Lobatus gigas*. The apex (*arrow*) of the shell was formed first and is at the caudal aspect of the shell, at the tip of the spire. The final whorl (W) of the shell opens to form the aperture (*asterisk*) through which the head and foot protrude and may be closed by an operculum. The aperture usually opens on the right side, as pictured here, and is bordered by inner and outer (*arrowhead*) lips.

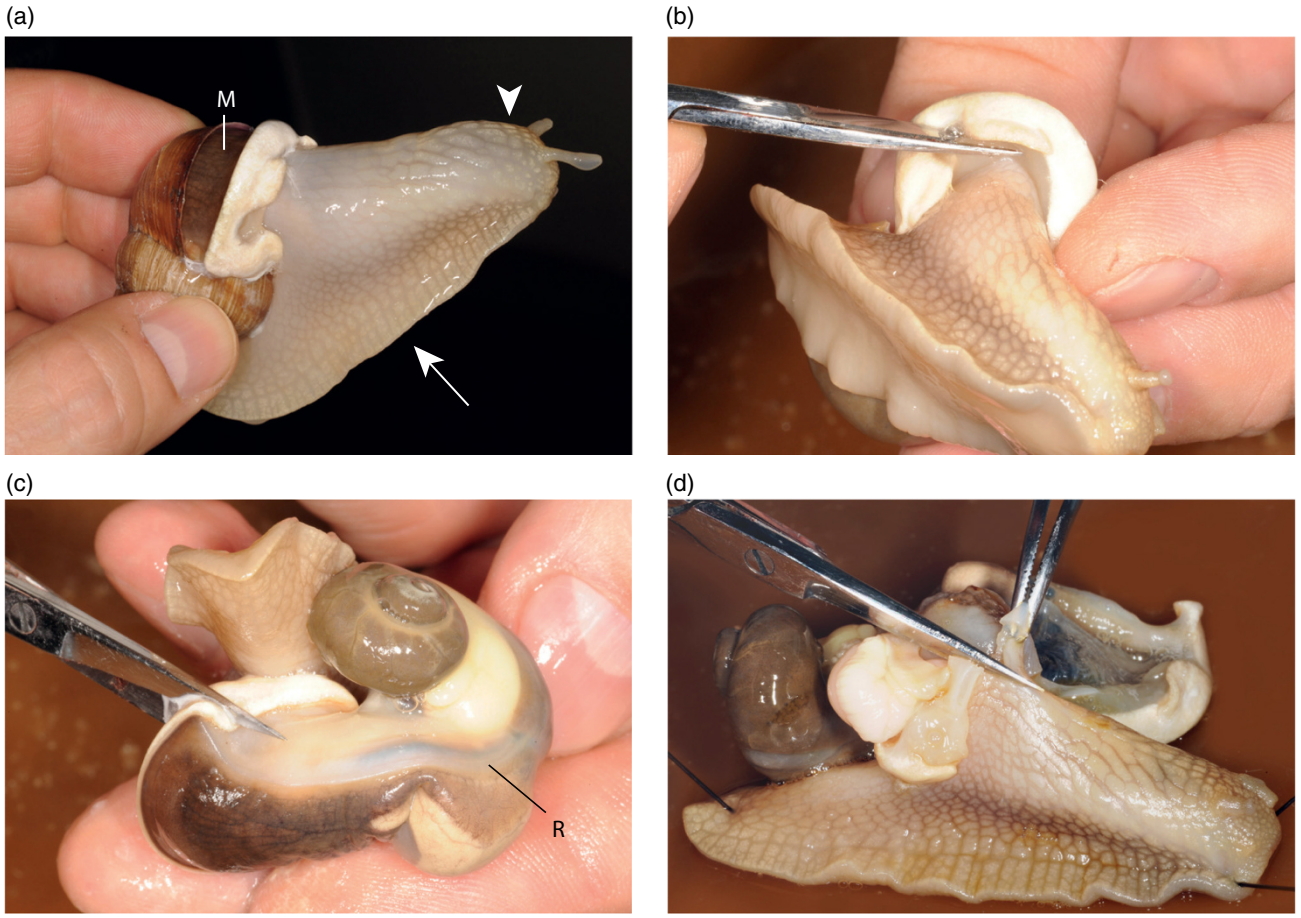


Figure 4.2 (a) *Helix pomatia* in anesthetized state. The head (*arrowhead*) and foot (*arrow*) of the gastropod merge to form the body, which extends beneath the mantel (M). (b–d) The necessary cuts to dissect the body. (b) The first cut of the dissection under the mantle edge. (c) The second cut follows the rectum (R) to the highest level of the pulmonary chamber. (d) The third cut goes from the visceral mass forwards through the body wall along the dorsal midline to the dorsal lip of the mouth.

opisthobranchs. It is typically composed of calcium carbonate as aragonite, sometimes containing calcite, in an organic protein matrix termed conchin. An operculum, a horn-like or calcareous process attached to the posterior foot, serves to close the shell's aperture in prosobranchs (Voltzow 1994).

Typically, the gastropod head bears a pair of eyes, tentacles (or rhinophores), and buccal mass (or proboscis, i.e., mouth and pharynx) (Figure 4.2). Within the final whorl of the shell, the mantle lines the inner shell, forming a space which encloses the head, gill (or lung), and openings of the digestive, excretory, and reproductive tracts; this space is termed the mantle (or pallial) cavity (Figure 4.3). The mantle that forms the roof of the mantle cavity contains the pallial complex (collectively, the gill[s], osphradium, and hypobranchial glands). The nervous system consists of paired ganglia connected by a nerve loop throughout the head, foot, and visceral mass (Hyman 1967; Little 1965). The gastrointestinal tract courses in a U-shape from the buccal mass, through the body, then the visceral mass, finally terminating in the mantle, near the head.

Gastropods have an open circulatory system where hemolymph flows through sinuses rather than endothelium-lined capillaries (Jones 1983). The heart, made of a single ventricle and auricle and lying within a pericardial sac, works to direct hemolymph flow from the organs to the kidney and gill, and then back to the heart. Hemocyanin is the copper-based oxygen-carrying pigment that gives hemolymph its blue tinge. Marine gastropods have a gill for respiration, whereas terrestrial and freshwater gastropods have a pallial lung (Figure 4.4) (Luchtel et al. 1997). The lumen of the kidney is continuous with the pericardial space through the renopericardial pore, and empties into the mantle cavity through the nephridiopore, adjacent to the anus (Voltzow 1994).

There is profound variation in the reproductive anatomy of gastropods such that only generalizations can be provided here (Hyman 1967). The gonad (or ovotestis in hermaphrodites) is always solitary and confluent with the digestive gland. In lower gastropods that fertilize externally, the reproductive system consists of the gonad and a

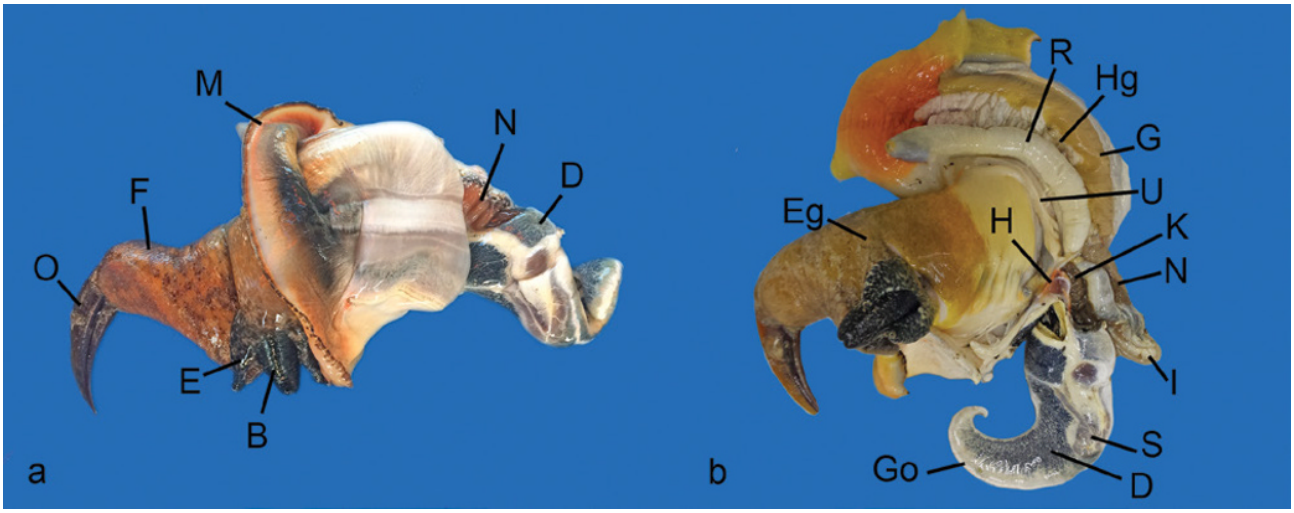


Figure 4.3 Two female *Lobatus gigas*, removed from shell; (a) mantle cavity unopened, and (b) mantle cavity opened. The operculum (O) is attached to the foot (F) which is continuous with the head, bearing two eyestalks (E) and a buccal mass (B). A verge is present on the body of males, and an egg groove (Eg) on the body of females. The visceral mass is viewed once the animal is removed from the shell and from its exterior, the digestive gland (D), stomach (S), and gonad (Go) are evident. The mantle (M) forms a cavity that contains the gill (G), hypobranchial glands (Hg), rectum (R), and distal reproductive tract (U). Further dissection is required to reveal the heart (H), kidney (K), nephridial gland (N), and intestine (I).

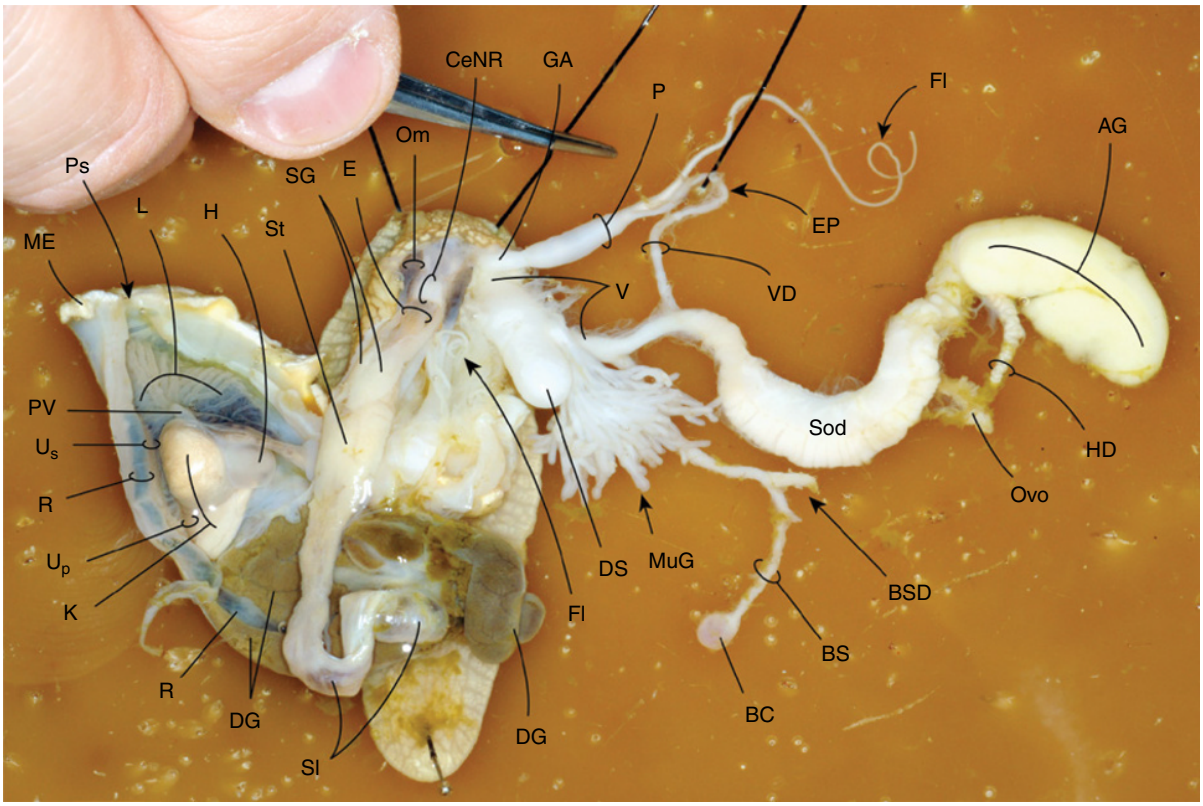


Figure 4.4 Dissected *Helix* in water cover displaying the internal organs. AG, albumen gland; BC, bursa copulatrix; BS, bursa stalk; BSD, bursa stalk, diverticulum; CeNR, circumesophageal nerve ring on pharynx; DG, digestive gland; DS, dart sac; E, esophagus; EP, epiphallus; Fl, flagellum; GA, genital atrium; H, heart in pericardium; HD, hermaphrodite duct; K, kidney; L, lung; ME, mantle edge; MuG, mucous gland; Om, ommatophore; Ovo, ovotestis; P, penis; Ps, pneumostome; PV, pulmonary vein; R, rectum; SG, salivary gland; Sl, small intestine; St, stomach; Sod, spermoviduct; U_p, ureter, primary; U_s, ureter, secondary; V, vagina; VD, vas deferens.

gonoduct that opens into the kidney. In male gastropods that fertilize internally, sperm pass through a sperm duct (or vas deferens) to the prostate, where mucous secretion suspends sperm (Reed 1995a). Sperm exit through the gonopore, and in some species, through a penis (or verge) on the right side of the head. The penis attaches to the female bursa copulatrix. In the female, ova pass through a duct (oviduct) from the gonad to a uterus (or ampulla, pallial oviduct) (Hyman 1967; Reed 1995b). There may be an adjacent seminal receptacle (or spermothecae) where sperm can be stored. Secretion from nidamental glands (variously termed mucus, jelly, albumen, or capsule glands, depending on the nature of the secretion) encapsulates eggs and forms the egg mass. The uterus may store fertilized eggs until they pass down the ovipositor groove at oviposition. In the hermaphrodite, the ovotestis produces eggs and sperm simultaneously (Duncan 1958). A hermaphrodite duct leaves the ovotestis and branches into a sperm duct and oviduct. The sperm duct will proceed to a prostate and penis, whereas nidamental glands, a bursa copulatrix, and potentially a seminal receptacle, supply the oviduct.

4.3 Histology

This section provides an overview of the microscopic anatomy of each major tissue with the ambitious aim of summarizing the basic features across a broad and diverse taxonomic group. There is a need for research that clarifies fundamental differences among clades and readers should be mindful that some species may not strictly confine to the generalizations made here. The well-studied pulmonate Roman snail (*Helix pomatia*), a subject of interest for the chapter's authors and arguably one of the most economically important gastropods, is used as a prototypical example.

4.3.1 Integument

Integument covers the entire surface of the gastropod's soft tissues, including head, foot, body, mantle and, although not apparent grossly, even the outer visceral surfaces. The role of the integument is to provide a protective barrier. It may also facilitate respiration sensation, locomotion, absorption of nutrients, osmoregulation, secretion of substances, and reproduction. Secretions include mucus, defensive substances, and shell components.

The integument consists of an epithelial surface, commonly referred to as epidermis. Regardless of location, histologically the epidermis comprises a single layer of tall cuboidal to columnar epithelial cells supported by a basement membrane (Figure 4.5). The epithelium consists of epidermal cells (the generic columnar cell of the epidermis), ciliated cells, and mucocytes, all of which bear a microvillus border that may be difficult to discern histologically. There may also be other types of secretory cells, pigmented cells, and sensory cells. Similar to other molluscs, pigments present in gastropod tissues including the epidermis are diverse and can include carotenoids, indigoids, melanin, porphyrins, and bilichromes (Fox 1983). Secretory cells may form intraepidermal glands (for example, hypobranchial gland, Figure 4.6), or may invaginate to form tubules or subepidermal glands. In opisthobranchs, a wide variety of secretory cells are observed. Some may project well into the subepidermal connective tissue, and be relatively large and distended with discrete vesicles such that they might not be recognizable as cells unless the nucleus is in section. In some species, epidermal cells or associated glands may show bioluminescence (Tsuji 1983) and in others, harbor symbiotic dinoflagellates (zooxanthellae) (Wägele and Johnsen 2001). A dermis is not distinct in gastropods and thus, the epidermis is supported by connective tissue blending with and similar to that found throughout the body. Although not readily apparent with HE stains, sensory neurons and their processes lie just beneath the epidermis.

The body surface of *Helix* is covered with a single-layered columnar epithelium equipped with both cilia and microvilli. Large mucus-secreting and mucus-storing cells extend deep into the subepithelial matrix, surrounded by individual smooth muscle cells (SMC) and connective tissue.

4.3.2 Connective Tissue

The body, visceral mass, foot, mantle, and shell are bound together by connective tissue. In prosobranchs connective tissue is typically fibrous and frequently contains abundant mucus-producing and glycogen-storing cells called "Leydig" (or vesicular or chondroid) cells (Figure 4.7) (Luchtel and Deyrup-Olsen 2001; Voltzow 1994). Cells containing deposits of calcium and other minerals are also not uncommon. In opisthobranchs, connective tissue is similar. Stellate cells predominate, intermingled with glycogen-storing Leydig cells and a few muscle fibers, and may also contain calciferous cells, plasmatic cells, rhogocytes (Wägele 1998), and pigmented cells (Figure 4.7b) (Gosliner 1994). Nerves and hemolymphatic vessels are present throughout connective tissue.

Comparable special cell types have been identified in the connective tissue of terrestrial gastropods including rhogocytes, granular cells, and hemocytes. Rhogocytes are large, usually spherical to elongate in shape, but occasionally branched and somewhat irregular in form. Their most characteristic feature is the cell surface forming a system of surface cisternae that is partly enclosed yet communicates

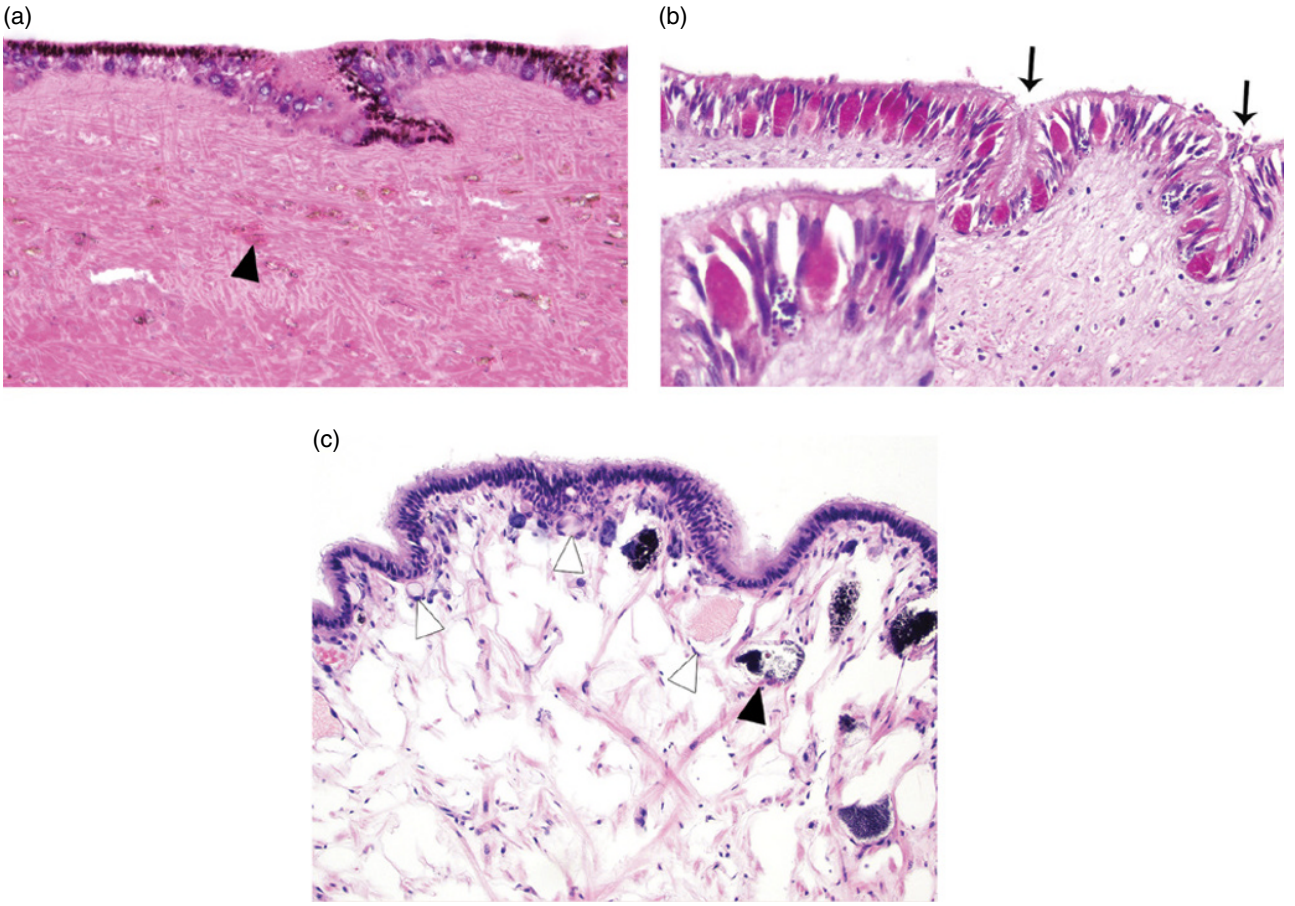


Figure 4.5 Comparison of integument in (a) mantel of *Littorina ziczac*, 400×, HE; (b) body of *Coralliophilia* sp., 400×, HE (inset: 1000×, HE); (c) body of *Aplysia californica*, 200×, HE. The epidermis consists of simple columnar ciliated epithelial cells, including many secretory cells and pigmented cells. Inset of (b) shows higher magnification of epidermal cells and more than one type of secretory cell. The epidermis may be sinuated forming gland-like invaginations (*arrows*). Opisthobranchs often have subepidermal gland cells (*white arrowheads*). The subepidermal connective tissue is similar to that found throughout the body, fibrous and often interspersed with pigment cells (*black arrowheads*). Source for (c): Elise LaDouceur.

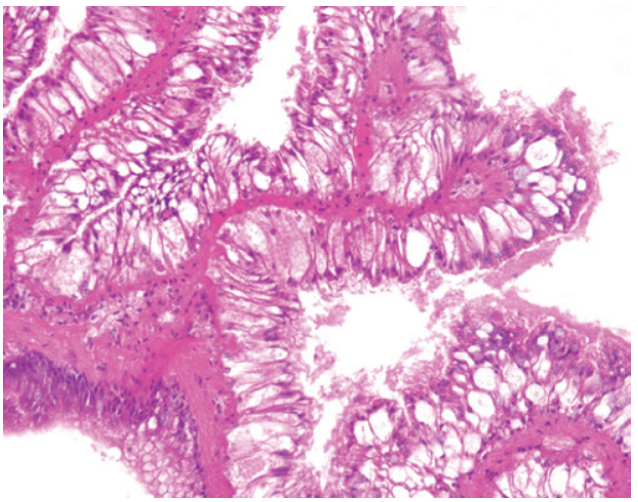


Figure 4.6 Hypobranchial gland of *Cittarium pica*. The gland is composed of columnar epithelial cells and mucocytes that form fronds supported by connective tissue septae. 400×. HE.

with the extracellular space. The name rhogocyte – from Greek *rhogos* which means "slit" – refers to the slits on the cell surface leading to these cisternae. The slits are bridged with fine, fibrillar diaphragms. Rhogocytes possess numerous lysosomes, lipid droplets, granules, and abundant rough endoplasmic reticulum. They may have extensive glycogen stores that can displace the spherical nucleus to a peripheral position. These cells play an important role in metal ion metabolism, possibly involved in the recycling of respiratory pigments and in metal detoxification. The large amount of glycogen found in rhogocytes suggests that these cells may also be involved in transport or storage of nutrients (Skelding and Newell 1975). Granular cells are characterized by cytoplasm that contains glycoprotein granules that sometimes pack the cells enough to deform the nuclei. The granules originate from the Golgi apparatus and are secreted by exocytosis (Steinbach 1977). Hemocytes are described in section 4.3.8.2.

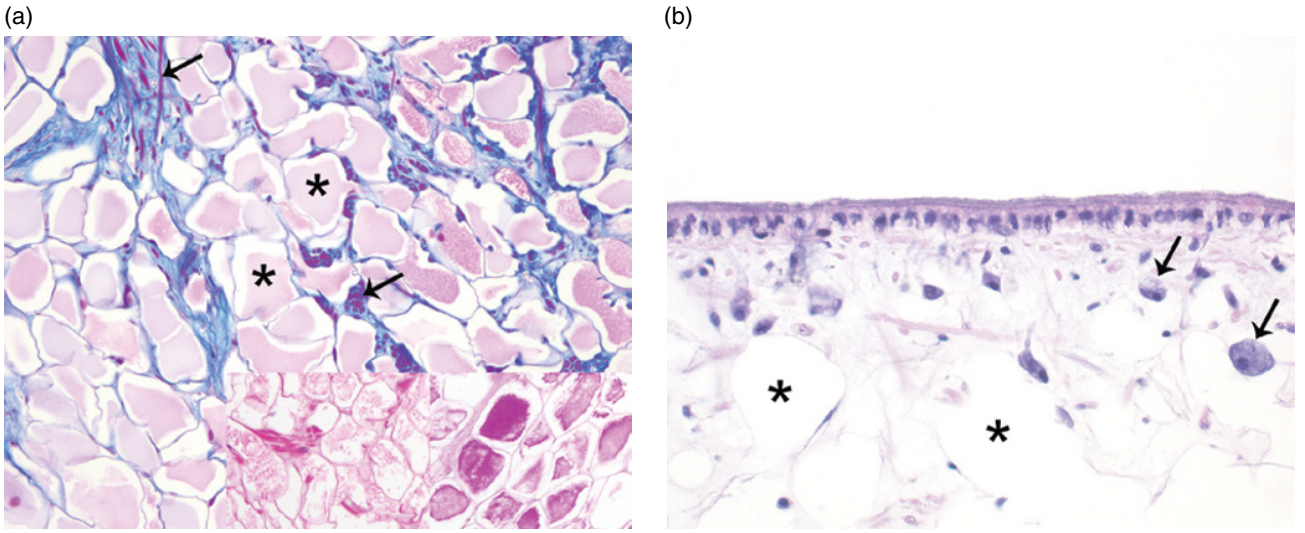


Figure 4.7 (a) Connective tissue of the body of *Lobatus gigas*. Fibrous connective tissue contains many vacuolated Leydig cells (*asterisks*) and myocytes (*arrows*). 400×. Masson's trichrome stain. Inset left: Myocytes are difficult to differentiate from collagen with HE stain. 400×. HE. Inset right: Leydig cells contain glycogen. 400×. PAS. (b) Connective tissue of the body of *Aplysia californica*. Fibrous connective tissue containing Leydig cells (*asterisks*) and plasmacytic cells (*arrows*). 400×. HE. Source for (b): Elise LaDouceur.

4.3.3 Mantle

The mantle is a fold of body wall that covers the visceral mass. It consists of muscle and connective tissue with an integument surface. In shelled gastropods, the mantle lines the inner shell surface of the last whorl and, at its anterior aspect, forms a skirt around the mantle cavity. It may also form a siphon to draw water into the mantle cavity, bear tentacles or papillae, or have lobes that can extend to cover the shell. In opisthobranchs, the mantle can similarly extend to form flaps. In addition to being a protective barrier from the environment, it is responsible for shell formation, mucus production, sensation, and may have roles in respiration and assimilation of nutrients (Voltzow 1994).

Histologically, the mantle epidermis composition varies by location (Figures 4.8 and 4.9). On the inner or ventral surface of the mantle, cells are typically heavily ciliated and more generously interspersed with other specialized cells types, including pigmented and secretory cells (Hyman 1967). The dorsal mantle epidermis comprises mainly epidermal cells with fewer specialized cell types and may be thrown into folds resembling glands. The connective tissue supporting the mantle is similar to connective tissue throughout the body.

Mantle-edge glands are responsible for production of the shell (Bevelander and Nakahara 1970; Kapur and Gibson 1967). These typically consist of acini of tall cuboidal epithelial cells and secrete to the surface without a clearly defined duct (Figure 4.10). Potentially to provide added protection where the shell has been lost, opisthobranchs may have defensive glands within the mantle tissue, with a variety of names, locations, compositions, and secretions (Àvila and Durfort 1996; Bezerra et al. 2006; Klussmann-Kolb 2004; Wägele 1998). In *Aplysia*, an ink gland may be present, composed of large flask-shaped secretory cells that contain granular to clear vesicles of secretory material, released through a short duct lined with cuboidal epithelial cells at the secretory cell's apex (Figure 4.11) (Bezerra et al. 2006; Prince et al. 1998). These are surrounded by muscle and collagen fibers, and other "dispersed" epithelioid cells (Bezerra et al. 2006).

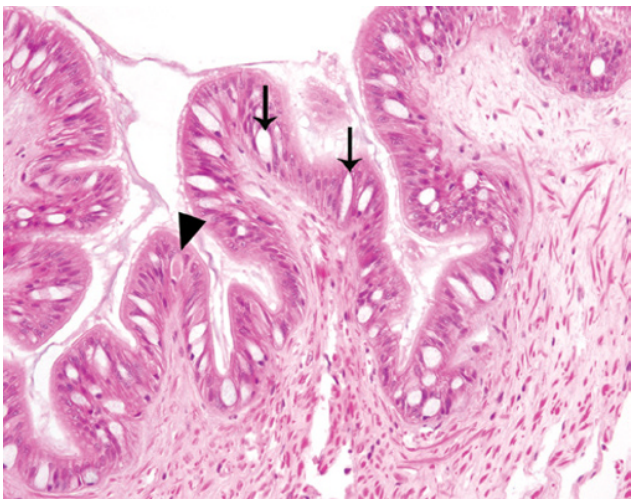


Figure 4.8 Ventral (interior) mantel of *Lobatus gigas*. Epidermal cells are ciliated and interspersed with many mucocytes (*arrows*) and other secretory cells (*arrowhead*). Beneath the epidermis is fibrous connective tissue containing many myocytes. 400×. HE.

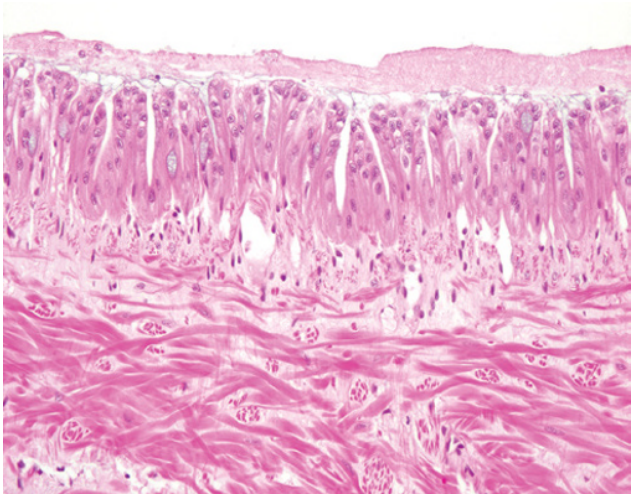


Figure 4.9 Dorsal (exterior) mantel of *Lobatus gigas*. Epidermal cells are less specialized but more heavily sinuated. 400×. HE.

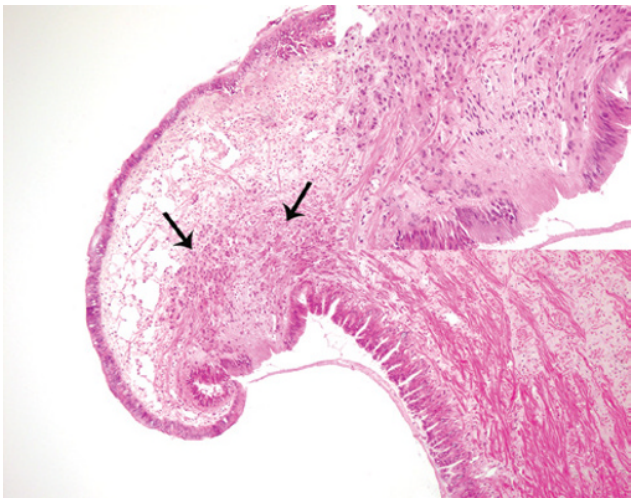


Figure 4.10 Mantle edge of *Lobatus gigas*. Arrows designate the mantle edge gland. 100×. HE. Inset: Higher magnification revealing detail of the gland, acini of tall cuboidal epithelial cells that empty to the surface without a clearly defined duct. 200×. HE.

Although the mantle in *Helix* does not have folds, the mantle edge can be subdivided into three zones (Figure 4.12).

1) An *inner glandular zone* is distinguishable because of the presence of numerous glands underneath a cuboidal epithelium. There are five types of gland cells.
   - Glandular epithelial cells: pyramidal shape, low microvilli, secretory surface; inclusions in the cytoplasm: probably protein crystalloids; ciliated cells are irregularly distributed
   - Mucus cells: sack-shaped mantle glands with granular secretion (MC1) and flask-shaped glands with a colorless, transparent secretion (MC2)
   - Calcium glands
   - Lipid cells
   - Protein glands
2) In the middle there is the *periostracal groove* or gland. It runs parallel to the mantle edge and is composed of tall columnar cells
   - Epithelial cells are pyramidal, with secretory surfaces less broad than basal regions; closely packed microvilli on the secretory surfaces; The nuclei are nearly central; masses similar to the droplets but not bound

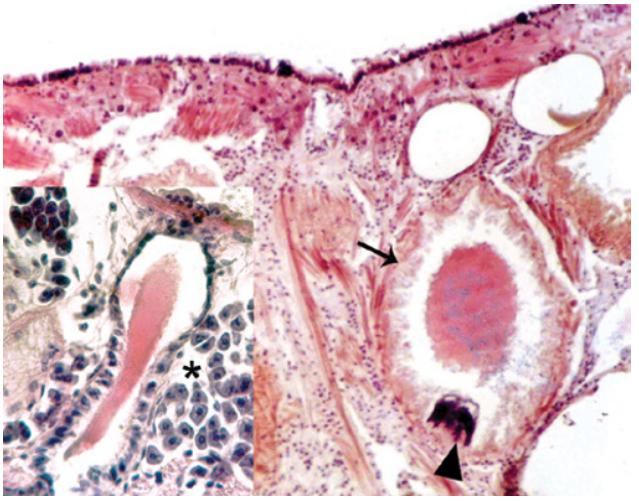


Figure 4.11 Ink gland of mantle edge of *Aplysia dactylomela*. A large flask-shaped secretory cell (*arrow*) contains a vesicle filled with ink secretion. Arrowhead designates the cell nucleus. 100×. HE. Inset shows detail of the duct, lined by cuboidal epithelial cells, and marginated by dispersed epithelioid cells (*asterisk*). 400×. HE. *Source:* Luis E. A. Bezerra and Vânia M. M. Melo.

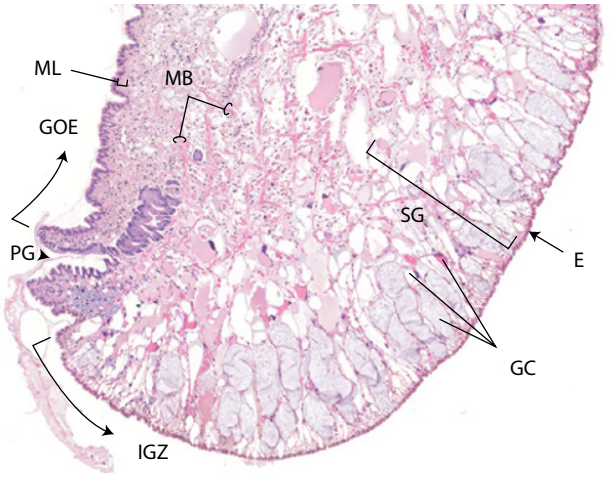


Figure 4.12 Overview of the mantle collar with zones of *Helix*. E, epithelium; GC, gland cells; GOE, general outer epithelium; IGZ, inner glandular zone; MB, muscle bundles; ML, muscle layer; PG, periostracal groove; SG, subepithelial glands. 40×. HE.

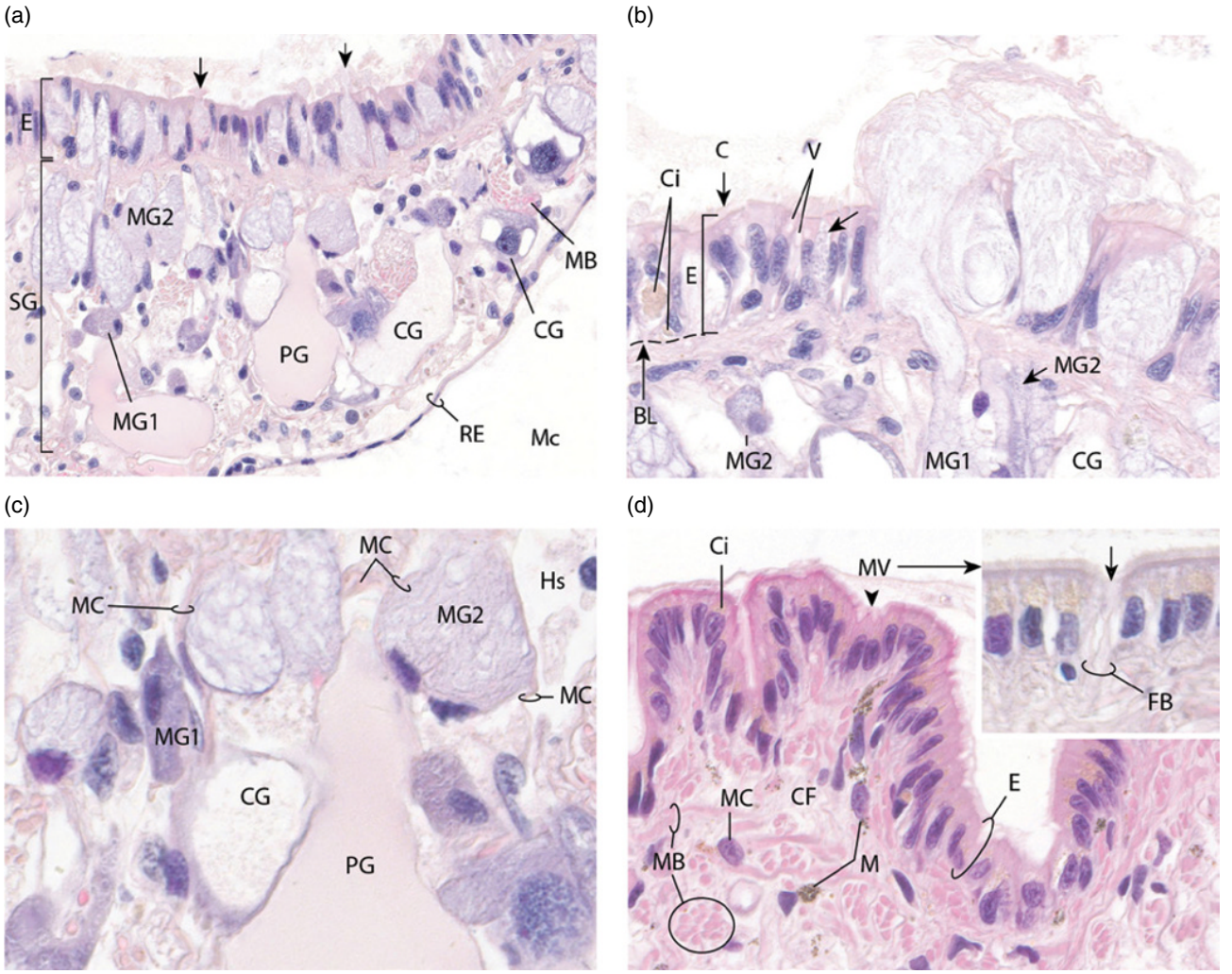


Figure 4.13 Histologic structure of the mantle collar of *Helix*. (a) Mantle collar (at the lung) with unicellular gland types. 300×. HE. (b) Ciliated epithelium near the pneumostome. 500×. HE. (c) Mantle collar gland cell types. 900×. HE. (d) General outer epithelium at mantle collar and beneath the shell (inset). 60×. HE, inset 600×. BL, basal lamina; C, cilia; CF, collagen fibers; CG, calcium gland; Ci, cytoplasmic inclusion; E, epidermis; FB, fibrous bundles for attaching to the shell; Hs, hemolymph sinus; M, melanocytes; MB, muscle bundles; Mc, mantle cavity; MC, muscle cell; MG1, mucous gland with granular secretion; MG2, mucous gland with transparent secretion; MV, microvilli; PG, protein gland; RE, respiratory epithelium; SG, subepithelial glands; V, vacuoles; arrows, secretion.

by membranes were always between microvilli. The presence of this secretory material between microvilli shows its movement toward the lumen of the groove and suggests that secretory material may contribute to shell secretion.

- The periostracal groove seems to indicate its secretory nature and involvement in the secretion of certain components of the shell (Figure 4.13). Although the name suggests that the groove may elaborate periostracum, there is little evidence in support of this. A better name would be "shell gland."

3) The *general outer epithelium* consists of tall columnar cells near the periostracal groove and low columnar cells in the proximal part of the mantle.

- The outer epithelial cells are tall columnar cells with light brown inclusions near the secretory surface: these are either lipofuscin or melanin.
- The cells contain characteristic lamellar organelles: these may be transformed mitochondria.
- The cells of the outer mantle epithelium which form shell (Saleuddin 1970).

The shell of *Helix* consists of calcium carbonate ($CaCO_3$) in the aragonite phase. Morphologic analysis showed the microstructure of the shell, composed of parallel lamellae and a very different appearance of the inner and outer shell surface. The inner surface is covered by longitudinal structures, whereas the outer surface is covered by a layer of

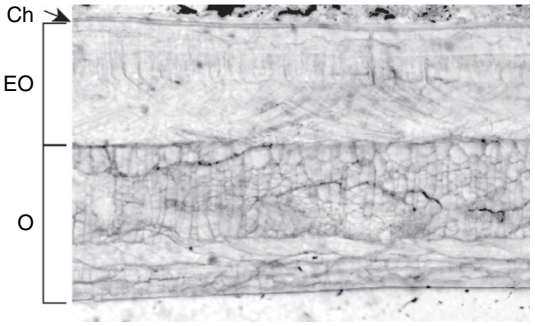


Figure 4.14 Undecalcified thin ground section of the shell of *Helix*. Ch, conchiolin; EO, epiostracum; O, ostracum. 200×.

small granules (Bächle et al. 2006). Figure 4.14 shows undecalcified thin ground section of the snail shell. The multilayered construction of the biomaterial can be seen with the crossed lamellar structures of parallel laths.

4.3.3.1 Hypobranchial Gland

The ventral aspect of the roof of the mantle may bear one or two hypobranchial glands that run parallel to the gill on its right side, between the gill and rectum. Its role is to secrete mucus to facilitate clearing of debris from the mantel cavity, and in Muricidae it produces a purple pigment that may be incorporated into egg masses.

Histologically, the gland consists of modified mantle epidermis where simple to pseudostratified tall columnar epithelial cells are often ciliated and heavily interspersed with mucocytes and several different types of other secretory cells (Figure 4.6) (Westley et al. 2010). Some of the secretory cells secrete acid mucin that stains metachromatically with toluidine blue (Voltzow 1994).

4.3.4 Musculoskeletal

As gastropods lack a body cavity, muscle and connective tissue form the interior of the animal and, like other molluscs, gastropods lack a skeletal system. Although muscle fibers are present throughout all parts of the body, the two main muscles of the gastropod are the columellar muscle and the tarsos muscle of the foot. The columellar muscle forms the bulk of the body of the gastropod. It originates at an attachment to the columella (the central axis) of the shell and becomes continuous with the muscle of the foot. Its main function is to draw the animal into its shell. The gastropod uses its muscular foot (tarsos muscles) for locomotion, mating, ovipositioning, defense, and prey capture. In some cases, particularly in marine prosobranchs, the foot muscle inserts on to the operculum.

Histologically, muscle cells are fusiform and unicellular and may be smooth or striated (Chantler 1983). They may be organized into fibers that weave in all directions and are heavily intermingled with connective tissue. In some instances, muscle cells may be highly ordered into anatomically consistent bundles or layers.

4.3.4.1 Foot

The foot is composed of highly interwoven and branching bundles of myocytes. The supporting connective tissue and epidermis are also similar to that of the mantle; only the latter can be heavily sinuated, particularly on the sole. Epithelium of the sole may be more heavily ciliated, whereas the epithelium of the sides and dorsum is not. In some species, intra- or subepidermal glands may supply the foot, for which the locations are highly variable. A pedal or suprapedal gland, formed by subepidermal acini of oval epithelial cells, may be present to supply the ventral foot with a secretion thought to facilitate locomotion (Shirbhate and Cook 1987).

The general appearance of the foot of *Helix* is a reticulum of grooves subdividing the surface into grayish polygonal areas (see Figure 4.2). Microscopically, apart from the grooving, the structure is remarkably reminiscent of the mantle collar and the same kinds of glands are present (Figure 4.13). The main difference is their smaller size, particularly on the dorsal aspect. There are isolated patches of ciliated cells in the epidermis, but the majority of the surface bears no trace of them. Type 1 mucus glands occur commonly in this region. Protein and calcium glands are less abundant than in the mantle, particularly on the dorsal surface. Those cells containing either pigment or lipid occur sparsely as before.

The ventral foot of *Helix* is divided into two layers parallel with the body surface: the superficial layer and deep subepithelial layer (Tonar and Markos 2004; Wondrak 2012). The body wall epithelia include several regions with mixtures of gland cell types, each combination being characteristic of the specific region. Their cell bodies containing the nucleus are situated underneath the epithelium at different depths within connective tissue (unicellular exoepithelial glands), an adaption to terrestrial life to avoid dehydration, and empty their secretions through their necks directly onto the surface of the body wall. Besides such polytypic glandular regions, the foot has two monotypic gland-cell regions.

- *The pedal protein gland* is situated at the anterior margin of the foot, in the bottom of the corresponding peripodial groove. The main part of this gland occupies the area between the anterior dorsal surface of the foot and the floor of the funnel-shaped cavity (Figure 4.15a). The cells of the pedal protein gland empty their content directly into the peripodial groove. The gland cells stain

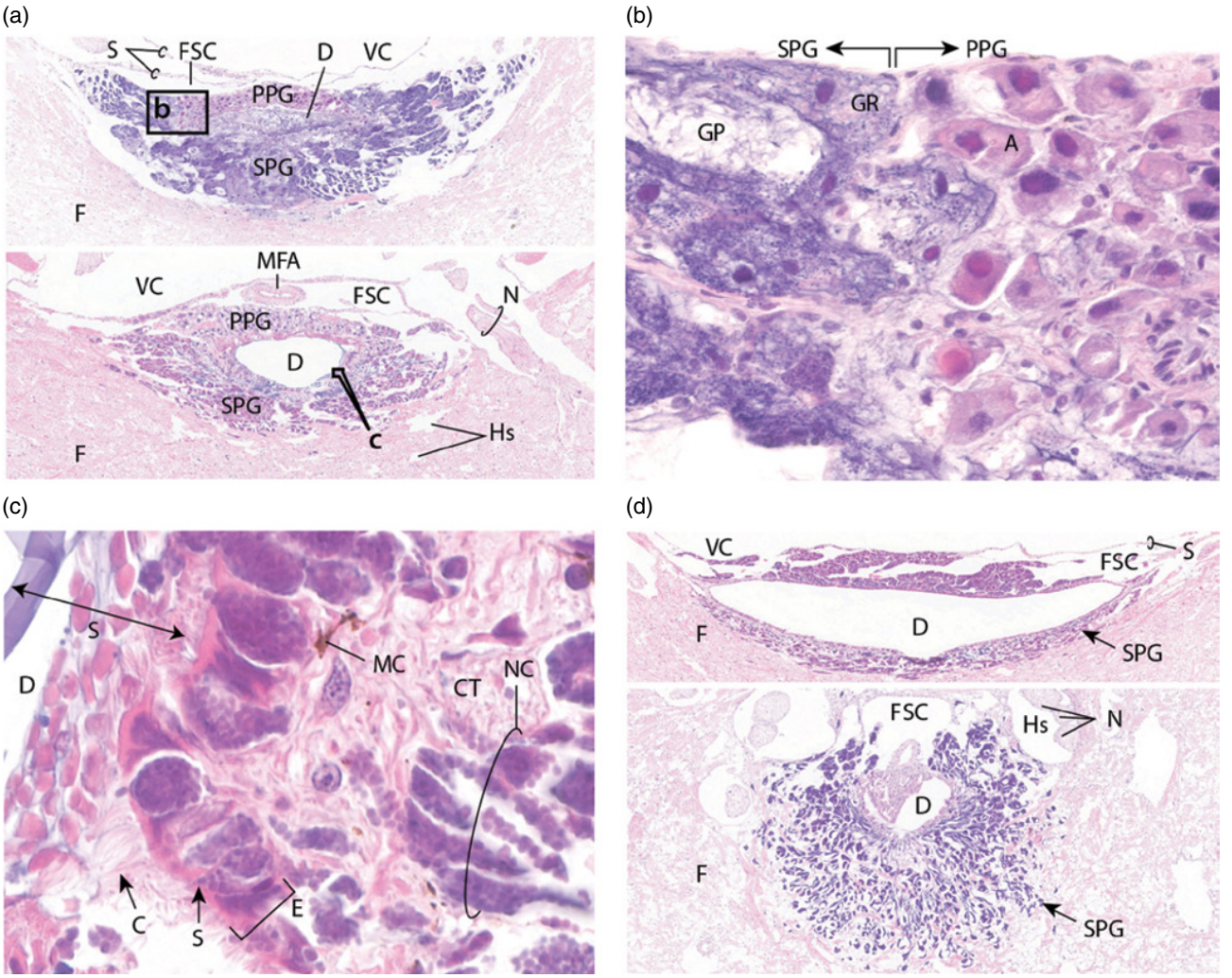


Figure 4.15 Pedal glands of *Helix*. (a) Overview of pedal protein gland and suprapedal gland (plane of the upper section is anterior to the lower one). 30×. HE. (b) Cell types of pedal protein gland and suprapedal gland. 300×. HE. (c) Ciliated cells of the duct of suprapedal gland and secretion. 900×. HE. (d) Overview of the suprapedal gland (planes of the sections are posterior to section in panel a, most caudal is bottom one). 30×. HE. A, acidophil cytoplasm; C, cilia; CT, connective tissue; D, duct of the suprapedal gland; E, epithelium of duct; F, foot; FSC, funnel-shaped cavity; GP, agranular cell cytoplasm; GR, granular cell; Hs, hemolymph sinus; MC, melanocyte; MFA, middle foot artery (branch); N, nerve; NC, neck of gland cell; PPG, pedal protein gland; S, secreted material; SPG, suprapedal gland; VC, visceral cavity.

eosinophilic with HE (Figure 4.15a,b) and react strongly positive to PAS, but they are negative with alcian blue. These properties point to the presence of neutral mucosubstances. The cell bodies of these exoepithelial gland cells with apocrine secretion lie at different depths beneath the epithelium and their thin necks open at the surface between the epithelial cells.

- *The suprapedal gland* surrounds a funnel-shaped recess which invaginates between the dorsal part of the sole and the ventral surface of the head (Figure 4.15d). The gland cells empty their mucous secretion onto the surface of this invagination (Figure 4.15c). The invagination serves as the secretory duct of the gland and delivers the mucus to the surface of the foot. The contents of secretory cells are basophilic and stain dark blue with HE (Figure 4.15c,d) and are PAS negative. The suprapedal gland is dorsally separated by a septum from the body cavity.

4.3.4.2 Columellar Muscle

The columellar muscle has a composition similar to the foot, including the predominance of muscle cells, supported by connective tissue and lined by a surface epidermis. The muscle cells are typically arranged into thick bundles to form orthogonal latticework (Voltzow 1994) (Figure 4.16).

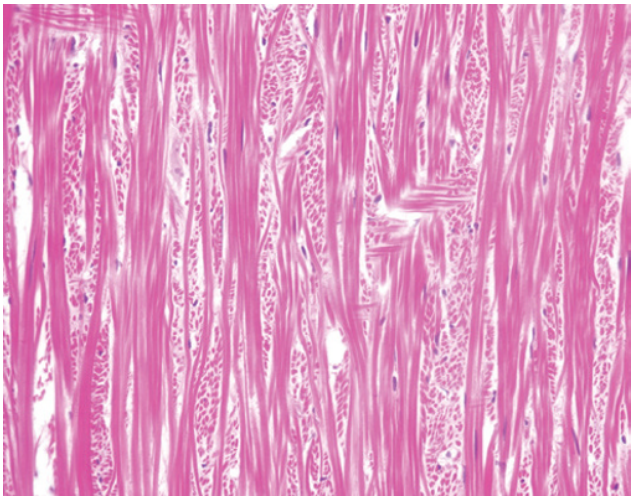


Figure 4.16 Columellar muscle of *Triplofusus papillosus*. Myocytes are arranged into intersecting bundles. 400×. HE.

4.3.5 Digestive System

The digestive system of gastropods is highly adapted to diet, and thus shows significant variation. It is typically U-shaped, particularly in torted gastropods (i.e., those that do not detort after the torsion process during the veliger stage). A protrusible radular apparatus lines the buccal mass, consisting of radula (a set of chitinized teeth), radular membranes, and odontophore (or jaw). The esophagus is a thin-walled organ that courses through the columellar muscle toward the visceral mass where it joins the stomach. Near the buccal mass, it is flanked by salivary glands (SG) and may give rise to a compartment (crop) near the stomach. The stomach often has a cuticularized shield and functions to grind food. It is supplied by a digestive gland (or hepatopancreas, digestive cecum, or midgut gland), and also a style sac in microherbivores. The style sac creates a current to move ingesta and may also contain a crystalline style, a cylindrical gelatinous structure that rotates and facilitates mechanical breakdown of ingesta. The digestive gland is responsible for enzymatic digestion and absorption of nutrients. A gizzard is present in some gastropods. In opisthobranchs, a gizzard is a distal compartment of the esophagus with a thick muscular wall and a heavy cuticle with teeth or plates, whereas in pulmonates, the gizzard refers to a stomach, which has a thick muscular wall. The intestine serves to package feces rather than absorb nutrients. In carnivores, it is often short and straight, whereas in herbivores it is longer and loops or coils near the kidney and heart. The intestine courses through the mantle as the rectum. The anus empties into the mantle cavity close to the gill.

4.3.5.1 Alimentary Canal

4.3.5.1.1 Mouth and Radula A cuticle covers the inner surface of the buccal mass. The radula consists of a thin chitinous band with teeth-like ridges (Figures 4.17 and 4.18). Odontoblasts, pseudostratified tall columnar epithelial cells at the posterior end of the radula, form new teeth. Two rows of similar columnar epithelium, the supraradular and subradular epithelium, together form the radular sheath that encapsulates the radula. Anteriorly, muscle bundles are attached to the radula for its manipulation, and the radula bends ventrally where it

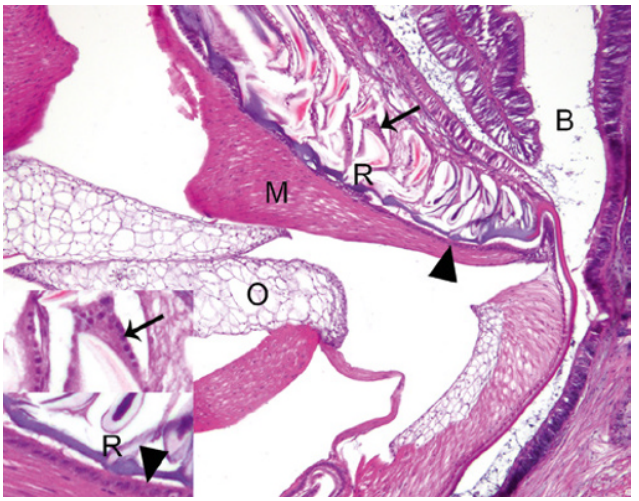


Figure 4.17 Radular apparatus of *Cerithium* sp. Within the buccal cavity (B), the radula (R) is ensheathed by supraradular epithelium (*arrows*, inset above) and subradular epithelium (*arrowheads*, inset below). Muscle (M) connects to the radula and associated odontophores (O). 200×. HE.

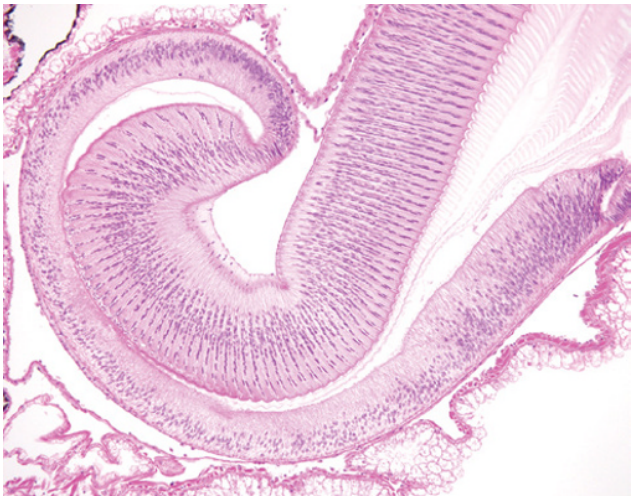


Figure 4.18 Posterior radular apparatus of *Nerita peloronta*. Odontoblasts are pseudostratified tall columnar epithelial cells that form new radula. 200×. HE.

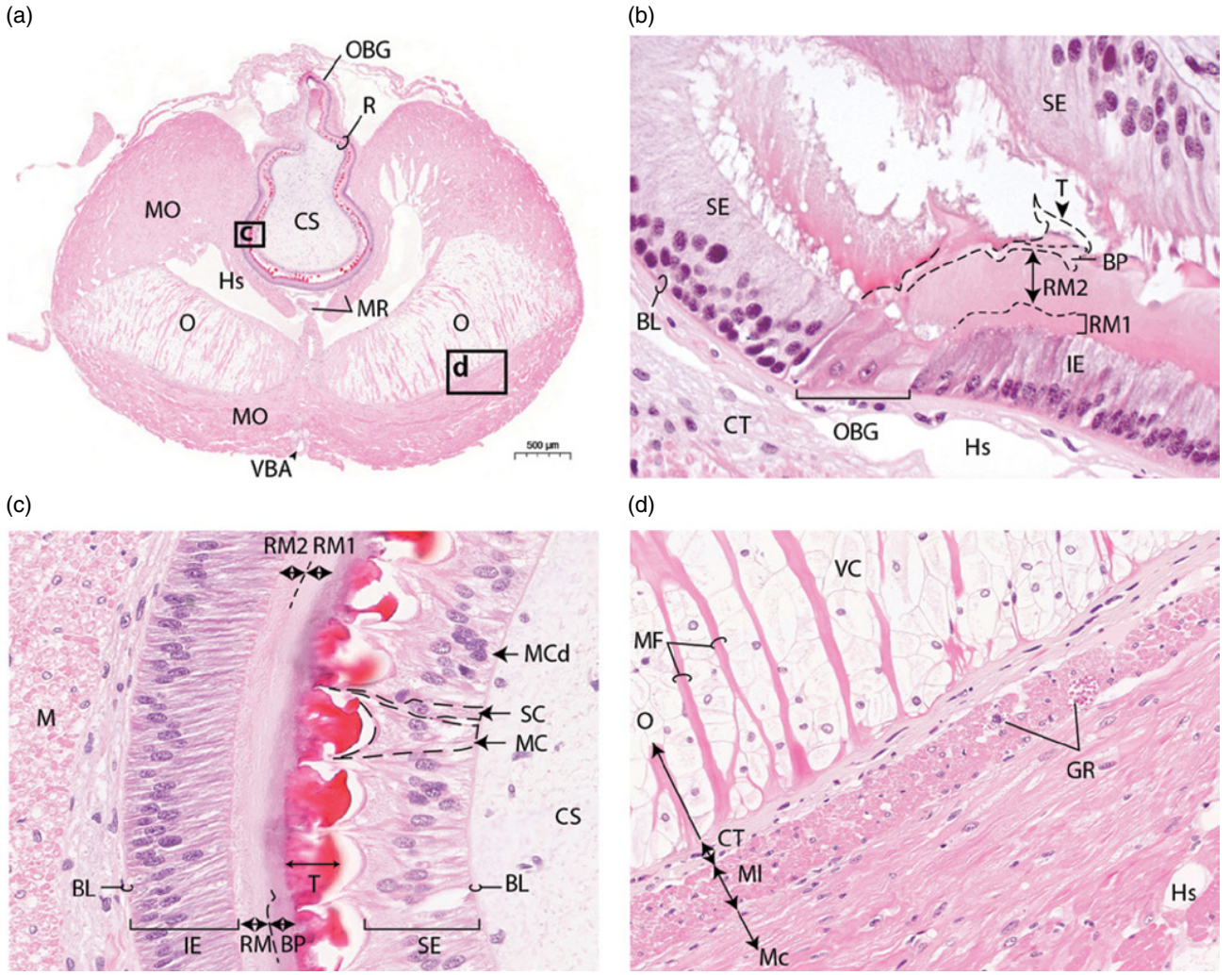


Figure 4.19 Pharynx and radula of *Helix*. (a) Overview of the cross-section of pharynx. 20×. HE. (b) Cross-section of an odontoblast group. 400×. HE. (c) Cross-section of radula with matured teeth. 350×. HE. (d) Cross-section of the odontophore. 200×. HE. BL, basal lamina; BP, basal plate; CS, collostyle; CT, connective tissue layer; GR, granulocytes; Hs, hemolymph sinus; IE, inferior epithelium; M, muscle cells (cross-section); Mc, circular muscle layer; MC, mineralizing cells; MCd, degenerated mineralizing cells; MF, muscle fibers; Ml, longitudinal muscle layer; MO, muscles of the odontophore; MR, muscles of the radula; O, odontophore; OBG, odontoblast cell group; R, radula; RM, radular membrane; RM1, RM2, layers of the radular membrane; SC, supporting cell; SE, superior epithelium; T, tooth; VBA, ventral buccal artery; VC, vesicular connective tissue cell.

contacts the odontophores, dense plates of connective tissue resembling cartilage, only containing myoglobin and polyhexose sulfate instead of chondroitin sulfate (Lash 1959).

In *Helix* the buccal mass is arranged in three concentric and integrated envelopes (Figure 4.19).

- Muscles between the body wall and buccal mass: these assist in protraction and retraction of the mass.
- In the middle, muscles pass from the inferior walls of the buccal mass to the odontophoreal cartilage. These assist in rotation, protrusion, and retraction of the odontophore.
- Muscles originate from the odontophore and insert on the radular ribbon. These elements generate the reciprocal movements of the radula, which occur during feeding.

In pulmonates, the multidenticular radula is generated by numerous groups of a few voluminous cells (situated in odontoblast groups). Pulmonates produce the radula matrix by microvilli, cytoplasmic protrusions, and apocrine secretions (Mackenstedt and Märkel 1987). The posterior part of the radula is located in a blind pouch, the radular sac or radular sheath. It is a U-shaped groove and the lateral teeth are directed inwards. The collostyle protrudes into the groove from above. The radular sheath consists of two

epithelia, the superior and the inferior epithelium, which are linked with the radula. In pulmonates, the few odontoblasts are located at the lower posterior end of the sheath. Odontoblasts are concentrated into groups of a limited number of cells, each secreting the teeth of one longitudinal row. The odontoblasts form the matrix of the radula, whereas the superior and the inferior epithelium contribute to the maturation and the transport of the radula.

Cells of the odontoblast group include the following (see Figure 4.19a,b).

- *Membranoblast*. The membranoblast is located anteriorly in each odontoblast group and is clearly distinguished from the cells of the inferior epithelium by its height. Numerous pinocytotic vesicles can be observed at the apical surface of the membranoblast and their content probably forms the ground substance of the radular membrane, in which the microvilli are embedded. Membranoblasts produce the radular membrane continually.
- *Basal plate cells*. The basal plates connect the teeth with the radular membrane and cause the regular distance between following rows of teeth. Basal plate cells show cyclical activities. The basal plates are composed of microvilli, secretory droplets, and protrusions of the apical surface of the cells, filled with secretion substances. These protuberances are pinched off and disintegrate into the ground substance of the basal plates. The lateral region of the basal plates is thought to form flexible connections between the teeth, which may function as joints.
- *Odontoblasts*. The real odontoblasts terminate the group and border on the superior epithelium. The apical surface of the odontoblasts is modified with numerous microvilli, orientated in a parallel or diagonal direction to the transverse axis of the odontoblasts. The tooth matrix consists of microvilli, secretion products, and cytoplasmic protrusions, which are pinched off. The odontoblasts define the shape and size of the teeth, with the latter remaining throughout life.

The superior and inferior epithelium are migrating epithelia. There are mineralizing and supporting cells in the superior epithelium (see Figure 4.19b,c). The mineralizing cells deposit minerals and organic compounds into the newly formed teeth to make them hard. The change in the chemical composition is illustrated by change of the staining properties. The matrix of the newly formed teeth consists of protein and mucopolysaccharides, mainly chitin, but an intussusception of minerals and organic compounds causes hardening of the teeth. After fulfilling their purpose, mineralizing cells die. Supporting cells reach to the basal plate between the teeth. They do not deposit minerals so the areas between the teeth remain flexible. Electron microprobe analysis shows that mainly calcium is incorporated into the teeth of the radula, whereas the radular membrane lacks minerals (Mackenstedt and Märkel 1987).

The radular membrane covers the migrating inferior epithelium in the radular sheath. At the boundary between two basal plates, the radular membrane becomes thinner whereas the parts of the membrane just beneath the basal plates remain thick. Functional aspects can explain this – for example, the thicker part of the membrane supports the teeth during feeding and protects the inferior epithelium against tensile forces. The radular membrane reveals two distinct layers in *H. pomatia*.

4.3.5.1.2 Esophagus Salivary glands supplying the anterior esophagus are typically simple tubular glands composed of columnar epithelial cells that form tubules or acini. Acini may be lined by two cell types: a basilar pyramidal vacuolated cell and a columnar ciliated cell (Andrews 1991). The wall of the esophagus is typically very thin, consisting of muscle cells arranged in a circular orientation around the lumen. The mucosa is composed of simple columnar microvillated epithelial cells, some bearing cilia, and an adventitial surface comprises loose fibrous tissue containing many nerve bodies and hemolymphatic vessels. Often the mucosa is arranged into longitudinal folds, and these may contain greater numbers of mucocytes at their tips (Figure 4.20). These folds are typically reduced or absent in the posterior esophagus. There may be discretely vacuolated cells of unknown function just beneath the mucosa (Lobo-da-Cunha et al. 2011). Morphologically, the crop and esophagus are similar (Howells 1942).

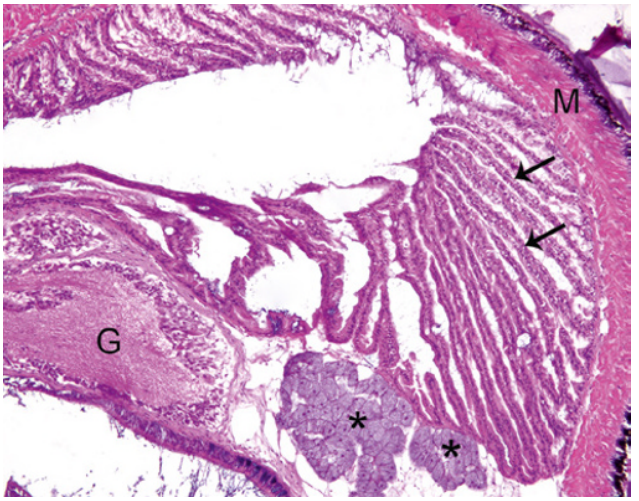


Figure 4.20 Esophagus of *Cerithium* sp. The esophagus has a very thin wall consisting of a circular layer of myocytes (M) and a mucosa arranged into longitudinal folds (*arrows*). At its anterior, it is often flanked by salivary glands (*asterisks*) and ganglia (G). 200×. HE.

The *Helix* esophagus wall is lined with a simple columnar epithelium (Figure 4.21), covered with a thin cuticular layer, the microvilli. The nuclei of the epithelial cells are located at various levels, usually closer to the base. Among the epithelial cells, there are two types of glandular cells (MU1 and MU2), characterized by a bottle-like shape with distinct "necks." Their nuclei are located basally, in their widest part. They differ in their secretion: mucin with no staining and acidic mucopolysaccharide with blue staining. Below the epithelium, there is connective tissue and muscle fibers (Koralewska-Batura 1999).

In *Helix*, the terminal part of the esophagus widens gradually to form a crop (Figure 4.22) which is often regarded as part of the stomach. The crop wall is lined with columnar epithelium, which also contains glandular cells (see Figure 4.22). Below the layer of epithelial cells there is connective tissue interspersed with muscle fibers that run in various directions. In the crop of *Helix*, the food is subject to preliminary enzymatic treatment (Koralewska-Batura 1999).

In *Helix*, the SG consist of numerous acini and the saliva secreted is forwarded from the acini into the intralobular (SDi) and then the interlobular ducts that converge on the paired muscular primary salivary ducts (SD), which finally open into the buccal cavity (see Figure 4.22). In SD, the saliva is driven by the peristaltic contractions of the muscle fibers of the duct toward the buccal mass. The cell types of the SG are as follows.

- *Mucocytes* are frequent and full of secretory vesicles. The number of mucocytes is higher near the blood vessel.
- *Vacuolated ells* are also frequent. The number of vacuolated cells is higher in the vicinity of the SD, and is higher in inactive animals than in active ones. Among the gland cells (see Figure 4.22), large cells of vesicular shape prevail, with oval nuclei. Inside them, a foam-like cytoplasm is visible.
- *Granular cells* are full of dark granules. The number of granular cells is lower in inactive animals than in active ones.

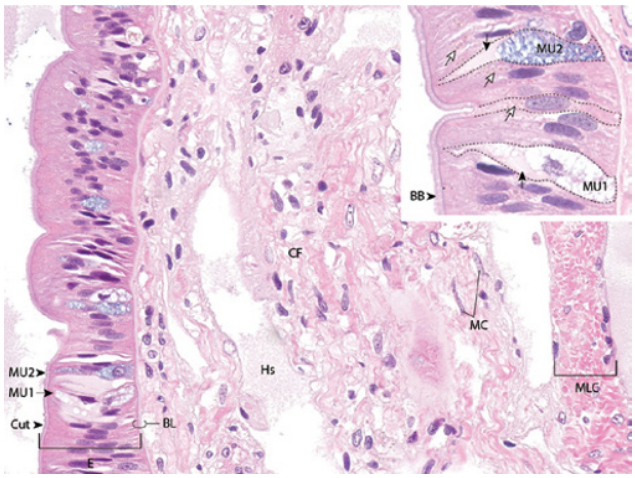
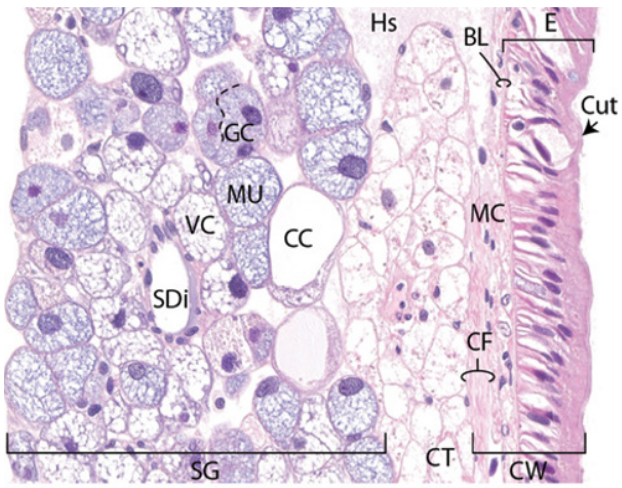


Figure 4.21 Longitudinal section of the esophagus of *Helix*. BB, brush border; BL, basal lamina; CF, collagen fibers; Cut, cuticular layer; E, epithelium; Hs, hemolymph sinus; MC, muscle cells in longitudinal layer; MLC, muscular layer, circular; MU1, mucus cell type 1; MU2, mucus cell type 2; white arrows, brownish granules in apical domain of supporting cells; black arrows, apical domain of mucus cells. 250×. HE; inset 700×.

(a)



(b)

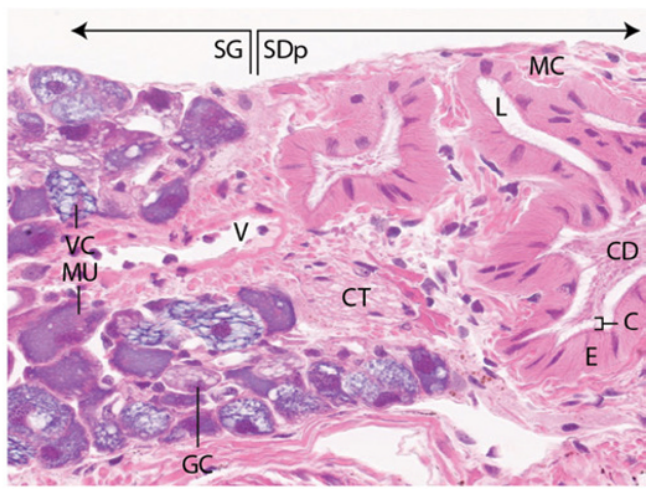


Figure 4.22 Crop and salivary gland in *Helix*. (a) Longitudinal section of the crop. 300×. HE. (b) Cross-section of the salivary gland and primary salivary duct. 350×. HE. BL, basal lamina; C, cilia; CC, cystic cell; CD, cell debris; CF, collagen fibers; CT, connective tissue; Cut, cuticular layer; CW, crop wall; E, epithelium; GC, granular cell; Hs, hemolymph sinus; L, lumen of the primary salivary duct; MC, muscle cells; MU, mucocyte; SDi, salivary duct, intralobular; SDp, salivary duct, primary; SG, salivary gland, lobe; V, hemolymph vein; VC, vacuolated cell.

- *Cystic cell*. Several authors refer to a cystic cell type in the SG of *Helix*, which is characterized by a single large secretory vacuole surrounded by a thin peripheral layer of cytoplasm. Cystic cells occur regularly in active animals and are never present in inactive ones. Cystic cells probably represent the last stage of vacuolated cells, rather than an additional, fourth cell type of the *Helix* SG.

Neutral and acid mucins lubricate the food, while the proteins constitute the enzymatic supply in the early phase of digestion (Pirger et al. 2004).

4.3.5.1.3 Stomach and Style Sac The gastric mucosal surface may be covered by a cuticularized chitinous gastric shield along its dorsal and left walls (Figure 4.23). The mucosa is lined by simple columnar epithelial cells with a microvillus border and is variably populated by mucocytes. Ridges and folds in the mucosa corresponding to typhlosoles may be present near the esophagus or small intestine and in these areas the epithelium is often ciliated and may be pseudostratified or bilayered. The submucosa consists of connective tissue seen throughout the body, but perhaps containing greater amounts of hemolymphatic vessels and hemocytes. The muscularis blends with the submucosal connective tissue and is composed of muscle cells arranged in a circular orientation around the longitudinal axis of the lumen. The external surface of the stomach comprises epidermis, similar to the mantle and other body surfaces.

In the stomach of *Helix* there are three large, very characteristic folds. Two of them, one located in the dorsal and the other in the central part of the stomach, are much larger than the third. They form a gutter – the typhlosole running toward the intestine. The third fold is located between the two outlets of the digestive gland lobes. Between them, there are much smaller folds. The stomach wall is lined with a ciliated columnar epithelium with numerous glandular cells. Below the epithelium there is connective tissue, its main component being lipid cells and relatively numerous muscle fibers. The stomach muscular layer is built of circular, longitudinal, and oblique fibers. Two ducts of the digestive gland open to the stomach. Digestion mechanisms in the stomach of pulmonate snails result in only the finest food particles, not exceeding 1 µm, getting into the digestive gland where they are absorbed. Larger particles are moved toward the intestine (Koralewska-Batura 1999).

The style sac mucosa consists of tall cuboidal to columnar epithelial cells with long cilia (Figure 4.24). There are often folds corresponding to a typhlosole with a central intestinal groove. This area is lined by pseudostratified tall columnar ciliated epithelial cells (Mackintosh 1925). If present, the crystalline style is poorly stained and appears as amphiphilic homogenous acellular substance.

4.3.5.1.4 Small Intestine and Rectum The mucosa of the small intestine may also be dominated by typhlosoles (Figure 4.25). Similar to the style sac and stomach, where present these bear pseudostratified to bilayered tall columnar ciliated epithelial cells. In the furrows of the ridges, the mucosa is lined by simple columnar epithelial cells. The rectal mucosa is composed of simple columnar epithelial cells but may also contain granular mucocytes. The anal mucosa consists of pseudostratified ciliated columnar epithelial cells, heavily interspersed with mucocytes (Figure 4.26). The submucosa, muscularis, and external surface of all segments of intestine are similar to the stomach. The external surfaces of the rectum and anus

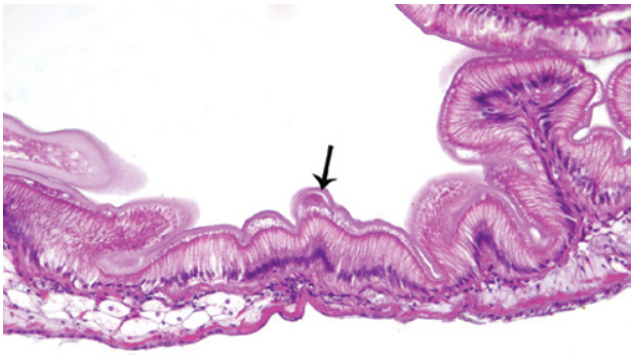


Figure 4.23 Stomach of *Cerithium* sp. In areas, the gastric mucosa is covered by a cuticle (*arrow*). 400×. HE.

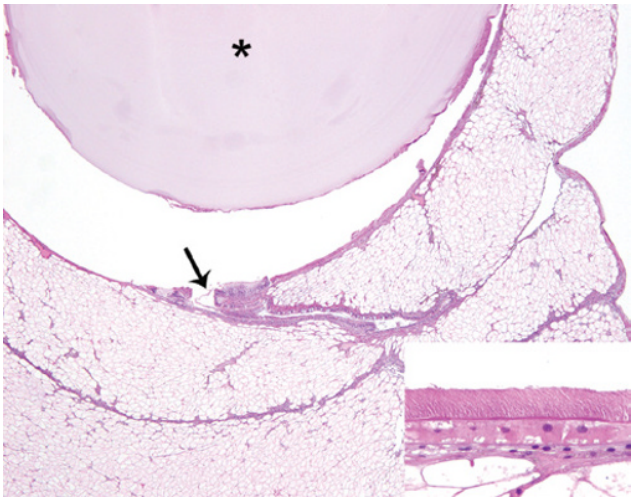


Figure 4.24 Style sac of *Lobatus gigas*. A distinct fold in the wall is a reflection of a typhlosole. The crystalline style (*asterisk*) is composed of homogenous acellular substance. 40×. HE. Inset: Higher magnification shows detail of mucosa, consisting of simple cuboidal epithelial cells with long cilia. 400×. HE.

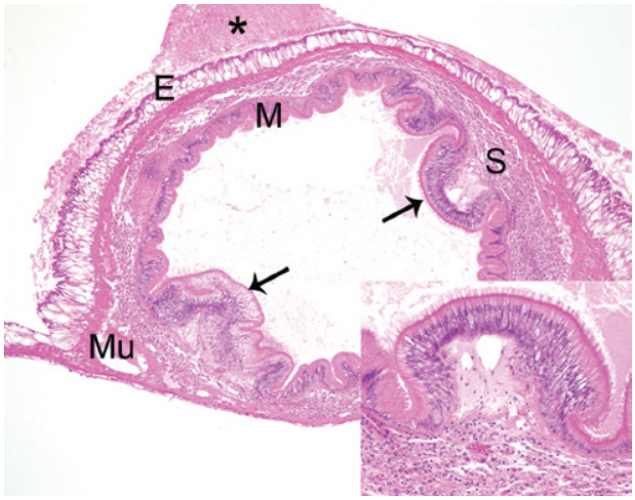


Figure 4.25 Intestine of *Cittarium pica*. Prominences reflect typhlosoles (*arrows*). The mucosa (M), submucosa (S), and muscularis (Mu) form distinct layers, although in many species the muscularis is not so distinct. The exterior comprises epidermis (E), in this instance bearing many mucocytes that have coated the specimen in mucoid secretion (*asterisk*). 100×. HE. Inset: Higher magnification of a typhlosole shows bilayered columnar ciliated epithelial cells. 200×. HE.

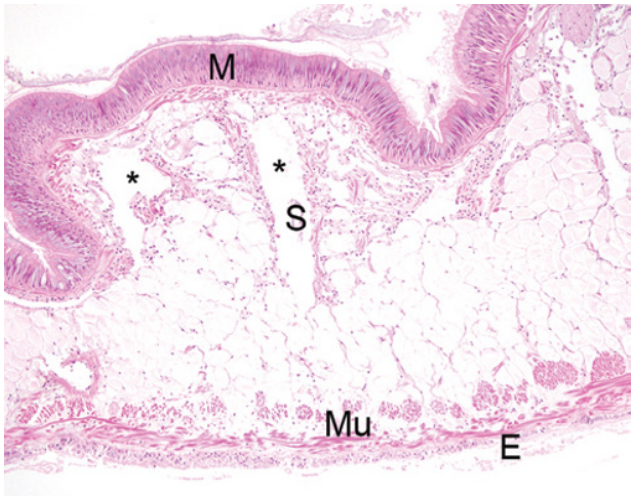


Figure 4.26 Anus of *Lobatus gigas*. The mucosa (M) is lined by simple to pseudostratified columnar epithelial cells with many mucocytes and ciliated cells. The submucosa (S) is similar to the stomach, small intestine, and rectum, being composed of loose fibrous connective tissue containing many hemolymph vessels (*asterisks*) and myocytes blending in from the muscularis (Mu). The exterior comprises epidermis (E). 100×. HE.

are integrated with the mantle and are often coated in mucus derived from the hypobranchial gland.

The intestine of *Helix* consists of the small intestine and rectum. Both these sections are lined with columnar epithelium with numerous glandular cells. In the folds of the glandular part of the small intestine there occur numerous goblet cells (Figure 4.27) which stain metachromatically blue with hematoxylin. The mucus produced in this part of the alimentary tract facilitates defecation. Resorptive cells proliferate between the folds and differentiate toward the top of the folds. Nuclei of young resorptive cells are light, euchromatic, whereas the older become darker, with more heterochromatin. At the top they detach from the basal lamina and are digested in the lumen of the gut.

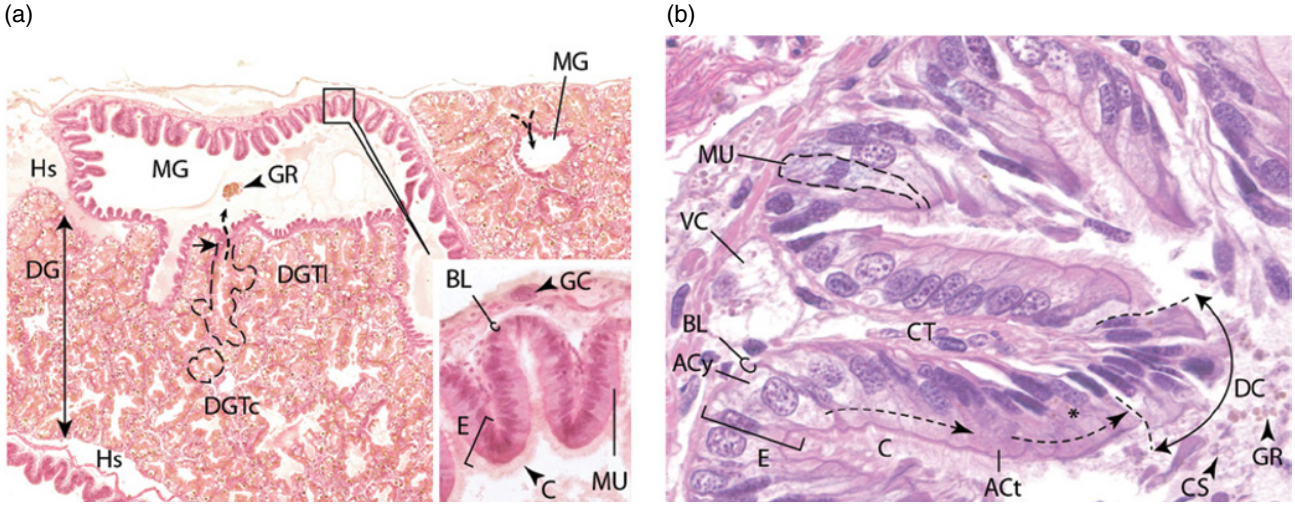


Figure 4.27 Midgut of an active *Helix*. (a) Overview of the midgut embedded in the digestive gland (hepatopancreas) of an inactive snail (Bouin fixation). 40×. HE, inset: midgut wall 300×. (b) Midgut epithelium of an active snail. 600×. HE. ACy, young absorptive cell; ACt, terminally differentiated absorptive cell; BL, basal lamina; C, cilia; CS, calcite spherules; CT, connective tissue; DC, dying cells; DG, digestive gland; DGTc, digestive gland tubule, cross-section; DGTl, digestive gland tubule, longitudinal section; E, epithelium; GC, ganglion cell; GR, granules; Hs, hemolymph sinus; MG, midgut; MU, mucocyte; VC, vacuolated cell. Asterisk, granules in terminally differentiated absorptive cell; black arrow, boundary between acinus and duct; dashed arrows indicate secretion in panel a, and maturation in panel b.

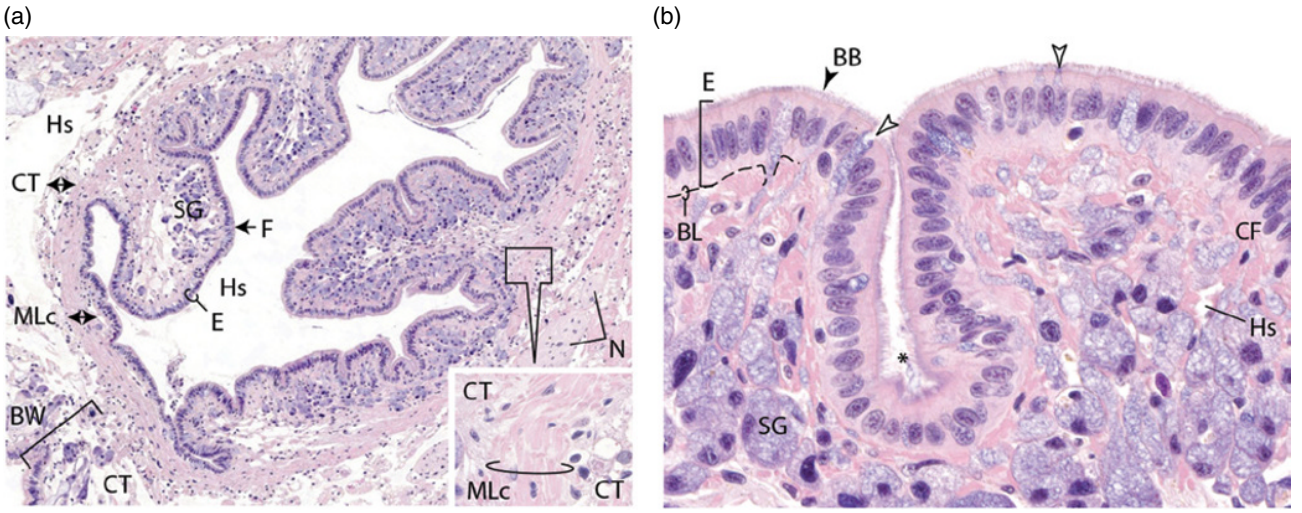


Figure 4.28 Rectum of *Helix*. (a) Overview of cross-section of the rectum. 50×. HE; inset: 600×. (b) Mucous layer of the rectum. 400×. HE. BB, brush border; BL, basal lamina; BW, body wall; CF, collagen fibers; CT, connective tissue; E, epithelium; F, fold of mucous layer; Hs, hemolymph sinus; MLc, muscle layer circular; N, nerve; SG, subepithelial glands; asterisk, secreted material; white arrowheads indicate secretion.

In the rectum of *Helix*, the mucus epithelial lining is folded with a profusion of subepithelial mucus cells secreting acid mucopolysaccharides (Figure 4.28). The apical portion of the subepithelial mucus cells reaches the surface among epithelial cells and empty directly into the lumen. There are muscle cells and hemolymph sinuses in the underlying connective tissue of the folds. The next layer is a circular muscle layer that is connected to other organs or the body wall with a connective tissue.

4.3.5.2 Digestive Gland (Hepatopancreas)

The digestive gland is a tubuloacinar gland consisting of simple pyramidal to columnar epithelial cells arranged into branching tubules supported by connective tissue. Cross-sections of the acini reveal an obscure triangular shape where the lumen indents at the corners, referred to as crypts (Figure 4.29). The literature on the gastropod digestive gland is inundated with confusing and inconsistent terminology of the digestive gland epithelial cells. The shorter cone-shaped or pyramidal cells in the crypts are best termed *secretory cells*, whereas the predominating columnar to club-shaped cells not within the crypts are referred to as *digestive cells* (Voltzow 1994). The latter have a microvillus border. Other cell types may be present, depending on species. Digestive gland epithelial cells often contain granules, spherules, or other inclusion bodies with a variety of staining characteristics. Their function is unknown but may be related to detoxification of metal ions or mineral homeostasis. Secondary ducts supply glandular tubules and are lined by microvillated simple columnar epithelial cells that show apocrine secretion. These ducts

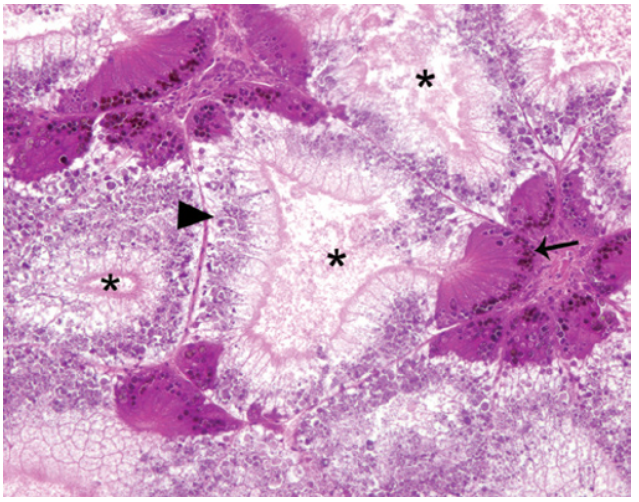


Figure 4.29 Digestive gland of *Cittarium pica*. Acini (*asterisks*) are lined by secretory cells (*arrows*) and digestive cells (*arrowhead*). The differential staining of the two cells types is especially pronounced in this species. A variety of pigmented or calcareous granules or inclusion bodies are often present within either cell type. 400×. HE.

continue on as larger primary ducts that empty into the stomach and are lined by pseudostratified ciliated columnar epithelial cells and mucocytes. In opisthobranchs, zooxanthellae may reside within the digestive gland epithelium and lumen (Wägele and Johnsen 2001), and in certain sacoglossans that feed on algae, chloroplasts may be retained within the digestive gland epithelium and the marginating stomach wall epithelium (Hirose 2005).

The acini of the digestive gland of the *Helix* are separated by interlobular loose connective tissue containing hemolymphatic vessels and hemocytes. Each tubule is provided externally with a circular muscle layer (Figure 4.30b,c). Nerve fibers are observable in and above the basal lamina (Hamed et al. 2007). In the digestive gland of *Helix*, amylase, maltase, and lactase, as well as cellulase activity were detected. It is also the place of absorption of digested food and accumulation of reserve materials, such as glycogen, whose quantity increases especially in the period preceding hibernation. Copper is also present within the tubules of the digestive gland of *Helix*, which suggests its participation in hemocyanin synthesis (Koralewska-Batura 1999).

In the digestive gland (hepatopancreas) of *Helix*, three cell types are present.

- *Digestive cells*: major cellular component of the digestive gland epithelium. They show morphofunctional alterations according to the digestive activity of the animal: absorptive and digestive phases; columnar cells with a slightly rounded apical surface with well-developed brush border; two types of granules in the cytoplasmic vacuoles (endo-/heterolysosomes and residual bodies); cytoplasm possesses abundant vacuoles; basally located nucleus round or oval (Hamed et al. 2007) (Figure 4.30b–d).

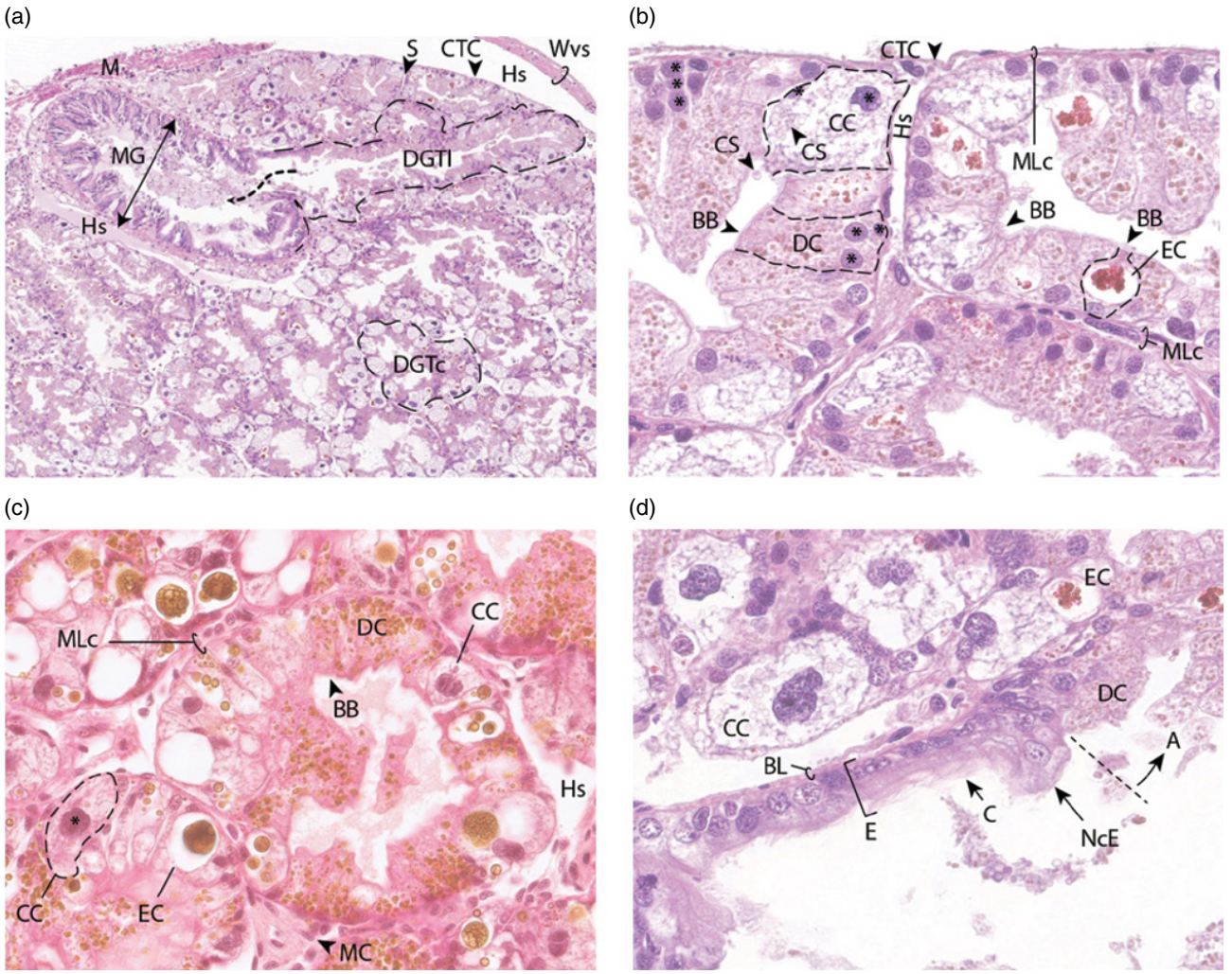


Figure 4.30 Digestive gland of *Helix*. (a) Overview of the digestive gland and midgut of an active snail. 60×. HE. (b) Acini of the digestive gland of an active snail. 400×. HE. (c) Acini of the digestive gland of an inactive snail after Bouin fixation. Note: there are no visible calcium spherules in the picture as the acetic acid content of Bouin fixative dissolved them away. 400×. HE. (d) Short duct of the digestive gland of an active snail. 400×. HE. A, acinus of digestive gland; BB, brush border; BL, basal lamina; C, cilia; CC, calcium cell; CS, calcium spherule; CTC, connective tissue capsule; DC, digestive cell; DGTc, digestive gland tubule, cross-section; DGTl, digestive gland tubule, longitudinal section; E, epithelium; EC, excretory cell; Hs, hemolymph sinus; M, columellar muscle; MC, muscle cell; MG, midgut; MLc, muscle layer, circular; NcE, nonciliated epithelium; S, interlobular loose connective tissue; Wvs, wall of visceral sac; asterisk, nuclei of multinucleated cells.

- *Calcium cells*: occur either singly or in pairs in the "corners" (crypts) of the tubules; they have a pyramidal shape with a narrow end and broad base; calcium spherules are light, refractive bodies; calcium cells possess secretory granules and large rounded nuclei; the apical surface is covered by a brush border (Figure 4.30b–d). Calcium cells are multinucleated (Mackenstedt and Märkel 1987) (Figure 4.30b asterisk). The granules of calcium cells contain large quantities of ions, usually Ca, P, and Mg. Calcium is an essential element in the biology of the snail, and its importance in the construction and regeneration processes of the shell is obvious. It is also, along with phosphorus, the most significant ion in the regulation of luminal pH. Besides these three main ions in the granules, traces of other metal ions such as Zn, Fe, Cd, and Pb have also been detected in quantities that in other zoological groups could be lethal. This suggests that these granules comprise a very sophisticated mechanism of cell detoxification, which eliminates highly toxic metals from the environment where they are found (Almendros and Porcel 1992).
- *Excretory cells*: globular shape; single large vacuole, filling nearly the whole volume of the cell; excretory products accumulated in the vacuole and form a large brown body; surface of the cell possesses a well-developed brush border; nucleus is small and pressed flat against the cell base (Hamed et al. 2007) (Figure 4.30b–d).

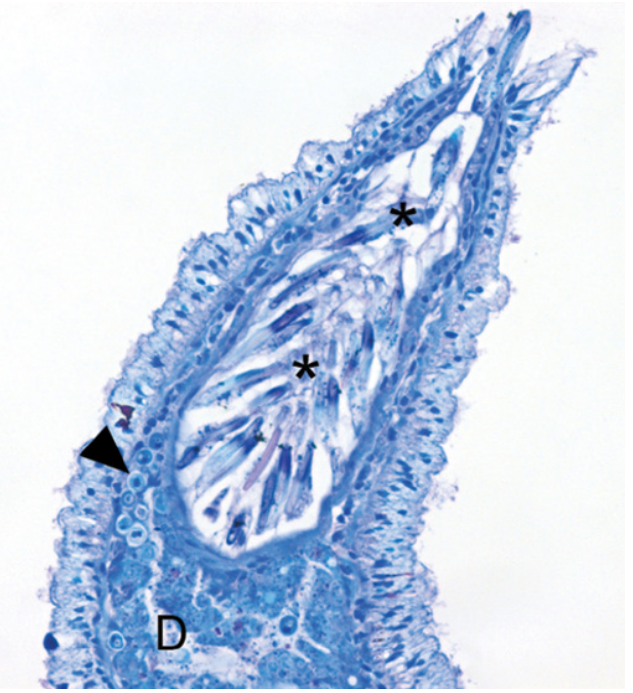


Figure 4.31 Ceras of *Limenandra confusa*. Cerata are extensions of the mantle that contain digestive gland (D) at the base and cnidosacs filled with nematocysts (*asterisks*) at their tips. Note the presence of zooxanthellae within the digestive gland (*arrowhead*). 400×. Toluidine blue stain. *Source:* Jessica Goodheart.

4.3.5.3 Cerata

Cerata are found in some opisthobranchs. Histologically, they are extensions of the mantle that may contain digestive gland at their base and cnidosacs at their tips. Nematocysts ingested from cnidarian prey pass through the digestive gland and are stored in the latter. The cnidosac lumen contains nematocysts, is variably lined by plump cuboidal to columnar epithelial cells (termed "cnidophages"), and often has a wall of smooth muscle and connective tissue (Figure 4.31) (Goodheart et al. 2018).

4.3.6 Excretory System

The kidney functions to remove waste products from the hemolymph and excrete them into the external environment through the nephridiopore (in most species), which is a separate opening near the anus. Hemolymph is filtered through the wall of the cardiac auricle(s), in some species involving a pericardial gland in the pericardial wall or on the epicardial surface, which is not a true gland but a filtration organ (Andrews 1981). The filtrate moves from the pericardial space to the kidney(s) through the renopericardial ducts. In monotocardians, there is a single (right) kidney, filtration occurs through the wall of a single auricle, and a nephridial gland near the kidney and heart may supplement the formation of urine. In diotocardians, there is also a smaller left kidney and filtration occurs through the walls of two auricles. Typically, nitrogenous wastes are excreted by the right kidney, particularly uric acid and/or ammonia in marine gastropods, and may include urea in freshwater gastropods (Little 1981). The right kidney may also contribute to osmoregulation in terrestrial gastropods. The left kidney and nephridial gland function in resorption of nutrients and possibly osmoregulation (Andrews 1985, 1988). In many gastropods, partitioning of nitrogenous waste excretion, osmoregulation, and resorption is not so well defined (Andrews and Little 1972).

4.3.6.1 Kidney

The kidney is highly lobulated and is composed of tubular folds of simple pyramidal to columnar epithelial cells (nephrocytes) interspersed with a few mucocytes (Figure 4.32). Delicate stroma densely populated by hemolymphatic sinuses supports the basal aspect of epithelium. The epithelial cells have basal nuclei and apical microvilli, and are often highly vacuolated, the latter thought to represent excretory function. Where renal tissue is involved in

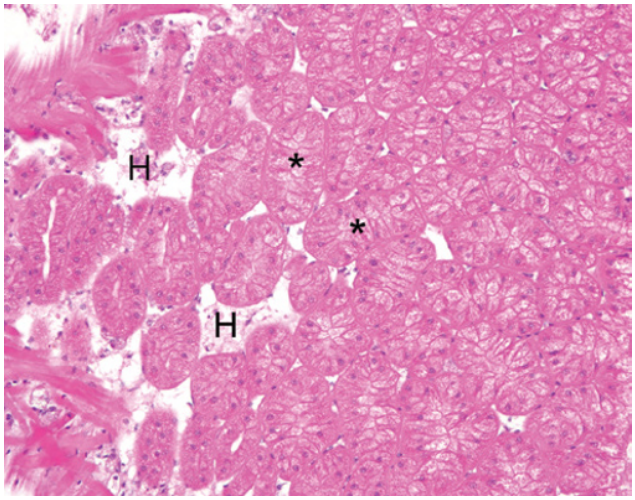


Figure 4.32 Kidney of *Tegula excavata*. Hemolymphatic spaces (H) separate tubules lined by simple pyramidal epithelial cells (*asterisks*). 400×. HE.

resorption of nutrients, epithelial cells may be less vacuolated, reflecting pinocytosis, and may also feature absorptive cilia. Clusters of pigmented cells may be found in the interstitium (Tiley et al. 2019). Large yellow-brown granules (excretory concretions) may be within the cytoplasm of nephrocytes (LaDouceur et al. 2016), whereas they may be absent in some gastropods (Tiley et al. 2019).

The kidney of *Helix* is a yellowish, triangular organ in the rear segment of the mantle close to the pericardium. The kidney consists of two parts: the renal sac, where the excrements are secreted in the form of yellow granules, and the primary ureter, which is coiled up forming a compact organ. This joins then to a second part, the secondary ureter, which runs parallel with the rectum. Both secondary ureter and rectum open into the pneumostome at the mantle collar (see Figure 4.4). As this species lacks a nephridiopore, the pneumostome serves as a common opening for the urinary and digestive systems. In other species with a nephridiopore, the urinary system has a dedicated opening to the external environment (i.e., nephridiopore) that is separate from the anus.

In the renal sac of *Helix*, the epithelium is composed of cells about 5 µm high which contain a large central nucleus, and an apical brush border (Figure 4.33). The cytoplasm of the cells bears clearly defined vertical striations. Below this epithelium lies a layer of connective tissue, which forms a capsule around the renal sac. The connective tissue capsule contains blood vessels, muscle fibers, collagen fibrils, wandering hemocytes, nerves, fibers, rhogocytes (pore cells), vesicular connective tissue cells (Leydig cells), and fibroblasts.

The superficial regions of the kidney contain blood capillaries of two types. In the first, the endothelium lining the lumen is complete while in the second it is fenestrated. The epithelium is composed of a single cell type, called nephrocytes. The nephrocytic epithelium is folded in a complex manner, making U-turns as it approaches the outer surface of the kidney. In this way, lamellae covered by nephrocytes are formed, which contain an axis of connective tissue containing most of those elements present in the connective tissue capsule (Skelding 1973).

Nephrocytes are columnar cells with a large, central vacuole containing a single excretory concretion. The spherical

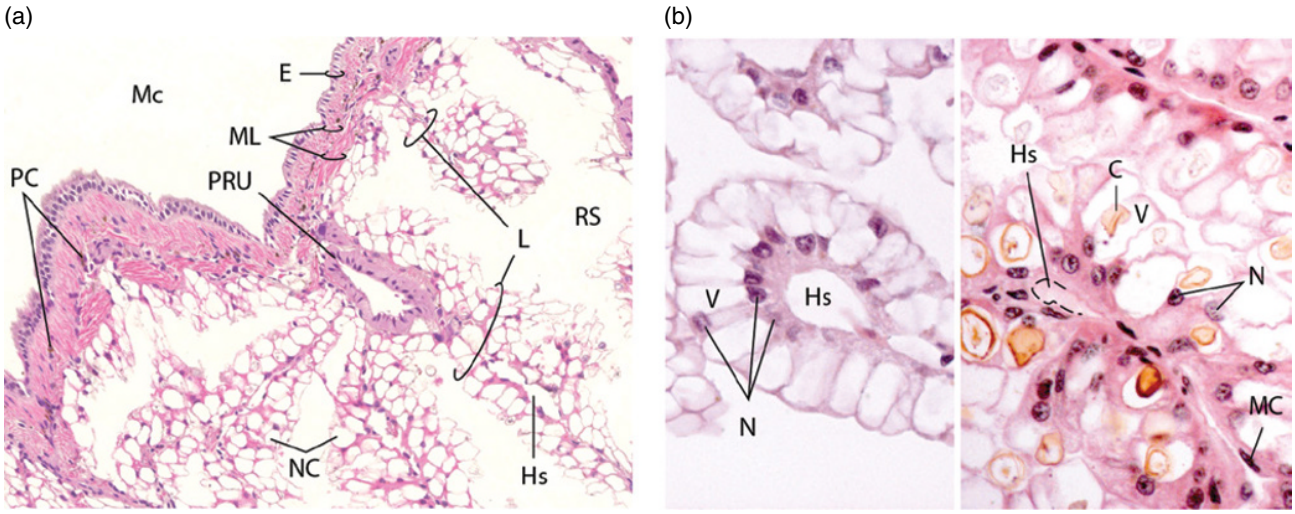


Figure 4.33 Histology of renal sac of active and inactive *Helix*. (a) Histologic structure of renal sac in summer. 200×. HE. (b) Nephrocytes in summer (*left*) and winter (*right*). Fixations: left, Karnovsky, right, Bouin. 750×. HE. C, excretory concretion; E, epithelium; Hs, hemolymph sinus; L, lamellae; Mc, mantle cavity; MC, muscle cell nucleus; ML, muscle layers; N, nucleus; NC, nephrocytes; PC, pigment cell; PRU, primary ureter; RS, renal sac cavity; V, vacuole.

nucleus lies in basal cytoplasm. Matured nephrocytes at the apex of the lamellae contain larger concretions and numerous small dense bodies (Figure 4.33b). Young cells at the base of the lamellae bear microvilli on their luminal surface. Residual bodies in the basal cytoplasm are discharged into the urine when the excretory concretion is liberated by the nephrocyte. Glycogen rosettes are observable predominantly in the basal cytoplasm. The basal cell membrane is folded.

The release of the vacuole concretion or excretory spherule into the renal lumen appears to occur by destruction of the nephrocyte. An unspecific endocytic mechanism has been suggested to occur in the basal region of *Helix* nephrocytes. Such an activity would allow incorporation of substances that, after a process of intracellular digestion, would incorporate into the excretory vacuole (Sanchez-Aguayo et al. 1989).

4.3.6.1.1 Primary Ureter The primary ureter epithelium of *Helix* is a simple columnar epithelium formed by principal cells, which have both a well-developed microvillar border and some cytoplasmic projections (Figure 4.34a). Ultrastructurally, pyramidal ciliated cells can be observed intercalated between these cells. Among them can occasionally be observed polymorphous cytoplasmic projections, granular in appearance and lacking organelles, which are orientated toward the lumen of the organ. One of the most striking ultrastructural characteristics of the principal cells of the ureter is the presence of deep mitochondria-associated basal folds, which can reach as far as the superior region of the cell. The nucleus, which is voluminous and irregular, has a different location depending on the cell type under consideration. Thus, in the principal

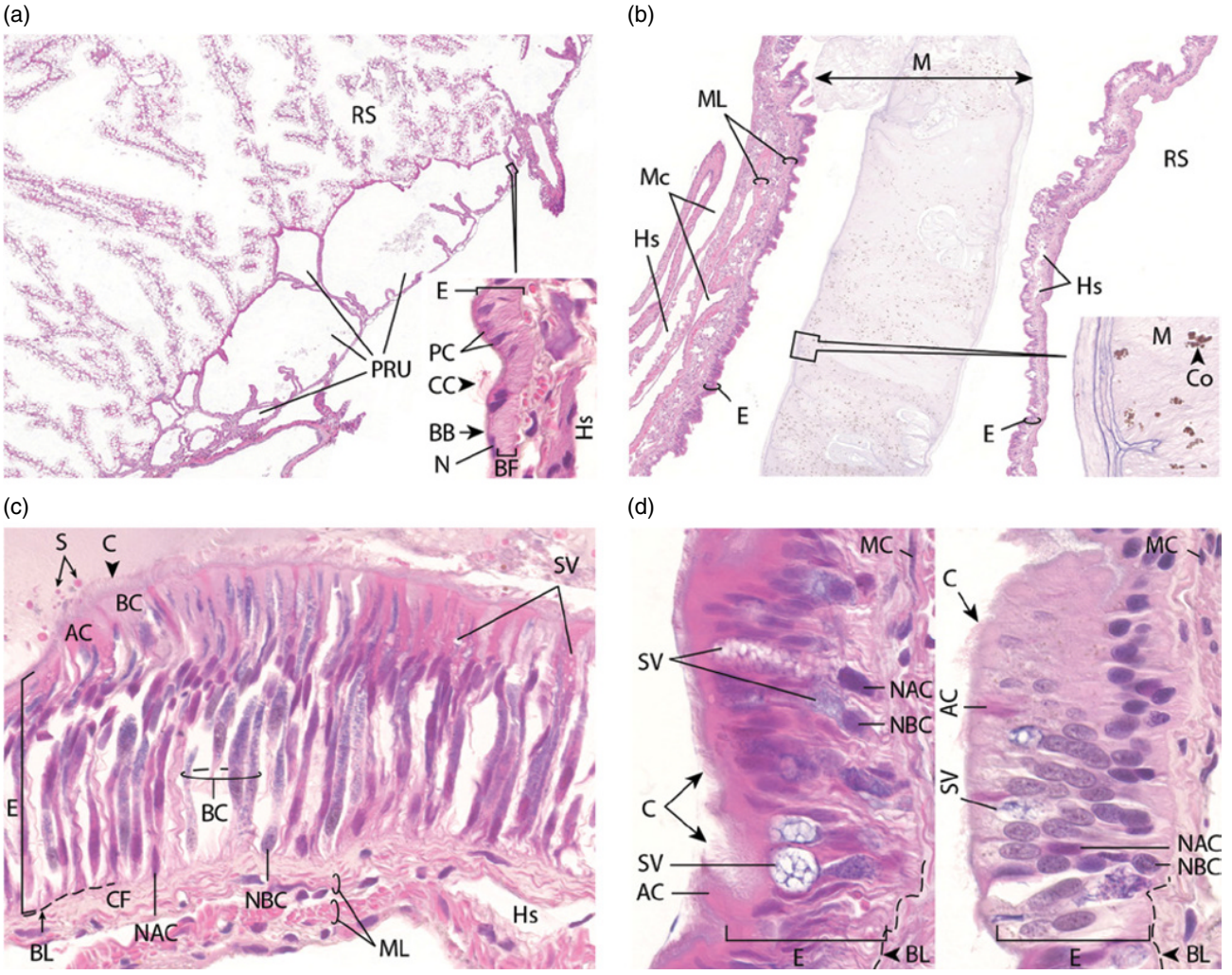


Figure 4.34 Renal sac and ureters in *Helix*. (a) Overview of primary ureter and cell types of its epithelium (inset). 40×. HE, inset 1000×. (b) Overview of secondary ureter and its content (inset) of *Helix*. 50×. HE, inset 1000×. (c) Epithelial cell types of secondary ureter. 600×. HE. (d) Acidophil and basophil cell groups in the secondary ureter. 750×. HE. AC, acidophil cell; BB, brush border; BC, basophil cell; BF, basal folds; BL, basal lamina; C, cilia; Co, concretions; CC, ciliated cell; CF, collagen fibers; E, epithelium; Hs, hemolymph sinus; M, mucus; Mc, mantle cavity; MC, muscle cell; ML, muscle layers; N, nucleus; NAC, nucleus of acidophil cell; NBC, nucleus of basophil cell; PC, principal cell; PRU, primary ureter; RS, renal sac; S, secretion; SV, secretory vesicles.

cell it is displaced toward the apical region by the basal folds, whereas in the ciliated cells the nucleus is situated in the medial or basal region of the cell. The principal cell could thus be considered specifically involved in the recovery of certain molecules and ions, which might be lost during excretion. The presence of basal folds suggests active mechanisms of ionic reabsorption. The ciliated cells appear to be involved in the outward displacement of the luminal content on the ureter. The energy required for the ciliary activity may be supplied by the mitochondria located in the basal folds of the cell (Sanchez-Aguayo et al. 1987).

4.3.6.1.2 Secondary Ureter The secondary ureter epithelium of *Helix* is a simple columnar epithelium containing two cell types: one with acidophil cytoplasm and the other with basophil cytoplasm (Figure 4.34b–d). Both of these cells are ciliated. Secretory vesicles and secretion can be observed in both. The secretion of acidophil cells is dissolved during histologic preparation, while the secretion stains blue in basophil cells.

4.3.6.2 Nephridial Gland

The nephridial gland is microscopically similar to and potentially indistinguishable microscopically from the resorptive kidney (Figure 4.35). Its cells are variably vacuolated and may have apical cilia.

4.3.7 Circulatory System

The circulatory system consists of a heart, arteries, veins, and hemolymph vessels. The hemocoel (i.e., body cavity containing circulatory fluid) is not considered to be a coelomic derivative but rather a vascular sinus related to the stroma that fills the spaces between the viscera. The heart resides within a pericardial sac and has a single ventricle, one or two auricles, and in some species an aortic bulb (or bulbous aortae). The pericardial cavity is a fluid-filled true coelom lined by endothelial-type cells (Fernandez 1971). A valve typically separates these compartments, promoting unidirectional flow of hemolymph. Circulatory patterns vary among gastropods but in general, blood passes from the heart through the aorta to open hemolymphatic spaces (or visceral sinuses) in most tissues. The hemolymph then passes to the kidney, then to the mantle and gill, allowing oxygenation before returning to the heart.

The fluid of the circulatory system is referred to as hemolymph rather than blood, because there is no separation of interstitial fluid and intravascular fluid. Hemolymph is typically isosmotic or mildly hyperosmotic, approximating the ionic composition of the gastropod's environment (Little 1981). Hemocyanin is the circulating respiratory pigment dissolved in the plasma of hemolymph.

4.3.7.1 Heart

All chambers of the heart are similarly layered. The bulk of the organ consists of myocardium, lined on the outside surface by epicardium, and lacking an endocardium on its inner surface. The myocardium consists of muscle cells that are typically striated and form trabeculae supported by collagenous matrix, sparsely populated by fibrocytes and nerve processes, particularly near the lumen (Figure 4.36). The ventricular myocardium is typically thicker than the

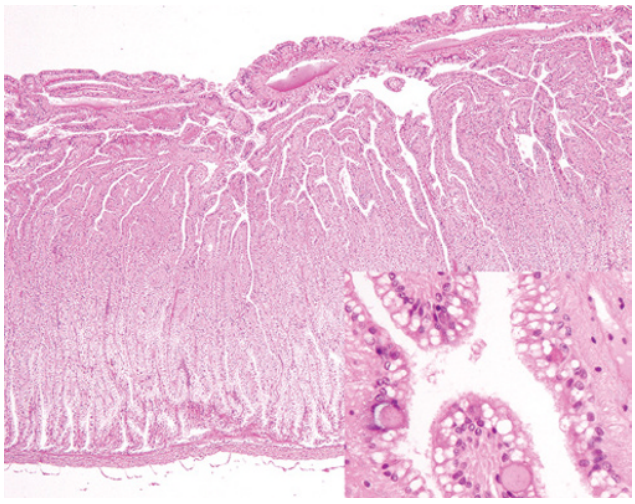


Figure 4.35 Nephridial gland of *Lobatus gigas*. Tubular folds are lined by simple pyramidal to columnar cells interspersed with mucocytes. 40×. HE. Inset: Higher magnification demonstrates detail of epithelial cells that commonly show distinct cytoplasmic vacuoles. 400×. HE.

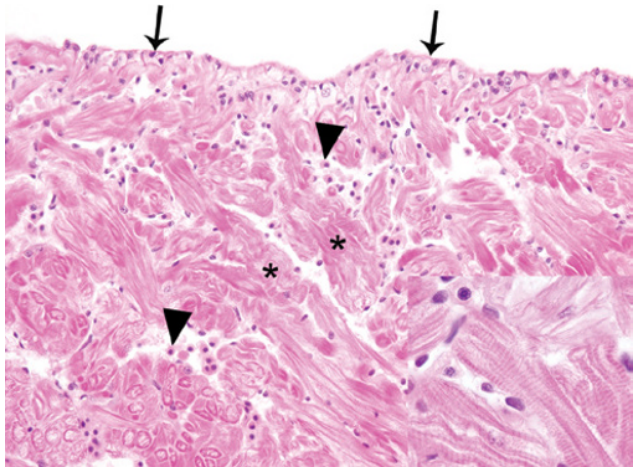


Figure 4.36 Heart of *Lobatus gigas*. The myocardium consists of trabeculae (*asterisks*) of muscle cells. The lumen contains hemocytes (*arrowheads*) and is not lined by endothelium. The epicardium (*arrows*) consists of simple cuboidal to squamous epithelial cells. 400×. HE. Inset: Higher magnification demonstrates cross-striation in muscle cells. 1000×. HE.

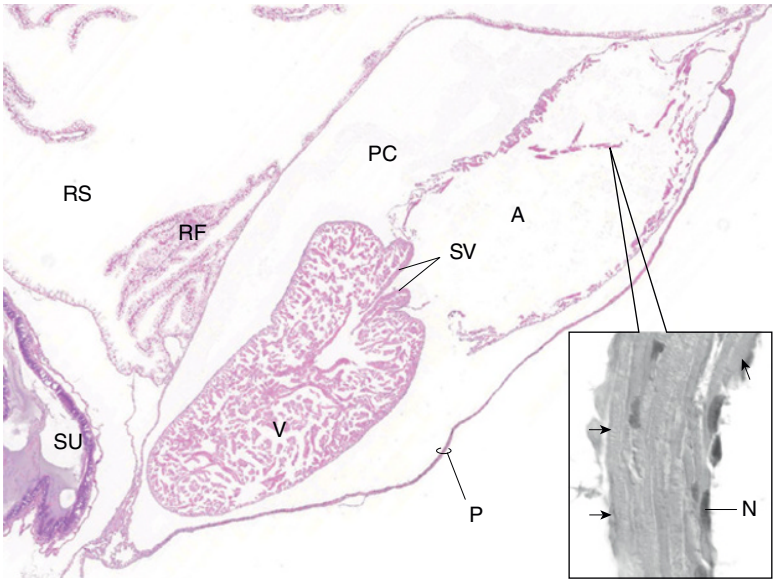


Figure 4.37 Overview of heart in pericardium and striated heart muscle of *Helix*. A, atrium; N, nucleus of muscle fiber; P, pericardium; PC, pericardial cavity; RF, renal folds; RS, renal sac; SU, secondary ureter; SV, semilunar valves; V, ventricle; black arrow, striation of muscle fibers. 13×. HE, inset 900×, HE grayscale.

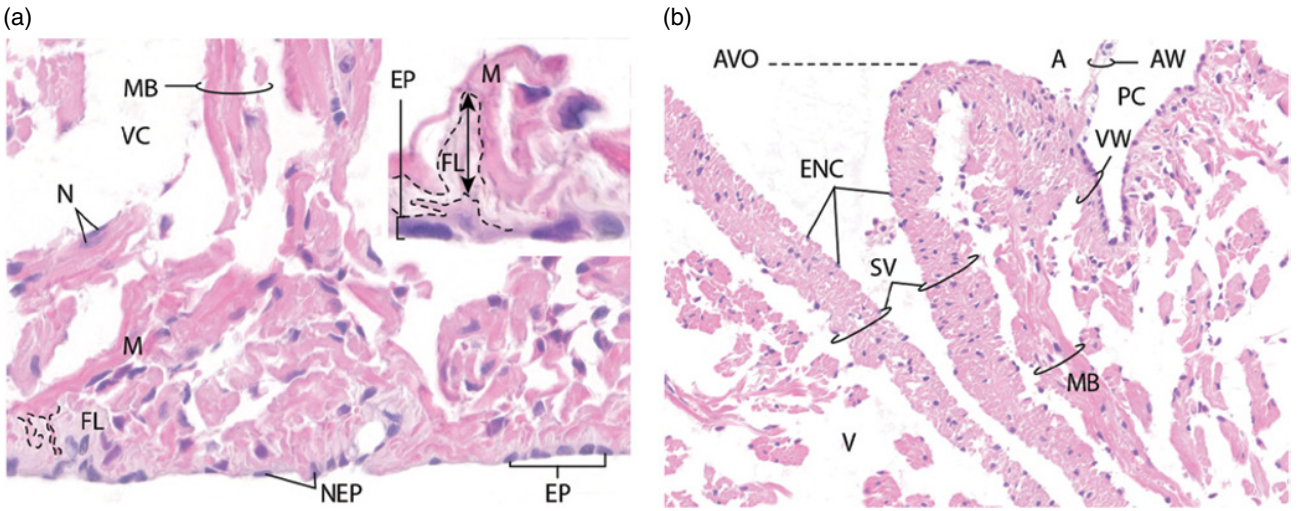


Figure 4.38 Histologic structure of the heart of *Helix*. (a) Ventricular wall. 300×, inset 1000×. HE. (b) Semilunar valves at the atrioventricular orifice. 200×. HE. A, atrium; AVO, atrioventricular orifice; AW, atrial wall; ENC, endothelial cells; EP, epicardial cells; FL, fibrous layer; MB, muscle bundles; M, muscle fibers; N, nuclei of muscle fibers; NEP, nuclei of epicardial cells; PC, pericardial cavity; SV, semilunar valves; V, ventricle; VC, ventricular cavity; VW, ventricular wall. Dotted lines, boundaries of fibrous layer.

auricular myocardium, and the aortic bulb is histologically similar to the ventricle.

Squamous to cuboidal epithelium make up the epicardium. In the auricle, these cells are typically highly specialized (termed "podocytes"), demonstrating intricate foot processes and fenestrations on electron microscopy (Andrews 1985), and having an important role in formation of the urinary filtrate. In some gastropods, these cells form folds, fimbriae, or pouches supported by a greater amount of connective tissue, referred to as pericardial glands (Andrews 1981; Schrödl and Wägele 2001).

The heart of *Helix* is composed of a thin-walled auricle and a strongly muscled ventricle (Figures 4.37 and 4.38). The two cavities communicate by a small orifice guarded by a pair of very efficient semilunar valves, projecting into the cavity of the ventricle. On the border between the ventricle and the main aorta, there is only one valve. The contractile tissue of the heart consists of unmistakable striated muscle. The muscle is composed of elongated spindle-shaped cells closely superimposed and very intimately attached to each other. Without careful examination, the cellular structure is not observable. The ventricle laid open shows them passing in all directions and interlacing in an elaborate and irregular manner. The valves are attached to the opening by the continuity of their fibers with those of both chambers of the heart. Sections of the heart stained

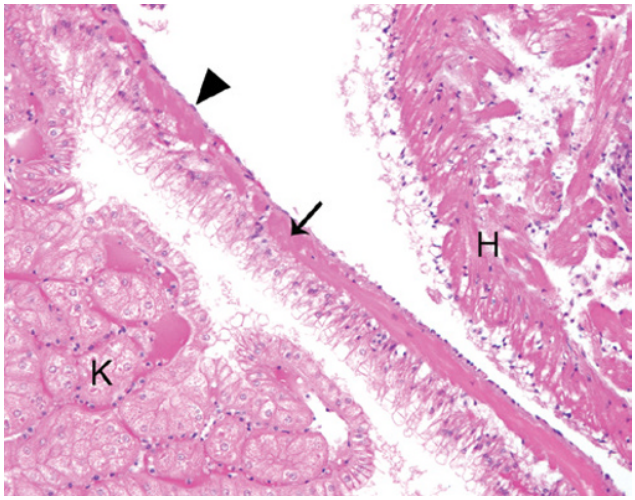


Figure 4.39 Pericardium of *Cittarium pica*. The pericardium consists of a membrane (*arrow*) of dense collagenous connective tissue that contains the heart (H) and is in close proximity to the kidney (K). It is internally lined by simple squamous epithelium (or endothelium) (*arrowhead*). 1000×. HE.

with hematoxylin present a curiously dotted appearance under a low power; this is chiefly due to the connective tissue corpuscles with which the muscle abounds. These bodies are about 9 µm in diameter, and contain a large nucleus embedded in a scanty protoplasmic body that stains in chloride of gold. They are often pyriform in shape and may be seen in profile projecting from the side of a muscular bundle or sending fine processes to be intimately distributed among the muscle cells. The pulmonary vein is lined with a regular endothelium and an epithelium coats the inner surface of the aorta. Analogy would suggest that the same membrane that lines the vessels also lines the cavity of the heart internally. The free, luminal surface of epicardial cells bears an irregular microvillus border.

4.3.7.2 Pericardium

The pericardium consists of dense collagenous connective tissue populated by muscle cells and fibrocytes (Figure 4.39) (Økland 1982). On its external surface, it may become continuous with surrounding muscle. It has an inner surface of simple squamous epithelium (or endothelium), potentially including podocytes similar to those of the epicardium (Fahrner and Haszprunar 2002). Hemocytes are often produced in the anterior pericardial wall (APW) near the base of the aorta, in some gastropods forming a distinct hemocyte (amebocyte)-producing organ (or blood gland). The hemocyte-producing organ (HPO) of *Helix* is described in section 4.3.8.1.

In *Helix*, the pericardium is muscular and lined by a beautiful endothelial mosaic of cells (Darwin 1876; Fernandez 1971). A thin basal lamina and a thin layer of extracellular connective tissue fibrils appear beneath the pericardial endothelial cells. Exterior to this, a smooth muscle layer is located (Figure 4.40) (Jeong et al. 1983).

4.3.7.3 Hemolymphatic Vessels

Microscopically, arterial and venous hemolymphatic vessels appear similar. Walls are composed of circular layers of SMCs and connective tissue. In larger vessels such as aorta, there may instead be longitudinally oriented layers, and the muscle layer may be within a connective tissue sheath. Generally, endothelium is considered to be lacking from hemolymphatic vessels, although simple squamous (endothelial-like) cells may discontinuously line the internal surface of hemolymphatic vessels of some species.

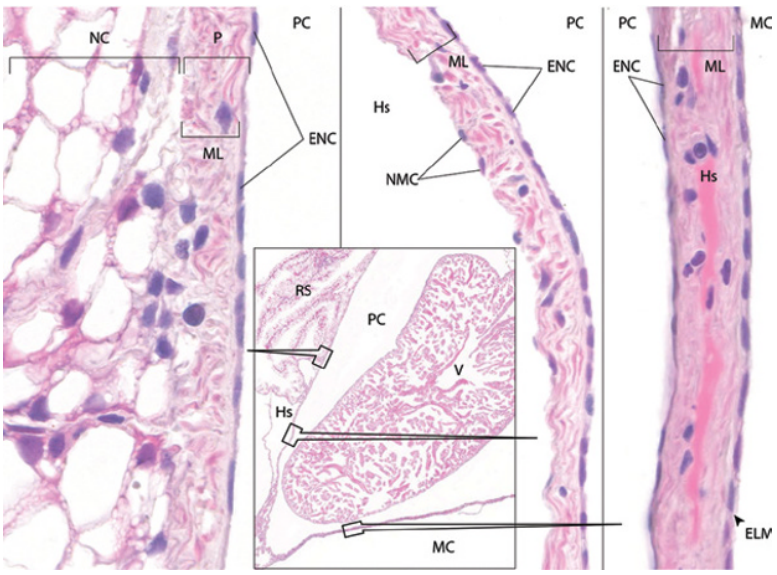


Figure 4.40 Different areas of the pericardium of *Helix*. ELM, epithelial layer of the mantle; ENC, endothelial cells; Hs, hemolymph sinus; MC, mantle cavity; ML, muscle layer; NC, nephrocytes; NMC, nuclei of muscle cells; P, pericardium; PC, pericardial cavity; RS, renal sac; V, ventricle. 600×. 400×. 400×. HE.

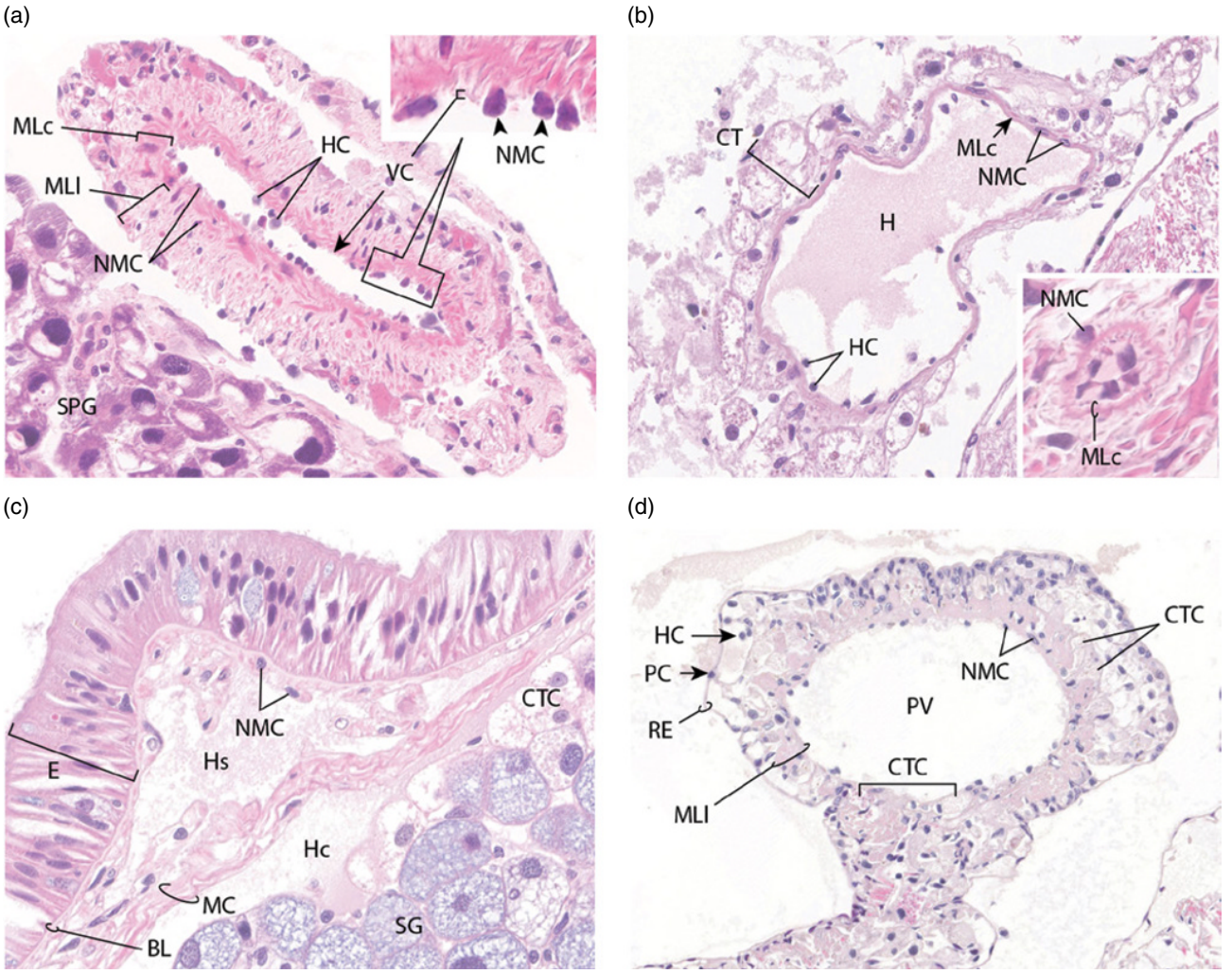


Figure 4.41 Hemolymphatic vessels in *Helix*. (a) Artery above subpedal gland. 300×. HE, inset 1500×. (b) Small artery and capillary (inset) of *Helix*. 250×. HE, inset 900×. (c) Hemolymph sinus in the crop wall of *Helix*. 400×. HE. (d) Pulmonary vein of *Helix*. 200×. HE. BL, basal lamina; CT, connective tissue; CTC, connective tissue cell; E, epithelium; H, hemolymph; Hc, hemocoel; HC, hemocytes; Hs, hemolymphatic sinus; MC, muscle cell; MLc, circular muscle layer; MLl, longitudinal muscle layer; NMC, nucleus of muscle cell; PC, pneumocyte; PV, pulmonary vein; RE, respiratory epithelium; SG, salivary gland; SPG, subpedal gland; VC, vascular coat.

Where hemolyphatic vessels taper to a small scale within a tissue, they may be referred to as sinuses, or capillaries when lined by endothelium.

In *Helix* the layer of single muscle cells at the hemocoel border of the neural sheath is discontinuous and lined by an extracellular material called the vascular coat (Figure 4.41a). This layer is 0.1–0.25 µm thick and consists of a basement membrane and scattered collagen fibrils. A vascular coat also lines the lumen of the blood capillaries and aorta. Capillaries appear as flattened ducts (lumen 0.1–0.3 µm in diameter) (Figure 4.41b). The capillary wall has an irregular outline and is formed by a single layer of muscle cells whose thickness varies from 0.1 to 5 µm. One of the most peculiar features of these capillaries is the discontinuous character of their walls. The muscle cells in this wall may be as long as several microns. Adjacent muscle cell profiles are separated by either narrow slits (150 Å) or large gaps (0.2–0.4 µm) which freely connect the capillary lumen with the extracellular space of the sheath. Desmosomes may be attached to the membranes of the capillary slits while hemidesmosomes, associated with thin filaments, sometimes anchor to the membranes of the capillary gaps. At the level of the gaps, the nonluminal basement membrane of the muscle cells is continuous with that of the vascular coat. The hemocoel and blood vessels consistently lack an endothelial lining (Fernandez 1971) (Figure 4.41c,d).

4.3.8 Immune System

The immunobiology of gastropods is poorly understood (Loker 2010). An important component of their innate immunity is the predominance of mucus-producing cells

and ciliated cells among tissue surfaces. Hemocytes (formerly referred to as amebocytes) make up the cellular component of hemolymph and the innate immune system. Hemocytes have roles in defensive reactions (such as phagocytosis, encapsulation, parasite counteraction), production of cytotoxic molecules, wound healing and tissue repair, blood coagulation, transplant rejection, and potentially postspawn dissolution of gonads (Adamowicz and Bolaczek 2003; Loker 2010; Pila et al. 2016). They may also be involved in digestion, absorption, and excretion, and are probably involved in the transport of hemocyanin as well as other proteins (Fernandez 1971). In gastropods, hemocyte production is thought to occur primarily in the pericardial sac, sometimes involving a region termed the hemocyte-producing organ, but hemocytes may also retain the ability to divide in other locations (Pila et al. 2016). Hemocytes may migrate from the hemolymph into tissue in response to injury. Hemocyte-like phagocytic cells may also be "fixed" residents of a tissue (Matricon-Gondran and Letocart 1999).

4.3.8.1 Hemocyte-Producing Organ (HPO)

Between the mantle and pericardium of the APW lie loose connective tissue and a hemolymph sinus. Typical cells of the connective tissue are present, including fibroblast-like cells, pore cells (rhogocytes), and hemocytes (Figure 4.42a,b). Additionally, isolated or confluent nodules of small, mitotically active basophilic cells (hemocyte precursors) are found attached to the basal surface of the pericardial endothelium, and based on histologic, histochemical, and ultrastructural evidence appear to be hemocyte precursors. Consequently, this structure has been named the HPO. In addition to structural evidence, transplantation studies support a hematopoietic function of the HPO (Dos Santos Souza and Andrade 2006; Lie et al. 1975; Pila et al. 2016).

Pseudopodial extensions of peripherally and internally located SMCs and extensions from few small fibroblasts appear to form a stroma or reticular network in which the developing blood cells lie (Figure 4.42b,c). Cytoplasm of SMCs contains actin and paramyosin filaments. Extracellular collagen-like fibrils surround muscle cells and their extensions. This is the first description of a structure in *Helix* that is anatomically and histologically similar to the HPO of *Biomphalaria* sp. (Jeong et al. 1983), which has also been reported in a number of other gastropods.

Hemocytes appear in small clusters in the quiescent HPO (Figure 4.43). The infrequent mitotic figures observed in the quiescent HPO are most often associated with the ameboblast layer next to the pericardial epithelium.

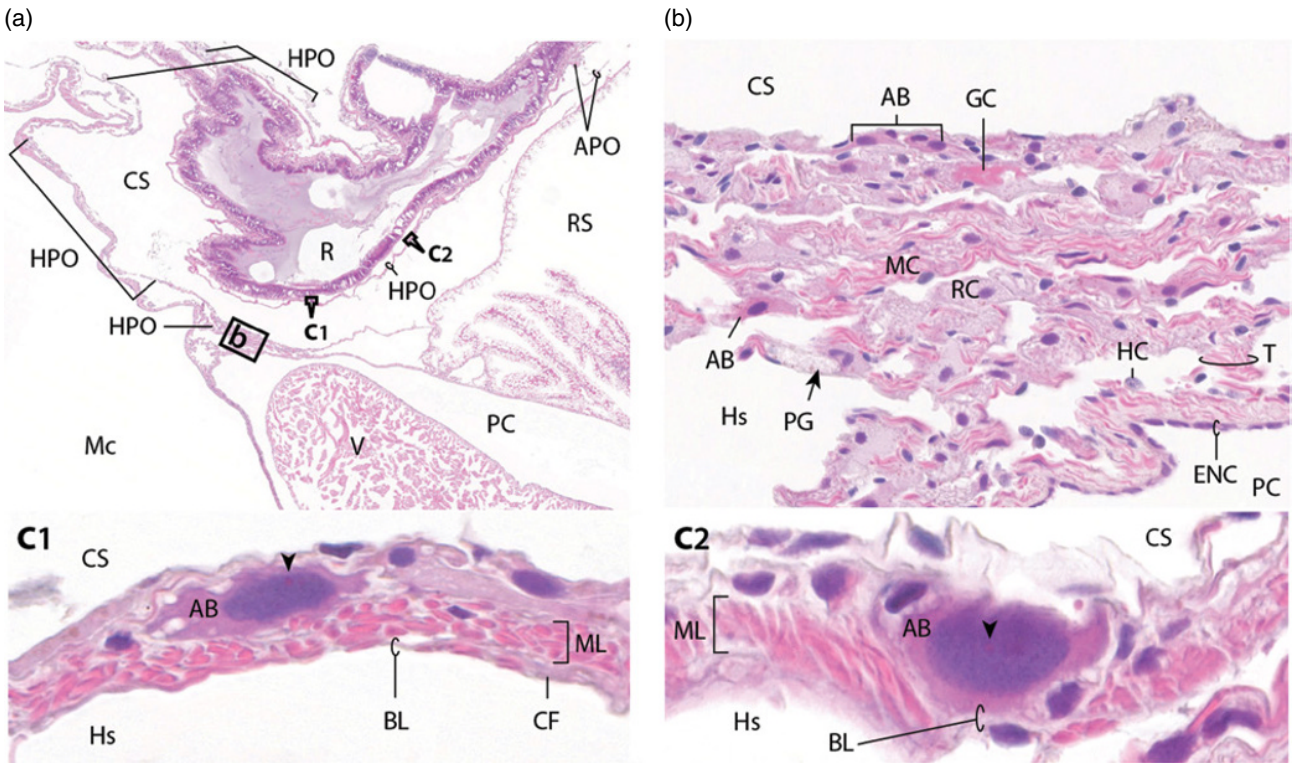


Figure 4.42 Hemocyte-producing organ in *Helix*. (a) Overview of hemocyte-producing organ between renal sac, pericardium, and rectum. 20×. HE. (b) Microstructure of hemocyte-producing organ of *Helix*. 300×. HE. (c) Ameboblasts of *Helix*. 1000×. HE. AB, ameboblasts; BL, basal lamina; CF, collagen fibers; CS, central sinus of HPO; ENC, endothelial cells; GC, granule cell; HC, hemocyte; HPO, hemocyte-producing organ; Hs, hemolymph sinus; MC, mantle cavity; Mc, muscle cells; ML, muscle layer; PC, pericardial cavity; PG, pigment granules; R, rectum; RC, rhogocyte (pore cell); RS - renal sac; T, trabecula; V, ventricle; black arrowheads, nucleoli of ameboblasts.

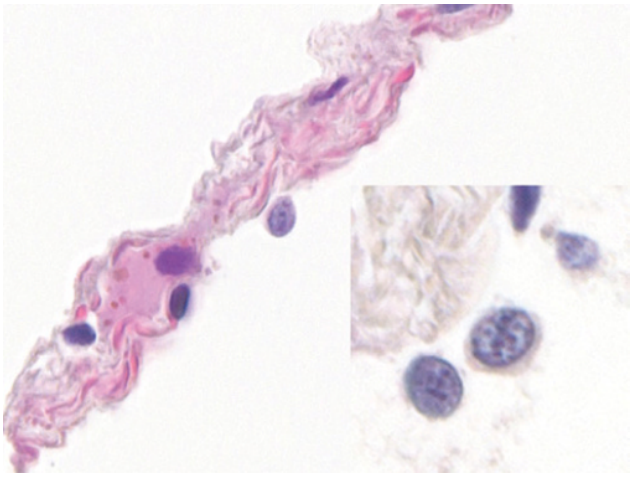


Figure 4.43 Type I hemocytes in the hemocyte-producing organ and in the lung (inset) of *Helix*. 1000× HE, inset 1500×.

Ameboblasts are hematogenic blast cells, progenitors of hemocytes. Small fibroblast-like cells and extensions, occasionally containing pigment granules, form stroma surrounding primary ameboblasts (Jeong et al. 1983).

4.3.8.2 Hemocytes

Hemocytes are classically categorized into two main cell types: granulocytes (or type I hemocytes) and hyalinocytes (or type II hemocytes) (Accorsi et al. 2013). Granulocytes (~15–25 µm dimeter) have cytoplasmic granules and constitute the majority of cells. They are similar to spread hemocytes (see below) and they have filopodia. They show a tendency to form aggregates of up to a dozen cells, which may suggest a function associated with preventing loss of body fluids, wound healing, and cell defense reactions. Ultrastructural studies on granulocytes have revealed that their cytoplasm contains numerous mitochondria, rough endoplasmic reticulum, phagosomes and lysosomes, which indicates their phagocytic abilities. Hyalinocytes (~6 µm dimeter) are poorly granulated and account for <15% of cells (Pila et al. 2016; Voltzow 1994). They have a high nuclear–cytoplasmic ratio, and limited capability to form pseudopodia and to spread on the substratum. They form short lobopodia only rarely and have a stable form. Type II hemocytes have two subpopulations. The first subpopulation contains small and agranular cells. They present the characteristics of blast-like cells, with an agranular and basophilic cytoplasm. The second one displays larger size and a more articulated internal organization. Some of these hemocytes display numerous granules (Adamowicz and Bolaczek 2003).

However, the relevance of the distinction between granulocytes and hyalinocytes is debated as they may reflect morphologic forms of one basic type of hemocyte (Sminia et al. 1983). Many studies instead classify hemocytes according to size (Martins-Souza et al. 2009; Matricon-Gondran and Letocart 1999), or as round cells and spread cells (Adamowicz and Bolaczek 2003). Round cells, making up ca. 5% of the total population of hemocytes, are small, with a high nuclear–cytoplasm ratio. The spread cells, constituting ca. 95% of the hemocyte population, are polymorphic cells with large pseudopodia, polymorphic nuclei, and numerous granules in the cytoplasm. The two types of hemocytes most probably represent different development stages of one basic type. The round cells would represent young, blastic cells, while the spread cells would constitute a population of mature cells.

Histologically, hemocytes may be difficult to distinguish. They may be associated in groups or scattered within the sheath and vascular channels, appearing as round nucleated cells with abundant cytoplasm, similar to a macrophage (Figures 4.43 and 4.44).

4.3.9 Respiratory System

Similar to other systems, much variation exists in the respiratory anatomy of gastropods. In marine prosobranchs, the gill(s) (or ctenidium/ctenidia) is located along the dorsal mantle cavity, next to the osphradium and hypobranchial gland. In contrast, in terrestrial prosobranchs the gill may extend outside the mantle cavity or be lacking altogether. Gill filaments typically consist of triangular leaflets supplied by afferent (dorsal) and efferent (ventral) hemolymphatic vessels in an arrangement that creates cross-current gas exchange, maximizing oxygenation. Opisthobranchs have a plicate gill consisting of folds of mantle wall resembling a fan or plume that is variably exposed, depending on the extent to which the mantle is reduced (Fischer et al. 2006; Schrödl and Wägele 2001). The gill is often located on the right side, but there is a great degree of variation, for example the circumanal gills found in doridacean nudibranchs. Opisthobranchs may also respire through cerata, finger-like structures that decorate the mantle which also function in digestion and defense. In terrestrial or amphibious gastropods, the gill is often reduced or lacking completely, and is replaced by a vascularized pouch of mantle referred to as lung.

4.3.9.1 Gill (Ctenidium)

Gills consist of a filament (or septum or axis) that bears triangular lamellae (or leaflets) on either one side (monopectinate gills) or both sides (bipectinate gills). In marine prosobranchs, cores of loose fibrous connective tissue containing small hemolymphatic vessels support lamellae. Simple cuboidal epithelial cells, and fewer granular gland cells and mucocytes, line the lamellae (Figure 4.45). Lamellar tips flare and epithelial cells become columnar

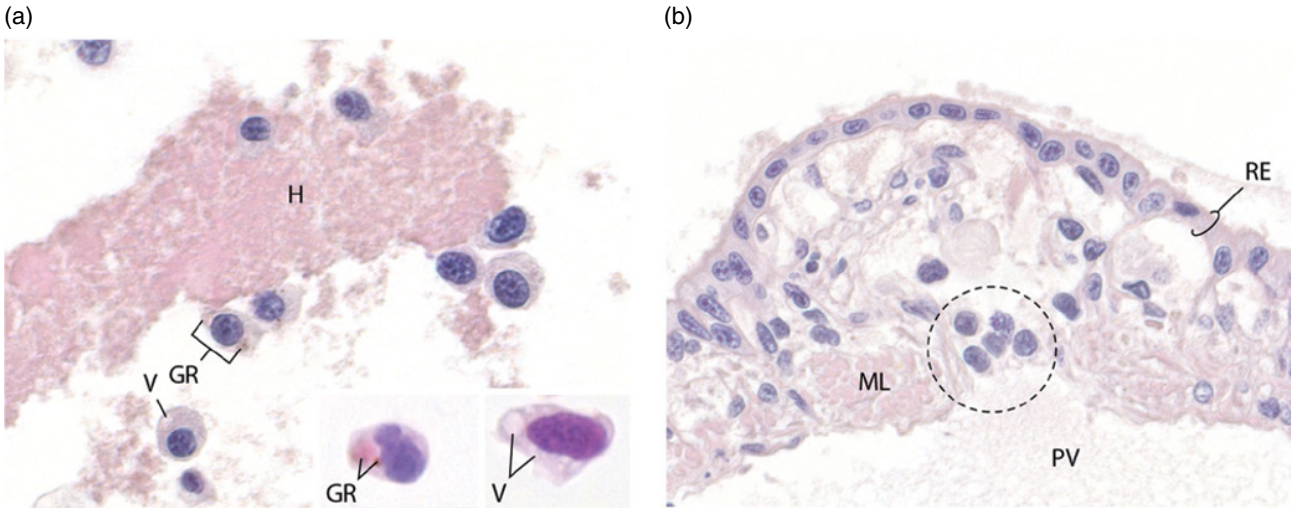


Figure 4.44 Type II hemocytes of *Helix*. (a) Type II hemocytes in the lung and in the hemocyte-producing organ (insets). 900×. HE, insets 1000×. (b) Group of type II hemocytes (*circle*) in the lung of *Helix*. 600×. HE. GR, granules; H, hemolymph; ML, muscle layer; PV, pulmonary vein; RE, respiratory epithelium; V, vacuoles.

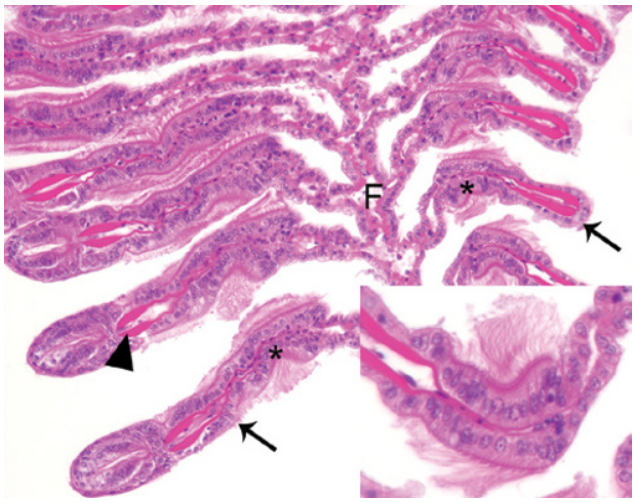


Figure 4.45 Gill of *Tegula excavata*. The gill is bipectinate, bearing lamellae (*arrows*) on each side of the filament (F). Near the tips of lamellae, epithelial cells (*asterisks*) become columnar and bear long cilia, and skeletal rods (*arrowhead*) are present. 400×. HE. Inset: Higher magnification of lamellae showing columnar epithelial cells with long cilia. 1000×. HE.

and heavily ciliated. The tips of the lamellae hold "skeletal rods," conspicuous deposits of basement membrane (Chitramvong et al. 2002; Tiley et al. 2019). This region of lamellae may also contain nerve fibers and muscle cells. In opisthobranchs, gill folds consist of thin trabeculae of connective tissue and muscle cells, with central hemolymphatic vessels (Schrödl and Wägele 2001). These are lined by simple squamous to cuboidal, sometimes ciliated epithelium. There may be glands at the base of gill folds. In some species, successive branches may form from the primary filament.

4.3.9.2 Lung

The lung is a thin-walled vascularized pouch in the dorsal mantle. The respiratory surface of the lung of *Helix* is lined with a squamous epithelium (Figure 4.46). The surface of respiratory epithelial cells (pulmonocytes) presents microvilli. The lung parenchyma is filled mostly with bundles of muscle fibers arranged in various planes, and a small quantity of connective tissue. There are numerous intertissue spaces representing hemolymphatic sinuses. Their inner surface is not lined with endothelium, similar to other hemolymphatic vessels.

Special gland cells, containing and secreting lamellar bodies similar to those of vertebrates (type II pulmonocytes), are found in the snail lung. The secreted material shows biochemical properties consistent with those observed in surfactants from "lower" vertebrates. Surfactant is characterized by the presence of disaturated phospholipid (DSP), especially disaturated phosphatidylcholine (PC). Snail surfactant has a relatively weak surface activity, defined as poor surface tension-lowering ability, but has good film spreadability (Daniels et al. 1999).

4.3.10 Nervous System

The central nervous system consists of paired cerebral ganglia connected by commissures or nerves. These form a circumesophageal ring containing the paired cerebral and buccal ganglia, a pair of longitudinal cords that ventrally supply the paired pedal ganglia of the foot, and a loop that dorsally supplies the paired intestinal and pleural ganglia, and unpaired visceral ganglion. The circumesophageal ring is concentrated around the esophagus, just aboral

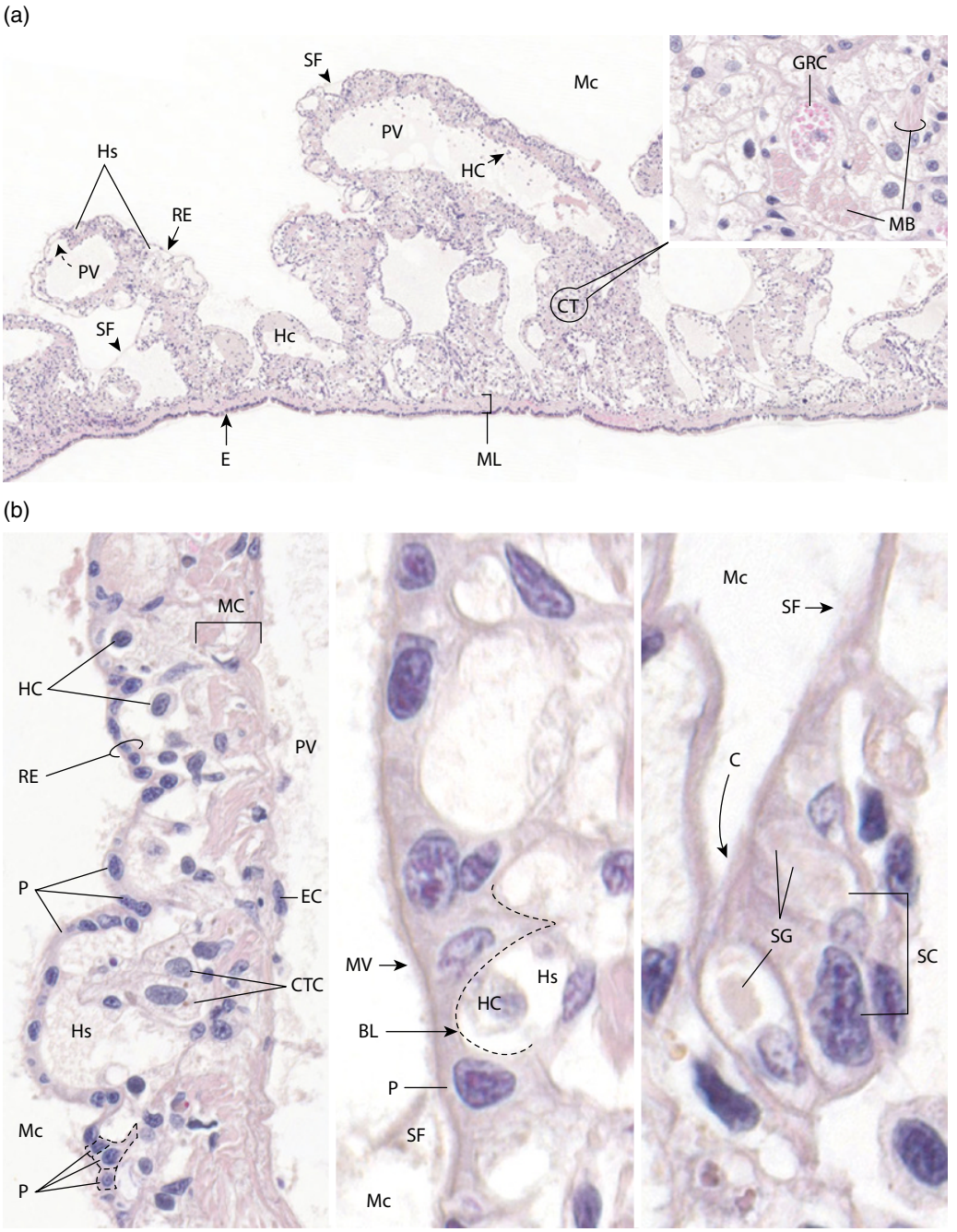


Figure 4.46 Lung of *Helix*. (a) Overview of cross-section of the lung. 30×. HE, inset 650×. (b) Respiratory surface, secretory cells of *Helix*. 300×, 1000×, 1000×. HE. BL, basal lamina; C, cilia; CT, connective tissue; CTC, connective tissue cell; E, epidermis; EC, endothelial cell; GRC, granular cells; Hc, hemolymph canal; HC, hemocytes; Hs, hemolymph sinus; Mc, mantle cavity; MC, muscle cells; ML, muscle layer; MV, microvilli; P, pulmonocyte; PV, pulmonary vein; RE, respiratory epithelium; SC, secretory cells; SF, surfactant layer; SG, secretory granules.

from the buccal mass. The cerebral ganglia tend to be the largest and are sometimes referred to as the brain. The ganglia give off nerves that supply particular body parts and organs (Koralewska-Batura 1999).

The cerebral ganglia of *H. pomatia* are large (see Figure 4.50). They are connected with each other by a very short commissure, and with the pedal and pleural ganglia by connectives. Each cerebral ganglion gives off five anterior nerves. Four innervate the region of the mouth, mouth lobe, tentacle, the upper part of the ommatophore, and the eye. The tentacle that bears an eye is called the ommatophore or eyestalk. The fifth nerve runs to the basis of the

ommatophore, and on the right part of the body, its branch reaches the terminal parts of the reproductive organs. The pedal ganglia are also large and connected by a very short commissure. They give off several nerves that go to the sole.

The parietal ganglia of *Helix* are connected by short connectives with the pedal and parietal ganglia. The latter are connected with the unpaired visceral ganglion. These three ganglia are so tightly connected that the borders between them are completely obliterated. The visceral ganglion gives off several very long and asymmetrically arranged nerves, which run to the body walls and the internal organs of the visceral sac. A pair of small buccal ganglia is located above the esophagus, in an invagination at the outlet of the pharynx to the esophagus. They are connected with each other by a fairly long commissure and by long connectives with the cerebral ganglia. They innervate the pharynx, radula, esophagus, and SG. A transverse section through the complex shows several groups of neurons, each corresponding to a different ganglion. In each of these ganglia, there is a superficial cortex, which contains the soma and proximal axon segment of the nerve cells, and a punctiform or fibrillated central core, named the neuropile, made up of nerve fibers.

4.3.10.1 Nerves

Nerves are composed of delicate fibers, sparsely populated by nuclei of support (glial) cells, and arranged in parallel to form bundles (Figures 4.47 and 4.48) (Peretz and Estes 1974). Some have a dense fibrous connective tissue

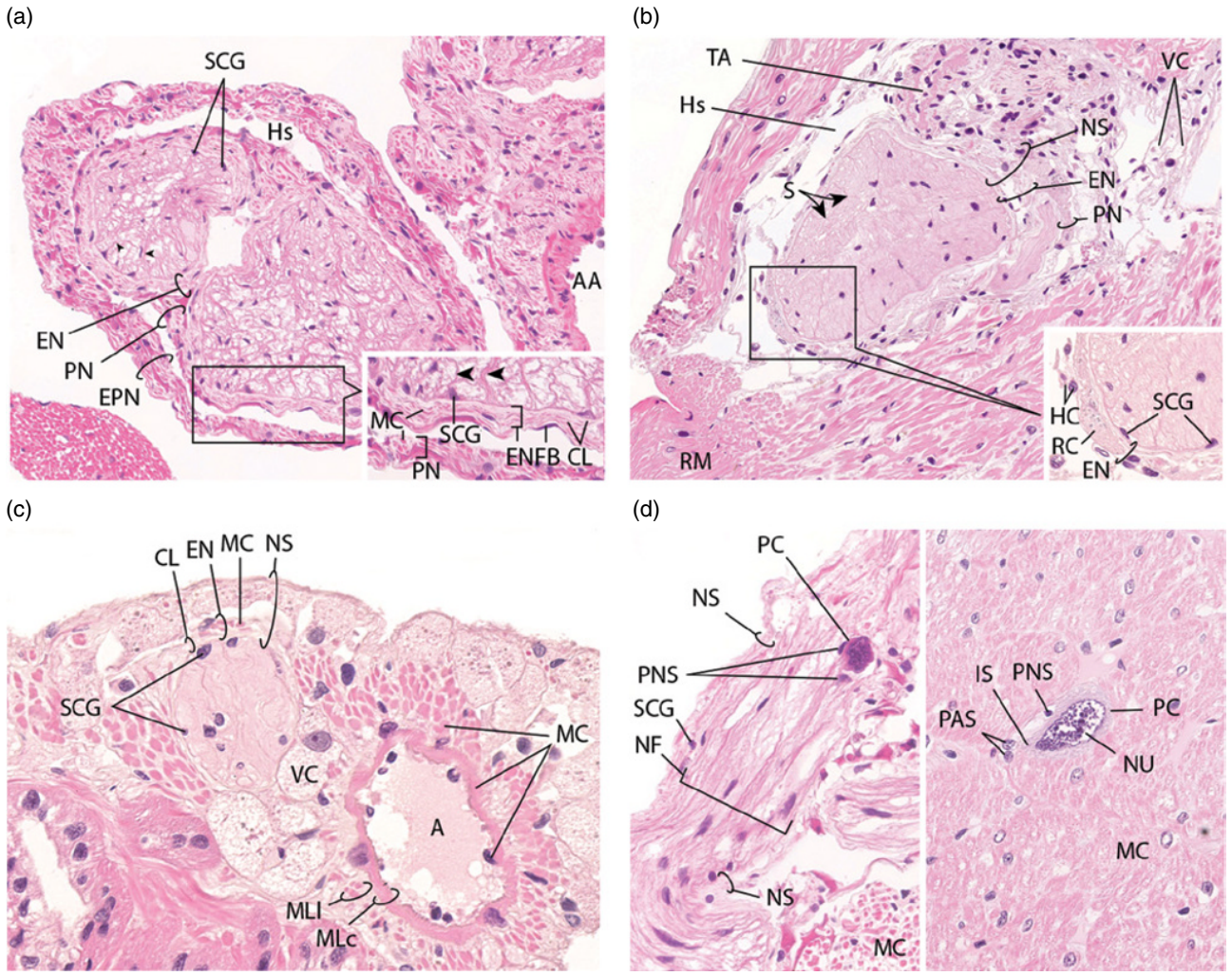


Figure 4.47 Nerves of *Helix*. (a) Large nerve from subesophageal ganglion of *Helix* with neural sheaths and cell types. 300×. HE, inset 600×. (b) A nerve in ommatophore of *Helix*. 300×. HE, inset 1000×. (c) Thin nerve along bursa copulatrix stalk of *Helix*. 600×. HE. (d) Ganglion (neuron) cells in nerve (*left*) and pharyngeal muscle (*right*) of *Helix*. 400×. HE. A, artery; AA, anterior aorta; CL, collagen lamellae; EN, endoneurium; EPN, epineurium; FB, fibroblast; HC, hemocyte; Hs, hemolymph sinus; IS, initial segment of axon; MC, muscle cells; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; NF, nerve fibers; NS, neural sheath; NU, nucleus; PAS, periaxonal satellite; PC, perikaryon; PN, perineurium; PNS, perineural satellite; RM, retractor muscle of tentacle; RC, rhogocytes; S, septa; SCG, subcapsular glial cells; TA, tentacular artery; VC, vacuolar cell; arrowheads, septa.

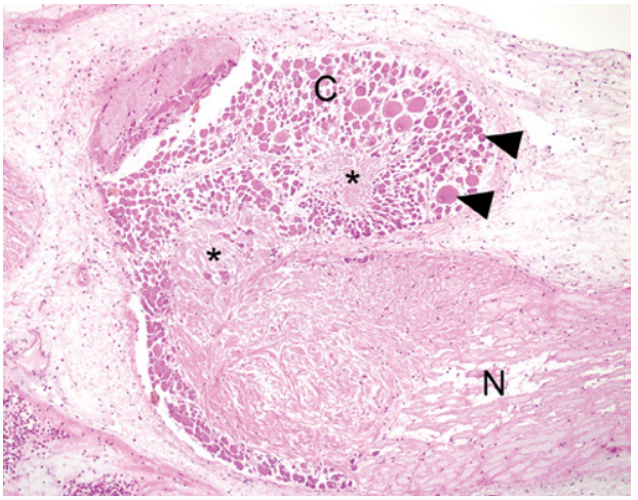


Figure 4.48 Ganglion and nerve of *Lobatus gigas*. Nerves (N) are composed of variably sized fibers arranged mostly in parallel. The ganglion has a cortex (C) of large and small neurons (*arrowheads*) which surrounds a central zone of neuropil (*asterisks*). 100×. HE.

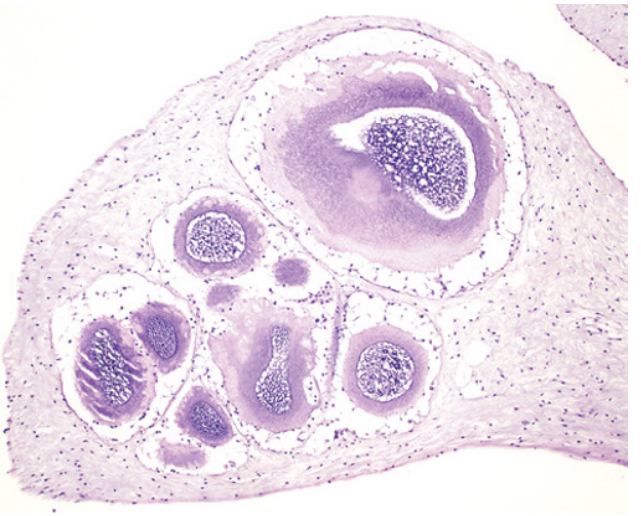


Figure 4.49 Ganglion of *Aplysia californica*. Large neurons predominate. 100×. HE. *Source:* Elise LaDouceur.

sheath. Axons are of variable diameter, and in cross-section have a glassy homogenous eosinophilic circular profile. Some are surrounded by clear space suggesting the presence of a myelin, or similar, sheath.

Large nerves coming from the subesophageal ganglion of *Helix* have three concentric linings: endoneurium, epineurium, and perineurium (Figure 4.47a). The endoneurium wraps the nerve directly. It consists of basal lamina, collagen sheets, and embedded muscle cells (Figure 4.47a–c). The epineurium contains mostly muscle cells (Figure 4.47a). The perineurium bundles neighboring nerves and interweaves with the fibrous layers of endoneurium (Figure 4.47a,b). There are vacuolar connective tissue cells (Leydig cells) and rhogocytes (pore) cells in the wall of the neural sheath (Figure 4.47b,c). Subcapsular glial cells make septa dividing the nerve into sections (Figure 4.47b). The sheath of smaller nerves appears simpler (Figure 4.47c).

4.3.10.2 Ganglia

Ganglia typically have a cortex of neurons and a central core of neuropile, each populated by glial cells (see Figure 4.48) (Tremblay et al. 1979). The neuronal composition of the cortex is variable. Giant neurons over 100 µm in diameter are present in many gastropods, particularly opisthobranchs where they may be over 500 µm in diameter (Figure 4.49) (Dorsett 1967; Gillette 1991). Dense fibrous connective tissue may border or encapsulate ganglia in the form of a neural sheath. Neurons may be referred to as neurosecretory cells where neurosecretory granules are demonstrated.

In *Helix*, neurons can be divided into four groups according to size (Elekes and Nässel 1990): giant neurons (over 100 µm), large neurons (80–100 µm), medium-sized neurons (4–70 µm), and small neurons (12–30 µm). They are distributed in either groups or clusters (typical of small neurons) or in solitary form or in groups comprising 2–3 cells (typical of large and giant neurons). Giant and large neurons reveal only limited arborizations in the neuropile, but rich branching toward and in the peripheral nerves. Giant neurons can be found in the cerebral ganglion (Figure 4.50). Medium-sized neurons are located mostly in the mesocerebrum, where they occupy the outer layer of the cortex. Some of the small neurons have extensive arborizations of varicose fibers in the neuropil. They may therefore play some role in integratory processes.

The superficial region of the neural sheath constitutes about two-thirds of its total width (Figure 4.50a). It contains many closely packed spheroidal cells with a diameter of 30–40 µm. These are vacuolar connective tissue cells (Leydig cells) because of the empty appearance of their cytoplasm. The deep region of the sheath is dense and lamellate. The cells of this part of the sheath show only their nuclei, which appear as dense horizontal chromatic rods. Muscle fibers of the sheath are cylindrical structures. In the surface region they are embedded in the intercellular cement which surrounds the globular cells. In the deeper regions, they follow the direction of the lamellate components of the sheath. In some regions, they can be seen to leave the sheath and join extraganglionic muscles. There are special cells – possibly hemocytes – in the sheath of the cerebral ganglion containing large granules in their cytoplasm with a diameter of 4–7 µm (Figure 4.50a, inset). They are numerous in the ventral region of the sheath.

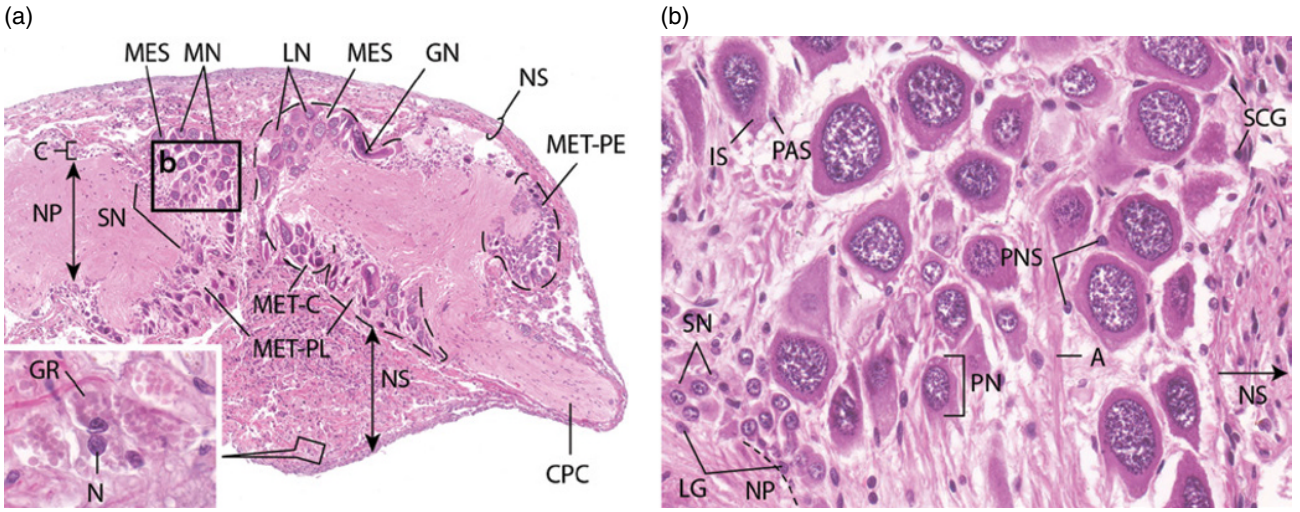


Figure 4.50 Cerebral ganglia of *Helix*. (a) Lobes and cell types and neural sheath. 60×. HE, inset 850×. (b) Glial cell types in the ganglion. 400×. HE. A, axon; C, cortex; CPC, cerebropleural connective; GN, giant neuron; GR, granules; IS, initial segment of axon; LG, limiting glial cell; LN, large neuron; MES, mesocerebrum; MET-C, metacerebrum, commissural lobe; MET-PE, metacerebrum, pedal lobe; MET-PL, metacerebrum, pleural lobe; MN, medium-sized neurons; N, nucleus; NP, neuropile; NS, neural sheath; PAS, periaxonal satellite; PN, perikaryon of neuron; PNS, perineural satellite; SCG, subcapsular glial cells; SN, small neurons; dotted line, boundary between neuropile and cortex.

The nonneuronal elements of the ganglia, the glial cells, are named for their location: subcapsular gliocytes under the basal lamella of the neural sheath, perineural satellite cells surrounding the neuron body, periaxonal satellite cells surrounding the initial segment of the axon, and limiting glial cells at the cortex–neuropile boundary. Glial cells have oval and hyperchromatic nuclei with a diameter of 6–8 µm (Figure 4.50b). The cells are widely spaced from one another. Those immediately beneath the basement membrane of the neural sheath are called subcapsular gliocytes (Figure 4.50b; see also Figure 4.47). They lie between the neural sheath and the cortical neurons. Subcapsular gliocytes often belong to perineural satellites, which form several coats around the soma of the neuron, the number of coats depending on the size of the neuron (Figure 4.47d). The periaxonal satellites are arranged around the axon in several layers, the number depending on axon caliber (Figure 4.47d). As they pass through the neuropile, the axons lose most of their glial coats; only the voluminous axons retain a thin covering. Limiting glial cells are located at the cortex–neuropile boundary, send fine ramifying cytoplasmic processes into the cortex of the ganglion, and whorl around some axons of the neuropil (Figure 4.50b). They may be indistinguishable from periaxonal satellites. There are a few gliocytes within the neuropile (Fernandez 1966).

In *Helix*, large groups of neurosecretory cells are found in the mesocerebrum. They secrete substances of neurohormonal character, which take part in the regulation of most reproductive processes. Collar cells are neurosecretory cells that surround the tentacular ganglion. These cells are larger than the other neurons within the ganglion. The collar cells are bipolar; one process extends to the base of the epithelium that covers the tentacle, and the other merges into the ganglionic mass (Figure 4.51). The cytoplasm of the collar cells is difficult to study by light microscopy because of the many spheroidal inclusions that fill the cell body (Lane 1964).

4.3.11 Reproductive System

Given the variability of gastropod reproductive biology, only generalizations can be described for the microscopic anatomy of the reproductive tract. The gonad usually integrates with the edge of the digestive gland and is coiled into the shell's spire. The genital duct courses from the visceral mass into the mantle, parallel to and on the right side of the rectum where the mantle adjoins the body, then may continue on the body surface (i.e., the floor of the mantle cavity). It is here where gametes, or zygotes in species with internal fertilization, are prepared and packaged. The ducts are flanked by accessory sex glands that produce secretion to support the gametes and progeny; accessory sex glands include the prostate in males and nidamental glands in females. The latter may produce mucoprotein, mucus, protein, or mineral secretions. The glands and ducts may become substantially enlarged during periods of reproductive activity.

4.3.11.1 Gonad

In sexually immature individuals or those in an inactive stage of gonadal development, sex may be difficult to histologically distinguish. The gonads consist mostly of connec-

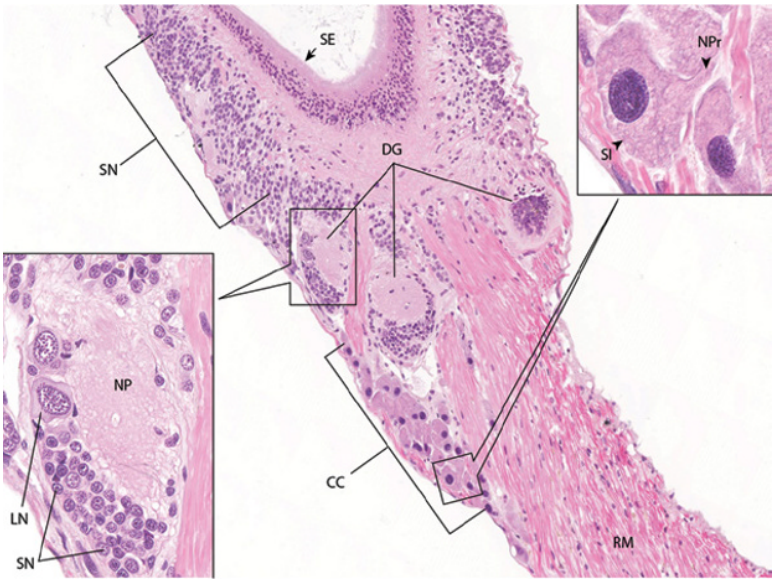


Figure 4.51 Overview of ommatophore with digitate ganglion and collar cells of *Helix*. CC, collar cells; DG, digitiform ganglion; LN, large neurons; NP, neuropile; NPr, neuronal process; RM, retractor muscle of tentacle; SE, sensory neurons; SI, spherical inclusions; SN, small neurons. 100×. HE, insets 600× and 1000×.

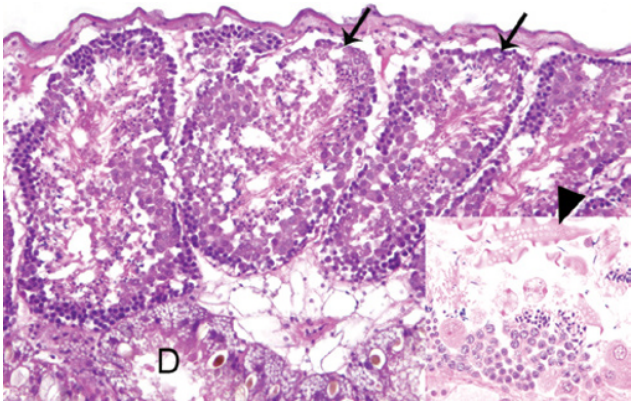


Figure 4.52 Male gonad of *Cerithium* sp. The gonad marginates digestive gland (D) and is composed of seminiferous tubules (*arrows*) lined by spermatogonia that centrally mature to form spermatozoa. 400×. HE. Inset: Comparison to male gonad of *Lobatus gigas* demonstrating presence of paraspermatozoa (*arrowhead*). 400×. HE.

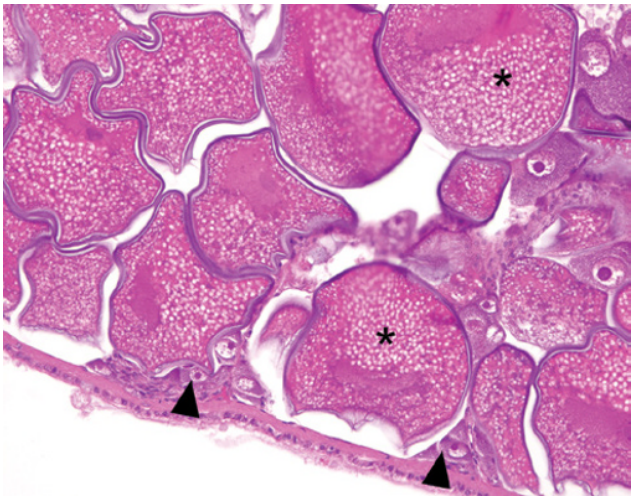


Figure 4.53 Female gonad of *Tegula ecavata*. Vitellogenic oocytes (*asterisks*) dominate the active female gonad and displace oogonia peripherally (*arrowheads*). 400×. HE.

tive tissue and potentially few tubules resembling empty follicles, lined by undifferentiated low cuboidal germinal epithelial cells.

In the male gonad, seminiferous tubules are present during spermatogenesis (Figure 4.52). These are lined by spermatogonia and may contain spermatocytes, spermatids, and/or spermatozoa. Mature spermatozoa may cling to paraspermatozoa (Figure 4.52, inset), also termed atypical spermatozoa, or nutritious cells, large acrosome-lacking spermatozoa incapable of fertilization and thought to provide nutritive support to mature spermatozoa or facilitate transfer of the mature spermatozoa to the female reproductive tract. Developing spermatozoa may also be supported by Sertoli cells (Buckland-Nicks and Chia 1986). Seminiferous tubules supply ducts of the vas deferens. These are lined by ciliated simple columnar epithelial cells and may be engorged with spermatozoa during spermatogenesis.

In the female gonad, follicles are present during oogenesis (Figure 4.53). These are lined by oogonia, which centrally differentiate into oocytes. The mature vitellogenic oocytes have intracytoplasmic bright eosinophilic vitelline granules, representing the presence of yolk protein. Follicles supply ducts of the oviduct. These are lined by ciliated simple columnar epithelial cells and may become distended with vitellogenic oocyte during oogenesis.

The ovotestis of *Helix* consists of lobes, each of which contains numerous ovoid finger-shaped tubular acini (Figure 4.54a). The outer layer, termed the tunica propria of

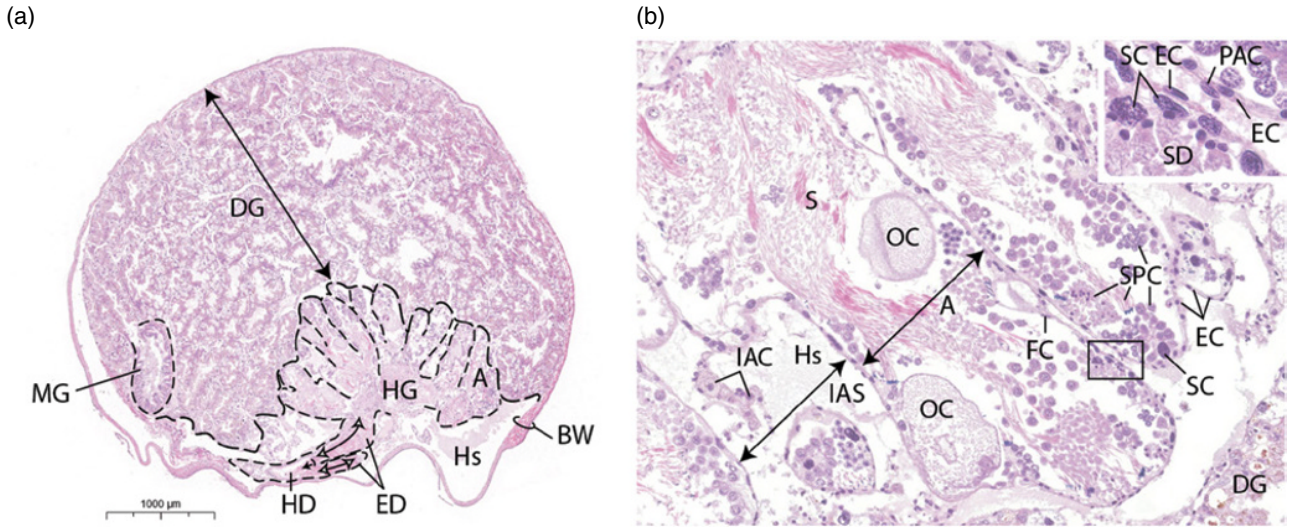


Figure 4.54 Ovotestis of *Helix*. (a) Overview of the gonad. 15×. HE. (b) Basic structure and cell types. 100×. HE. A, acinus; BW, body wall (visceral sac); DG, digestive gland; EC, epithelial cells (wall of acinus); ED, efferent ductules; FC, follicular cell; HD, hermaphrodite duct; HG, hermaphrodite gland, circular; Hs, hemolymph sinus; IAC, interacinar cells; IAS, interacinar space; MG, midgut; OC, developing oocytes; PAC, periacinar cell; S, mature sperm cells; SC, Sertoli cell; SPC, spermatocytes; SD, spermatids.

Semper, consists of connective tissue cells that often are full of pigment. Interacinar spaces consist of loose connective tissue, blood vessels and hemolymph sinuses, nerve elements, and mesenchyme. There are special cells among the acini in the interacinar space – the interacinar cells (Figure 4.54b). Their functions might be similar to those of mammalian Leydig cells. Cells around the wall of acini, the periacinar cells, are flattened and occupied largely by the nucleus (Figure 4.54b). The cells form a thin layer with smooth muscle around each acinus. Muscle cells were observed distributed along the outer boundary of each acinus; they probably promote the movement of developing spermatozoa through the acinar lumen (Roy et al. 2018). The function of the periacinar cells is supposed to be similar to that of mammalian peritubular myoid cells of seminiferous tubules, and probably produces peristaltic waves to release the mature sperm into the acinar lumen (Roy et al. 2016).

The basic unit of the ovotestis is the acinus, which is a rounded tubular sac, closed at the basal end but opening into an efferent ductule at the other end (Figure 4.54a). All the ciliated efferent ductules combine together to form the ovotestis (or hermaphroditic) duct. In Stylommatophora, both germ cell types originate and differentiate in every part of the gonad and mature on site. Each acinus is completely lined with a basement membrane that forms a layer outside and another layer inside. The inner epithelium, one or two cells thick, is the acinar epithelium. This epithelium is the same as (and continuous with) the epithelium of the hermaphroditic duct and the latter can regenerate the whole ovotestis in young snails.

4.3.11.1.1 Spermatogenesis Sperm cell clusters arise from a single primary spermatogonium (Figure 4.55). Spermatogonial cells are round with a prominent centrally placed circular nucleus. The nucleus contains homogeneous nuclear materials. The developing spermatocytes are large, irregular in shape, and contain large nuclei with prominent hetero- and euchromatin. The chromatin materials are dispersed throughout the nucleoplasm. Spermatocytes become attached to the Sertoli cells in the first meiotic prophase. The Sertoli cells are large columnar cells stretching parallel with the basement membrane. The nuclei of the cells are large, but their shape varies depending on the functional state of the cells (Roy et al. 2016). The different developing stages of spermatogenic cells adhere tightly to the cytoplasm of Sertoli cells. The spermatocytes are large, with an irregular cell boundary and a nucleus containing prominent euchromatin as well as heterochromatin components. Spermatocytes are distributed in the acinar lumen as cell aggregates due to prominent syncytial cytoplasmic bridges between adjacent developing cells. First meiotic prophase generates secondary spermatocytes, which are smaller cells with narrow cytoplasm round nuclei. The nuclei of haploid spermatids are more condensed than spermatocytes and arranged in an anteroposterior axis. Spermatids aggregate in bundles and exhibit different stages of maturation. The nuclear materials of developing spermatids are packed and residual bodies are formed. The spermatozoa within the ovotestis acini are aggregated, with their pointed nuclear heads inserted into the Sertoli cell and the tails free within the acinar lumen (see Figure 4.55).

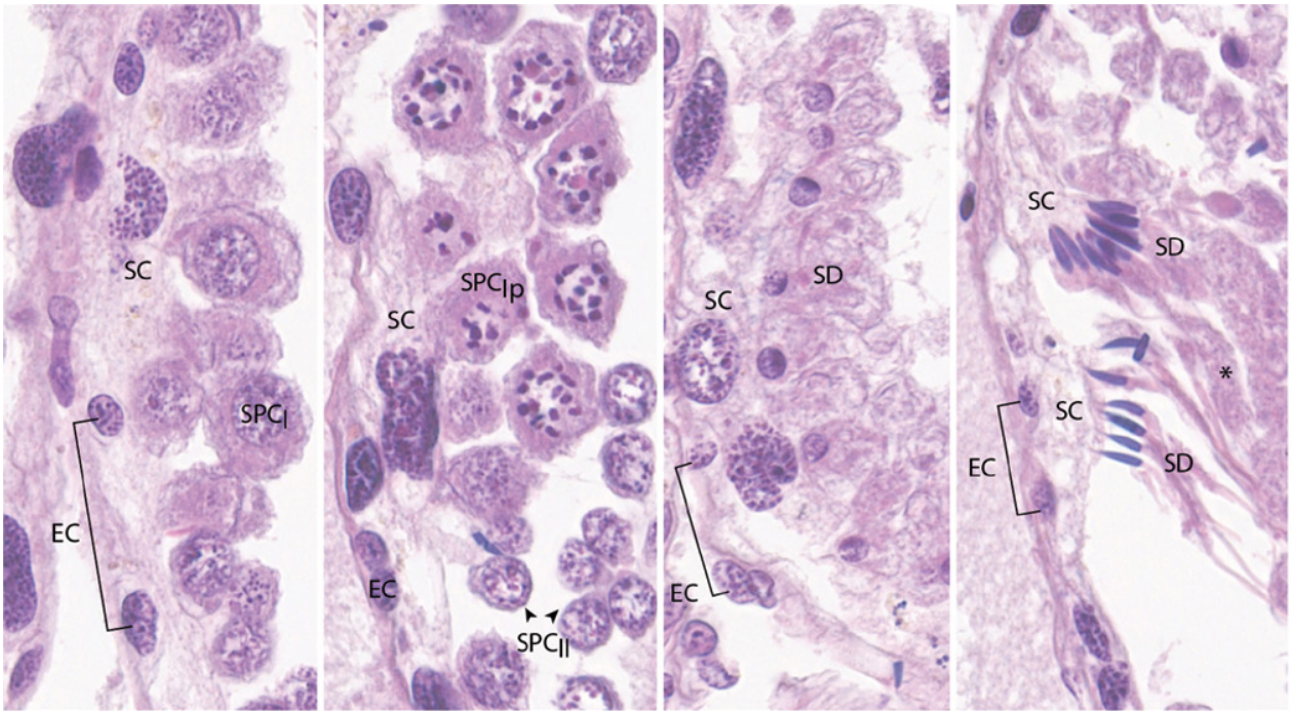


Figure 4.55 Spermatogenetic stages of *Helix*. EC, epithelial (nutritive) cells; SC, Sertoli cell; SPC$_I$, spermatocyte at the beginning of meiotic prophase; SPC$_{Ip}$, primary spermatocyte in meiotic prophase; SPC$_{II}$, secondary spermatocyte; SD, spermatid; asterisk, residual bodies. 600×. HE.

4.3.11.1.2 Oogenesis A distinct follicular cell layer surrounds the oocytes and separates them from the rest of the acinar components (Figure 4.56) (Griffond and Bolzoni-Sungur 1986). The follicle cell layer comprises minute, flattened cells, each with a small, elongated nucleus. The ooplasm contains a prominent nucleus with a small round nucleolus. One face of each oocyte is intimately associated with the acinar boundary, while the other face is directed toward the acinar lumen. A very thin layer of follicle cells covers each oocyte. Although often isolated, the oogonia can constitute clusters of 2–4 cells, incompletely separated from each other due to extensions of the adjoining epithelial cells. The nucleus, irregular in outline, possesses one nucleolus and small clusters of heterochromatin. At the beginning of the premeiotic stage, the oocytes are still adjacent but neighboring epithelial cells with dark nuclei insinuate extensions to isolate them from each other and also from the acinar lumen. They gradually constitute a follicular envelope. Each oocyte, however, remains in contact with the basal lamina. During the previtellogenic stage, the long axis of the oocyte is generally oriented parallel to the acinar wall. The cytoplasm becomes less basophilic. At the apical and lateral sides of the oocyte, the intercellular spaces are regular at first; they then enlarge and some intermediate junctions connect the oocyte to the follicular cells. The cytoplasm of oocytes is filled with clear vesicles in the vitellogenic stage. The intercellular spaces gradually enlarge between the follicle cells and the oocytes move toward the hermaphroditic duct.

4.3.11.2 Spermoviduct

The spermoviduct is a multifunctional part of the hermaphroditic reproductive system of *H. pomatia* and other pulmonates. It is a very long, strongly folded, yellowish-white duct, of mean length 30–37 mm and 5 mm thick. On its transverse section (Figure 4.57a), two parallel, contacting grooves are visible. One is the oviduct, the other is the spermduct. In the distal part, the spermoviduct diverges, forming a free vas deferens, while the rest of it becomes a free oviduct. The oviduct canal serves both eggs migrating downwards and the allosperm moving upwards to the fertilization chamber. The oviduct groove is spacious, and its wall is highly folded and lined with epithelium. The cells of this epithelium have large, centrally located nuclei. The female part of the spermoviduct is extremely glandular and forms the perivitelline membrane and shell of the egg. In cross-section, this female duct wall consists of three layers: an inner epithelium, an elaborate middle glandular layer, and an outer connective tissue capsule. The epithelial cells are rarely ciliated (Figure 4.57a, lower inset). The gland cells in the middle region, which form the bulk of the female tract, are the uterine glands that form the eggshell.

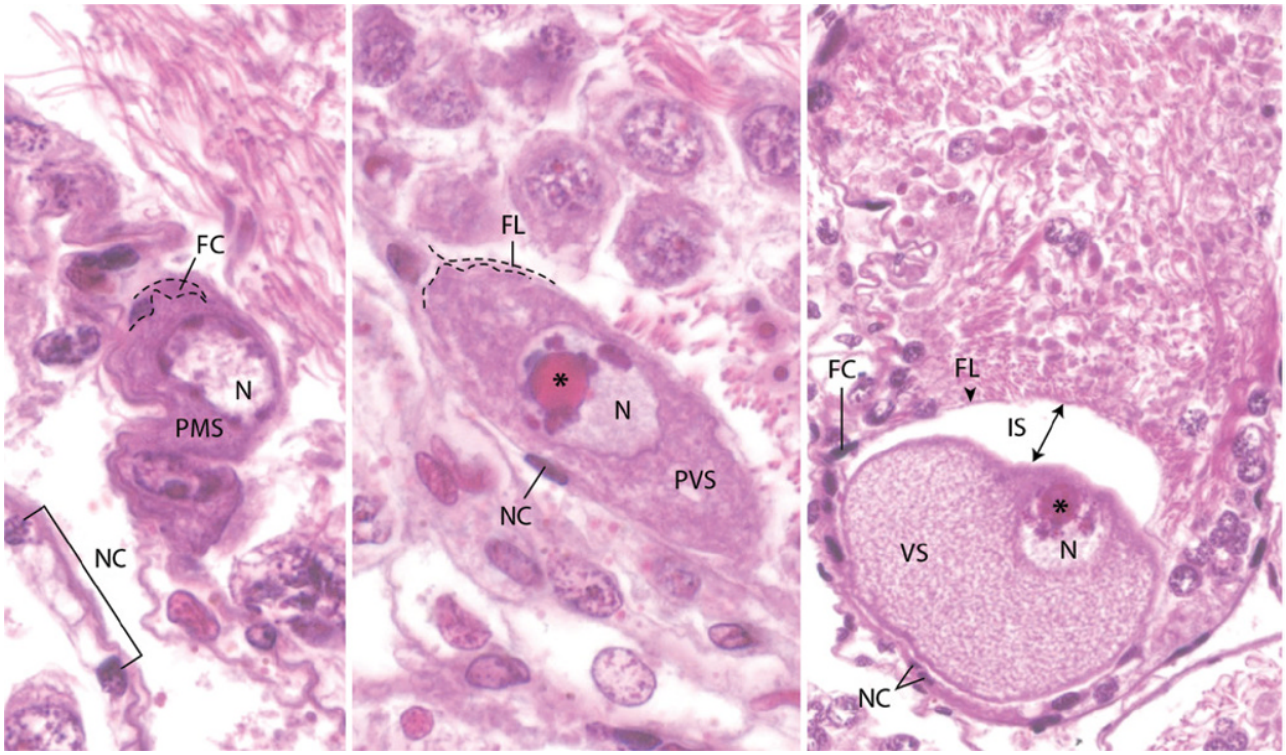


Figure 4.56 Oogenic stages of *Helix*. FC, follicular cell; FL, follicular cell layer; IS, intercellular space between the oocyte and follicular layer; N, nucleus; NC, nutritive cells; PMS, premeiotic stage of primary oocyte; PVS, previtellogenic stage; VS, vitellogenic stage; asterisk, nucleolus. 200×. HE.

The spermduct groove wall is lined with ciliated columnar epithelium (Figure 4.57a). The groove is adjoined by the prostate gland (Figure 4.57a). The prostate gland is a thin, long, tubular gland connected to the sperm groove along much of the length of the spermoviduct. This gland consists of glandular cells of large, basally located nuclei (Figure 4.57a, upper inset). Its secretion products are found in the lumen of the spermduct gutter. The prostate gland provides fluid for autosperm during mating. In *H. pomatia* the prostate gland is in a constant secretory phase during the whole activity period (i.e., all time outside of winter hibernation), and not only during mating. In addition to the prostate gland, the posterior spermoviduct contains another set of male glands, the flask cells. These are unicellular and discharge their products between the epithelial cells in the groove. The flask gland secretions appear to contain polysaccharides, possibly providing an energy source for the sperm.

4.3.11.3 Vas Deferens and Flagellum

The spermduct groove passes into a thin vas deferens near the terminal part of the prostate gland in *H. pomatia*. It runs freely in the snail's body, though at half its length it is suspended with connective tissue on the genital atrium wall. On transverse sections, its inner wall is strongly folded and lined with ciliated columnar epithelium of large, centrally located nuclei (Figure 4.57b). The cilia of the epithelium are dense and so long that they almost completely obliterate the spermduct lumen (Figure 4.57b, inset). In the region of the outlet of the spermduct to the epiphallus, the cilia get shorter. Below the spermduct epithelium, there is a thin layer of longitudinal muscle fibers, interspersed with connective tissue.

The epiphallus and the flagellum are spermatophore-producing organs. The lumen of the flagellum along its whole length is folded into a hook-shaped, longitudinal fold (Figure 4.57b). The flagellum wall is lined with columnar epithelium of elongate, basally located nuclei. The epithelium is surrounded by muscle fibers. Among them, there are numerous glandular cells with mucus secretion.

4.3.11.4 Bursa Copulatrix

Sperm received from the mating partner (allosperm) travel up the spermoviduct to reach the tubules of the spermatheca, where they are stored until fertilization. The vast majority of allosperm, however, is transferred into the sacculate reservoir of the bursa copulatrix (van Osselaer and Tursch 2000). The bursa was originally thought to function as the site of allosperm storage or sperm activation. More recently, it was discovered that it serves to dissolve and

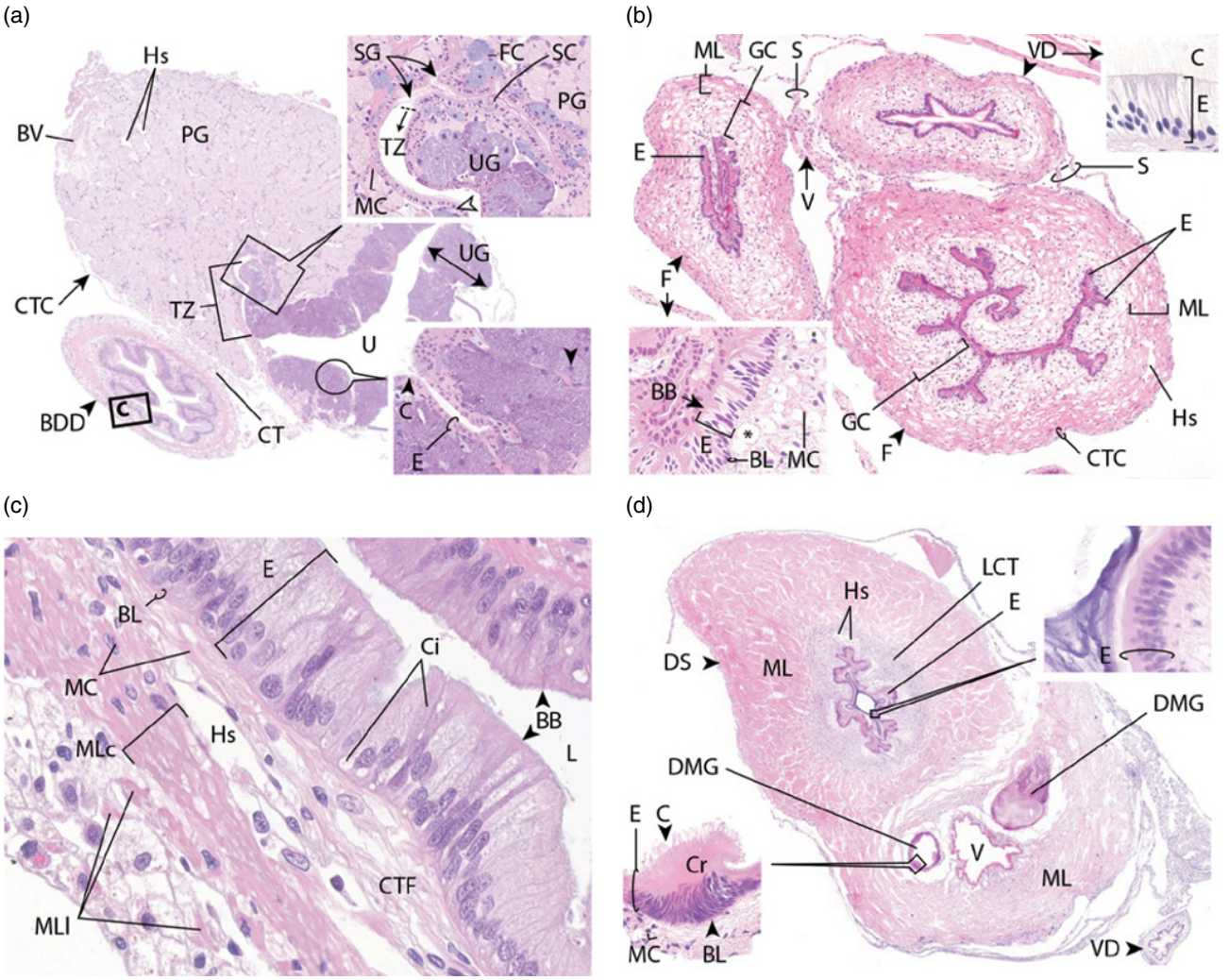


Figure 4.57 Gonoducts and dart sac of *Helix*. (a) Histologic structure of the spermoviduct. 20×. HE, insets 200× and 200×. (b) Vas deferens and flagellum. 60×. HE, insets 600× and 600×. (c) Bursa copulatrix duct diverticulum. 750×. HE. (d) Overview with the dart sac and mucous gland ducts. 20×. HE, insets 400× and 600×. BB, brush border; BL, basal lamina; BDD, bursa copulatrix duct diverticulum; BV, blood vessel; C, cilia; Ci, cytoplasmic inclusions; Cr, crest; CT, connective tissue; CTC, connective tissue capsule; CTF, connective tissue fibers; DS, dart sac; DMG, duct of mucous gland; E, epithelium; F, flagellum; FC, flask cell; GC, gland cells; Hs, hemolymph sinus; L, lumen; LCT, loose connective tissue; MC, muscle cell; ML, muscle layer; MLc, concentric muscle layer; MLl, longitudinal muscle layer; PG, prostate gland; S, septum; SC, sperm cells; SG, sperm groove; TZ, transition zone between sperm groove and uterus; U, uterus; UG, uterine gland; V, vagina; VD, vas deferens; asterisks, gland cells; black arrowhead, nucleus of uterine gland cell; white arrowhead, neck of flask cell.

resorb excess sperm. The evidence, such as detection of enzymes in the bursa (Németh and Kovacs 1972), supports this theory. The bursa generally contains dissolved or dissolving spermatozoa, which are probably both allosperm (from copulation) and autosperm (from itself). In *Helix*, the spermatophore substance dissolves by fluids containing digestive enzymes that are released into the lumen shortly after mating. Subsequently, the cells of the bursa copulatrix absorb and accumulate the digested material. Sometimes the stalk has a lateral diverticulum (Figure 4.57a,c), which is especially long. The name *diverticulum* is unfortunate because it implies that the organ is an annex of the bursa stalk, as it appears in some illustrations (Beese et al. 2006).

4.3.11.5 Dart Sac

The dart sac is an organ that secretes a calcareous spicule, "love dart," which is used during courtship in land pulmonates. In *H. pomatia*, when a pair of snails are intertwined, one member drives its dart into the body wall of the other. Copulation follows this rather drastic form of stimulation. A new dart is secreted later. In *Helix*, the dart sac is large, strongly muscular, of whitish color and sac-like shape. Its length is about 5 mm, the diameter ca. 3 mm. The inner surface of the sac is lined with columnar epithelium (Figure 4.57d). The cells of this epithelium have elongate, basally located nuclei. Under the epithelium, there is a fairly thick layer of loose connective tissue. Then, there is a

thick muscle layer, with circularly and longitudinally arranged fibers. Calcium concretions are found in the sac tissues. The love dart is formed as a result of the secretory function of the inner surface epithelium of the sac. It is built of aragonite. Inside the stem of the love dart there is a canal which is spacious and rounded in outline. The surface of the dart is rough, covered with numerous tubercles (Koralewska-Batura 1999).

4.3.11.6 Accessory Sex Glands (Nidamental Glands and Prostate)

Where nidamental glands are present, tubules supply the uterus (or pallial oviduct) and the uterine lumen may distend. Tubules consist of simple or pseudostratified cuboidal to columnar secretory epithelial cells (Figure 4.58). They may be interspersed with cuneiform ciliated cells. Mucocytes secreting acid mucopolysaccharide are common among nidamental glands, but the predominating type of secretory cell varies by gland and species (Burton 1978; de Jong-Brink et al. 1979; Klussmann-Kolb 2001; Lee et al. 2015; Rajalakshmi Bhanu et al. 1982). The secretory epithelium of the albumen gland often contains eosinophilic or basophilic PAS-positive granules.

Similarly, the spermduct lumen becomes expanded where the prostate is present. The prostate may integrate with the spermduct or remain separate. The gland is lined by simple or pseudostratified cuboidal to columnar secretory epithelial cells (Figure 4.59). The secretory cells often have fine cytoplasmic granules and are interspersed with cuneiform ciliated cells.

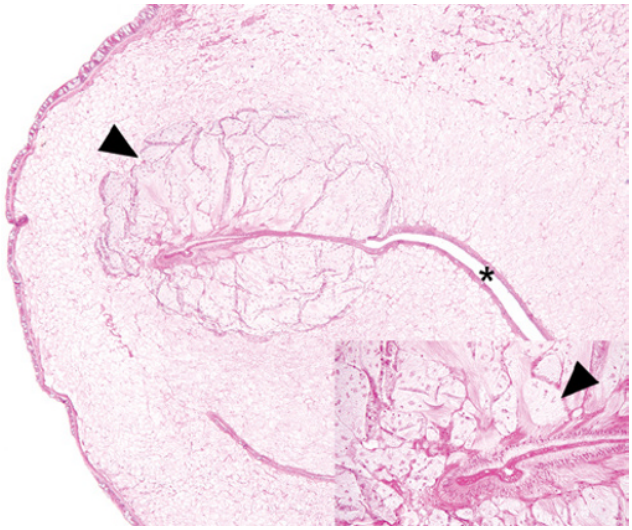


Figure 4.58 Nidamental gland of *Lobatus gigas*. Mucopolysaccharide-secreting cells form tubules (*arrowheads*) that supply the uterus (*asterisk*). 40×. HE. Inset: Higher magnification of glandular detail and ciliated columnar cells lining the uterus. 400× HE.

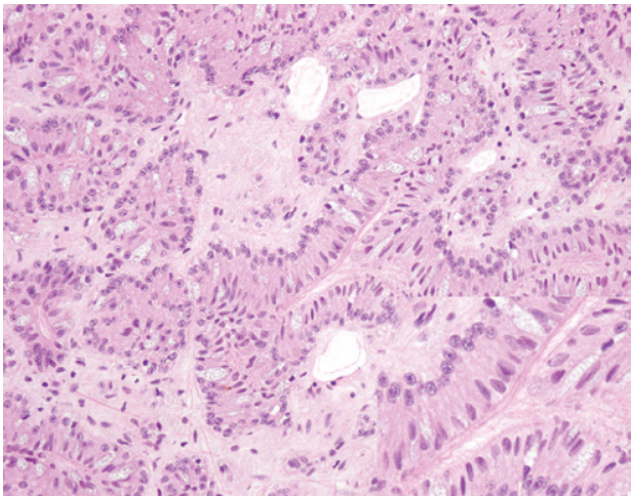


Figure 4.59 Prostate of *Lobatus gigas*. Tubules are lined by simple columnar epithelial cells. 400×. HE. Inset: Higher magnification demonstrating cuneiform ciliated cells apically interspersed with columnar cells. 1000×. HE.

In general, nonglandular ductular components of the reproductive tract are lined by simple cuboidal to columnar epithelial cells that are typically ciliated and supported by a connective tissue sheath. Tubular and ductular lumina are often distended with mucus secretion during spawning.

There are special mucous glands in *Helix* which have a spacious, circular lumen, filled with milky mucus and built of columnar epithelium whose cells adhere closely. The epithelium of the mucous gland is surrounded by muscle fibers pressed into a wall built of connective tissue. The secretory ducts of mucous glands run next to the dart sac (see Figure 4.57d) and open into the vagina (see Figure 4.4). The secretion of the mucous glands is transferred to the vagina prior to copulation and facilitates movements of the love dart during its shooting (Koralewska-Batura 1999).

4.3.12 Special Senses

Gastropods have neurosensory organs for touch, light, chemicals, and gravity. Chemoreception is especially important for locating food, mates, and predators. The osphradium is a chemoreceptive organ located on the central aspect of the roof of the mantle, to the left of the gill and next to the siphon, when present. It is usually more developed in aquatic than terrestrial gastropods and varies from resembling a gill to an inconspicuous ridge. Similar chemosensory structures may also be found within projections of the integument (Emery 1992), and may be particularly well developed in opisthobranchs lacking an osphradium (Faller et al. 2008; Gobbeler and Klussmann-Kolb 2007). These include the cephalic shield, Hancock's organ, lip organ, papillae or tentacles (cephalic, oral, optic epipodial), oral

lappets, or rhinophores. Those involving the head are often together referred to as cephalic sensory organs (CSOs). Some of these organs may also sense mechanical stimuli and light (Faller et al. 2008). Eyestalks attach the eyes to the lateral aspect of cephalic tentacles in many gastropods. Differences in microscopic anatomy of the eye are plentiful and reflect the habitat of the host (Bobkova et al. 2004). The statocysts are gravity receptors used for orientation and equilibrium (Budelmann 1988). They are present as paired organs near the pedal ganglia, adjacent to the pharynx and innervated by cerebral ganglia.

4.3.12.1 Eye

A well-developed eye consists of a cup of collagenous connective tissue, internally lined by retina, centrally containing a vitreous body and lens, and closed on its surface by a cornea (Figure 4.60). In less developed eyes, the cup may be open and comprise a depressed and modified continuation of the epidermis, lacking a cornea and lens.

The corneal surface is variably lined by simple squamous, cuboidal, or columnar epithelial cells that may have a microvillus border. There is a subjacent thin band of connective tissue that forms the corneal stroma. The retina may consist of photoreceptor cells (sensory cells) only or may also contain support cells. Photoreceptor cells have layered membranous projections or complex microvillous formations resembling rhabdomeres, rods, or discs (Hughes 1976; Mayes and Hermans 1973; Seyer 1994; Tonosaki 1967). There may be more than one morphologic type of photoreceptor cell; however, retinal cell types are usually not distinct under light microscopy. Rather, the retina may have distinguished layers representing the cell types present and their processes (Bobkova et al. 2004). For example, the photoreceptor processes may form an internal photoreceptor layer. Pigment within the cytoplasm of support cells and sometimes photoreceptor cells may form a pigmented layer, and the nuclei of both cell types may form a nuclear layer deep to the pigmented layer (Bobkova et al. 2004; Mayes and Hermans 1973; Seyer 1994; Zhukov et al. 2006). Some species have another layer formed by accessory neurons (Gibson 1984). The optic nerves form at the base of the eyes and supply the pleural ganglia.

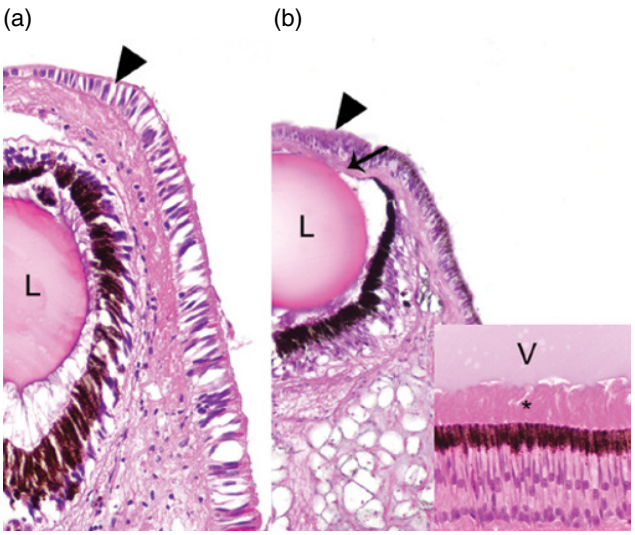


Figure 4.60 Comparison of eyes. (a) *Corallophilia* sp. 400×. HE. (b) *Littorina* sp. 400× HE. The integument becomes attenuated as it transitions to corneal epithelium (*arrowheads*). The corneal stroma consists of a narrow collagenous band (*arrow*). Centrally, refractive media consist of lens (L) and vitreous (V). The retina lines the inner posterior eye and may show a photoreceptor layer, pigmented, and nuclear layers. Inset: Higher magnification of the retina of *Lobatus gigas* demonstrating a rhabdomeric photoreceptor layer (*asterisk*) and well-defined pigmented and nuclear layers. 1000×. HE.

The eye structure of *H. pomatia* is shown in Figure 4.61. Above the eye vesicle, there is a translucent epithelium forming the external cornea. Below it, there is an internal cornea, colorless also, that forms the anterior wall of the eye vesicle. The retina is formed of optic (photoreceptor) and pigment (accessory) cells. The latter surround the optic cells and form rods together with them. Fibers of the optic nerve reach the bases of optic cells. In the middle of the vesicle, there is a large, spherical lens (Koralewska-Batura 1999). At the base of the optic tentacular (digitiform) ganglion, there is a group of large monopolar cells containing numerous secretory inclusions. The optic tentacles of various Stylommatophora contain distinctive cells (collar cells) arranged singly or in groups in the dermomuscular walls abutting against the lateroventral margins of the tentacular ganglion (Rogers 1969). The base of the tentacle is covered with sensory epithelium. Under this epithelium, there are numerous subepithelial gland cells, a network of ganglion cells and hemolymph sinuses.

4.3.12.2 Statocyst

The statocyst is a spherical to ovoid structure, typically <0.5 mm in diameter, with a wall of connective tissue and muscle cells that is lined by simple cuboidal epithelial cells (Stahlschmidt and Wolff 1972; Voltzow 1994). The latter may form syncytia, be ciliated or bear short bristles ("hair cells") or be concentrated into thickened macules. Fluid-containing calcareous bodies fill the statocyst. Calcareous bodies consist of either a solitary statolith (or otolith) or multiple smaller statoconia (Hyman 1967).

In *Helix*, on each pedal ganglion, a shiny, roundish statocyst is located. Despite its position, the corresponding cerebral ganglion with which it is connected by a long nerve running between the cerebropleural and cerebropedal connectives innervates it.

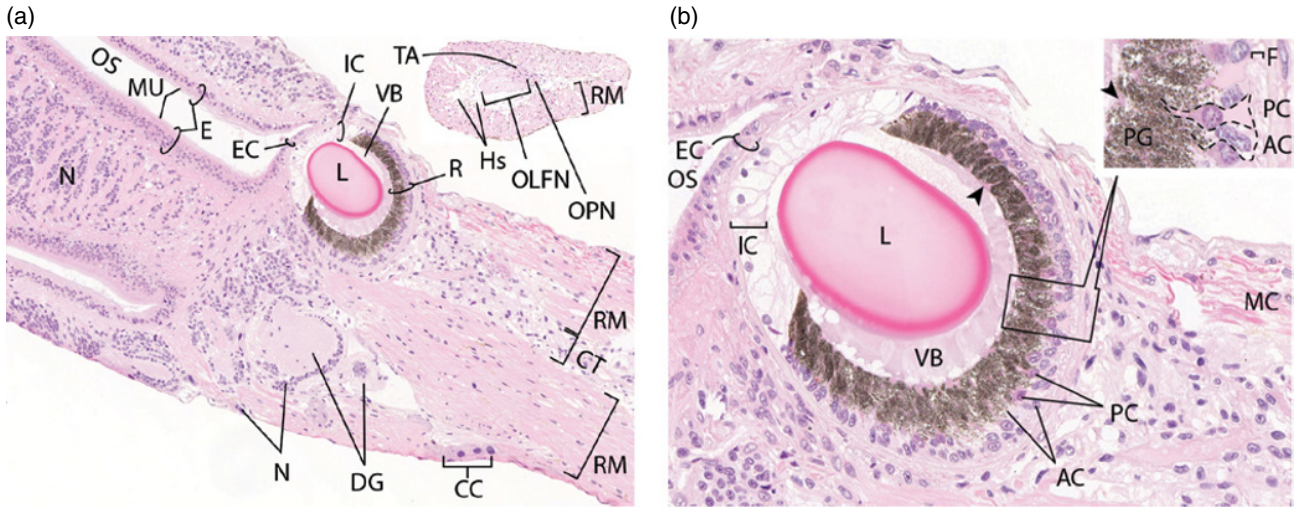


Figure 4.61 Eye and tentacular ganglion complex of *Helix*. (a) Overview with eye, ganglion and collar cells; inset: cross-section of ommatophore. 100×. HE, inset 20×. (b) Eye and retinal cells of *Helix*. 300×. HE, inset 1000×. AC, accessory cell; CC, collar cell; CT, connective tissue; DG, digitate ganglion; E, epidermis; EC, external cornea; F, fibers of optic nerve; IC, internal cornea; Hs, hemolymph sinus; L, lens; MC, muscle cells; MU, mucus layer; N, neurons of tentacle ganglion; OLFN, olfactory nerve; OPN, optic nerve; OS, outer space; PC, photoreceptor (optic) cell; PG, pigment cell; R, retina; RM, retractor muscle of tentacle; TA, tentacular artery; VB, vitreous body; arrowheads, brush border.

4.3.12.3 Osphradium

The osphradium is the olfactory organ of gastropods. When well developed, the osphradium consists of paired lamellae (or leaflets) that line a central rod or axis, similar to the gill. It is lined by simple to pseudostratified columnar epithelial cells with microvilli representing a heterogenous population of ciliated or bristled cells, unspecialized cells, mucocytes, other secretory cells, and pigment-containing cells (Figure 4.62) (Haszprunar 1985). In some gastropods, the distribution of each cell type may follow a zonal pattern, partitioned into ridges, cups, or clefts (Hyman 1967). Sensory dendrites from subepidermal neurons innervate the epithelial cells, although this is not histologically evident. Centrally within the leaflets is a chain of ganglia and connecting nerves. While other integumentary chemoreceptor organs are diverse, there is a similar microanatomic theme. Neurosensory epithelial cells with apical cilia or microvilli are supplied by dendrites from sensory neurons residing within or subjacent to the epidermis, or in a nearby ganglion (Emery 1992; Wertz et al. 2006; Yi and Emery 1991).

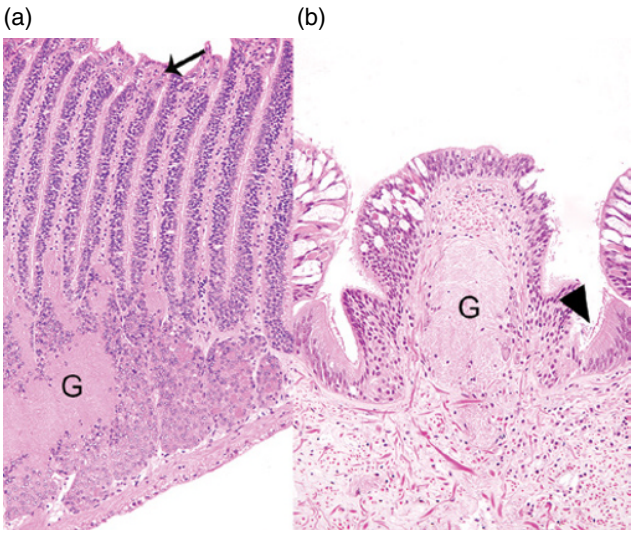


Figure 4.62 Comparison of osphradia. (a) *Nerita peloronta*. The well-developed osphradium bears lamellae (*arrow*), similar to a gill. Chains of ganglia (G) supply its base. 200×. HE. (b) *Lobatus gigas*. The regressed osphradium consists of a central ridge flanked by grooves on each side. Within the grooves are ciliated pseudostratified columnar epithelial cells (*arrowhead*). 200× HE.

4.4 Histology Processing Techniques

(Table 4.1)

Ideally, histology samples are collected soon after death or euthanasia to minimize postmortem decomposition. If a live gastropod is to be euthanized for histologic assessment of tissues, it is advisable to use humane euthanasia methods and to keep the animal in a stress-free environment beforehand. This will minimize the stress response and prevent the animal from fully retreating into the shell. Copious mucus production is a common response of stressed gastropods and this can confound histology of a number of organs, not limited to the integument. For aquatic gastropods, a two-step anesthesia and pithing approach is recommended by the American Veterinary

Table 4.1 Organs for histologic evaluation in Gastropoda.[a]

Organ system		Organs
Body wall		Mantle and mantle edge
Muscular		Foot, body
Digestive	Alimentary canal	Esophagus, stomach, style sac, small intestine, large intestine
	Digestive organs	Digestive gland (*hepatopancreas*)
Excretory		Nephridial gland, kidney
Circulatory		Heart, blood vessels
Immune		Hemocytes
Respiratory		Gill (*ctenidium*), lung
Nervous		Nerves, ganglia
Reproductive		Gonad (*hermaphrodite gland*)
Special senses/ organs		Eye, statocyst, osphradium

[a] Alternative names for organs are provided parenthetically, in italics.

Medical Association for euthanasia of aquatic invertebrates (Leary 2013). Pithing is done after extraction from the shell, if required. Efficacious anesthesia approaches for aquatic gastropods include immersion in 5% ethanol for 10 minutes or 2–24% magnesium sulfate for 5–45 minutes (Gilbertson and Wyatt 2016; White et al. 1996). Some gastropods may elicit a mucus-producing stress response in higher concentrations, in which case the concentration can be reduced (in some cases the reduction may need to be considerable), and then the anesthesia period prolonged (Tiley et al. 2019).

The use of an ice bath for anesthesia is not advised because it seems to ineffectively immobilize animals and at the same time it may produce artifactual changes, including formation of ice crystals and pooling of hemolymph in the viscera (Tiley et al. 2019). Immersing a live animal into a fixative solution without previous anesthesia is not an acceptable euthanasia method (Gilbertson and Wyatt 2016).

Once anesthetized (or euthanized), the methods used to extract a gastropod from its shell depend on the size. In smaller gastropods, removal from the shell can be done by cracking the shell using a D-shaped rotary-style ground clamp or a hammer. For those too large to crack the shell, a hole must be made through the dorsal shell spire to visualize the attachment of the columellar muscle to the columella. A knife is then inserted to transect this attachment, taking care to avoid incising the visceral mass at the same time. Once the columellar muscle attachment is severed, the foot is grasped (by the operculum, if present), and slow steady traction will extract the animal. Some gastropods are small enough to fix whole and decalcify the shell later, but this approach precludes dissection and gross examination of fresh tissues, and may prevent fixative from rapidly penetrating tissues inside the shell, obscuring histologic analysis.

For histologic work, the choice of fixative is important. In marine gastropods, tissues are preserved well in Davidson's solution (Howard and Smith 1983) and Bouin's solution. Ten percent neutral buffered formalin and 70% ethanol are also effective, but produce inferior preservation of tissues. Solutions prepared with saline or sea water generally produce better fixation for marine gastropods. Some gastropod tissues may produce copious mucus upon immersion in 10% neutral buffered formalin, in which case its use should be avoided (Tiley et al. 2019). For terrestrial and freshwater gastropods, Bouin's solution is used as tissue structure with a soft and delicate texture must be preserved. It is composed of picric acid (4%, v/v), acetic acid (1%, v/v) and formaldehyde (15%, v/v) in an aqueous solution. In order to retain the histologic structure of dense, highly proteinous organs another compound fixative is beneficial. This is composed of 1.5% (v/v) paraformaldehyde, 1% (v/v) glutaraldehyde, and 0.028% (w/v) $CaCl_2$ (w/v) in 0.1 M sodium cacodylate, pH 7.4; the tissues should be immersed in it for several days at 4 °C then washed out with two changes in 0.1 M sodium cacodylate. To get a perfect morphologic result in *Helix*, fixative should be injected into the mantle and body cavities before the removal of the shell.

The shell is composed mainly of calcium carbonate aragonite within an organic matrix (conchin or conchiolin) and thus can easily be dissolved using conventional decalcifying solutions (such as EDTA or formic acid) post fixation although a membrane of the organic component may remain.

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5

Mollusca

Cephalopoda

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5.1 Introduction

Cephalopods are predaceous marine molluscs with bilateral body symmetry, a prominent head and a set of arms or tentacles. The word *cephalopod* means "head-foot" and refers to the fact that these animals' arms branch directly off their heads. All cephalopods are carnivores feeding on a variety of crabs, fish, and molluscs.

In the phylum Mollusca there are two, only distantly related, extant subclasses of cephalopods: the ectocochleate (cochleate meaning shaped like a snail's shell, spirally twisted) Nautiloidea, represented by nautilus and allonautilus that have an external shell, and the endocochleate Coleoidea, where the molluscan shell has been internalized or is absent (Budelmann et al. 1997). The coleoids are divided into the Decapodiformes (cuttlefish and squid) and Octopoda/Octobrachia (octopus) orders.

While there are over 10 000 extinct taxa, more than 800 species of cephalopods are recognized today. Cephalopods are a diverse group of species but share common life history traits such as a rapid growth rate and relatively short life expectancy. Some species live for as little as six months while larger species, like the giant Pacific octopus (*Enteroctopus dofleini*), may live for up to five years under suitable circumstances.

Cephalopods are dioecious with well-developed gonads. During sexual maturation, one arm of the male is modified to form a hectocotylus used to transfer sperm to the female and can be disengaged from the main body of the male. Fertilization usually takes place within the mantle cavity of the female. Embryonic development is direct from the egg without any larval stages. Cephalopods are also semelparous, which means they only undergo one reproductive cycle before they enter senescence and die. Males die up to several months after mating, females after eggs are hatched. Once a female octopus lays her eggs, she guards and aerates them, rarely leaving the clutch. During this time she does not feed, resulting in massive loss of body weight and eventually her life. In contrast to the solitary octopus, squids feed, socialize, and mate in shoals or squads. Neither cuttlefish nor squid care for their eggs.

Senescence is a normal stage of an octopus's life cycle that often lasts a month or longer before death. During senescence, octopuses stop eating and become cachectic, such that the body shrinks and the eyes appear to bulge, they develop skin and eye lesions, self-mutilate, exhibit uncoordinated activity, and often succumb to opportunistic infections. In the wild, a senescent octopus is likely to be preyed upon, but in captivity it is critical for aquarists to recognize signs of senescence and differentiate them from husbandry and disease issues (Anderson and Wood 2002).

Cephalopods have no conservation status under the United States Endangered Species Act, IUCN, or CITES, but they are commercially important in many Asian countries where they are a major food source. The greatest threats to cephalopods include damaging fishing techniques like trawling.

5.2 Gross Anatomy

5.2.1 General Characteristics

The mantle is a highly muscled structure that houses all the cephalopod's organs. The mantle is rubbery and made almost entirely of muscle and collagen with no support

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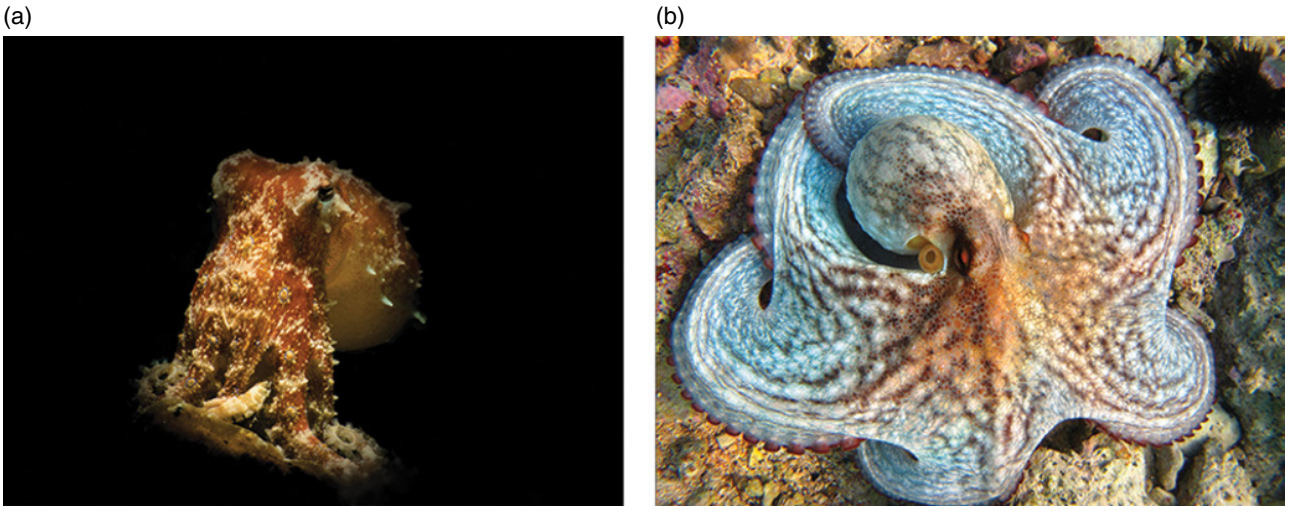


Figure 5.1 Color variation and patterns of *Hapalochlaena* spp. chromatophores. *Source:* Photo credit: Francesco Martini.

from hardened skeletal elements. Contractions of the mantle regulate water movement for respiration, waste excretion, and jet propulsion. In Nautiloidea, there is a hard, calcified external shell and the mantle is only a thin sheet of tissue between the body and the shell. Similar to other molluscs, the shell grows along the rim by calcified layers which are formed by gland cells of the mantle rim (Arnold 1992). This shell has numerous gas-filled chambers; the innermost one houses the body organs.

The mantle skin contains the chromatophores or chromatophore organs responsible for the rapid color-changing abilities of cephalopods. Yellow, orange, red, brown, and black colors exist (Figure 5.1). Under nervous control, fine muscle contractions and relaxations change the dispersion of pigment granules in cytoelastic sacs, changing how the pigments are displayed (Messenger 2001). While most chromatophores are independently controlled by the nervous system, radial muscles of adjacent chromatophores can be linked so contraction or relaxation may spread synchronously, which enables a high degree of complexity in the display of colors. Some cephalopods may also possess silvery iridophores and reflective cells to enhance and reflect the pigment color, white leukophores to further accentuate the dramatic color changes, and/or luminescent symbiotic bacteria or intracellular photophores. Chromatophore color change can even persist postmortem, due to prolonged muscle contractions. Nautilus skin lacks many of these morphologic specializations because it is internal to the shell.

In cephalopods, there is a single siphon or funnel positioned ventral to the head. This muscular tube, or flap in the nautilus, is part of a mantle collar and moderates water movement in and out of the mantle cavity (Figure 5.2). It also serves as the exit route for all reproductive products, waste products, and ink (Gestal et al. 2019).

All coleoids except incirrate (classic benthic and pelagic) octopods have fins (Figure 5.2). Fins are lateral and vary in form and size (Bermingham 1987). Undulating movements of the muscular fins are used for slow swimming, hovering, and pivoting.

Arms that branch directly from the large head and surround the mouth are the most characteristic structures of cephalopods. These circumoral appendages can be classified as cirri, tentacles, and arms. The octopus, squid, and cuttlefish each has eight arms, while the chambered nautilus has many cirri (Figure 5.2). In males, there is a modified arm called a hectocotylus which is used to transfer spermatophores to females. Squid and cuttlefish have two additional modified arms. These tentacles are long and smooth with a terminal, broad, flattened pad bearing larger suckers and can be extended and retracted (Figure 5.3). In coleoids, suckers of the arms and tentacles can vary in size, shape, number, and arrangement. While Decapodiformes can have multiple rows of stalked and bilaterally symmetrical suckers that may have hooks (Figure 5.3), octopods often have sessile, radially symmetric double suckers (Figure 5.4).

Generally, the eyes are situated laterally and can be very large (Figure 5.5). Cephalopod eyes are large spherical to oval, closed organs with all major components of a complex vertebrate eye including cornea, pupil, iris, retina, and spherical lens. The nautilus has only a simple pinhole pupil, a retina, and no lens.

Cephalopods are carnivores with an active lifestyle and generally high metabolic activity. To seize prey, they can thrust out their highly mobile tentacles and bring the food

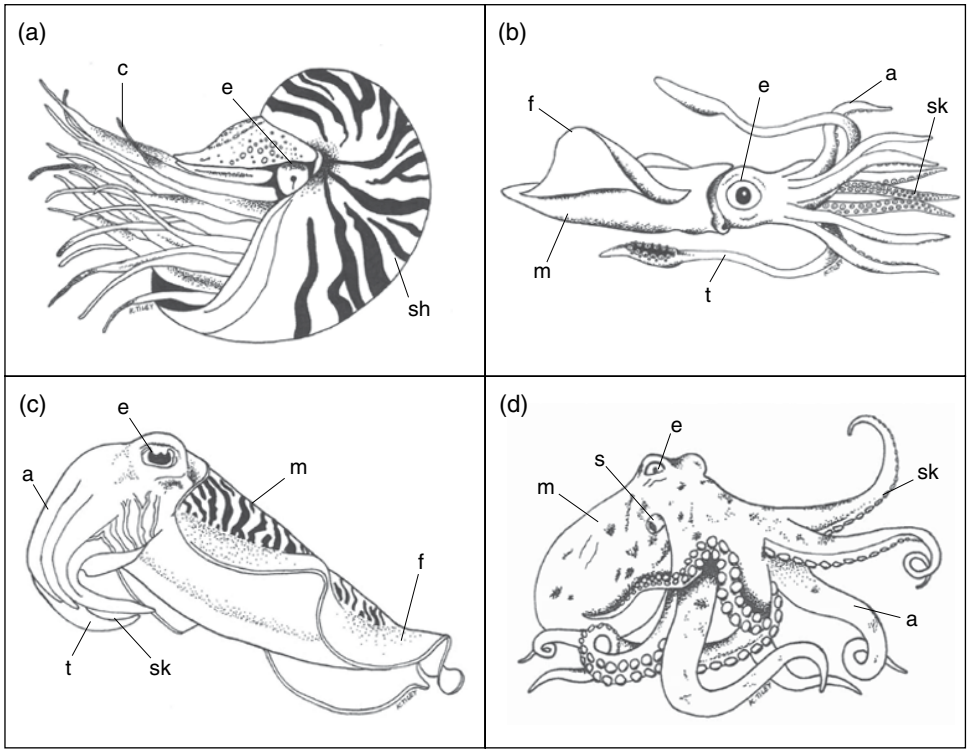


Figure 5.2 External anatomy of cephalopods. (a) Nautilus. c, cirri; e, eye; sh, shell. (b) Squid. a, arms; e, eye; f, fins; m, mantle; sk, suckers; t, tentacles. (c) Cuttlefish (*Sepia officinalis*). a, arms; e, eyes; f, fins; m, mantle; sk, suckers; t, tentacles. (d) Octopus. a, arms; e, eye; m, mantle; s, siphon; sk, suckers. *Source:* All original artwork produced by Katie Tiley.

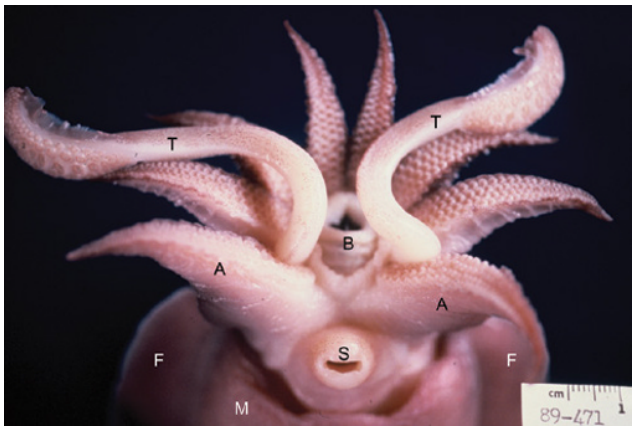


Figure 5.3 Decapodiforme, cuttlefish (*Sepia officinalis*) photograph showing the mantle (M), arms (A), tentacles (T), fins (F), siphon (S), and beak (B).



Figure 5.4 Giant Pacific octopus (*Enteroctopus dofleini*) with sessile, radially symmetric double suckers on the oral surface of the arms.

to their mouths. The arms aid in holding the prey while the powerful, beak-like jaws tear off large pieces of tissue, which can then be sheared and pulled into the buccal cavity by the radula. The buccal mass is a large ovoid bulb that can be rotated freely and is composed of lips, two beaks (mandibles), a radula, and salivary glands. The beak apparatus or mandible is composed of protein matrix and chitin (Hunt and Nixon 1981), and consists of articulated upper and lower beaks (Figure 5.6). The radula is a fundamental molluscan feature and is composed of varying rows of chitinous teeth, 7–9 in coleoids and 13 in nautiloids.

There are three types of salivary glands associated with the buccal mass. The submandibular or sublingual gland, which is lobed but nonpaired, lies within the buccal mass near the salivary papilla and radula. The anterior or lateral salivary glands are larger and paired. These glands are

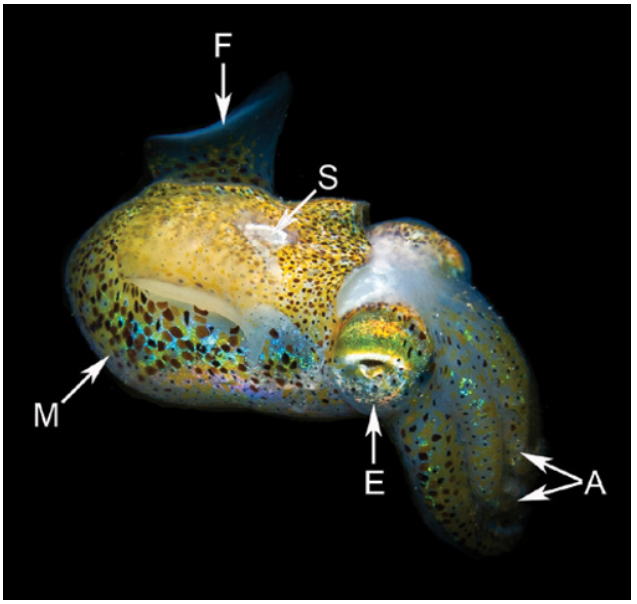


Figure 5.5 Cuttlefish (*Sepia* sp.) showing the mantle (M), eye (E), arms (A), fins (F), and siphon (S). *Source:* Photo credit: Francesco Martini.



Figure 5.6 Giant Pacific octopus (*Enteroctopus dofleini*) beak *in situ* (background) and *ex situ* (inset). The inset shows detail of the upper and lower beaks.

enclosed in the muscular lateral lobes in the nautiloids and sepioids but are located to the left and right side of the buccal mass in octopods. The posterior salivary gland or venom gland is paired in octopods and sepioids and located further aboral to the buccal mass. Secretions are used to immobilize

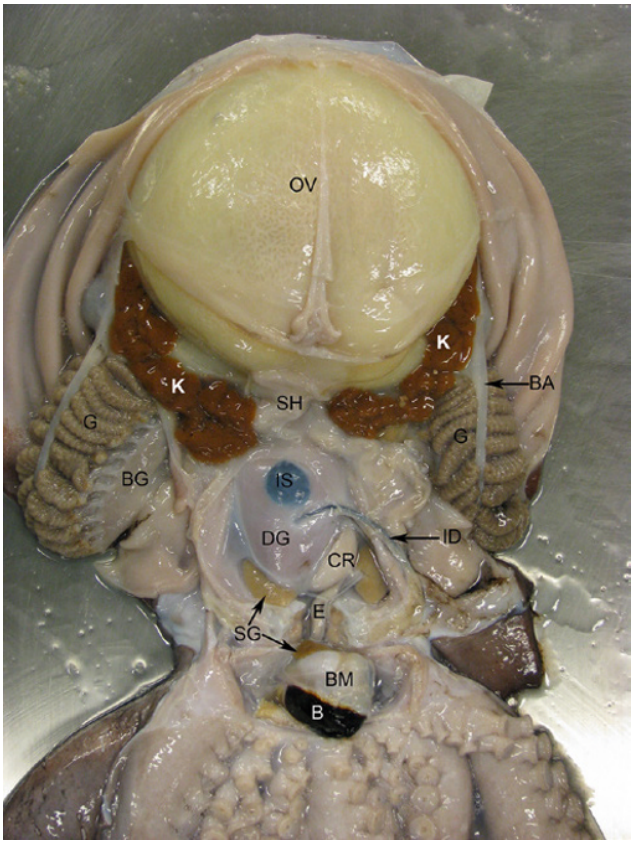


Figure 5.7 Giant Pacific octopus (*Enteroctopus dofleini*), the mantle of which has been opened for dissection. B, beak; BA, branchial artery; BG, branchial gland; CR, crop; DG, digestive gland; E, esophagus; G, gill; ID, ink duct; IS, ink sac; K, kidney; OV, ovary; SG, salivary gland; SH, systemic heart.

prey and maculotoxin produced by the deadly blue-ringed octopus (*Hapalochlaena* spp) is secreted from this gland. Nautiloids lack a posterior venom gland.

From the oral cavity, the esophagus conducts fluid by peristaltic action into the stomach. The esophagus, which includes a crop in some octopods and nautiloids, passes through the cranial cartilage and the brain, and runs between the buccal mass and stomach along the dorsal aspect of the digestive gland (Figure 5.7). Attached to the muscular stomach is a thinner-walled cecum with digestive and hepatopancreatic grooves. The cecum is a spirally coiled, pouch-like part of the digestive tract that functions in transport, sorting of ingested material, and digestion. Here, food is mixed with glandular secretions from the digestive gland, which empties through a common duct into the junction between the stomach and cecum. The digestive gland, historically termed the *hepatopancreas*, is the largest gland in the cephalopod body (Figure 5.7). The intestine is a short muscular channel and the anal opening is located mid-body.

The ink sac is one of the most characteristic glands of cephalopods. It is an exocrine gland that produces a dark brown to black pigment composed of melanin. The ink can also contain tyrosinase, a highly irritating substance. When necessary, the octopus can eject ink to serve as a decoy or to obscure a quick getaway. The ink gland complex is located near the digestive system and contains three parts: the ink gland that produces the ink, the larger ink reservoir/sac where ink is stored, and the ink sac duct (Figure 5.7). The duct runs parallel with the rectum and terminates with the anus.

Major excretory organs in cephalopods include the pericardial and renal appendages (also called kidneys), as well as the pancreatic appendages of sepioids. However, the gills and coelomic epithelia are also involved in excretory processes (Figure 5.8). The pericardial appendages (pericardial gland, branchial heart appendage) are a lacunar system originating from an outfolding of the apical coelomic epithelium of the branchial heart, surrounded by a pericardial sac (Figure 5.9). The sac may or may not cover the branchial heart, depending on the species of cephalopod. The pericardial gland's lumen is continuous with that of the branchial heart. The branchial appendage cells are responsible for catabolism of hemocyanin and other blood products (Gestal et al. 2019). The renal appendages, synonymous with kidney, renal organ and renal sac, are the central excretory kidneys. Two large renal sacs are present in the cranial portion of the visceral mass (Figure 5.8). A thin transparent membrane contains the delicate orange-red renal appendages. Each sac opens to the mantle cavity thorough a pore. Branches of the cephalic (anterior vena cava) vein project through the renal sacs, where they become convoluted and have renal epithelium, before reaching the branchial heart (Furuya et al. 2004). In the Nautiloidea, the pericardial appendages and the renal appendages are separated anatomically and functionally.

Cephalopods have a closed circulatory system and lack the extensive hemocoel system found in other molluscs. Octopods and cuttlefish have three hearts, one main

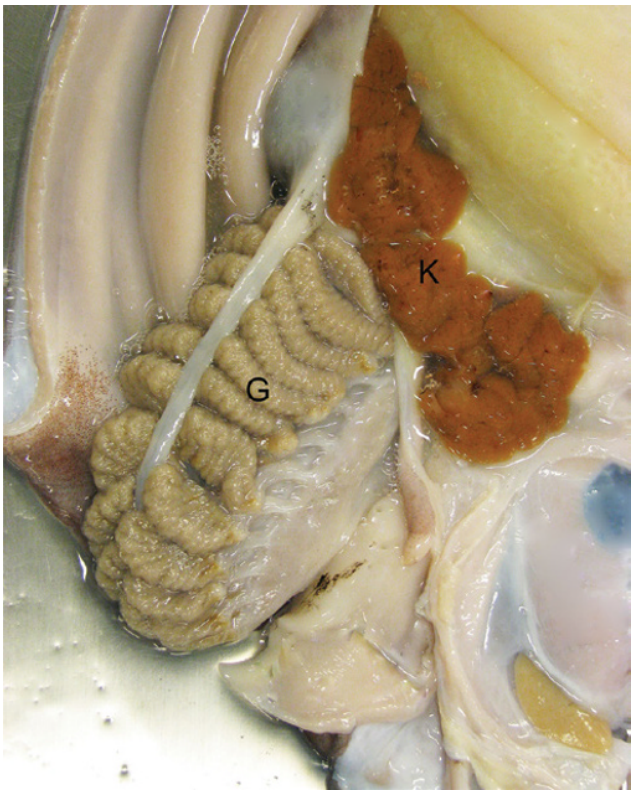


Figure 5.8 Excretory organs of a giant Pacific octopus (*Enteroctopus dofleini*) including kidneys (K) and gills (G).



Figure 5.9 Giant Pacific octopus (*Enteroctopus dofleini)* circulatory system. This consists of three hearts, including one systemic heart (SH) and two branchial hearts (BH). The gills (G), kidneys (K), oviduct (OD), and oviductal glands (OG) are also pictured.

two-chambered heart located centrally between the kidneys and two smaller branchial hearts located at the bases of the gills (Figure 5.9). The systemic heart is a powerful hollow muscle with two contractile auricles (four in nautiloids), valves, and a ventricle. A branchial heart and a branchial gland can be seen at the caudal end of each gill (Figures 5.9–5.10). The gills or ctenidia are bilateral folded, comb-like structures and the main respiratory organ of the cephalopod (Figures 5.7–5.10).

The nervous system of cephalopods is the largest and most complex of all invertebrates, with well-developed central and peripheral systems. The brain is composed of optic lobes, a supraesophageal mass, a subesophageal mass, and a periesophageal mass (Gestal et al. 2019). The optic lobes vary in size between species but are always the largest lobes of the brain. The supra- and subesophageal masses are divided into a number of lobes (Figure 5.11). Some of the supraesophageal lobes (vertical, subvertical, subpedunculate, precommissural, and frontal lobes), are involved in the analysis of sensory input, learning, and memory (Newth 1972). Other supraesophageal lobes, including the basal lobes and peduncle lobe, are higher motor centers for coordination of head, arm, and eye movement, swimming, respiration, and chromatophore control. Another supraesophageal lobe (superior buccal lobe) controls the motor activity of feeding (Budelmann



Figure 5.10 Formalin-fixed cuttlefish (*Sepia officinalis*), the mantle of which has been opened for dissection. Two branchial hearts (BH) are visible, but the systemic heart is obscured. DG, digestive gland; G, gills; IS, ink sac; S, siphon.

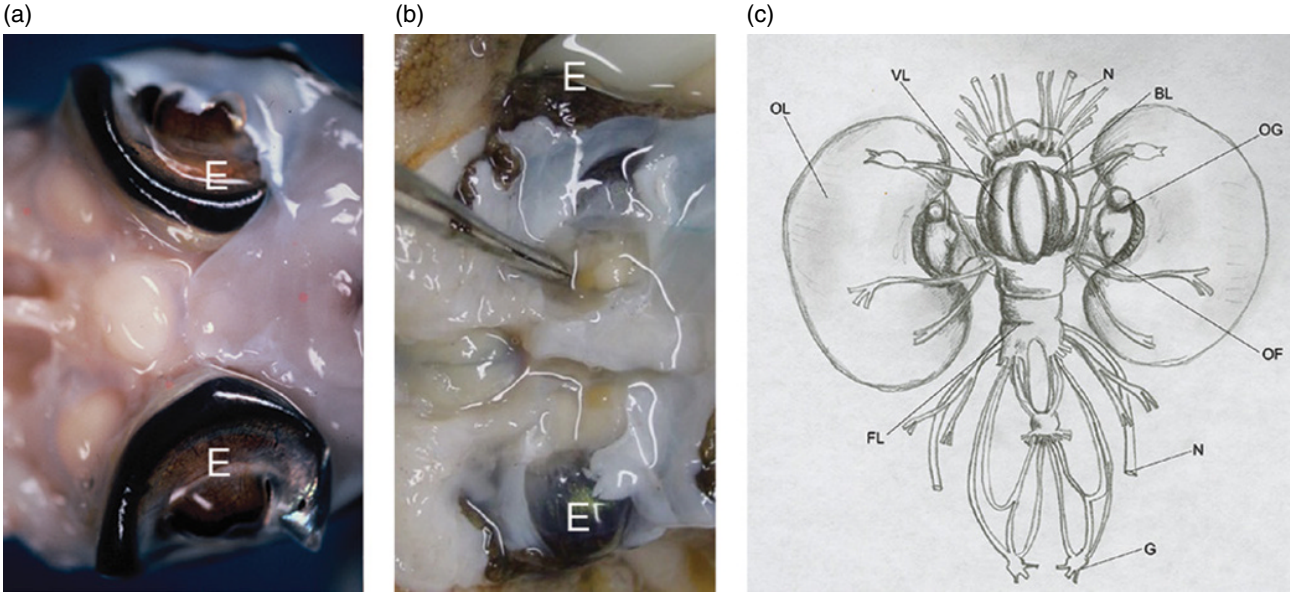


Figure 5.11 (a) Necropsy of a common cuttlefish (*Sepia officianalis*). Portions of the brain are visible between the eyes (E). (b) Brain and optic glands (held up with forceps) in a common octopus (*Octopus vulgaris*). *Source:* Photo courtesy of Dr Shane Boylan. (c) Line drawing of a dorsal view of the CNS of a common octopus. The supraesophageal masses are divided into multiple lobes. *Source:* Line drawing by Elisabeth Narkin. BL, basal lobe; E, eyes; FL, frontal lobes; G, ganglion; OF, olfactory; OG, optic gland; OL, optic lobe; VL, vertical lobes; N, nerves.

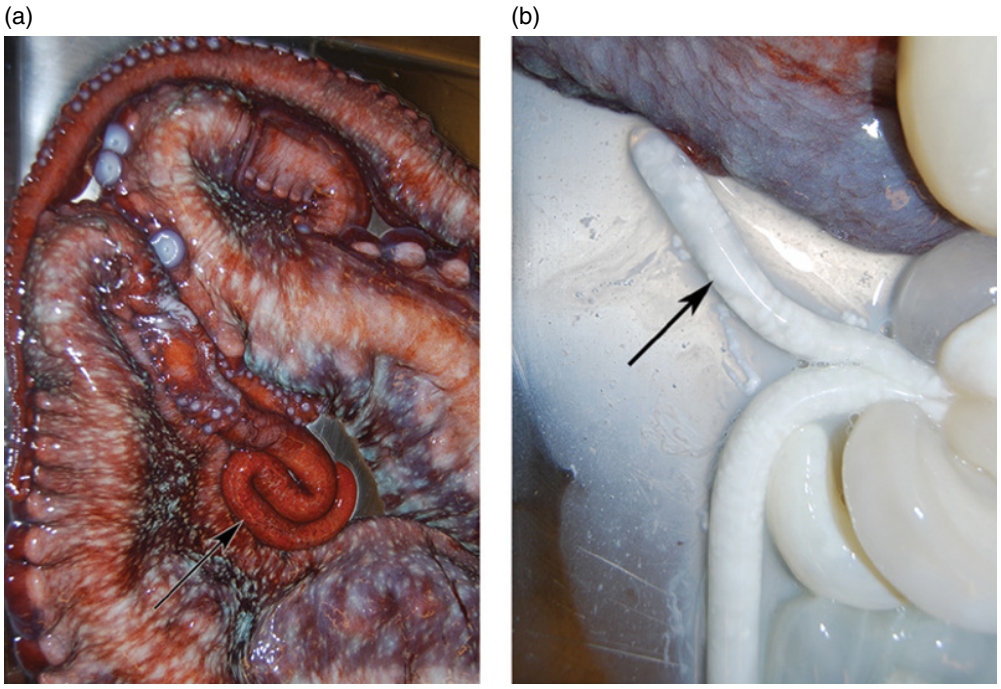


Figure 5.12 Giant Pacific octopus (*Enteroctopus dofleini*). (a) Hectocotylus arm (*arrow*) with modified suckers. (b) Spermatophore containing myriad sperm (*arrow*).

et al. 1997). The periesophageal magnocellular lobe is involved in defense and fast escape. The subesophageal lobes are all motor lobes. The nautiloid brain is much simpler and lacks higher learning centers as well as areas for control of arm movement and chromatophores.

The optic glands (optic body, spherical body, tubercle ganglionnaire, ganglion pedunculatum, ganglion chromatophoricum) are distinct tissues on the upper posterior edges of the optic tracts (Wells 1978). The pale yellow or orange glands are present in octopus, cuttlefish, and squids, and a similar tissue has been identified in nautiloids. Although debated, this endocrine organ has functions similar to the vertebrate pituitary, controlling gonadal maturation and possibly feeding and senescence (Wodinsky 1977; Wells and Wells 1969) (Figure 5.11).

Cephalopods are dioeceous and sexually dimorphic. The reproductive system includes the gonads, accessory glands, an oviduct in females, and a sperm groove and copulatory organs in males. The accessory glands and copulatory organs are extremely diverse among species. In the female, the ovary is located at the caudal apex of the coelomic cavity. When fully developed, it assumes most of the space inside the body (Figure 5.7). The ovary is an accumulation of mature and immature eggs with an oviduct(s) and oviductal gland (octopods) or nidamental gland (Decapodiformes). Secretions of the oviductal gland act as an egg cement, while those of the nidamental glands are responsible for the final encapsulation of eggs prior to release via the mantle cavity (Boletzky 1986). In octopods, the oviductal gland also stores sperm and is the site of fertilization (Gabr et al. 1998). The genital pore is located on the left side of the body and opens just cranial to the left gill. The male genital system consists of an unpaired testis, also located at the caudal tip of the body cavity, and a ductular system found on the left side (right side in nautiloids). Males also have a modified hectocotylus arm, which can vary in length and have modified suckers and a sperm groove (Figure 5.12). Macroscopic and microscopic anatomy undergo a variety of changes during subadult development, but even aged, senescent male and females should be identifiable.

5.2.2 Keys for Dissection/Processing for Histology

All cases should include information regarding clinical signs, environmental conditions, vitality of tissues, and gross photographs. Before beginning a dissection, identify any mantle lesions, such as erosions, and sample, including unaffected lesion margins. Organize the arms and if male, identify the hectocotylus arm. Position the animal in dorsal recumbency with the siphon directed up and incise the ventral mantle to open the body cavity (Figure 5.13). Most organs can now be identified, including the buccal mass, a majority of the intestinal tract, the digestive gland, ink sac, renal appendages, systemic heart, gills, and branchial glands.

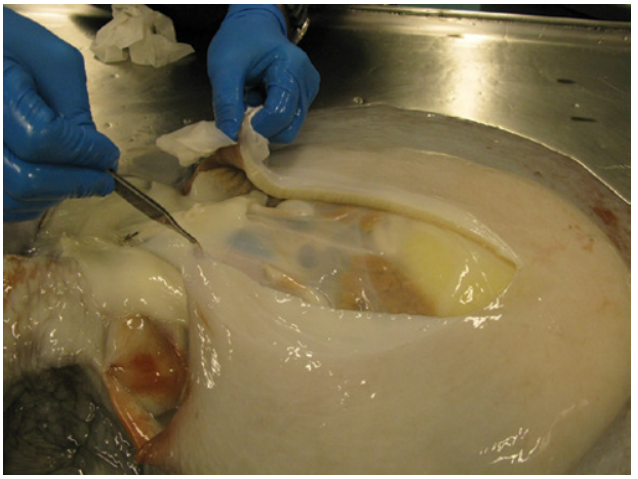


Figure 5.13 Mantle incision of a Giant Pacific octopus (*Enteroctopus dofleini*) when starting a cephalopod necropsy.

The gonads are generally very prominent and can fill the majority of the head. Be careful not to rupture the ink sac as it will cause artifacts grossly and histologically. Collect samples of all organs and fix in 10% neutral buffered formalin.

In the Decapodiformes, the dorsally located cuttlebone or pen (gladii) can now be identified. The cuttlebone of sepiids is composed of chitin heavily fortified by aragonite or calcium carbonate. The cuttlebone forms a shield to which some muscles of the mantle are attached and has thin chambers that contain fluid or gas. By regulating the relative amounts of both, the degree of buoyancy can be varied (Figure 5.14a). The pen in squid is uncalcified and made predominantly of chitin (Figure 5.14b). The pen can vary greatly in size and intricacy.

5.3 Histology (Table 5.1)

5.3.1 Body Wall/Musculoskeletal System

With no skeleton and unconstrained by joints, cephalopods can flex and move in unlimited directions due to a complex arrangement of antagonistic striated muscle groups and intermuscular connective tissues (Kier and Stella 2007; Kier 2016). The body plan of cephalopods has been called a muscular hydrostat, as it facilitates movement but also serves as its own support system. This is possible because the volume of a muscular hydrostat is constant and any decrease in one dimension will necessitate an increase in at least one other dimension. Elongation results from the contraction of transverse, circular or radial muscles, while shortening is produced by contraction of longitudinal muscles (Kier and Smith 1985). Connective tissue provides support and tensile strength while also allowing for flexibility. Components include fibroblasts, hyaline matrix, elastin, and collagen (Simkiss and Wilbur 1977). Locomotion includes hovering using fins, forcefully collapsing the mantle cavity to expel water and produce jet propulsion, and walking using rows of suckers on the underside of each arm. Suckers are complex muscular organs and each sucker is innervated by its own acetabular and brachial ganglion (Budelmann et al. 1997).

5.3.1.1 Mantle and Integumentary Structures

Musculature of the mantle is covered by an integument that serves a variety of functions including barrier defense and osmoregulation. When escape is not possible, neuronally controlled dermal chromatophores also provide almost

(a)



(b)



Figure 5.14 (a) Cuttlefish (*Sepia officinalis*). The cuttlebone attached to muscles. (b) Squid (unspecified species). The pen is uncalcified and made predominantly of chitin. It is not attached to any structures and can be easily removed once the mantle is opened.

Table 5.1 Organs for histologic evaluation in Cephalopoda.[a]

Organ system		Organs
Body wall/musculoskeletal		Arms (*tentacles*), funnel (*siphon*), suckers, mantle, chromatophores, shell, cuttlebone, pen (*gladii*)
Digestive	Buccal mass	Lips, beaks (*mandibles*), radula, salivary glands
	Alimentary canal	Esophagus, crop, stomach, cecum, digestive duct appendages (*pancreatic appendages*)
	Digestive organs	Digestive gland
Excretory		Branchial heart appendages (*pericardial glands, pericardial appendage*), renal appendages (*kidney, renal organ, renal sac*), ink glands
Circulatory		Hemolymph, systemic heart, branchial hearts and appendages
Immune		Hemocytes
Respiratory		Vascularized skin, gills, branchial gland
Nervous		Brain, peripheral nerves
Reproductive	Male	Hectocotylus arm, testes, vas deferens, accessory glands (*spermatophoric gland system*), spermatophore
	Female	Ovaries, oviduct, oviductal gland, nidamental gland
Special senses/organs		Eyes

[a] Alternative names for organs are provided parenthetically, in italics.

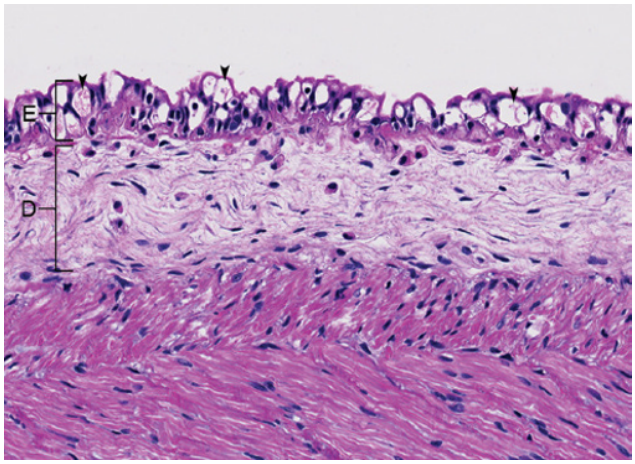


Figure 5.15 Cuttlefish (*Sepia officinalis*) mantle. Columnar epithelial cells make up the epidermis (E) overlying a loose collagenous dermis (D). The epidermis contains many mucous cells (*arrowheads*). 200×. HE.

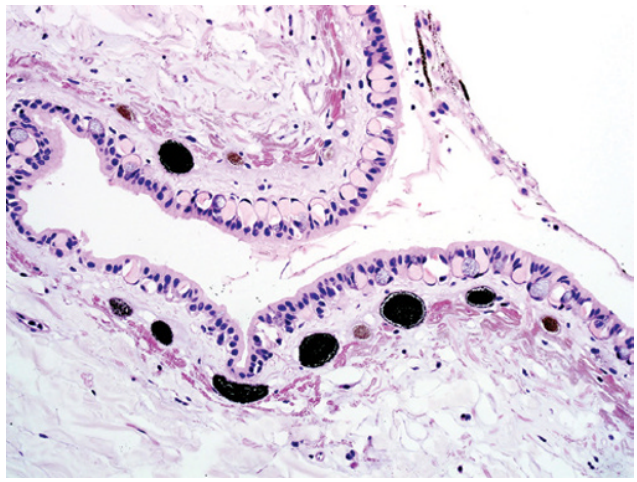


Figure 5.16 Cuttlefish (*Sepia officinalis*) skin showing melanophores and iridophores in the superficial dermis. 200×. HE.

instantaneous camouflage (Simkiss and Wilbur 1977). The integument consists of an epidermis and dermal connective tissue with chromatic elements. The transparent epidermis is a single-cell layer of simple cuboidal to columnar epithelial cells, depending on location, while the epithelium lining the mantle cavity and organs is cuboidal. On the external mantle surface, mucous cells are interspersed throughout the epidermis as well as ciliated cells of unknown function (Figure 5.15). The thick subjacent dermis is a loose network of collagenous fibers and fibroblasts. Numerous chromatophores (Figure 5.16) are located within the superficial dermis as well as bundles of nerve fibers and blood vessels and each chromatophore organ and pigment cell is surrounded by a packet of radial muscles.

5.3.1.2 Circumoral Appendages: Arms and Tentacles

In all coleoid appendages, a cuboidal to columnar epidermis overlays a complex arrangement of transverse, longitudinal and helical or oblique muscle groups associated with a central nerve core (Figure 5.17) (Kier and Stella 2007; Kier 2016). While the squid has two unilateral bundles of longitudinal muscles, the octopus has inner lateral oblique

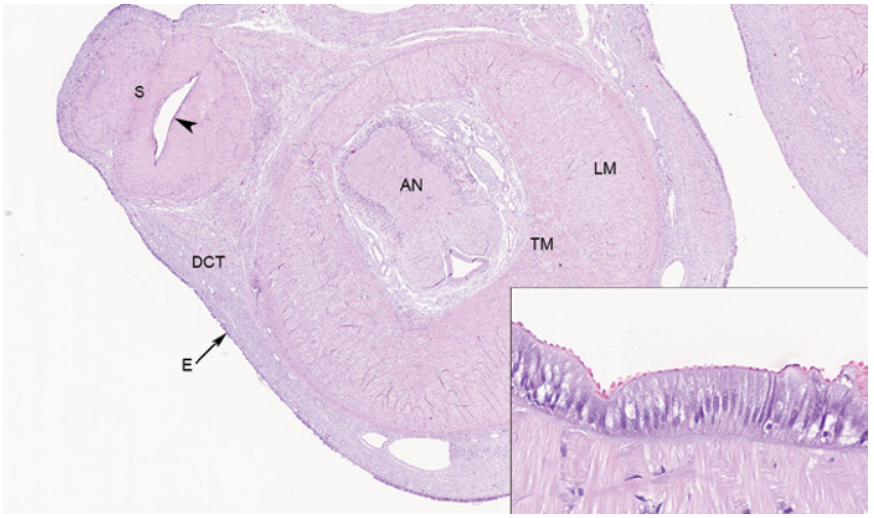


Figure 5.17 Arm of giant Pacific octopus (*Enteroctopus dofleini*). Note that the muscular cup of the sucker (S) is lined by a layer of epithelium (inset) without a cuticle. 40×; inset 400×. HE. EP, epidermis; DCT, dermal connective tissue; AN, axial nerve cord; LM, longitudinal muscle; TM, transverse muscle.

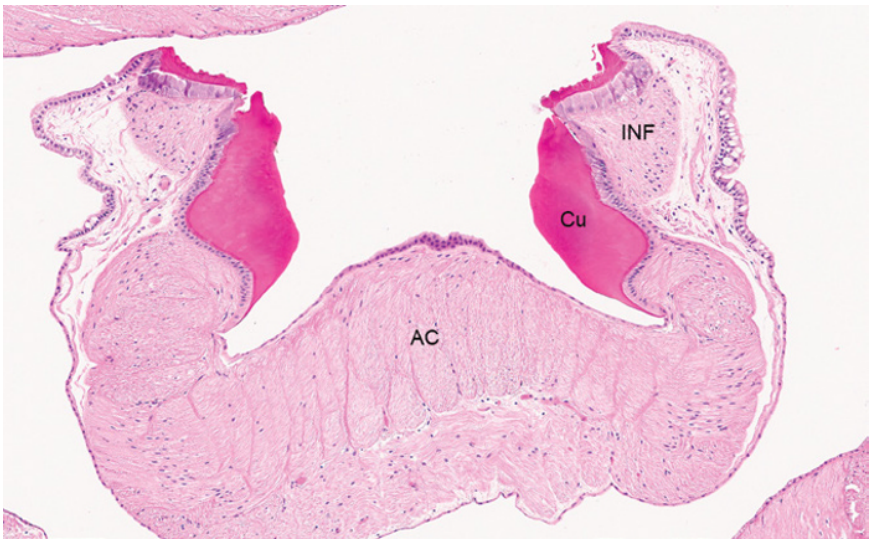


Figure 5.18 Section through a sucker on the arm of a cuttlefish (*Sepia officinalis*). The muscular cup is lined with bright eosinophilic cuticle (Cu). Note the thick layer of muscle, deep to the epidermis. AC, acetabulum roof and wall; INF, infundibular wall. 100×. HE.

muscles and a peripheral layer of circumferential fibers (Budelmann et al. 1997).

Many arms bear suckers arranged in rows. The sucker is made up of a cup-shaped acetabulum (sucker chamber), an infundibulum chamber, the surface of the sucker that attaches to the substrate, and an outer wall. Interactions of radial, circumferential, and meridional muscles support movement. The muscular floor is covered by a columnar epithelium and a chitinous inner ring or cuticle. Decapodiform suckers are equipped with a horny ring that can carry a tooth-like hook to cling to soft prey, while most octopod suckers use negative pressure and suction. The outer wall of the sucker is also covered a single layer of columnar epithelial cells (Kier and Smith 2002) (Figure 5.18).

5.3.2 Digestive System

The cephalopod digestive system is developed into several voluminous compartments with structural and functional specializations. The buccal mass has beaks for tearing and multiple salivary glands to aid digestion. In the stomach, food is mechanically mixed by muscle movements and liquefied by enzyme-rich fluid. The contents of the stomach are periodically released into the cecum along with additional enzymatic secretions from the digestive gland. The walls of the cecum have an elaborate series of spiral ciliated folds that sort and transport chyme, mucus, and nondigestible particles. The digestive gland functions in the synthesis and secretion of digestive enzymes, metabolism of nutrients, synthesis and storage of lipids, pigments and metals,

excretion and rejection of waste products, and in nautiloids, it may synthesize hemocyanin (Costa et al. 2014).

5.3.2.1 Buccal Mass and Salivary Glands

Microscopically, the beak and radula are composed primarily of bright eosinophilic chitin (Figure 5.19). Some beaks can have basophilic mineral. They are attached to muscle by a single layer of columnar cells called the beccublast epithelium (Budelmann 1996). The chitinous teeth of the radula are secreted by odontoblasts of a high columnar epithelium with a thick cuticle, and anchored in a muscular and cartilaginous odontophore (Messenger and Young 1999) (Figure 5.20). At times the salivary papilla, formed by muscle and a thick chitinous cuticle, is also identifiable near the radula, extending into the oral space and adjacent to the submandibular salivary gland. The submandibular or sublingual gland, found within the buccal mass and muscle, is composed of a mixture of mucus and serous cell acini (Figure 5.19). The anterior salivary glands are composed of serous acini with various types of basophilic staining granules. These glands release mucus into the buccal cavity and contain neutral glycoproteins, sialic acid, dipeptidase, and hyaluronidase (Budelmann 1996), The posterior salivary gland is composed of lobules subdivided into tubules separated by interstitium (Figure 5.21). Secretory cells contain basophilic and eosinophilic globules. In all salivary glands, the secretory units merge into ducts, which are lined by cuboidal to columnar epithelium and are surrounded by myoepithelial cells.

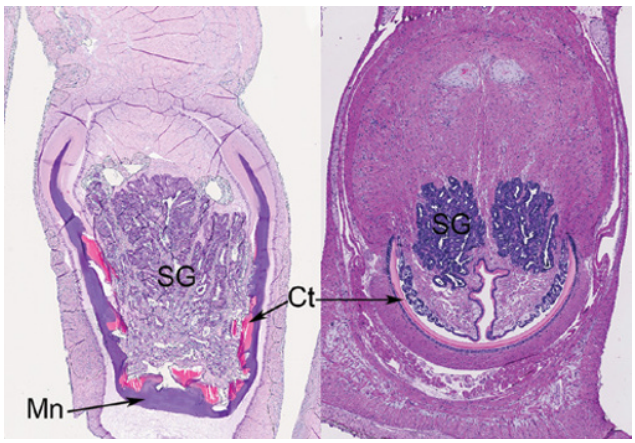


Figure 5.19 Section through the buccal mass of a giant Pacific octopus (*Enteroctopus dofleini*) (a) and common cuttlefish (*Sepia officinalis*) (b). The beak is composed primarily of brightly eosinophilic chitin (Ct) with some basophilic mineral (Mn) in the octopus. Salivary glands (SG) are adjacent to the beak. 20×. HE.

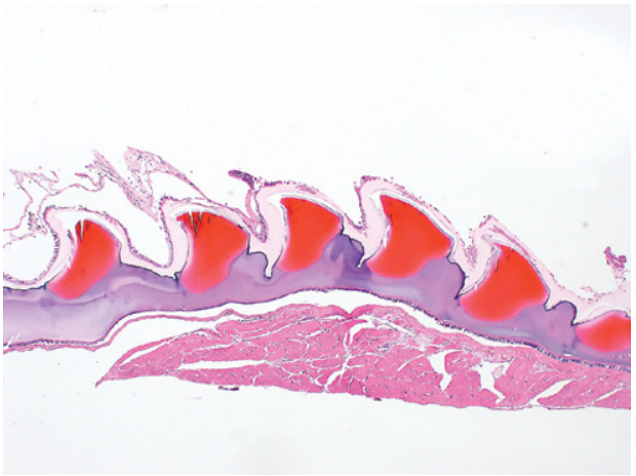


Figure 5.20 Radula bearing chitinous teeth in a chambered nautilus (*Nautilus pompilius*). 40×. HE.

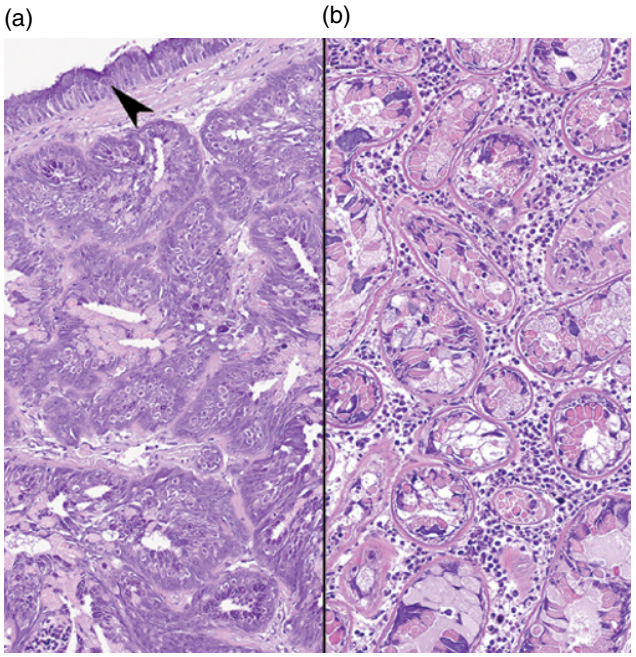


Figure 5.21 Types of salivary gland from giant Pacific octopus (*Enteroctopus dofleini*). (a) Anterior salivary gland. The duct (*arrowhead*) of the gland is in the top left of the image. 200×. HE. (b) Posterior salivary gland. 200×. HE.

5.3.2.2 Digestive Tube

The digestive tube includes the esophagus, crop, stomach, cecum, digestive duct appendages, and digestive gland. A chitinous cuticle lines the mucosa of the esophagus and stomach. The esophageal wall is thin and distensible. The adventitia or serosal epithelium and submucosa can be very vascular. The muscularis has distinct outer and inner longitudinal striated muscle layers that produce peristalsis. The mucosa forms longitudinal folds

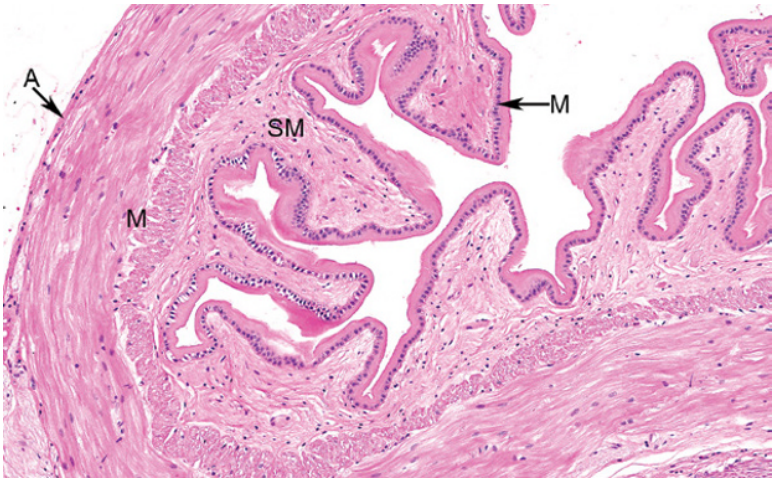


Figure 5.22 Esophagus of a northern flamboyant cuttlefish (*Metasepia pfefferi*). The wall is composed of an adventitia (A), muscularis (M), submucosa (SM), and mucosa (M), which has longitudinal folds and tall cylindrical epithelium overlain by a cuticle. 100×. HE.

covered by a tall cylindrical epithelium that contains goblet cells and an eosinophilic, chitinous cuticle that is easily identifiable microscopically (Figure 5.22). In coleoids, the wall of the stomach consists of thick obliquely striated circular muscles and an inner thin layer of irregularly arranged longitudinal fibers with an additional layer of interwoven muscle fibers and a subepithelial internal plexiform layer of muscle cells that is comparable to the tunica muscularis mucosa of vertebrates. The mucosa has longitudinal folds and a tall epithelium with numerous goblet cells, and is covered by a thick eosinophilic cuticle. The mucosa of the nautiloids has variable longitudinal folds and goblet cells.

The cecal folds or leaflets are lined by a columnar, ciliated epithelium with clusters of mucus-secreting cells at the tip (Figure 5.23). Small arterioles, capillaries, single muscle fibers, and nerve endings are identifiable in the connective tissue of leaflets. No secondary folds are present in nautiloids.

The intestine has an outer layer of circular and an inner layer of longitudinal densely innervated muscle fibers. The submucosa is well vascularized with longitudinal folds and is covered

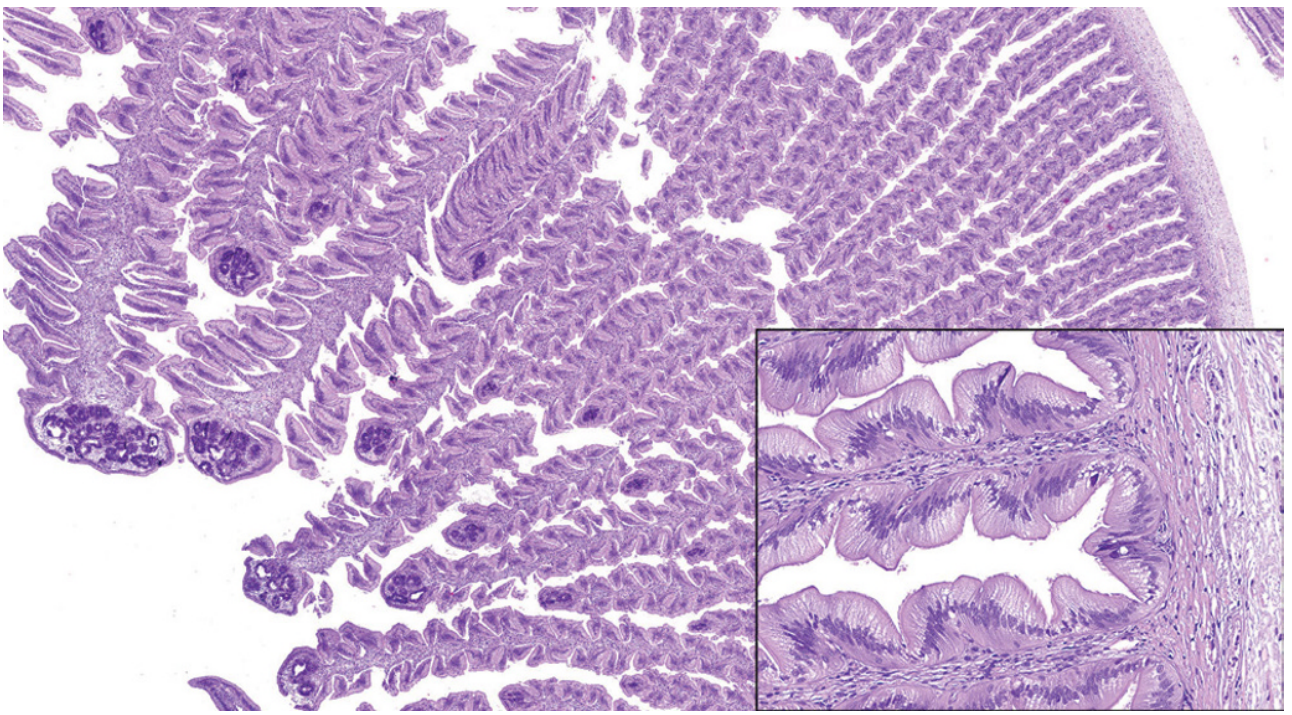


Figure 5.23 Cecum of a giant Pacific octopus (*Enteroctopus dofleini*). The mucosa has numerous spiral folds. The mucosa is lined by tall columnar epithelium without a cuticle (inset). 20×; inset 400×. HE.

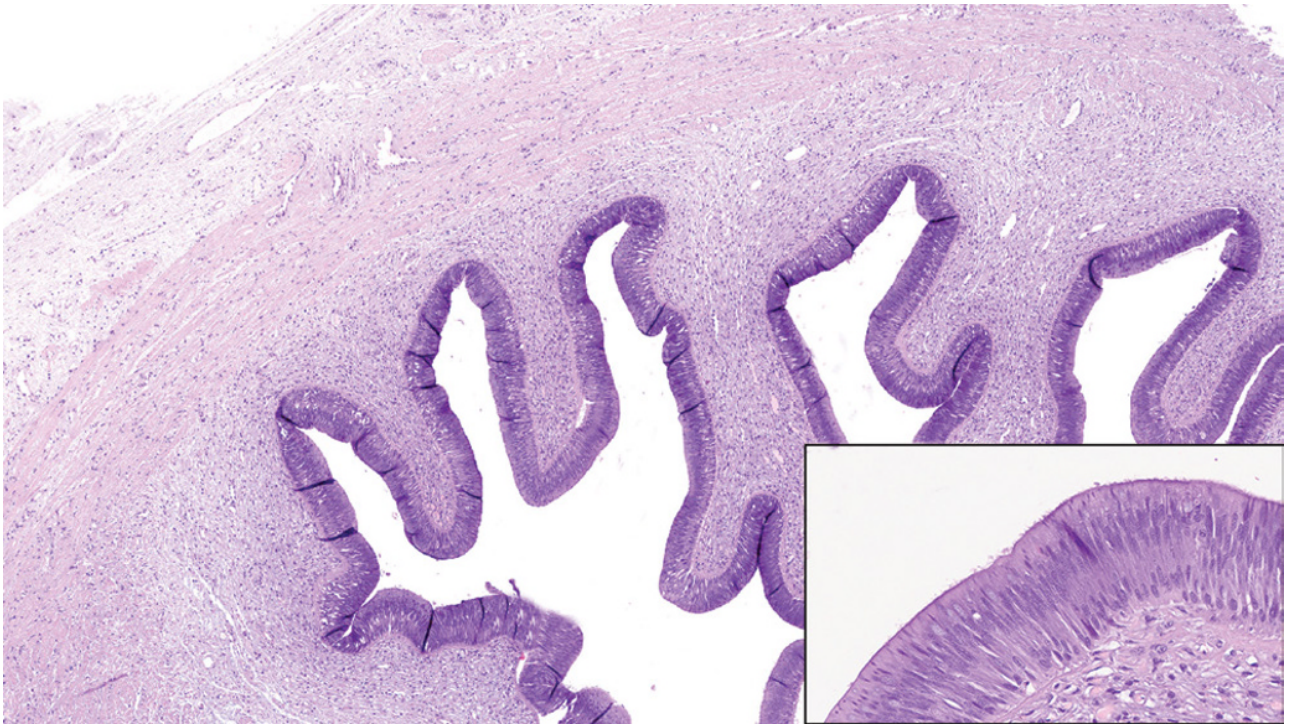


Figure 5.24 Intestine of a giant Pacific octopus (*Enteroctopus dofleini*). The mucosa forms folds and is composed of tall columnar epithelium without a cuticle (inset). 100×; inset 400×. HE.

by a cuboidal mucosal epithelium (Figure 5.24). In the nautiloids, the proximal intestine is divided longitudinally by folds into two canals. The intestinal tract ends at a muscular anus.

5.3.2.3 Digestive Gland

The digestive gland is paired in coleoids and has a thin capsule of connective tissue, while in nautiloids it has multiple lobes surrounded by coelomic epithelium. This organ contains densely packed tubules interspersed with arteries and veins, but little connective or muscular tissue (Figure 5.25). Tall columnar cells with a brush border line each tubule. Tubules vary in size and shape and can vary in appearance depending on the phase of digestion or with senescence. Digestive cells of the tubules contain myriad, membrane-bound, vacuole-like structures that stain brightly eosinophilic. During senescence, the digestive gland markedly atrophies grossly and microscopically with near-complete loss of tubular contents and structure (Figure 5.26).

5.3.2.4 Ink Sac Complex

The ink gland has convoluted fronds of cuboidal to polygonal, excretory epithelial cells that contain pigment and a lamina propria. Melanin granules vary in size and shape. The pigment produced is dark brown to black. The active glandular epithelium of the ink gland is surrounded by the dilated ink sac (Figure 5.27). The sac epithelium is a single layer of pigmented, simple cuboidal cells and there is muscle in the sac wall for rapid expulsion of contents.

5.3.3 Excretory System

The excretory system is not a single "kidney" but a system of organs and tissues with different functions. The pericardial appendages begin the process of urine production. An ultrafiltrate of the blood is first produced by the branchial appendages of the branchial hearts and distributed to the pericardial cavity. Branchial appendage histology is covered in the circulatory system (see section 5.3.4). The fluid is then conducted to the renal appendages via the pericardial canal, a long, ciliated duct. The renal appendages are the primary site of ammonia production and may also function in mineral metabolism (Budelmann et al. 1997). The pancreatic appendages or digestive duct appendages are tubular or follicular protrusions of the digestive duct. They are often contained within the digestive gland and are connected to the terminal epithelia of the renal appendages (Schipp and von Boletzky 1976; Schipp and Martin 1981).

5.3.3.1 Renal Appendages

The renal appendages are formed by convoluted tissue folds contained within a thin-walled sac and separated by blood sinuses. The folds are lined by a single layer of tall columnar

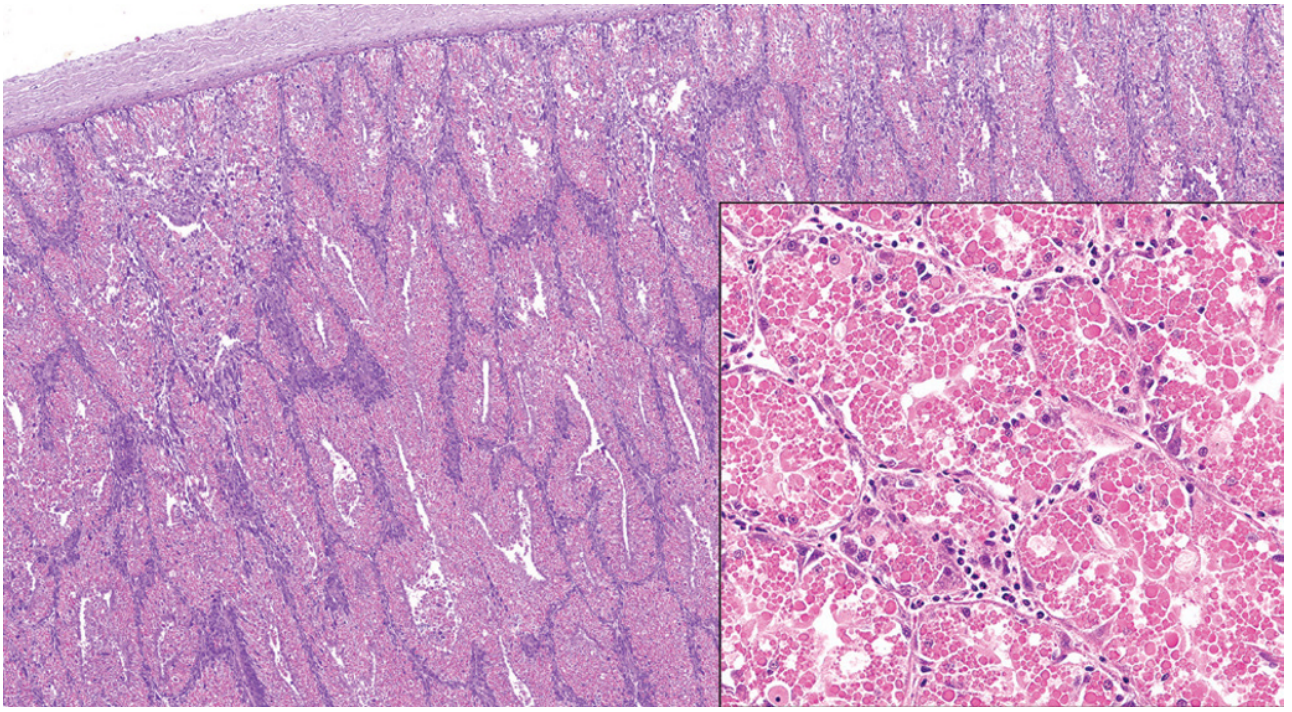


Figure 5.25 Digestive gland of a giant Pacific octopus (*Enteroctopus dofleini*) (background) and northern flamboyant cuttlefish (*Metasepia pfefferi*) (inset). The digestive gland is composed of tubules separated by fine fibrovascular stroma. Tubules are lined by large polygonal epithelial cells with numerous eosinophilic cytoplasmic globules. 40×; inset 400×. HE.

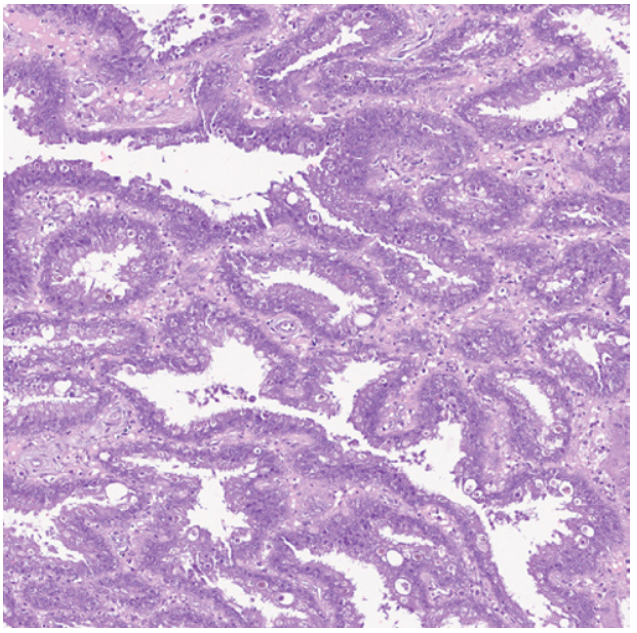


Figure 5.26 Atrophic digestive gland of a senescent giant Pacific octopus (*Enteroctopus dofleini*). Epithelial cells lack cytoplasmic eosinophilic globules seen in Figure 5.24. 100×. HE.

epithelial cells with basal nuclei, apical vacuolation, and a brush border (Figure 5.28). Epithelial cells are often mitotically active. Sinusoidal lumens can contain eosinophilic secretory product and often endosymbiotic dicyemids (Figure 5.29). Dicyemids are enigmatic mesozoans with elongate, simple bodies composed of approximately 40 cells and a nearly complete absence of differentiated tissues (Furuya et al. 2004).

5.3.3.2 Pancreatic Appendages

The pancreatic appendages are only identified in coleoids. The appendages are composed of tubular acini with single-layered columnar epithelium with microvilli (Figure 5.30).

5.3.4 Circulatory System

Cephalopods have a closed, low-volume circulatory system and a short circulation time. The system lacks the extensive hemocoel system found in other molluscs. There is one two-chambered systemic heart and two branchial hearts, with appendages, adjacent to the gills. Deoxygenated blood from the body is pumped by the branchial hearts across the gills, oxygenated, and then on to the systemic heart for distribution in to the general circulation. Vessels are differentiated into arterial and venous systems linked by capillaries, sinuses, and lacunae. The branchial appendages act as part of the excretory system and produce primary urine by ultrafiltration. They also utilize highly specialized "polygonal or round cells" to detoxify, as well as break down and store hemocyanin and other blood proteins.

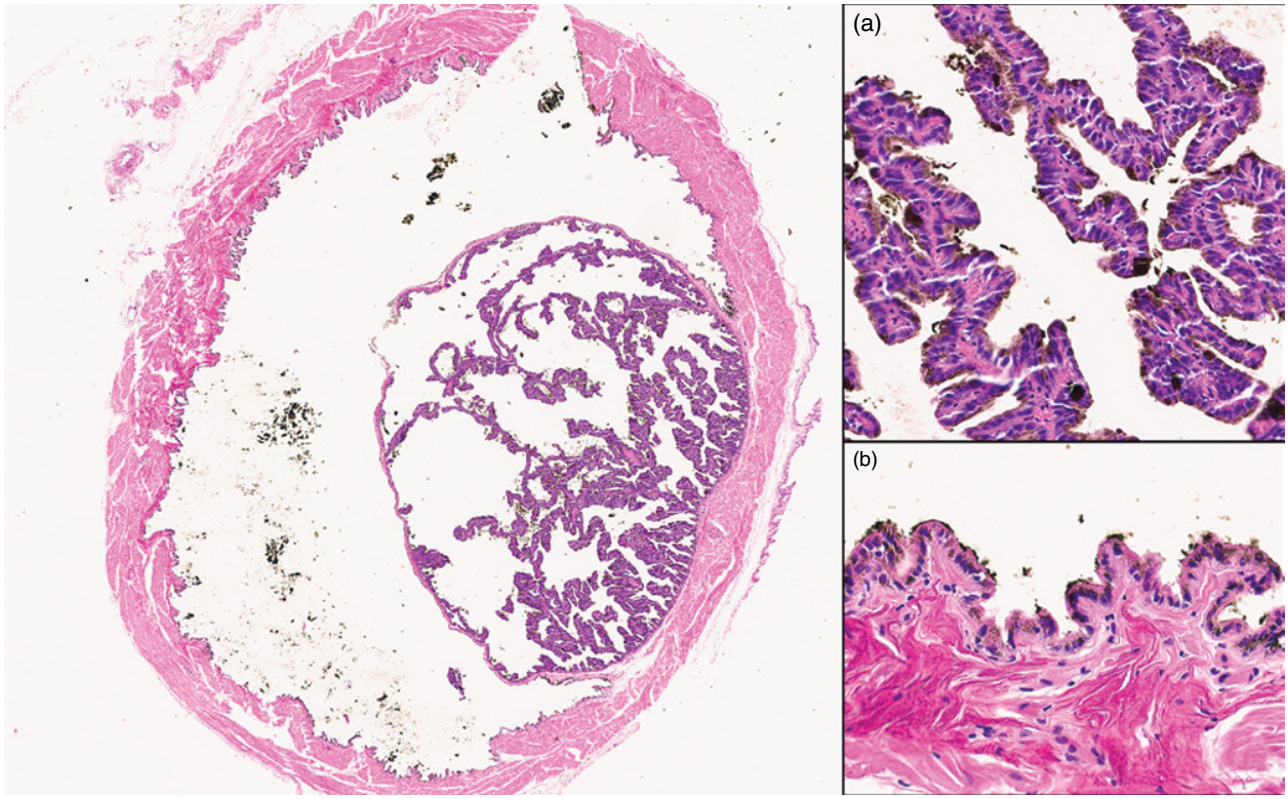


Figure 5.27 Ink sac complex of a northern flamboyant cuttlefish (*Metasepia pfefferi*). The ink gland has convoluted folds covered by pigment-producing epithelium (inset a). The ink sac contains luminal ink and is lined by a simple layer of pigmented, cuboidal epithelial cells and a muscular wall (inset b). 40x; inset 400×. HE.

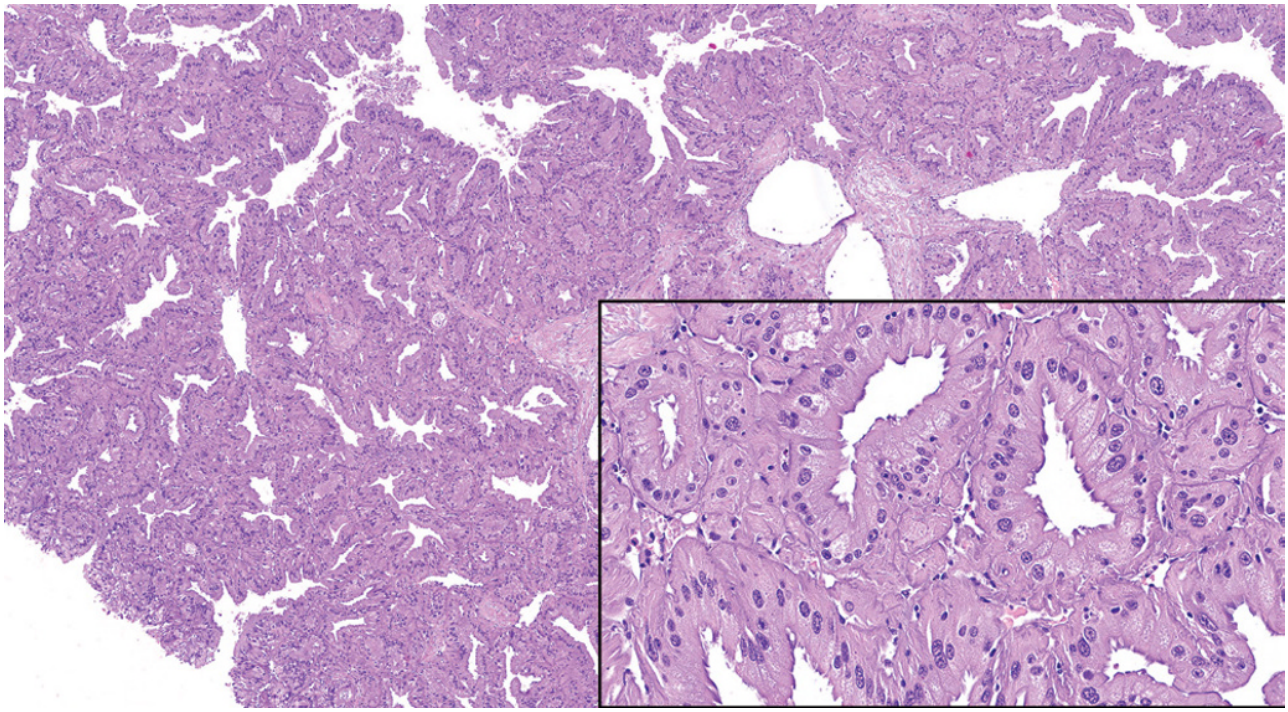


Figure 5.28 Renal appendages of giant Pacific octopus (*Enteroctopus dofleini*). Tall columnar epithelial cells with a brush border form numerous folded tubules. Tubules are separated by small sinuses. 100×; inset 400×. HE.

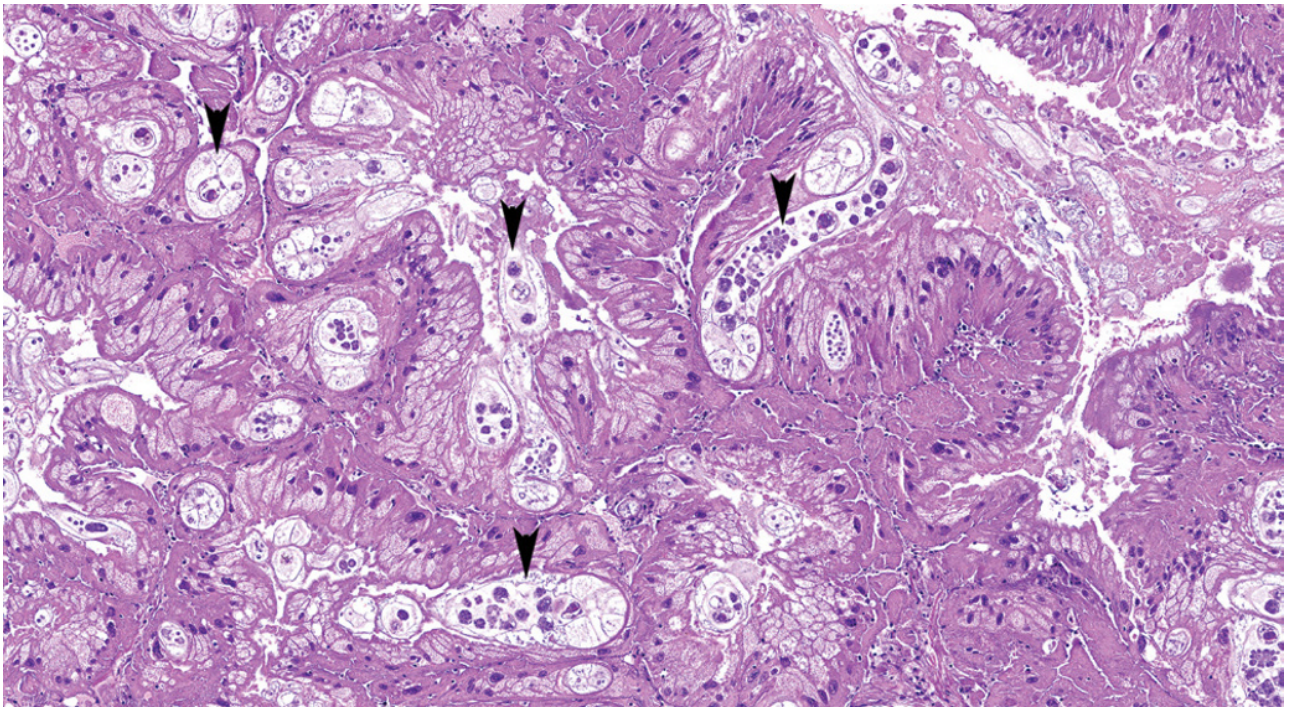


Figure 5.29 Renal appendage of a giant Pacific octopus (*Enteroctopus dofleini*) with commensal dicyemids (*arrowheads*) in tubular lumina. 200×. HE.

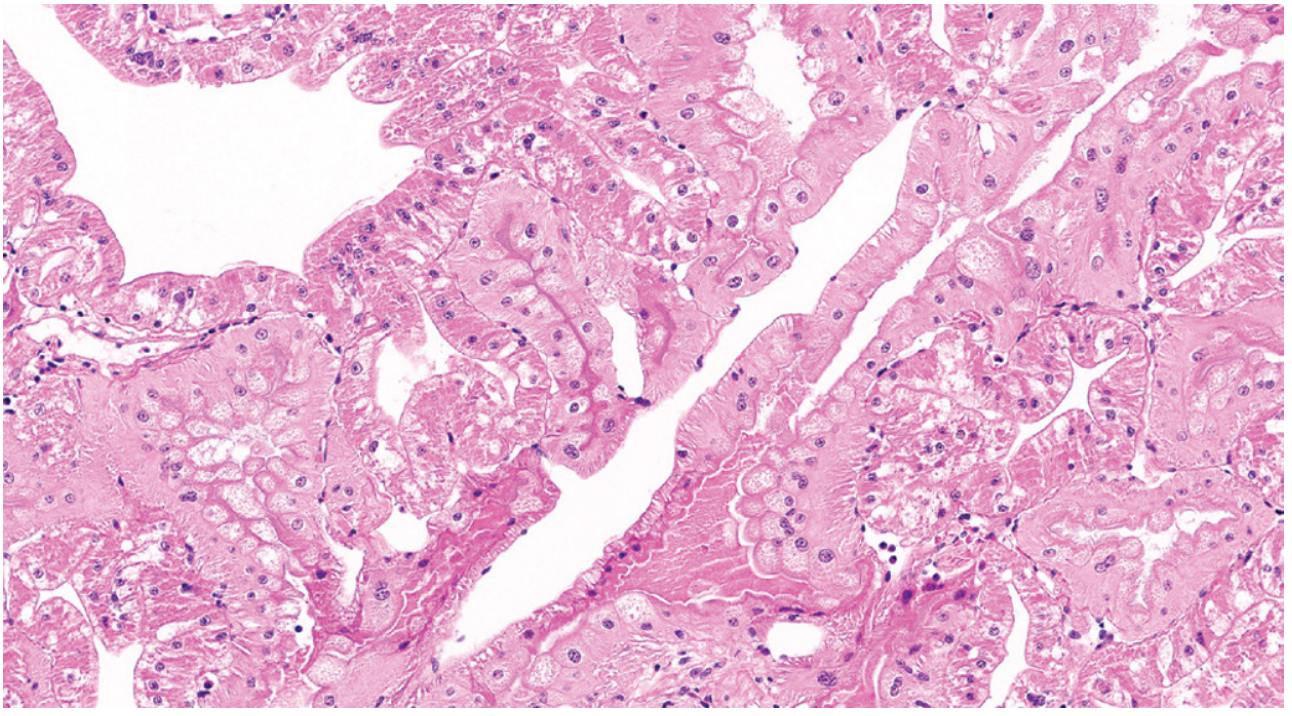


Figure 5.30 Pancreatic appendages of a northern flamboyant cuttlefish (*Metasepia pfefferi*). Branching tubules are lined by tall columnar epithelial cells, the apical aspect of which occasionally bulges into tubular lumina. 400×. HE.

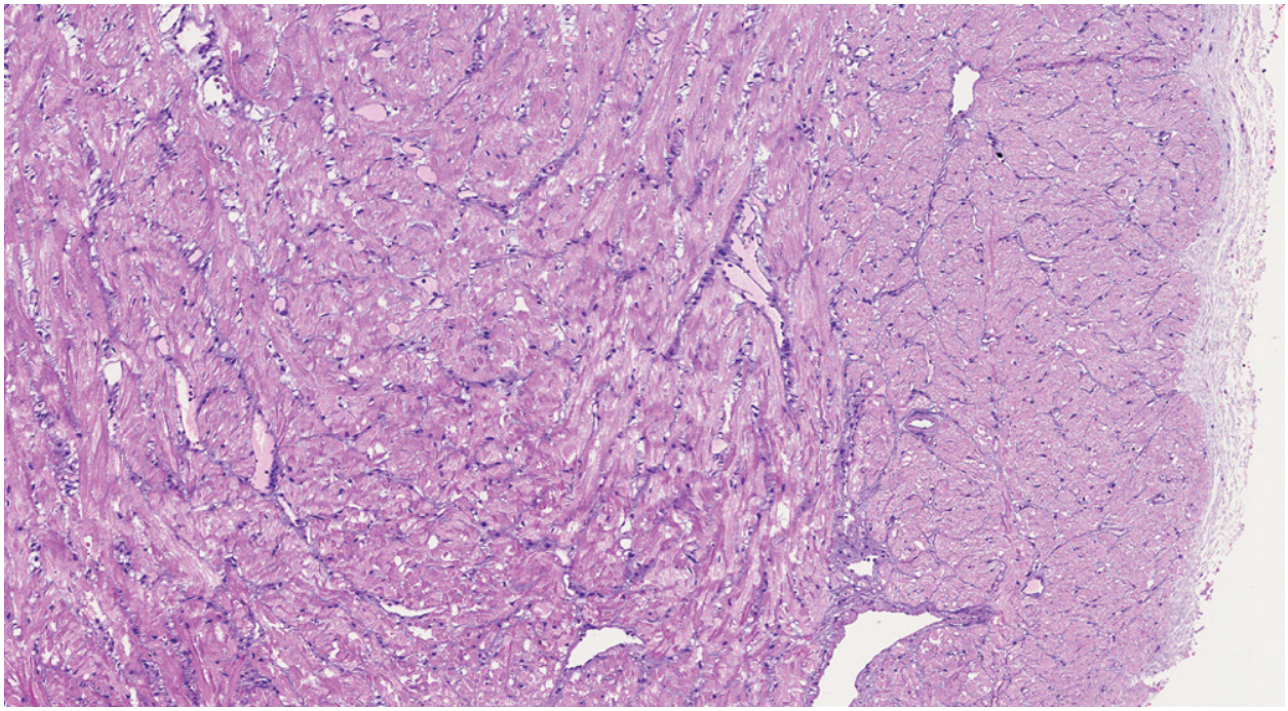


Figure 5.31 Systemic heart (i.e., cardiac muscle) of a northern flamboyant cuttlefish (*Metasepia pfefferi*). 100×. HE.

The circulatory system of molluscs contains hemolymph. In contrast to the red, iron-based hemoglobin of vertebrates, the primary oxygen transporter molecule of molluscs is hemocyanin, a copper-based protein that is blue when oxygenated. In cold conditions with low oxygen pressure, hemocyanin is more effective than hemoglobin at oxygen transportation. Blood-forming organs of cephalopods are the "white bodies" (corpora bianca), a pair of multilobed organs located in the optic sinuses between the medial external surfaces of the eyes and the optic ganglia (Cowden and Curtis 1981). Each white body is supplied by arterial blood from branches of the ophthalmic artery (Claes 1996).

5.3.4.1 Systemic Heart

The systemic heart is composed of striated cardiac muscle with an epicardium, myocardium, and endocardium similar to higher vertebrates (Figure 5.31). The myocardium of the auricles is formed by one or two layers of densely innervated muscle fibers embedded in an extendable collagenous network. The ventricle is a thick meshwork of longitudinal, circular, and diagonal muscle fibers.

5.3.4.2 Branchial Heart and Appendages

The myocardium and epicardium are similar to those of the systemic heart (Figure 5.32). Each branchial heart has an adjacent appendage. These are ductless, highly vascularized glands with columnar epithelial cells on their exterior. Polygonal cells form the bulk of cells that surround the lumen of the branchial appendage (Figure 5.33). These cells have a large, darkly staining, cytoplasmic inclusion that represent lysosomes. Nautiloids have no branchial hearts, but instead have contractile vessels and accumulations of polygonal cells.

5.3.4.3 Vessels

Vessels have three layers similar to vertebrates: the tunica intima (endothelium), tunica media (loose connective tissue and striated muscles fibers), and tunica adventitia (a collagenous network with nerve fibers) (Figure 5.34). In coleoids there can also be a vasa vasorum and in nautiloids a marginal blood sinus.

5.3.4.4 Hemolymph

Only a single type of blood cell is found in hemolymph, the hematocyte, also referred to as a hemocyte, granulocyte or amebocyte (Figure 5.35). Cells are generally round with large lobate, often peripheral nuclei and an abundance of cytoplasmic eosinophilic granules (Figure 5.36). A hemocytic response can be identified where increased numbers of hemocytes are concentrated on a focal lesion, such as an epidermal ulcer, or streaming through tissue, as seem with systemic *Aggregata* sp. coccidian infections.

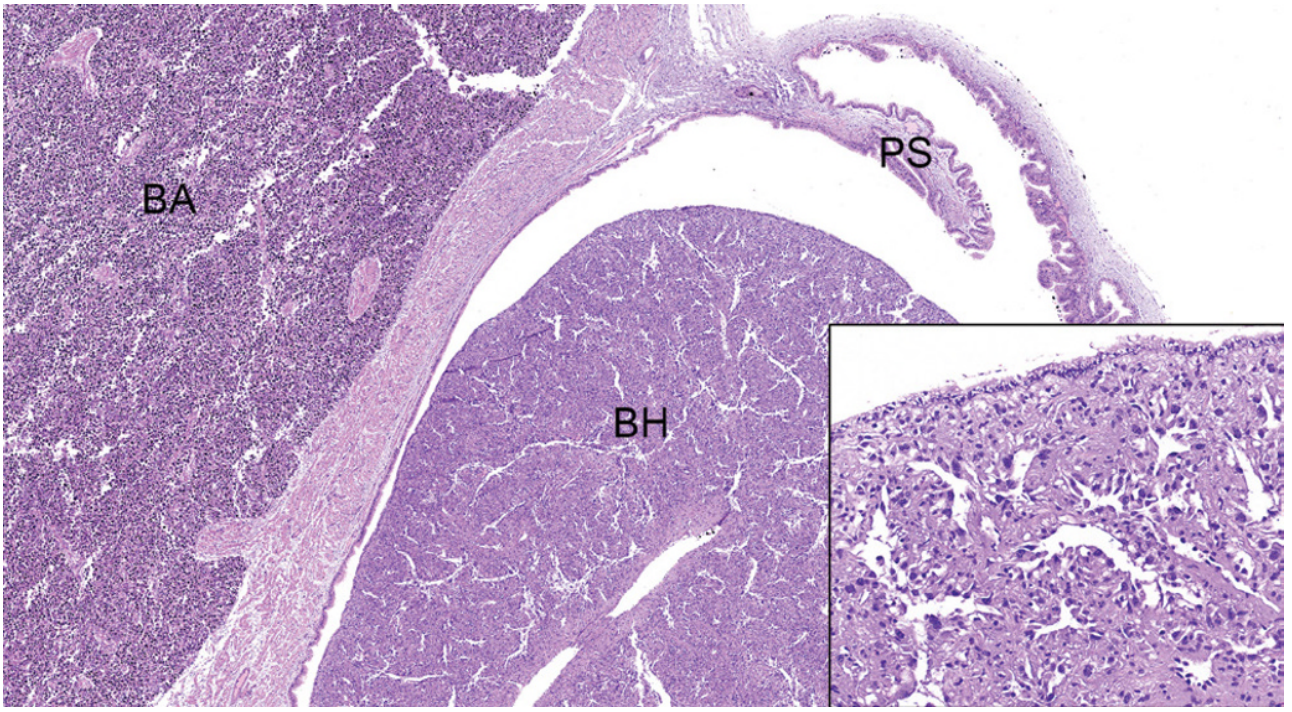


Figure 5.32 Branchial heart (BH) and branchial appendage (BA) of a giant Pacific octopus (*Enteroctopus dofleini*). A pericardial sac (PS) can also be identified. The branchial heart is composed of a spongy myocardium with numerous sinuses (inset). 20×; inset 200×. HE.

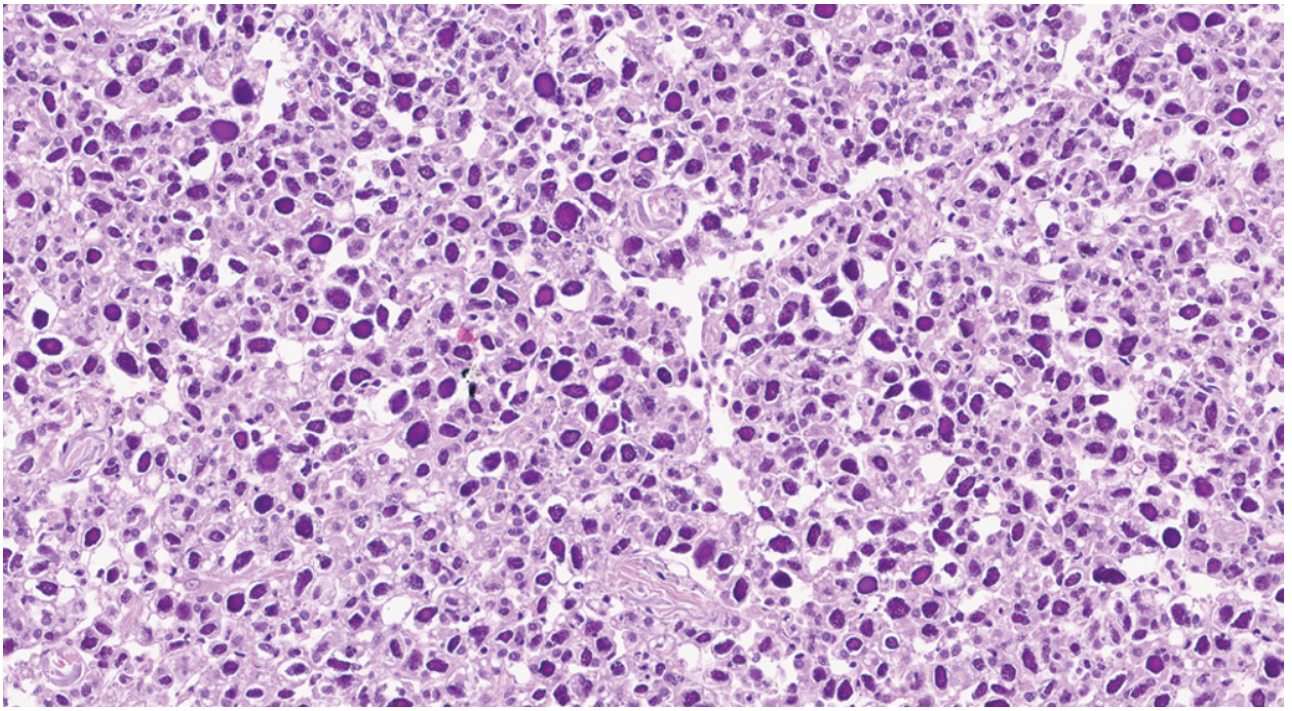


Figure 5.33 Branchial appendage of a giant Pacific octopus (*Enteroctopus dofleini*). The branchial appendage is composed of clusters of epithelial cells separated by small sinuses. Epithelial cells have cytoplasmic concretions that range from basophilic (as seen here) to dark brown. 200×. HE.

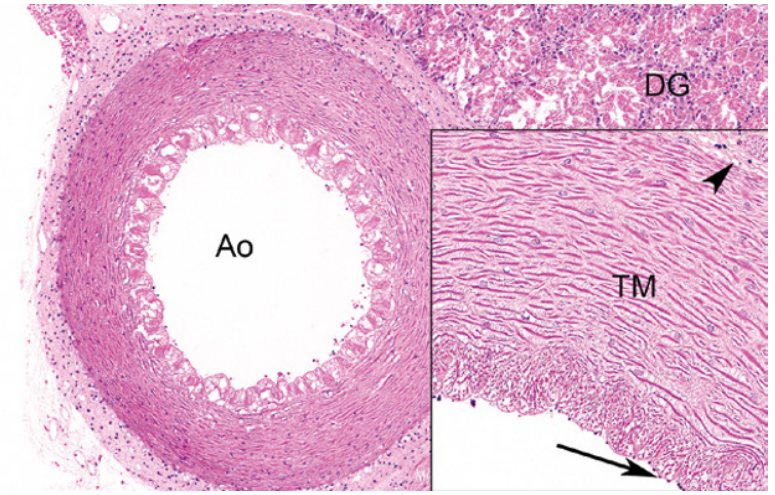


Figure 5.34 Aorta of a northern flamboyant cuttlefish (*Metasepia pfefferi*). The aorta (Ao) is adjacent to the digestive gland (DG). The aorta has a tunica intima with endothelium (*arrow*), thick tunica media composed of loose connective tissue and striated muscles fibers, and outer tunica adventitia (*arrowhead*). 100×; inset 400×. HE.

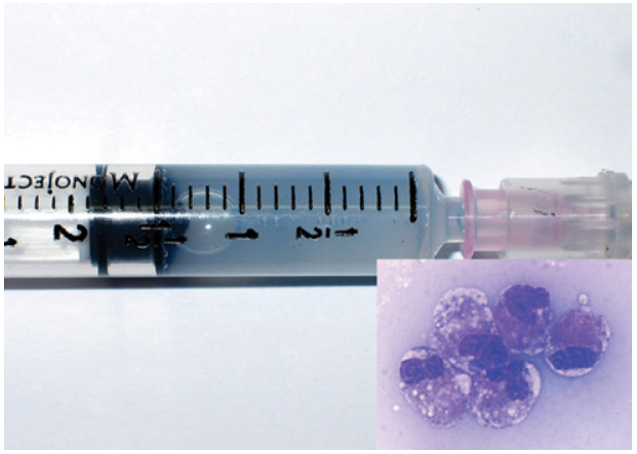


Figure 5.35 Hemolymph from a giant Pacific octopus (*Enteroctopus dofleini*) with cytology of hemocytes (inset). Inset 1000×. Diff-Quik stain.

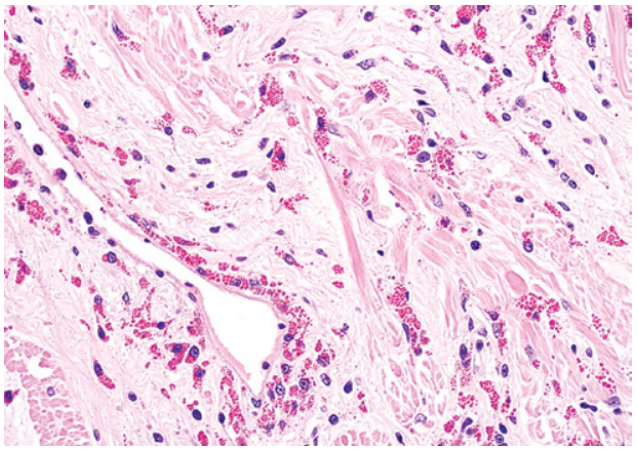


Figure 5.36 Body wall of a common octopus (*Octopus vulgaris*) with interstitial hemocytes, which have abundant, eosinophilic, cytoplasmic granules. 250×. HE.

5.3.4.5 White Bodies

The hematopoietic white bodies are composed of an external collagenous capsule and internal connective tissue network, in which dense sheets of round cells, presumed blood cell precursors, are located (Figure 5.37). Characteristic features of the round hemopoietic cells of this gland are their mitotic activity and heterogenous stages of development. Immature cells stain intensely basophilic.

5.3.5 Immune System

As indicated above, cephalopods possess only a single blood cell type, the hemocyte. Hemocytes exhibit a number of functions similar to vertebrate granulocytes, including the formation of cellular clots, phagocytosis, encapsulation, and inflammation. There is no evidence that they produce antibodies or have an immunologic memory.

5.3.6 Respiratory System

The vascularized skin and gills represent the main respiratory organs. The gills are more developed and efficient than those of other molluscs (Budelmann et al. 1997). They are ventilated by water movement when water is pulled into the mantle cavity (mantle expands, creating negative pressure) and flows across the gills.

5.3.6.1 Gills

Subjacent to the gills is the muscular gill ligament and the branchial gland (Figure 5.38). The gills are composed of extensively folded and well-vascularized lamellae covered in a respiratory epithelium with microvilli but no cilia (Figure 5.39). The columnar epithelium varies in height depending where on the folds it occurs. Goblet cells are

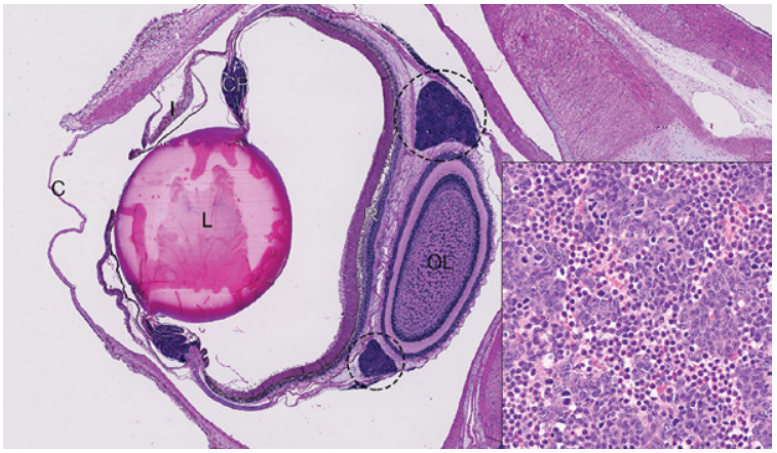


Figure 5.37 White body of a common cuttlefish (*Sepia officinalis*). The white body (*dashed circles*) is located posterior to the sclera of the eye and on either side of the optic lobe (OL). The white body is composed of hematopoietic tissue. Inset: Detail of a white body of a giant Pacific octopus (*Enteroctopus dofleini*). CP, ciliary papilla; C, cornea; I, iris; L, lens; R, retina. 10×; inset 400×. HE.

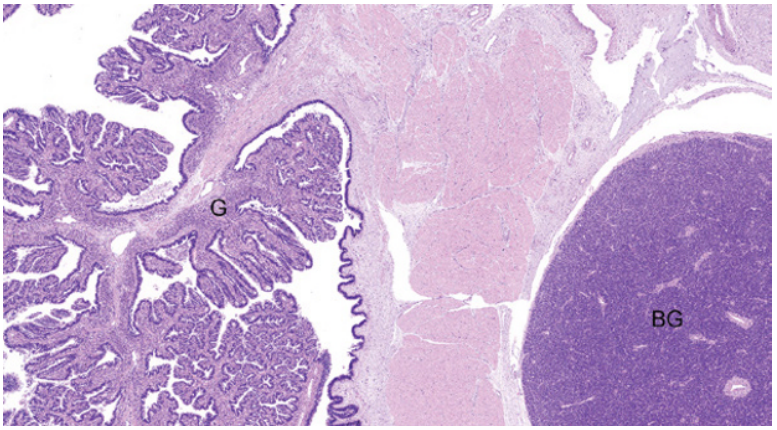


Figure 5.38 Branchial gland and gills of a giant Pacific octopus (*Enteroctopus dofleini*). The gills are composed of arborizing papillary structures on a fibrovascular stalk. BG, branchial gland; G, gill.

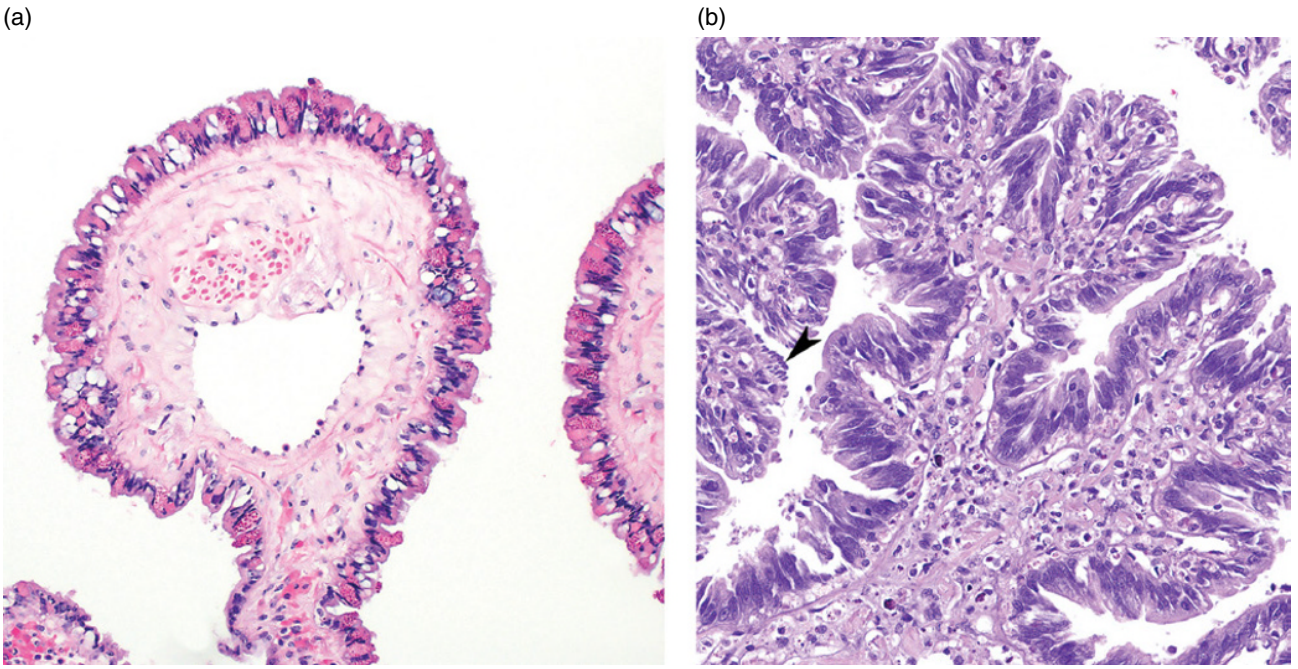


Figure 5.39 Gills of a chambered nautilus (*Nautilus pompilius*) (a) and a giant Pacific octopus (*Enteroctopus dofleini*) (b). The gills are composed of folded and well-vascularized lamellae overlain by respiratory epithelium interspersed with goblet cells. The gills of the octopus are infected with *Ichthyobodo*-like protozoa (*arrowhead*). 200×. HE.

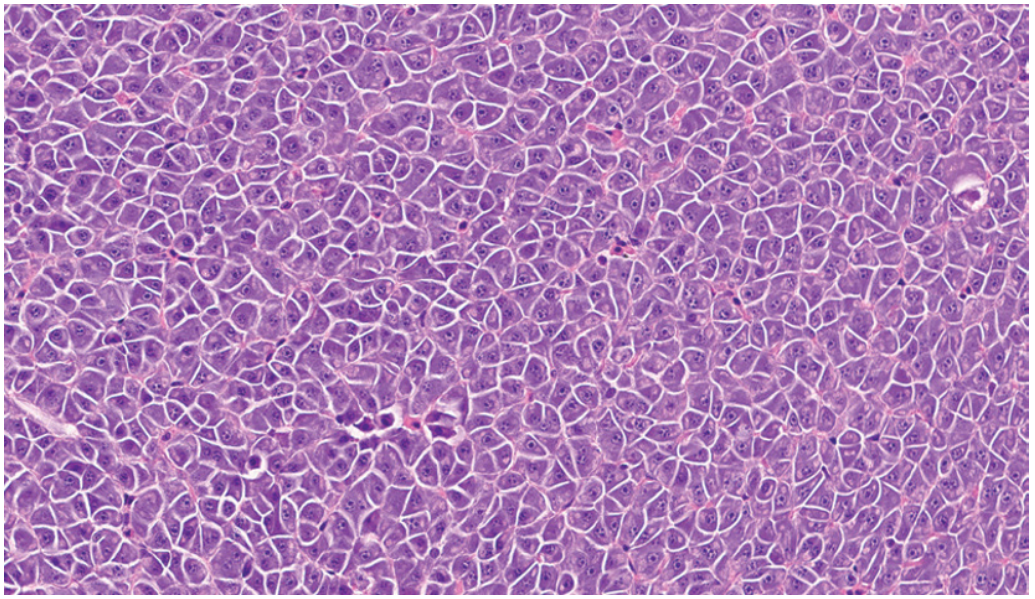


Figure 5.40 Branchial gland of a northern flamboyant cuttlefish (*Metasepia pfefferi*). Glandular cells are deeply basophilic and polygonal, and contain ovoid nuclei with distinct nucleoli. The cytoplasm has variable numbers of eosinophilic inclusions. 200×. HE.

interspersed within the epithelium. Bodonid flagellates can often be identified on the gills of senescent octopuses.

5.3.6.2 Branchial Gland

The branchial gland, previously called the "spleen" or "blood gland," lies within the dorsolateral suspensory ligament. There are no secretory ducts but this gland is well vascularized and proposed to function in hemocyanin synthesis. The glandular cells are deeply basophilic and polygonal and contain ovoid nuclei with distinct nucleoli (Figure 5.40). In Nautiloidea, there is no branchial gland.

5.3.7 Nervous System

The nervous system of cephalopods is the largest and most complex of all invertebrates, with well-developed central and peripheral systems. The brain is composed of numerous masses and lobes. The peripheral nervous system consists of multiple large branchial nerve cords and ganglia. The branchial ganglia are located in each arm and control the suckers. The inferior buccal ganglion controls movement of the buccal mass muscles and radula. The subradular ganglia control the salivary papilla. The gastric ganglion innervates the crop, stomach, cecum, and intestine. The fusiform ganglia innervate the systemic heart, while cardiac ganglia innervate the branchial hearts. The branchial ganglia innervate muscles of the gill lamellae and the stellate ganglia supply the mantle (Budelmann et al. 1997). The giant fiber system of squids and cuttlefish results in lateral synchronization and simultaneous contraction of

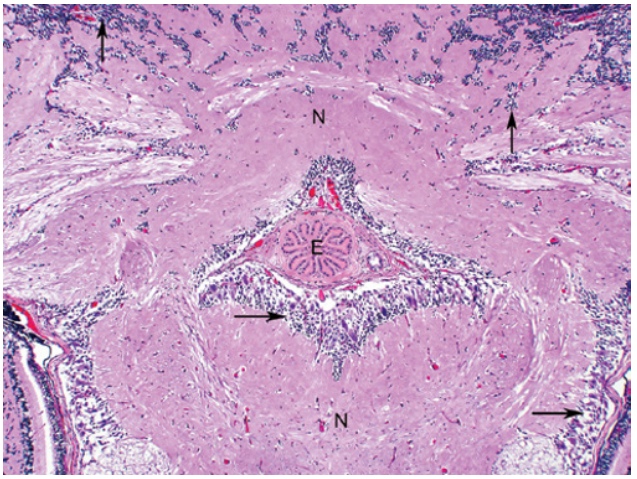


Figure 5.41 Brain of an unspecified cephalopod revealing the brain surrounding the esophagus (E). The neuropile (N) is eosinophilic with few cells. The neurons (*arrows*) are arranged in both lamina (layers; *horizontal arrows*) and aggregates (*vertical arrow*). 40×. HE.

muscle fibers. It includes a chain of three giant nerve cells on each side of the animal (Corning et al. 1975). Octopods lack a giant fiber system.

5.3.7.1 Central Nervous System

The brain is protected within a cartilaginous case located between the eyes and forms a ring around the esophagus (Figure 5.41). Large, oval or reniform optic lobes are present on either side of the brain separated by the optic tract (Figure 5.42). There is a distinct layered cortex and a

medulla containing large multipolar neurons. The cortical layers resemble the organization of deeper layers of the vertebrate retina (Figure 5.42 inset). Neuropile is a pale, acellular tissue composed of axonal processes. Basophilic neurons are found peripherally around the neuropile (Figure 5.43). Ganglia are composed of neuronal cell bodies and are arranged in both lamina (i.e., layers) and aggregates, depending on the region of the brain. The optic glands are located on the upper posterior edge of the optic tracts and composed of a well-vascularized, uniform mass of cells with no obvious formation of epithelia or vesicles (Budelmann et al. 1997) (Figure 5.43).

5.3.7.2 Peripheral Nervous System

All peripheral ganglia show characteristic ganglionic organization with a peripheral perikaryal layer and a central neuropile (Figure 5.44). The peripheral nerves of cephalopods are all unmyelinated. In the arms, a large branchial (axial) nerve cord is located in the center of four smaller intramus-

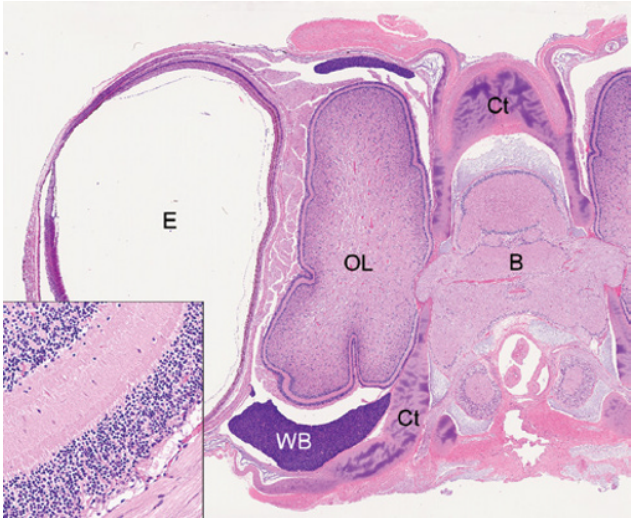


Figure 5.42 Brain of a giant Pacific octopus (*Enteroctopus dofleini*). The optic lobe (OL) is medial to the eye (E) and surrounded by the white body (WB). The brain proper (B) is encased in cartilage (Ct). Inset: Cortical layers of the optic lobe. 20×; inset 100×. HE.

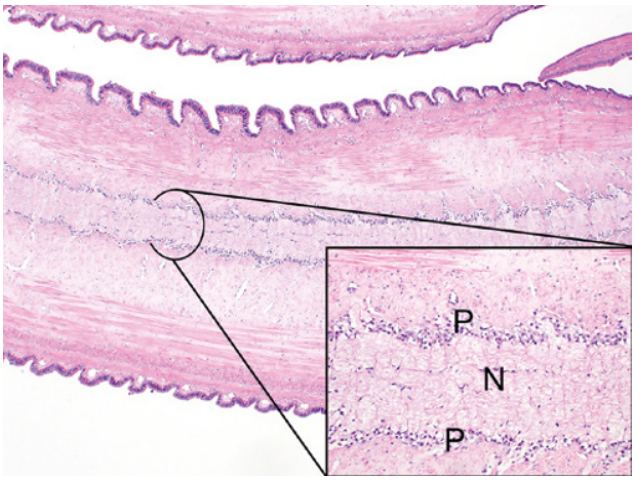


Figure 5.44 Peripheral nerve of a chambered nautilus (*Nautilus pompilius*). Peripheral nervous tissue extends down the length of the arm. On higher magnification, the central neuropile (N) and peripheral perikaryon (P) can be seen. 40×; inset 200×. HE.

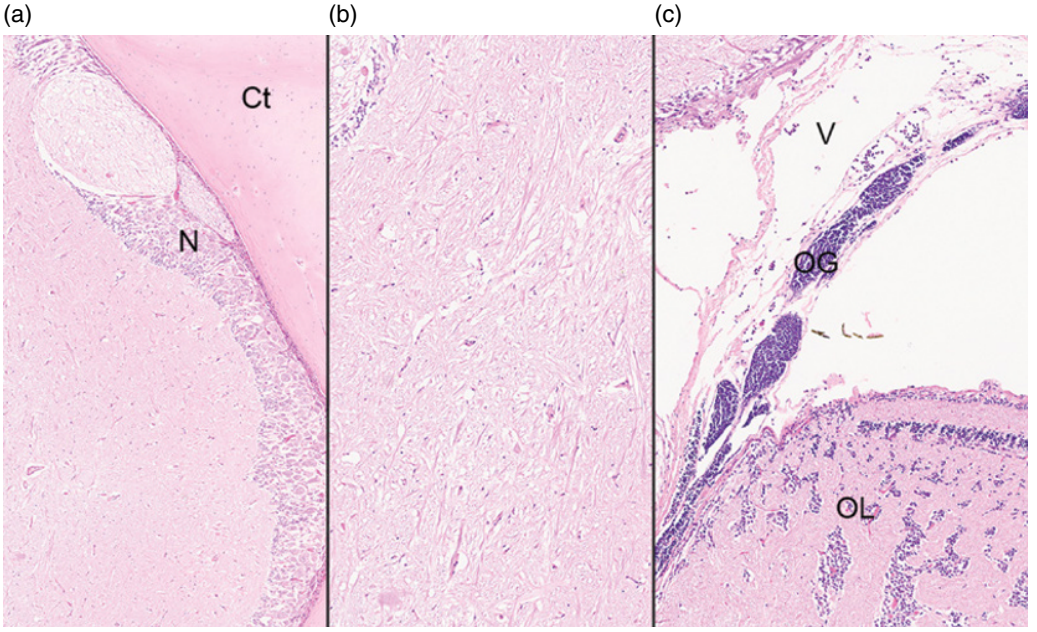


Figure 5.43 Central nervous system of a northern flamboyant cuttlefish (*Metasepia pfefferi*). (a) Basophilic neurons are found peripherally around the neuropile. Ct, cranial cartilage; N, neurons. (b) Neuropile is a pale, acellular tissue composed of axonal processes. (c) The optic gland (OG) is a uniform mass of cells. OL, optic lobe; V, vessels. 100×. HE.

cular nerve cords, with branchial ganglia occurring at regular intervals. One small sucker (acetabular) ganglion is associated with each sucker (see Figure 5.17).

5.3.8 Reproductive System

Copulation can take an hour or more and occurs when a male inserts the hectocotylus arm into the mantle cavity and oviduct of the female to implant spermatophores. Giant Pacific octopuses (*E. dofleini*) have the longest spermatophores, each containing about seven billion sperm (Budelmann et al. 1997). Female egg masses can be up to 25–40% of the body weight, equivalent to a 120 lb woman carrying a 48 lb baby (Boletzky 1986).

5.3.8.1 Female

Developing ova within the saccular ovary are surrounded by follicular epithelium and may contain a visible nucleus when previtellogenic. In some species, ova can be stalked and vascularized (Figure 5.45). As ova mature, the follicular epithelium folds and variably separates the yolk mass (Figure 5.46). Mature eggs travel down the oviduct, which has a deeply folded, ciliated, tall columnar epithelium (Figure 5.47), to the oviductal gland. Organized into frond-like lobules composed of densely packed tubules, the oviductal gland has an inner zone of pale-staining, mucus-secreting cells and an outer zone of cells with intensely eosinophilic granules (Figure 5.48). Nidamental glands are large, paired glandular structures in Decapodiformes and nautiluses (Bermingham 1987). The nidamental gland can have a similar microscopic anatomy as the oviductal gland with cuboidal epithelial cells and supporting stroma. The decapodids also possess an accessory nidamental gland composed of tubules with large secretion-filled lumens (Figure 5.49).

5.3.8.2 Male

The mature testis is a compact structure of seminiferous tubules (seminal cysts) that contain densely packed mature spermatozoa as well as earlier sperm stages (Figure 5.50). Spermatogenesis and supporting Sertoli cells can be identified. Spermatozoa vary greatly between species in thickness, acrosome size, and position of the flagellum. Sperm leave the testis and pass through a ciliated ampulla into the coiled vas deferens, which is a duct lined by ciliated

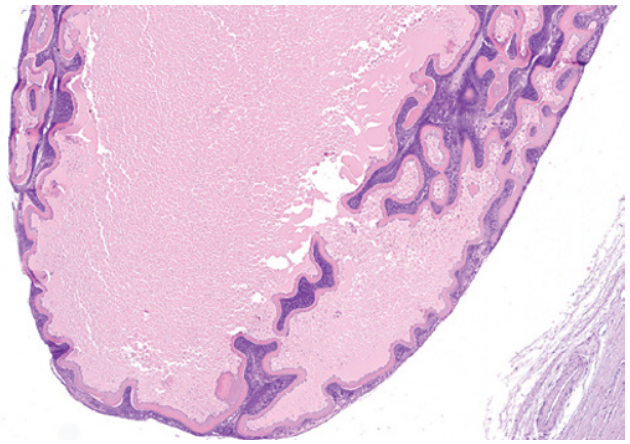
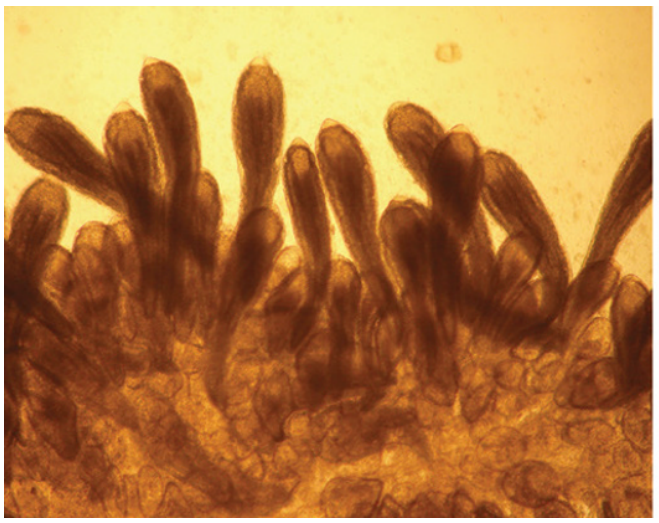


Figure 5.46 Mature follicle of a giant Pacific octopus (*Enteroctopus dofleini*). The follicular epithelium becomes folded as the yolk expands. 100×. HE.

(a)



(b)

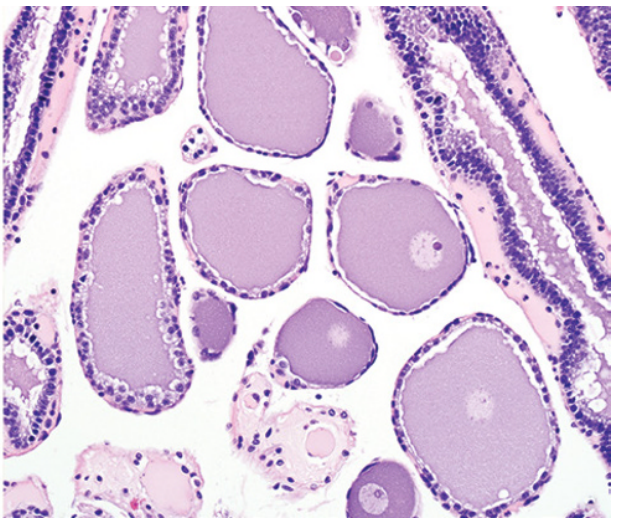


Figure 5.45 Saccular ovary of a common octopus (*Octopus vulgaris*) on wet mount (a) and histology (b). 120×. HE. Histologically, developing follicles are surrounded by follicular epithelium. *Source:* Impression photo courtesy of Dr Shane Boylan.

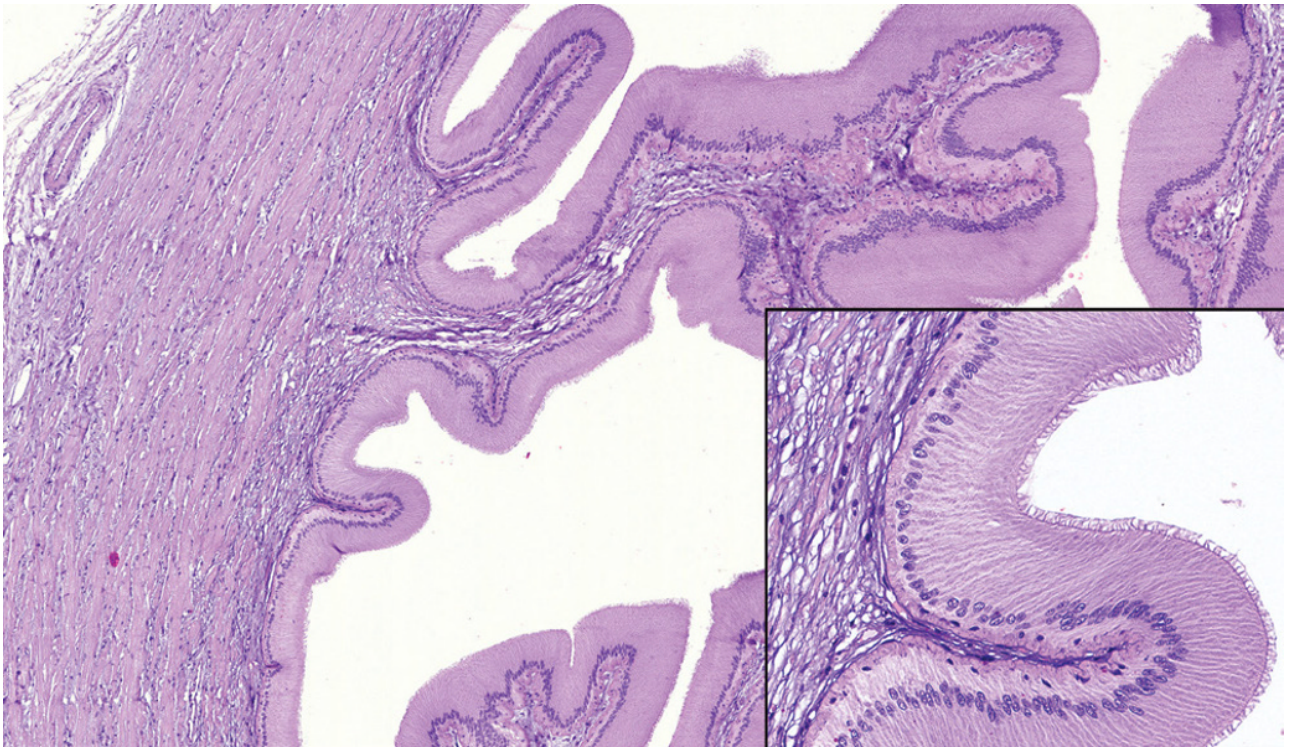


Figure 5.47 Oviduct of a giant Pacific octopus (*Enteroctopus dofleini*) has deeply folded mucosa with ciliated columnar epithelial cells. 100×; inset 400×. HE.

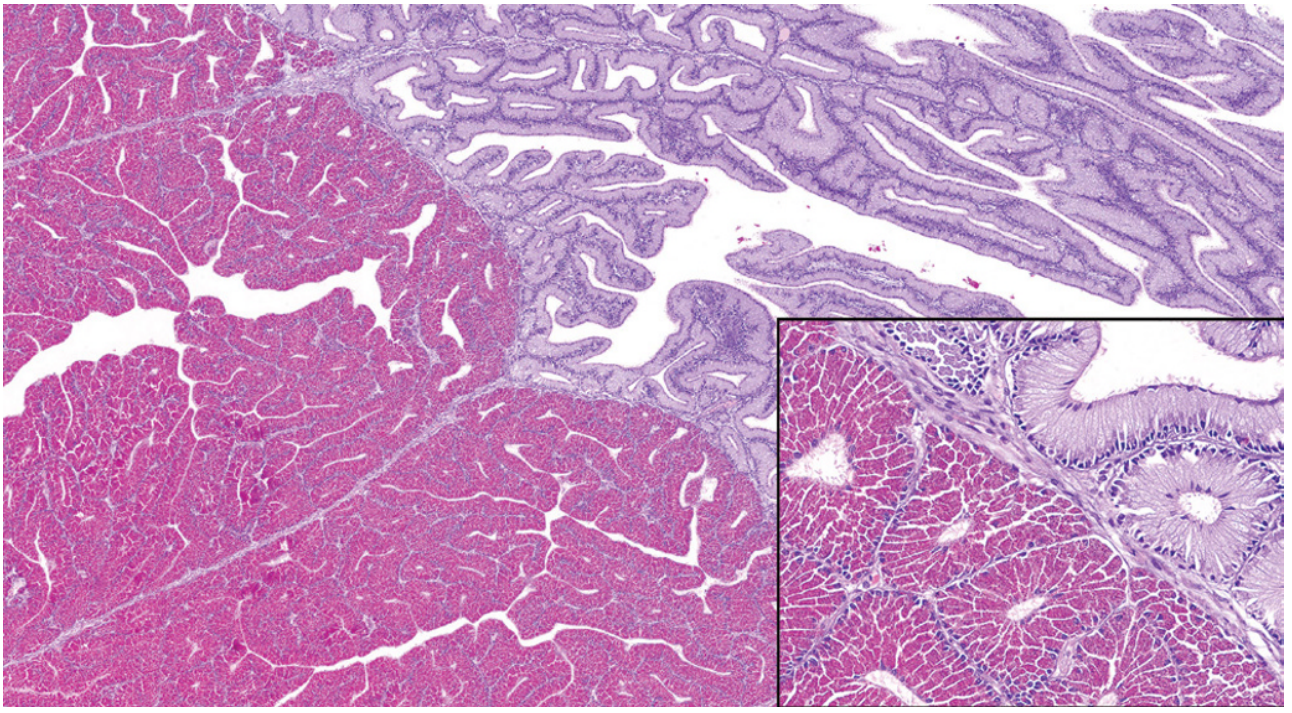


Figure 5.48 Oviductal gland of a giant Pacific octopus (*Enteroctopus dofleini*). The oviductal gland consists of two distinct zones representing the proximal and distal glandular units. The distal units have brightly eosinophilic globules. The proximal units have pale basophilic cytoplasm. 100×; inset 400×. HE.

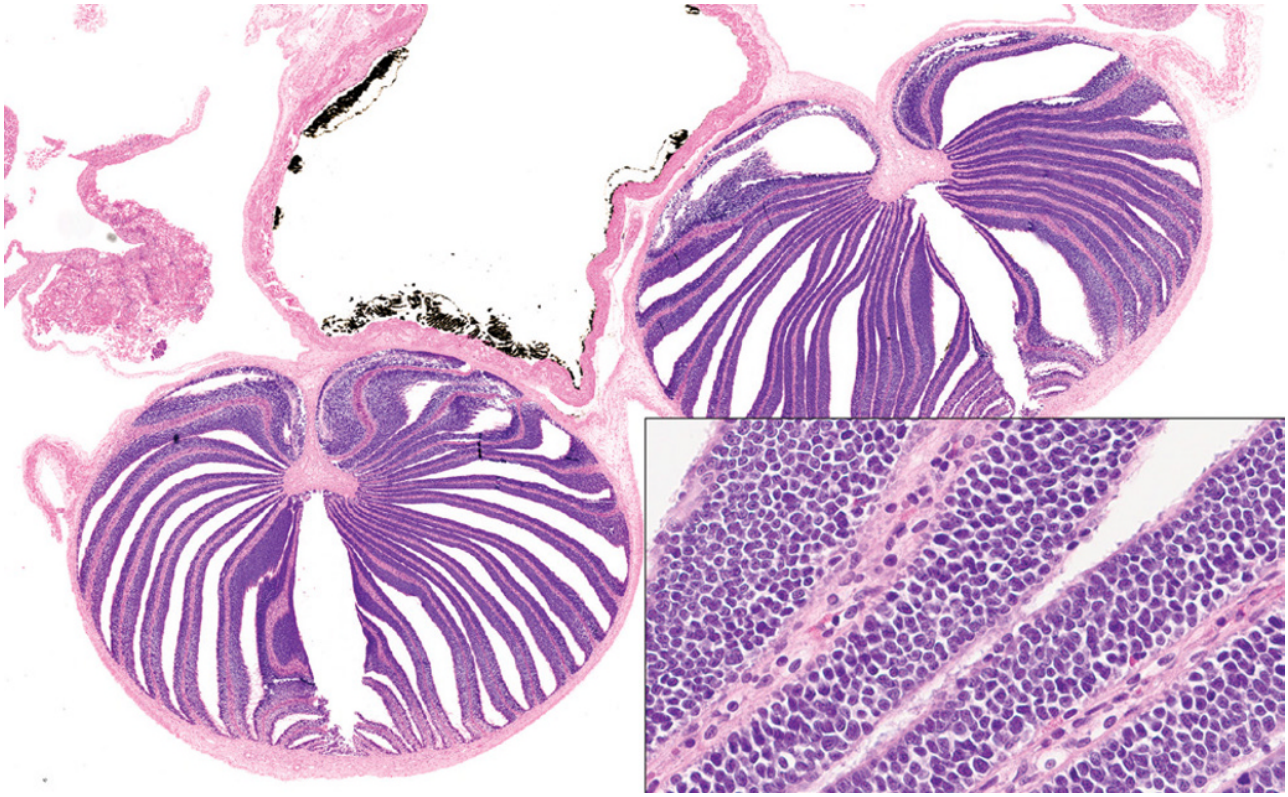


Figure 5.49 Paired nidamental gland in a northern flamboyant cuttlefish (*Metasepia pfefferi*). Inset: Ciliated epithelium. 40×; inset 400×. HE.

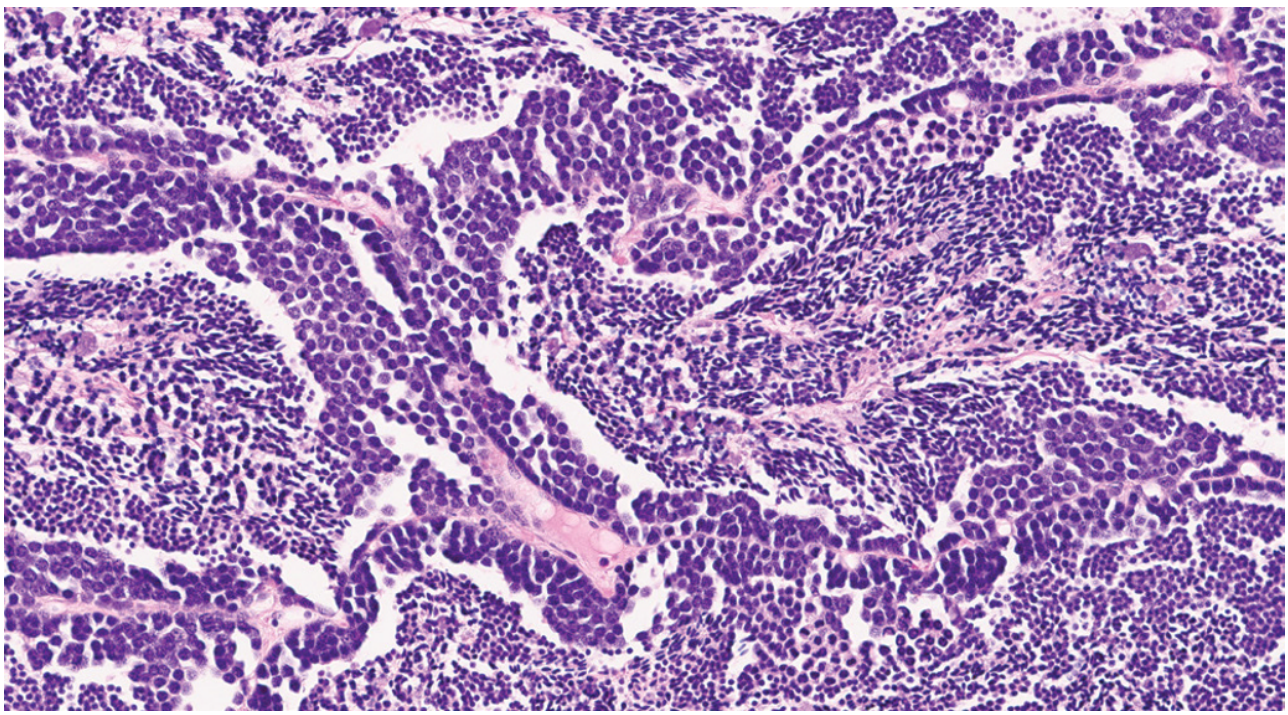


Figure 5.50 Male gonad in a northern flamboyant cuttlefish (*Metasepia pfefferi*). Testes are organized into lobules with a peripheral layer of spermatogonia that differentiate toward the central lumina into spermatocytes and spermatids. 300×. HE.

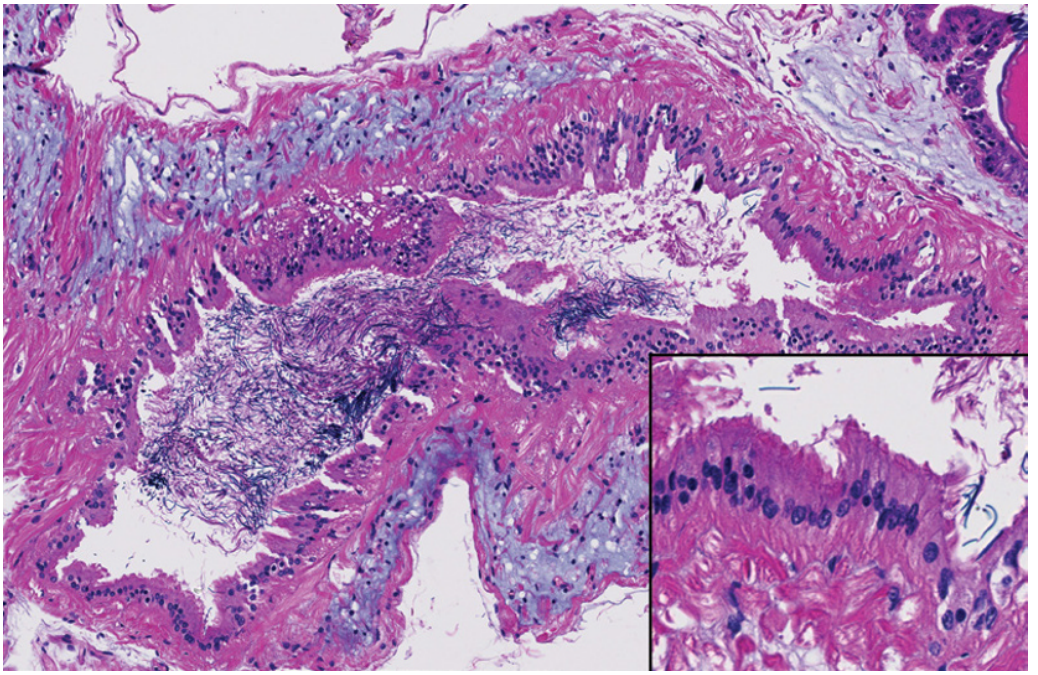


Figure 5.51 Vas deferens of a two-spot octopus (*Octopus bimaculoides*) is lined by simple, cuboidal ciliated cells. Numerous spermatozoa fill the lumen. Spermatozoa are slightly coiled in octopus compared to straighter spermatozoa in cuttlefish (see Figure 5.50). 120×; inset 400×. HE.

epithelial cells (Figure 5.51). Sperm emerge from the vas deferens compacted into a sperm rope with sperm heads aligned. The vas deferens terminally widens and becomes the spermatophoric organ, which consists of the seminal vesicle (also called spermatophoric gland I) and prostate (also called accessory gland and spermatophoric gland II) (Figures 5.52 and 5.53). The seminal vesicles and accessory glands are lined by glandular secretory cells that imbed the sperm rope in a mucilaginous secretion. In the seminal vesicles is an undulating to arborizing mucosa composed predominantly of glandular cells, which are ciliated in the apical position. The height of these columnar cells and eosinophilic secretory granules varies. The prostate has a glandular parenchyma with compound tubuloalveolar secretory segments abutting a central duct. The glandular epithelium as well as the duct epithelium can range from cuboidal to columnar in shape (Figure 5.54). Most of the thickness of the epithelium is formed by glandular cells with basal nuclei. Other cells are ciliated and have apical nuclei located near the central lumen. Completed spermatophores are stored in Needham's sac (spermatophoric sac) (Figure 5.55) and/or in the small blind sac located near the genital opening known as the diverticulum of the penis.

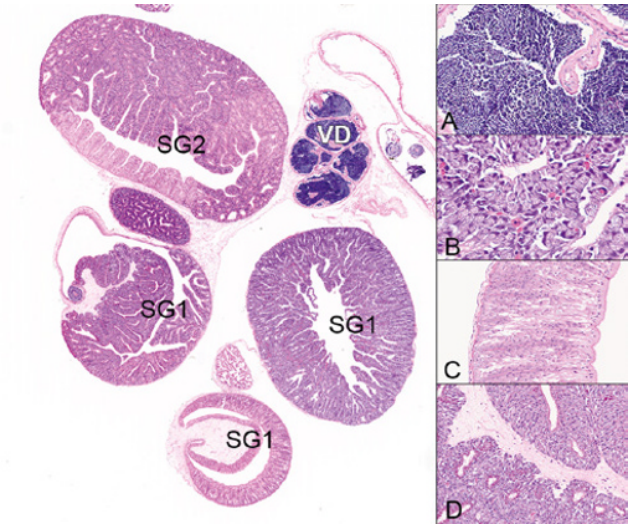


Figure 5.52 Male gonad in a northern flamboyant cuttlefish (*Metasepia pfefferi*). Accessory sex organs. Inset: (a) Vas deferens with ciliated epithelium and myriad sperm. (b) Spermatophoric glands I, glandular parenchyma. (c) Spermatophoric glands I, ciliated apical cells. (d) Spermatophoric glands I with eosinophilic secretory granules. 40×; insets 400×. HE. SG1, spermatophoric glands I; SG2, spermatophoric glands II; VD, vas deferens.

5.3.9 Special Senses

Cephalopods have well-developed eyes and complex visual behavior. While nautiloid eyes have no lens and a pinhole iris, the majority of cephalopod eyes include lids, cornea, iris, pupil, lens, and retina (Budelmann et al. 1997). Light penetrates the cornea and lens to reach a heavily pigmented retina. Ciliary muscles shift the lens forward and backwards to focus an image on the retina. In cephalopods, light excites retinal photoreceptors directly without

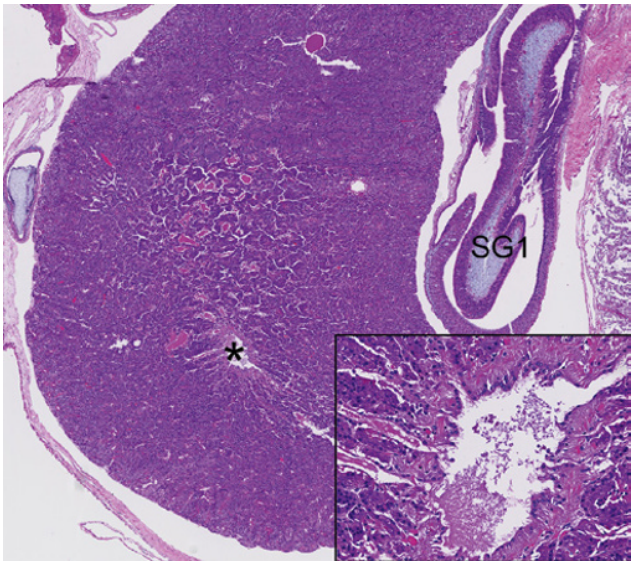


Figure 5.53 Spermatophoric glands I and II of a two-spot octopus (*Octopus bimaculoides*). The terminal, secretory portion of gland I is on the right of the image. Gland II has a central duct (*asterisk*), higher magnification of which is in the inset. 20×; inset 200×. HE.

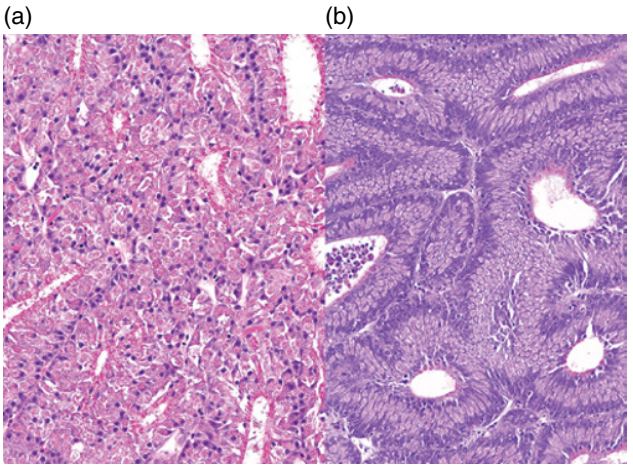


Figure 5.54 Spermatophoric glands II with glandular parenchyma with basal nuclei of a (a) northern flamboyant cuttlefish (*Metasepia pfefferi*) and (b) giant Pacific octopus (*Enteroctopus dofleini*). 400×. HE.

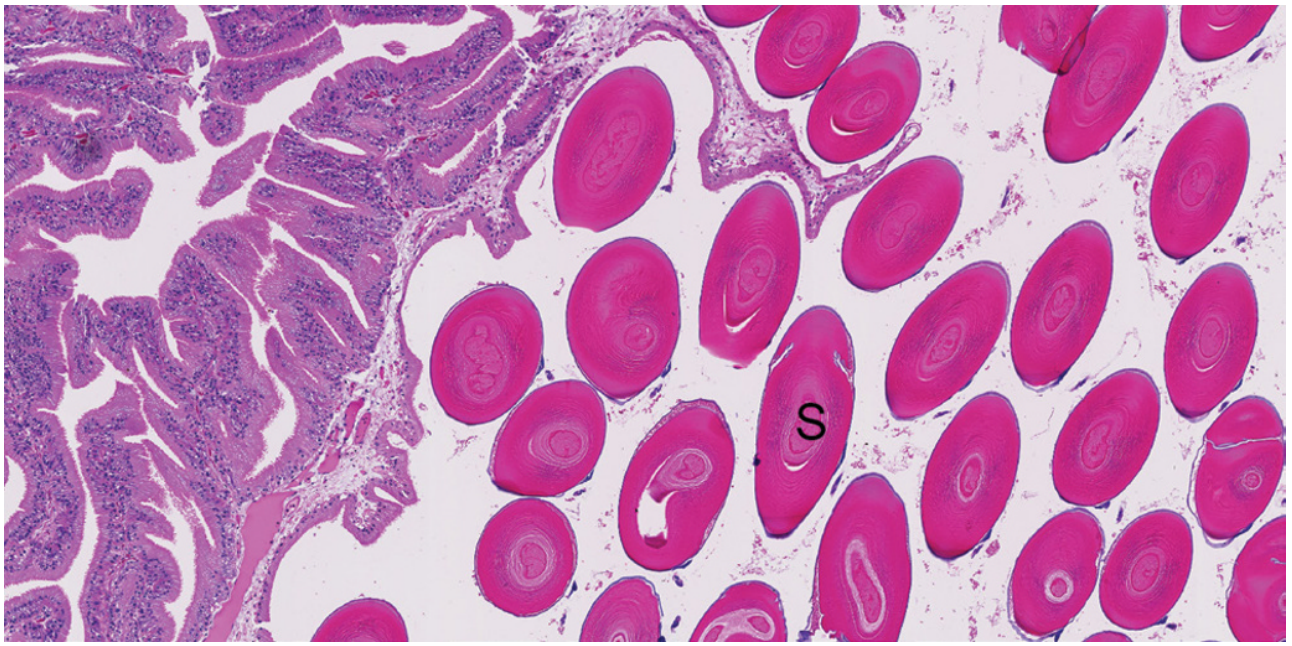


Figure 5.55 Needham's sac of a two-spot octopus (*Octopus bimaculoides*) contains numerous lamellated spermatophores (S). 50×. HE.

passing through other layers as in vertebrate species (Fernald 2004). As a result, the layers of the retina are in reverse order in cephalopods compared to those in vertebrates. The photoreceptor cells are directed toward the light, while a retinal nerve cell layer underlies the photoreceptors and sends fibers to the optic ganglion (Budelmann et al. 1997).

Cephalopods are thought to have most major sense organs including photoreceptors, chemoreceptors, and mechanoreceptor organs (Budelmann 1996). These equilibrium receptor organs can have statocysts with sensory hair cells and large neurons. Some cuttlefish, squid, and octopus hatchlings have lines of ciliated epidermal hair cells on their head and arms that are sensitive to water movements (Budelmann 1996, 2000).

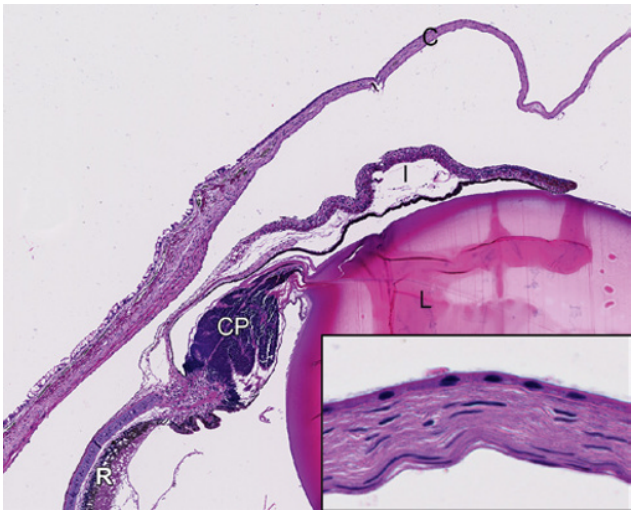


Figure 5.56 Anterior portion of the eye of a common cuttlefish (*Sepia officinalis*). The cornea (C) is the most external layer and is composed of a thin, fibrous tunic overlain by simple epithelium (inset). The pigmented epithelium covering the posterior aspect of the iris (I) is continuous with the anterior epithelium of the ciliary papilla. The inner cells of the ciliary papilla (CP) have cytoplasmic extensions that are continuous with the lens (L). The posterior pigmented cells of the ciliary papilla are continuous with the retina (R). 20×; inset 400×. HE.

5.3.9.1 Eyes

The cornea is a thin fibrous tunic overlain by a simple epithelium (Figure 5.56). The iris surrounds a horizontal slit-shaped pupil in most cephalopods and is composed of an external epithelium, chromatophore layer, iridophore layer, a layer of muscle and collagen fibers, and a pigmented epithelium (Figure 5.57). The pigmented epithelium covers the inner layer of the iris and is continuous posteriorly with epithelium of the ciliary papillae, and ultimately with the retina. Ciliary epithelial cells are small peripherally, becoming large and basophilic (with or without cytoplasmic melanin) centrally, forming cytoplasmic extensions continuous with the lens. Anterior and posterior lens components are separated by a septum created by cytoplasmic extensions of the ciliary epithelial cells. The simple retina is dominated by two layers (proximal and distal) that both contain photoreceptor cell processes. The proximal layer is thinner and at the deep aspect contains neurons of the photoreceptors. The distal layer is generally thicker. Deep to the two photoreceptor layers is a thin plexiform layer composed of inner processes of the photoreceptor cells (Figure 5.58). Periocular cartilage can be identified as well as extraocular muscles; there are four muscles in nautiloids, seven in octopods, and 13–14 in Decapodiformes.

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The views expressed in this chapter are those of the author and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or US Government.

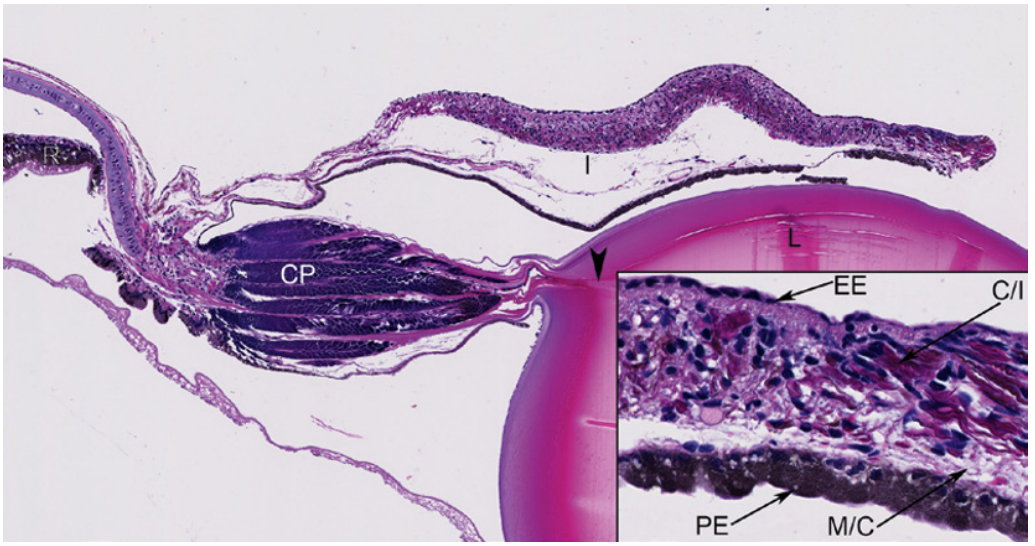


Figure 5.57 Anterior portion of the eye of a common cuttlefish (*Sepia officinalis*). The iris (I; inset) is composed of an outer external epithelium (EE), a pigmented layer (chromatophores and iridophores), a layer of muscle and collagen fibers, and an inner pigmented epithelium (PE). Posteriorly, the pigmented epithelium is continuous with the anterior epithelium of the ciliary papilla (CP). The inner cells of the ciliary papilla have cytoplasmic extensions that are continuous with the lens (L), and divide the lens into anterior and posterior portions; this dividing line is slightly basophilic (*arrowhead*). R, retina. 60×; inset 400×. HE.

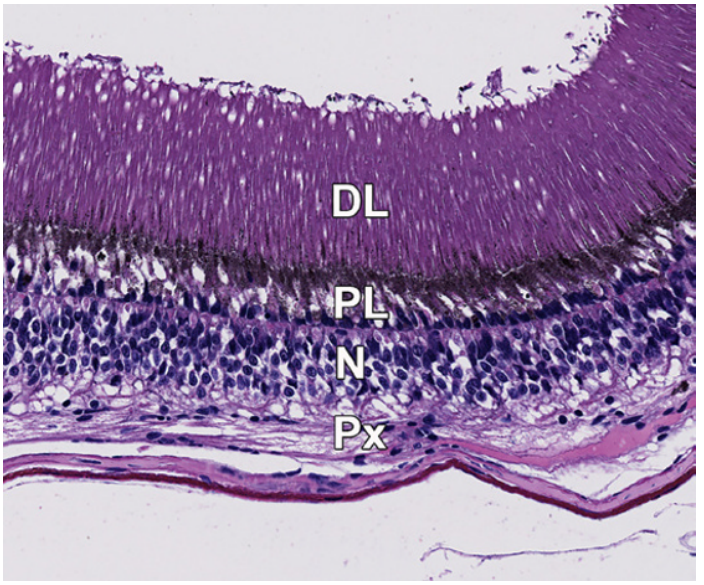


Figure 5.58 Retina of a common cuttlefish (*Sepia officinalis*). The distal layer (DL) is thicker and contains photoreceptor cell processes and pigment. The proximal layer (PL) is thinner and at the deep aspect contains the neurons of the photoreceptors (N). Deep to the distal and proximal photoreceptor layers is a thin plexiform layer (P×) composed of inner processes of photoreceptor cells. 400×. HE.

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6

Mollusca

Bivalvia

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6.1 Introduction

Bivalvia (Lamellibranchiata, Pelecypoda) is a large class of laterally compressed animals characterized by two calcified variably flattened to deeply cupped valves (which make up the shell) that are attached to each other at the dorsal surface with teeth (interlocking waves in the calcified shell, not true teeth) spanned by a flexible hinge ligament. The inner surfaces of the valves are lined by a thin mantle. The visceral mass (body) of the bivalve extends from the dorsal attachment into the space (pallial cavity/mantle cavity) between the mantle-lined valves.

There are more than 15 000 species of bivalves, most of which are marine. Because they are filter feeders, most species live on or in the sediment, or on rocky surfaces of relatively shallow microalgal rich marine or fresh waters. Based on their overall morphology, they are separated into five subclasses but are practically placed into the following groups: clams, cockles, mussels, scallops, and oysters. In the past, bivalves were an important human (and animal) food source. Importantly, bivalves are widely recognized as providing an ecosystem service as filter feeders by removing suspended material from the water column and either incorporating it into their bodies or depositing undigested materials on the sediment surface in mucus-bound composites. A single bivalve can filter between 1 and 4 L/h and calculations based on oyster populations in 1880 have determined that it only took 3.3 days to filter all of the water in the Chesapeake Bay (Rice 2001).

Today in many areas, bivalves, especially those species used as food by people, have either been fished heavily and/or have suffered from diseases, resulting in significant decreases in their populations. While wild capture is still commonly used for many species of edible bivalves, aquaculture was, and continues to be, a method of producing bivalves for human consumption. The species of bivalve that are routinely cultured varies by country and climate, but the most commonly cultured bivalves are oysters (*Crassostrea virginica*, the eastern oyster and *Crassostrea gigas*, the pacific oyster), clams (*Mercenaria mercenaria*, the northern clam, *Venerupis philippinarum*, the Manila clam and *Ruditapes decussatus*, the grooved carpet clam), mussels (*Mytilus edulis*, the blue mussel and *Mytilus galloprovincialis*, the Mediterranean mussel), the Yesso scallop (*Patinopecten yessoensis*), and the common cockle (*Cerastoderma edule*). As an example of the increased importance of aquaculture, the global production of Pacific oysters increased from 149 163 tons in 1950 to 625 925 tons in 2014 (FAO fact sheet; www.fao.org/fishery/species/3514/en).

6.2 Gross Anatomy

6.2.1 Larval Morphology

Bivalve larvae are amazingly similar from fertilization until metamorphosis which makes learning larval anatomy easy, but identification of larvae from different species difficult when samples are collected from the wild environment! Recently developed molecular identification methods are helping in that effort (Lutz et al. 2018). The stages of development prior to metamorphosis are (in order of development): fertilized egg, trophophore, Prodissiconch I (D-shaped larvae) (Figure 6.1) and Prodissiconch II. The Prodissiconch II stage is subdivided into early and late stages named sequentially umbo (Figure 6.2) and pediveliger stages (Figures 6.3 and 6.4). Once a larva becomes a fully developed pediveliger, it searches for an appropriate location to attach using small protein threads (byssal threads) extruded by a gland in the foot. In the Prodissoconch I and II stages (Carriker 2001; Elston 1980; Galtsoff 1964; Morse

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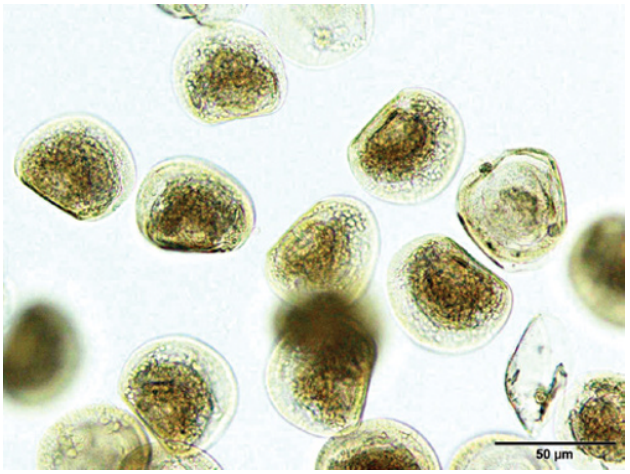


Figure 6.1 D-shaped eastern oyster (*Crassostrea virginica*) larvae.

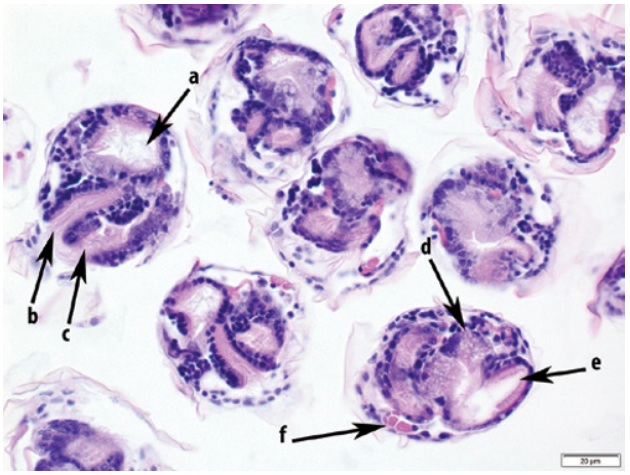


Figure 6.2 Early Prodissoconch II (umbo) stage of the eastern oyster. a, stomach lumen; b, esophagus; c, retracted velum; d, digestive gland cells; e, style sac; f, anterior adductor muscle.

and Zardus 1997; Tompson et al. 1996), the velum (consisting of two circular lobes, one inside the other, that are covered with rows of cilia and are adjacent to the mouth) is used both for movement in the water column and to filter feed. Both anterior and posterior adductor muscles, as well as velar retractor muscles, can be identified in pediveligers. The velum and soft tissues can be retracted into the thin translucent shell by the retractor muscles when startled. The esophagus is located adjacent to the posterior portion of the velum, is also heavily ciliated, and funnels food to the stomach.

In the Prodissoconch II stage, histologically, just ventral to the velum is a focus of distinct but undifferentiated cells referred to as a cerebral ganglion. The digestive gland, and its finely vacuolated absorptive cells, is prominent in the more developed larvae and is located around the stomach

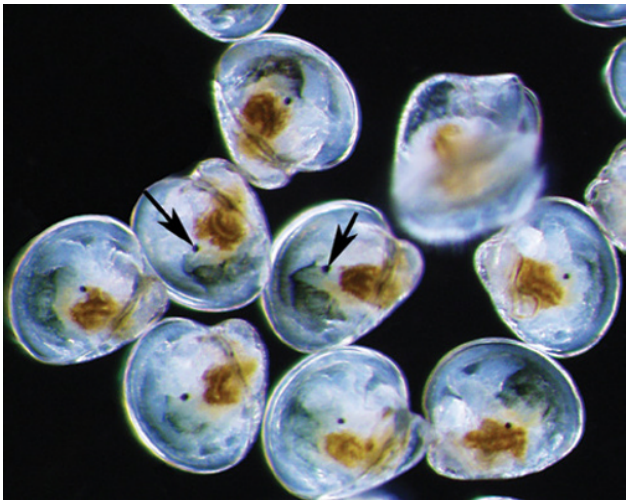


Figure 6.3 Pediveligers of hard clams (*Mercenaria mercenaria*). Arrows indicate eyes.

and esophagus. In Prodissoconch II larvae, the style sac and intestine can also be located extending from the stomach lumen. In the pediveliger stage of development, a rudimentary foot containing a byssal gland and rudimentary gills can be identified projecting into the pallial (mantle) cavity. The gills develop from the mantle tissue lining the inner surface of the valves (see descriptions of the mantle and mantle cavity in adult anatomy). At the pediveliger stage, paired statocysts appear with the body mass. These organs are formed by invagination of the ectoderm and are connected by a fine tube that empties into the mantle cavity surrounding the body. The statocysts are composed of two cell types: a nonciliated cell, which produces the thin translucent statoliths, and a strongly ciliated cell type with cilia that extend into the lumen of the statocyst. Processes from the ciliated cells form a nerve that connects to the pedal ganglia (Cragg and Nott 1977). Research demonstrates that statocysts are gravity receptors. Statocysts are retained in adult animals after metamorphosis in some species.

In late-stage Prodissoconch II (pediveliger), a pair of pigmented eyespots develop on the budding gill tissue (see Figure 6.3). The eyespots consist of a cell with pigmented granules that can be seen through the thin, translucent valves. It is thought the eyes provide directional photoreception (Cragg 2016). Aquaculturists often use the term "eyed up" to indicate the age of the larvae.

After attachment, the larvae undergo metamorphosis (also termed *setting*). At metamorphosis, depending on the species, some larval organs are destroyed and others are formed. Clams, cockles, and mussels undergo the least amount of change. At metamorphosis, clams lose the velum, and slightly rearrange the organs so that the mouth is anterior and the rectum posterior with gills suspended

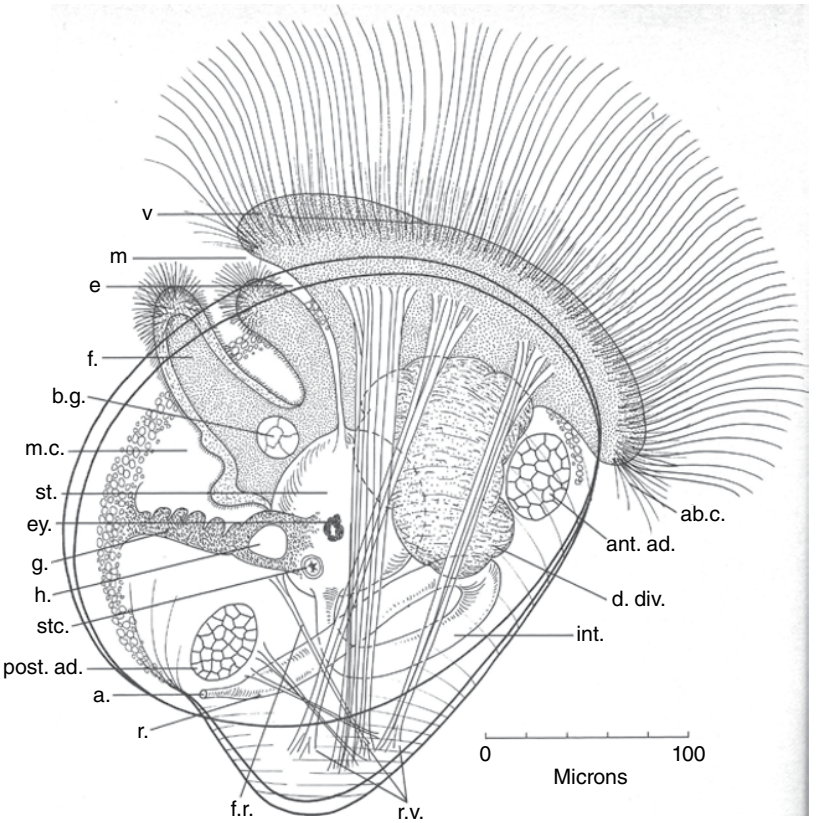


Figure 6.4 Diagram of a pediveliger of the European flat oyster (*Ostrea edulis*). a, anus; abc, aboral belt of cilia; ant.ad., anterior adductor muscle; a.p.o., apical sense organ and ganglion; b.g., byssus gland; cr.s, crystalline style sac; d. div, digestive diverticula; e., esophagus; ey., eye; f., foot; f.r., foot retractor muscles; g, gill rudiment; g.sh., gastric shield; h.r., heart and kidney rudiment; int., intestine; m., mouth; m.c., mantle cavity; p.g., pedal ganglion; post.ad, posterior adductor muscle; r., rectum; r.v., velar retractor muscles; st., stomach; stc., statocyst; v., velum; v.g., visceral ganglion. *Source:* Galtsoff (1964).

from the dorsal portions of the soft body in an anterior to posterior direction. Oysters undergo the most dramatic rearrangement of tissues and organs. They lose not only the velum but also the foot and the anterior adductor muscle. The mouth moves dorsally to a location just underneath the ligament, resulting in a J shape to the gills. Scallops also dramatically change. They lose the anterior adductor muscle and reorient organs to form a distinct kidney, gonad, and a C-shaped gill.

6.2.2 Adult Bivalve Gross Morphology

6.2.2.1 General Body Plan Post Metamorphosis

Post metamorphosis (even though often no larger than 450 μm in diameter!), bivalves take on the morphologic appearance identified for each subclass of bivalve as described above. In this work, we will discuss the anatomy of the clam (which changes the least at metamorphosis) (Eble 2001) and then describe important anatomic differences in oysters, scallops, mussels, and cockles (Benninger and Le Pennec 2016; Eble 2001; Eble and Scro 1996; Morton 2008).

Anterior, posterior, dorsal, and ventral directions can be identified in clams (Figure 6.5) (Gosling 2003; Elston 1980). Dorsal, embryologically, is the area where the valves

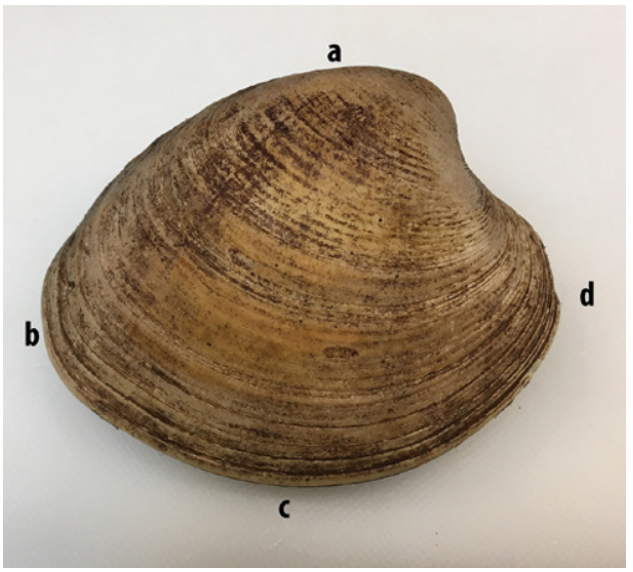


Figure 6.5 A hard clam with labeled directions. a, dorsal; b, posterior; c, ventral; d, anterior.

connect via the shell ligament and interlocking teeth (Figure 6.6). The connected valves together are termed a *shell*. The ligament is primarily composed of a firm to hard, black/brown laminated "resilium" that is secreted by the

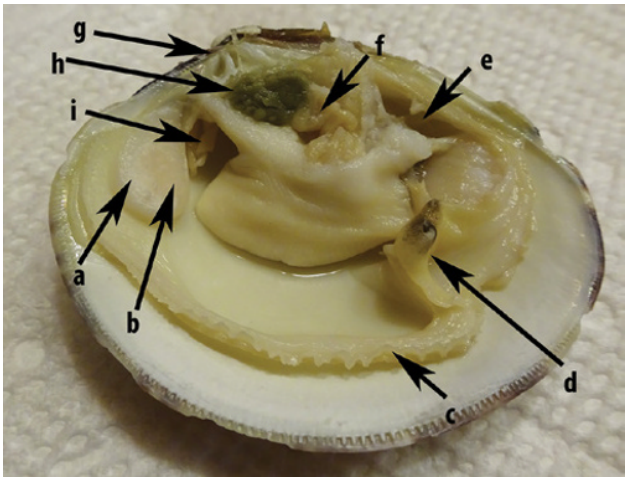


Figure 6.6 Fixed, shucked hard clam (left demibranchs and a portion of the visceral mass have been removed). Adductor muscle consisting of a, catch muscle and b, quick muscle. Other organs are: c, mantle edge; d, siphon; e, pericardial sac; f, gonadal and intestinal loop in visceral mass; g, teeth; h, digestive gland; i, palps.

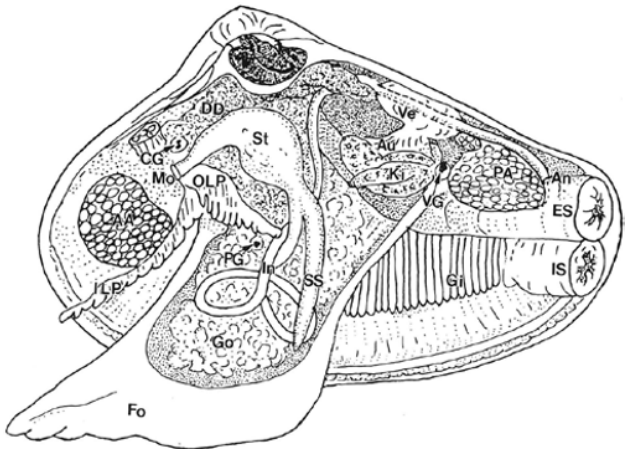


Figure 6.7 Diagram of an Atlantic surf clam (*Spicula solidissima*) with left valve and gill removed. AA, anterior adductor muscle; An, anus; Au, auricle; CG, cerebral ganglion; ED, excurrent siphon; Fo, foot; Gi, gill; Go, gonad; Ki, kidney; ILP, inner labial palp; In, intestine; IS, incurrent siphon; Mo, mouth; OLP, outer labial palp; Pa, posterior adductor muscle; PG, pedal ganglion; SS, style sac; St, stomach; Ve, ventricle; VG, visceral ganglion. *Source:* Morse and Zardus (1997). Reproduced with permission from John Wiley & Sons.

mantle and composed of elastic organic material. The resilium holds the two valves together but also acts to open the shell valves when adductor muscles are relaxed (Carriker 1996; Galtsoff 1964).

Bivalves lack a head but the mouth area is considered anterior. The rectum empties into the posterior area (Figure 6.7). In most adult bivalves, when examining the closed animals (valves shut tight), some portion of the outer surface of the shell hinge or adjacent phalanges extends in an anterior direction (see Figure 6.5).

Clams have both an anterior and posterior adductor muscle. While the interlocking teeth and hinge ligament hold the two valves of the shell together dorsally, it is the adductor muscles extending from the left valve to the right valve that close the shell (a fatigable action!). Extending from the dorsum of the shell on the inner surface of both valves is the mantle. This easily disregarded organ is responsible for production of the entire shell and the ligament. The mantle is divided into two portions: the thin membranous mantle (pallial mantle) that lines the inner surface of the valves and the mantle edge (mantle border), a thickening of the mantle that occurs along the outer circumference adjacent to the edges of the valves. The mantle is lightly connected to the inner surface of the shell from the dorsal ligament to the crescent-shaped pallial line which can be found from 1 mm to 3 cm (depending on species and size of animal) from the valve edge on the inner surface of the valve. It parallels the curvature of the valves except in the location of the siphon. From the pallial line to the edge of the shell, the mantle is detached from the inner surface of the valve and can either be extended over the edge of the valve or retracted into the closed shell. In some clam species, the mantle edge is fused along much of the circumference, leaving only a small opening for the foot. In these animals, the mantle cannot be fully retracted into the shell. Posteriorly, the mantle is fused in all clams and is modified to form a siphon, which may or may not be fully retractable, depending on the species.

The visceral mass contains most of the organs, including the foot, and extends from the dorsum into the mantle cavity (pallial cavity) of the shell. Laterally, on both sides of the visceral mass and attached at the dorsum between the mantle and the visceral mass, are two demibranches of longitudinally oriented gills. The siphon, a specialized area of the posterior mantle, is divided into two channels. The incurrent siphon (ventral to the excurrent siphon) is used to draw water into the mantle cavity, and the excurrent siphon eliminates water from the mantle cavity. Water (fresh or salt) enters the mantle cavity, flows over the gills and through small ostia (holes) in the surface of the gill. The water flows up the water tubules inside each of the lamellae of the gills (demibranchs) toward the dorsum of the clam where vertical water tubules empty into large, horizontally oriented, dorsal water tubules. The water then flows through the dorsal tubule posteriorly to the excurrent siphon where it is eliminated from an animal's mantle/gill cavities (Figure 6.8) (Pearse et al. 1987). While it appears that water is flowing through the gill and body tissues, it is really flowing through tubules surrounded by gill and body tissues. It does not enter the body tissues (structure of the gills is described under histology). Almost all bivalves are filter feeders and directed water flow provides a way to

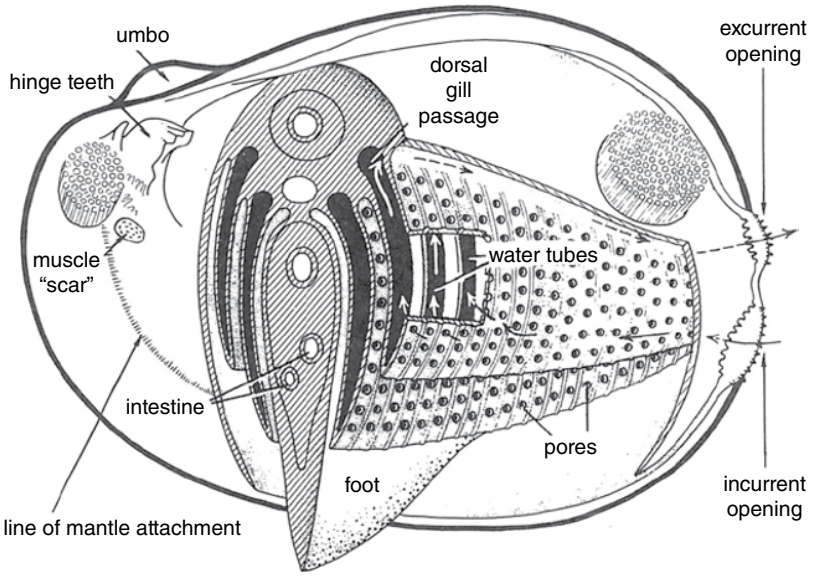


Figure 6.8 Diagram illustrating the direction of water flow from the mantle cavity through the gill to the dorsal gill passage. The anterior of the clam has been removed. The ostia (pores) of the gills are microscopic in live tissue and are enlarged here for illustrative purposes. *Source:* Pearse et al. (1987). Reproduced with permission from John Wiley & Sons.



Figure 6.9 Intact eastern oyster. a, anterior (ligament); b, dorsal.

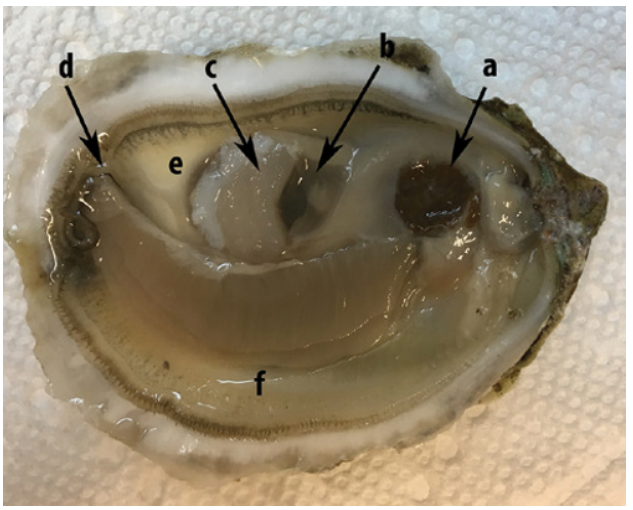


Figure 6.10 Shucked eastern oyster. a, digestive gland; b, pericardial sac with heart; c, translucent portion of adductor muscle; d, area of posterior mantle fusion forming the epibranchial (dorsal) chamber (e) and hypobranchial chamber (ventral) (f).

expose the surface of the gills (where particulate matter is gathered) to a continuous source of particulate (food, etc.)-containing water.

The surface of the visceral mass is smooth to wrinkled and the visceral mass itself, depending on the reproductive stage (or disease), will be firm and thick to thin and edematous. Within the visceral mass are found the gastrointestinal tract, the reproductive tract, excretory organs, most of the cardiovascular system, the neural system, and abundant variously oriented muscles.

Post metamorphosis (as noted above), the oyster's orientation is much different from that of other bivalves (Figure 6.9). The shell ligament becomes anterior and the ventral surface of the shell becomes posterior. Dorsal is now what would have been anterior in the larvae and only a posterior adductor muscle remains. Along the length of the oyster, both sides of the mantle fuse with the dorsum of the gills. The fusion extends past the posterior adductor muscle area to the mantle edge and is strongly adhered to the inner surface of the valves (Figure 6.10). Thus the mantle cavity is divided into two major sections, the hypobranchial chamber containing the gills and the epibranchial chamber (dorsal chamber) containing most of the visceral mass. Oysters do not have a foot. Instead, the inner surfaces of the gill lamellae (demibranchs) are directly adjacent to each other. Oysters also do not have siphons. In oysters, water enters the mantle cavity, flows into the ostia and travels dorsally up the tubules in the gill. Instead of emptying into dorsal water tubules, the water flows

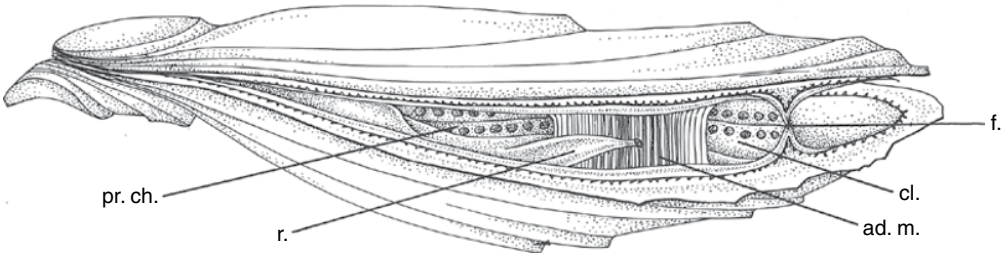


Figure 6.11 Diagram of an oyster showing the opening of the gill's water tubules into the epibranchial chamber (composed of the promyal and cloacal chambers). ad.m, adductor muscle; cl, cloaca; f, fusion of the mantle lobes and gills; pr.ch., promyal chamber; r, rectum. *Source:* Galtsoff (1964).

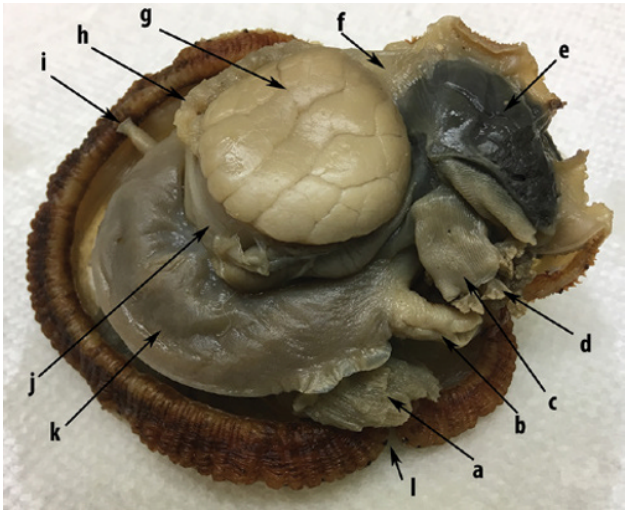


Figure 6.12 Shucked Atlantic sea scallop (*Placopecten magellanicus*), fixed in formalin. a, portion of the left gill (right gill removed); b, foot; c, palp; d, lips; e, digestive gland; f, pericardial sac; g, quick portion of the adductor muscle; h, catch portion of the adductor muscle; i, rectum; j, kidney; k, gonad; l, scallop eye.

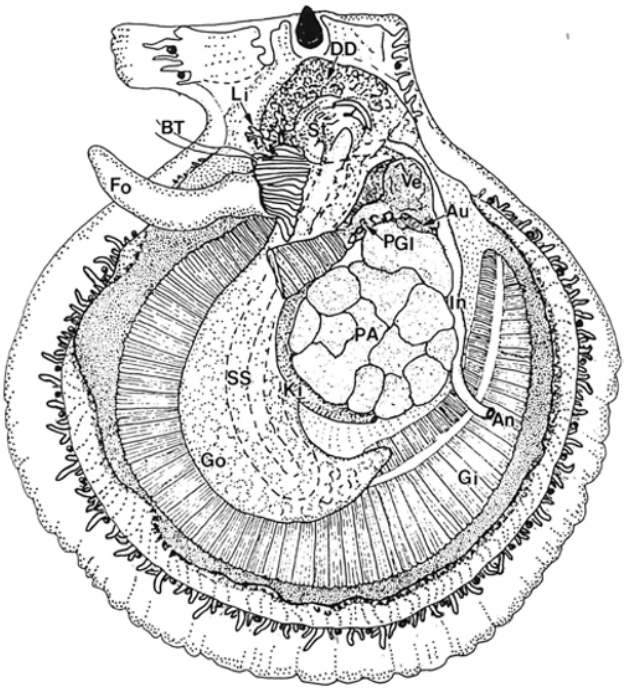


Figure 6.13 Diagram of a pink scallop (*Chlamys hastata*) with left valve and gill removed. An, anus; Au, auricle; BT, byssal threads; DD, digestive gland; Fo, foot; Gi, gill; Go, gonad; In, intestine; Ki, kidney; Li, peribuccal lips; PA, posterior adductor muscle; PGl, pericardial glands; SS, style sac; St, stomach; Ve, ventricle. *Source:* Morse and Zardus (1997). Reproduced with permission from John Wiley & Sons.

through a sieve-like structure located at the fusion of the mantles and gills and into the epibranchial chamber, where it is flushed out of the cavity by action of the adductor muscle opening and closing the valves (Figure 6.11).

Post metamorphosis, only the large posterior adductor muscle remains in scallops (Figures 6.12 and 6.13). It is in a central location in the midportion of the shell. The foot also remains but is very small and is located just ventral to the mouth and palps. The visceral mass is expanded and most of the mass is located anterior to the adductor muscle. There are no siphons, but ciliary action of the cells lining the mantle cavity creates directed flow from the anterior "wings" adjacent to the ligament of the dorsal scallop shell to the posterior wings of the shell.

Mussels are very similar to clams, but they are the most dorsal-ventrally compressed and anterior-posteriorly elongated of all the bivalves. Their mantles are sealed along most of the length of the valves. Small siphons extend from the posterior portion of the sealed mantle. About one-third of the way from the anterior end, the mantles are not sealed so that the foot can be extended through the gap to provide movement and access for byssal thread attachment (see section 6.3.9.2 for further detail on byssal thread).

6.2.2.2 Gastrointestinal System

In all bivalves, the gills function as a food collection organ as well as for respiration. Food is collected by ciliated cells on the surface of the gills. On the gill surface, the particulate matter is mixed with mucus and transported by directed ciliary motion to the ventral food groove of the

gills. Mucus balls of food are moved along the food groove/canal to the palp/mouth complex.

Bilaterally from either side of the mouth and extending into the mantle cavity are the palps (the mouth is small in size and usually cannot be seen grossly). The palps (thought to be highly sensory) break up the food-containing mucus balls, sort through the material and select appropriate food particles that are then transferred to the mouth. Nonfood particles are moved to the inner surface of the membranous mantle (usually adjacent to the incurrent siphon or a similar position in animals without siphons). Periodically, these nonfood particles, which can be seen grossly and are termed *pseudofeces*, are "burped" out through the incurrent siphon or are flushed out of the shell. In clams, palps are long oar-like structures that extend into the mantle cavity. The outer set of palps attach to the body above the mouth and the inner set attach below the mouth. The structure of the palps varies greatly between different types of bivalves. Oysters show a similar orientation of the palps, but they are much shorter and form small triangular flaps around the mouth. In scallops, the palps connect to the dorsally and ventrally oriented cauliflower-like lips. Scallop lips are especially unusual and are discussed further in the histology section.

Food particles enter the mouth, and move through the esophagus and into the stomach. The stomach is a thin-walled, irregular structure divided into two major compartments. Major ducts lead from the anterior compartment of the stomach into the digestive gland (where most digestion and absorption occur). Projecting into the posterior portion of the stomach is the style, a crystalline structure made of mucin-type glycoproteins that are continually produced by the epithelium of the blind-ended style sac. The jelly-like style can be grossly dissected out from the surrounding tissue. The projecting end of the style rotates against a gastric shield composed of chitin (a firm plaque on the dorsal surface of the stomach) that is located on the opposite side of the stomach lumen from the style sac. The two structures produce a mortar and pestle-like action that breaks down food particles.

The digestive gland, usually colored brown/black, surrounds the stomach on its ventral and lateral sides. Exiting from the posterior stomach is the descending intestine, which winds ventrally through the visceral mass then ascends to become the rectum. The rectum extends dorsally and posteriorly, enters into the pericardial coelom and, in clams, continues through the center of the heart's ventricle to become the rectum. The rectum leaves the heart and continues in a dorsal positon adjacent to the posterior aorta (and the aortic bulb, to be discussed later) where it exits the pericardial coelom and extends to a location dorsal to the posterior adductor muscle. The anus deposits feces in the excurrent siphon lumen.

In oysters, the rectum is attached to and/or is bilaterally surrounded by the dorsal side of the ventricle and the rectum deposits feces in the cloacal chamber (a posterior portion of the epibranchial chamber) on the dorsal side of the mantle fusion (as discussed previously). In scallops, the rectum curves from dorsal to ventral around the adductor muscle and empties into the posterior ventral mantle cavity.

6.2.2.3 Circulatory System

Bivalves have an open circulatory system. Bivalve "blood" is termed *hemolymph*. In bivalves, the heart consists of large right and left atria with poorly organized, thin muscle bundles that receive hemolymph from thin-walled veins. Atria empty into a single ventricle composed of thick trabeculated muscle bundles. An anterior aorta leaves the ventricle, divides and supplies the gill and anterior organs. A posterior aorta leaves the ventricle and supplies the posterior adductor muscle and siphon. Before leaving the pericardial coelom, the posterior aorta forms a muscular aortic bulb. The bulb is spongy and can contain large amounts of hemolymph. The bulb is thought to allow space for the "back-flushing" of hemolymph that occurs when the siphons contract in clams. The bulb is often not present in bivalves without siphons. The aortas and larger arteries distribute hemolymph to the body. Arterioles empty into sinusoids which surround and permeate the tissues. Hemolymph flows through the sinusoids and is carried back to the atria through large and poorly delineated veins.

6.2.2.4 Excretory System

The bivalve excretory system is divided into two parts and is unusual in that the pericardial sac and the atria are part of the system. Pericardial glands, which extend from the pericardial wall or are an extension of the atrial surface (often termed a green gland when see grossly), can be located between the pericardial sac and the dorsal ligament. In clams, the second grossly visual portion of the kidney, a thin brown tissue, underlies the pericardial sac. In oysters, it is not possible to see the second part of the kidney grossly. C-shaped, brown kidney tissues in scallops are prominently located between the anterior surface of the adductor muscle and the gonad tissues.

6.2.2.5 Reproductive System

Bivalves can be hermaphroditic or dioecious. The gonad is a branching tubular gland. It surrounds the digestive gland and extends into the foot (in clams and cockles). In scallops, the gonad is a distinct, slightly curved organ that lies anterior to the adductor muscle (and the intervening kidney). In mussels, abundant branching gonadal tubules extend into the membranous mantle, resulting in thickened mantles in ripe animals (bivalves containing gonadal

tubules full of mature eggs and sperm). Eggs and sperm are released into gonadal ducts that lead to bilateral gonadopores. Gonadopores are either separate from but adjacent to the renopore, or merge with the renopore to form a common opening, depending on the species. Among the different bivalve species, mature female gonads tend to be yellow-orange and mature male gonads tend to be white. Reproduction is often annual in animals primarily found in the northern climates, but in animals in warm climates with lesser changes in seasonal temperatures, reproduction can occur more than once per year. Spawning is usually induced by increased temperature or by spawning of adjacent animals of the same species. Fertilization occurs in the water column (termed *broadcast spawning*) in most bivalves.

In oysters, such as *Carpobrotus edulis*, that incubate eggs in the mantle cavity, once eggs are spawned into the epibranchial cavity, they migrate (are sucked) back into the water tubules (a direction opposite to the water flow through the gills) and exit into the hypobranchial chamber via the gill ostia. Fertilization occurs as sperm enters the hypobranchial chamber with incoming water and fertilized eggs are retained within the hypobranchial chamber until the trophophore stage. Rarely, other species of bivalve incubate fertilized eggs in unusual ways within the mantle cavity and gills (Foighil and Taylor 2000).

6.2.2.6 Hydromuscular System and Connective Tissues

The adductor muscle is the most obvious muscle of bivalves. Each adductor muscle is made up of two muscle types. A smaller, often crescent-shaped portion of the adductor muscle (the catch muscle) is responsible for slow movement and feeding. The larger portion of the muscle (sometimes taking on a pink color in clams), termed the quick or phasic muscle, is responsible for fast contraction. Two pairs of retractor muscles are prominent in clams (and cockles and mussels). They extend from the ventral foot to the anterior and posterior sides of the dorsal ligaments. These muscles can quickly withdraw the foot into the shell. Abundant muscles oriented in multiple directions can be found throughout the body of the animal and especially in the foot. Sinusoids extend throughout the body between the connective tissue cells and between and around muscles of the body. Contraction of muscles moves the hemolymph to other portions of the body, resulting in dilation of that portion and reduction of size of the body area in which the muscle contracted. This hydromuscular movement is especially noted in the siphon and foot where it can cause enormous expansion and collapse of the siphon and foot and allows for movement of those organs in many directions.

6.2.2.7 Neural and Sensory Systems

6.2.2.7.1 Nervous System Bivalves do not have a "brain." In clams, mussels, and cockles, there are three pairs of ganglia. The "cerebral" ganglia are noted just posterior and slightly dorsal to the anterior adductor muscle. The pedal ganglia are in the dorsal portion of the foot. The visceral ganglia are associated with the anterior side of the posterior adductor muscle. Neural commissures connect each pair of ganglia and fine nerves extend from the ganglia to the tissues of the body as well as to other ganglia. Ganglia and larger nerves often have a light yellow-tan to pink-orange color depending on the species of bivalve and, in some species, can be seen grossly.

Oysters lack the pedal ganglia. Their visceral ganglia, located on the anteroventral side of the adductor muscle, are fused. In scallops, the cerebral and pedal ganglia are fused and are located close to the esophagus. The scallop's visceral ganglia are fused and enlarged to form lobes. These fused, lobulated ganglia are located adjacent to the renopore of the right kidney. In scallops and oysters, the visceral ganglia innervate most of the animals' tissues.

6.2.2.7.2 Eyes The eyes of bivalves are of two basic types. First are the "cerebral eyes" noted on the gills of larval veligers. These disappear in most postmetamophic bivalves but do remain in the adult blue mussel, *M. edulis*, where they can be identified as small red spots on the anterior end of the inner gill (Rosen et al. 1978). Pallial eyes are the most common type of eye and are usually found on the edges or tentacles of the middle or outer lobe of the mantle edge or on siphon tentacles. Eyes in some species, such as the scallop, can be seen grossly, but in most cases pallial and tentacular eyes are noted only microscopically.

6.3 Histology (Table 6.1)

6.3.1 Integument

The epidermis of bivalves is composed of simple cuboidal to sometimes columnar, ciliated cells (Figure 6.14). The continuous epithelium surrounds and contains the dispersed internal organs. Interspersed in the epithelium are mucus and sensory cells. A basal lamina separates the epithelial cells from the underlying connective tissues.

6.3.2 Mantle

The mantle is divided into two zones. The central zone (also termed the pallial or membranous zone) is composed of two layers of epithelium separated by connective tissues (see Figure 6.14). The outer epithelial layer (shell epithelium) of the mantle produces the valves and consists primarily of

Table 6.1 Organs for histologic evaluation in Bivalvia.[a]

Organ system		Organs
Body wall/musculoskeletal		Mantle, pallial line, mantle edge, periostracum, adductor muscle, hinge ligament, teeth, umbo, foot, siphon, velum[b]
Digestive	Alimentary canal	Gills, palps, esophagus, stomach, style, gastric shield, intestine, rectum, anus
	Digestive organs	Digestive gland
Excretory		Heart-kidney complex
Circulatory		Heart, artery, vein, open vascular system
Immune		Hemocytes
Respiratory		Gills
Nervous		Three paired ganglia (paired cerebral ganglia, pedal ganglia, and visceral ganglia), circumpallial nerve
Reproductive	Male	Testes, gonoduct
	Female	Ovaries, gonoduct
Special senses/organs		Pallial tentacles, pallial eyes (*ocelli*), statocysts, cerebral eyes (*cephalic eyes*)

[a] Alternative names for organs are provided parenthetically, in italics.
[b] Organ present in larval stage only.

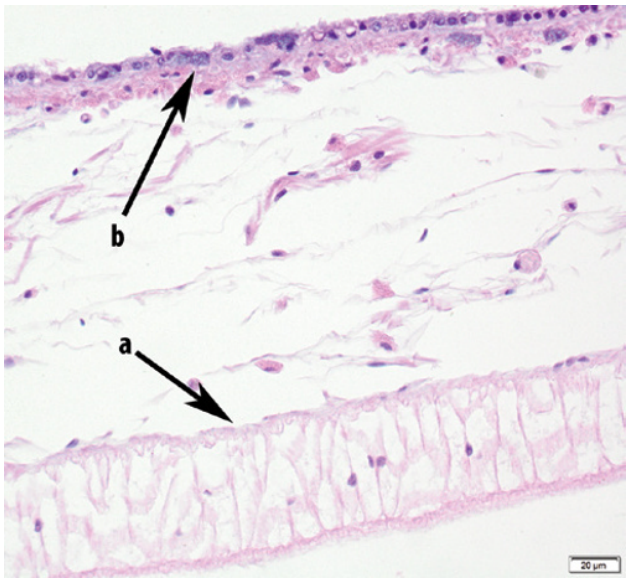


Figure 6.14 Pallial mantle (membranous mantle) of the central zone. a, shell epithelium; b, mantle cavity epithelium.

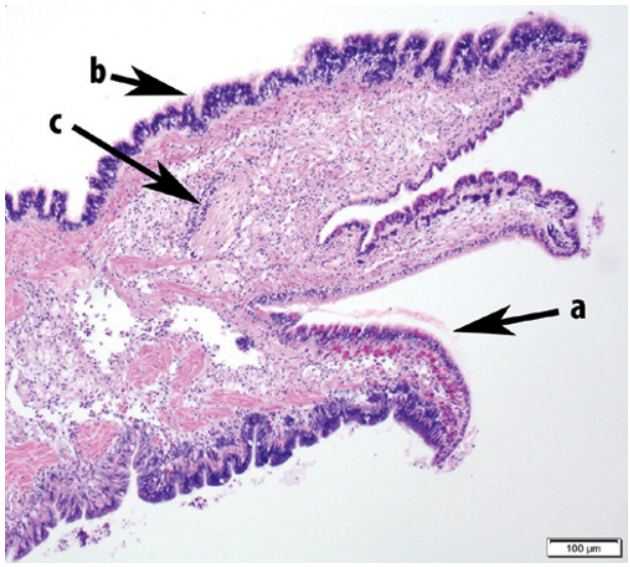


Figure 6.15 Mantle edge of an eastern oyster showing three lobes. a, periostracum between the outer (1st) and middle (2nd) lobe; b, 3rd (inner) lobe; c, circumpallial nerve.

simple, cuboidal to columnar cells with abundant, long, closely packed cilia. The ciliated inner cuboidal epithelium that lines the surface of the mantle facing the mantle cavity contains many mucus-producing goblet cells with numerous round inclusions in their cytoplasm. In the central zone, the outer (shell) epithelium of the mantle is attached to the inner surface of the shell (although the attachment can be easily broken without destroying the epithelial cells or damaging the cilia). The pallial line circumferentially demarcates the central zone of the mantle that is attached to the valve from the mantle edge that is not attached to the valve.

The mantle tissue beyond the pallial "line" is termed the mantle border (mantle edge) and is usually substantially thicker than the central zone of the mantle. In most bivalves, the mantle border consists of three projecting lobes – outer (1), middle (2), and inner (3) (Figure 6.15). At the base of the

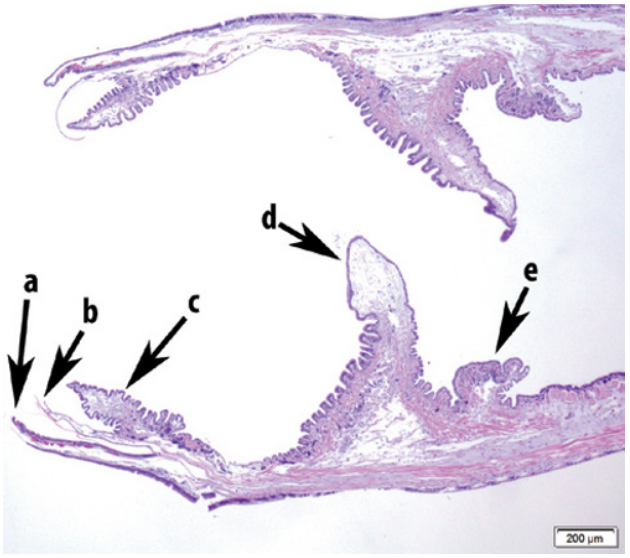


Figure 6.16 Mantle edges of a hard clam. a, lobe 1 (outer lobe); b, periostracal fold; c, lobe 2 (middle lobe); d, lobe 3; e, lobe 4.



Figure 6.17 Mantle edge of a hard clam. a, periostracal fold; b, periostracum.

periostracal groove, a recess between the outer and middle lobes (lobes 1 and 2), is the periostracal gland, which secretes periostracum. This thin, often flaky, brownish material, noted grossly on the outer surface of the shell and at the edge of the mantle, provides a template on the inner surface of which the rest of the shell is built. The periostracum is often rubbed off the outer shell surface in older areas of the shell. In clams (Figures 6.16 and 6.17) (which demonstrate an anatomic evolutionary exception from other bivalves in this instance), extending from the base of the middle (second) lobe is a thin fold of tissues termed the periostracal fold (which is still considered part of the larger middle/second lobe), that projects toward the first lobe. This fold, and not the bulk of the secondary lobe as in other bivalves, forms the inner surface of the periostracal groove. Also, in clams, a fourth lobe (often divided into sublobes) can be identified medially and dorsally to the third (outer) lobe (Eble 2001).

The marginal zone is the specialized area of the shell epithelium of the mantle of the outer lobe (lobe 1) that extends past the pallial line attachment. In the distal portion of the marginal zone, a secretion chamber is formed between shell epithelium lining the outer lobe and the periostracum that connects the edge of the outer mantle lobe to the periostracum on the exterior surface of the growing shell. Protein is secreted by the simple columnar, glandular epithelial cells in this area. The protein matrix is calcified within the chamber and forms the prismatic layer of the shell on the inner surface of the periostracum. As the circumferential edge of the animal grows, through the deposition of the periostracum and prismatic layers, shell epithelium of the central zone comes in contact with the inner surface of the prismatic layer and lays down nacre (also known as mother-of-pearl, which is the inner, iridescent layer of shells), increasing the depth of the shell. Nacre is produced by the shell epithelium of the mantle throughout the central zone, resulting in the thickest portion of the valve being closest to the ligament.

The middle and inner lobes of the mantle edge are punctuated by numerous small tentacles. Tentacles on the inner lobes are often long and the lobes themselves are often large. The inner lobes project at a right angle to the middle lobe. The inner lobe tentacles interdigitate and, when the shell is almost, but not quite, closed, form a semi-closed shell. This semi-closed formation provides a closed cavity that allows for water flow through the siphons and over the gills. Numerous small mucous glands with ducts extending to the surface are noted in the underlying connective tissue adjacent to the columnar epithelium of the inner lobes. As noted above, in some species of bivalves (usually clams such as *Mya arenaria*, the soft shell clam), evolution has resulted in a permanent seal between the two inner lobes of the mantle edge.

In addition to forming part of the periostracal groove, the middle lobe forms sensory tentacles and in some species the middle lobes can be divided in to sublobes. Scallops are unusual in the bivalve world. The middle, sensory tentacle is subdivided into four smaller sensory lobes. At the end of one of these smaller sensory lobes are located the beautiful scallop eyes.

The connective tissues found between the two epithelial layers of the mantle are composed of sinusoids, muscles, nerves, and supporting cells. Underlying the lobes in the connective tissues of the margin zone is a prominent circumpallial nerve and a circumpallial artery. Longitudinal, thick muscles extend from the pallial line to the tips of the lobes and function to retract the mantle edge into the shell when the valves are closed tightly.

6.3.3 Digestive System

The labial palps of clams (Figure 6.18) and oysters generally show a ridged inner and smooth outer surface. On both sides of the mouth, the inner sides of the palps sandwich the anterior most parts of the gills. These inner palp surfaces form ridged tracts responsible for sorting mucus balls containing food, received from the ventral groove of the gills. The tracts are lined by tall, ciliated, simple columnar epithelium punctuated with mucous cells. The outer sides of the palps (away from the gills) are lined by low simple cuboidal epithelium underlain by a thick layer of muscle. Sinusoidal connective tissue fills the spaces between the inner and outer sides of the palps. Scallops show an interesting variation from other bivalves. Palps are present as described above, but are reduced in size and funnel particles from the left and right to a mouth surrounded by upper and lower cauliflower-like lips. Histologically, the scallop's lips consist of orally (medially) oriented ciliated columnar epithelium with mucous cells separated by sinusoidal connective tissue from the outer (aborally) oriented lip epithelium consisting of nonciliated epithelium. It has been hypothesized that the lips wrap around the food particles to prevent food loss, which might occur otherwise due to water flow in the mantle cavity, and/or that the lips may function as absorptive epithelium.

The esophagus is thrown into rugae and lined by ciliated pseudostratified epithelium containing numerous mucous cells. The stomach is divided into anterior and posterior sections. The lining of the stomach varies but in most areas consists of ciliated simple to pseudostratified columnar epithelium underlain by a dense, thick layer of collagenous fibers that merge with the surrounding sinusoidal connective tissue. Numerous brown cells (fixed phagocytes) are noted in the adjacent sinusoids. The gastric shield in the posterior portion of the stomach consists of flat laminated, chitin-like material produced by tall ciliated columnar epithelium. The style sac extends from an outpouching of the stomach and is connected to the midgut along its length by a thin communicating channel (Figure 6.19). The style sac is lined by ciliated tall columnar epithelium and produces layers of the style which can appear onion-like on cross-section. The style is often missing in histologic sections.

In clams, the midgut intestine can be divided into descending, ascending, and rectal portions. The descending intestine, which extends into the foot, forms a prominent typhlosole (a fold of the wall into the lumen of the intestine) resulting in a C-like appearance of the midgut in cross-section (Figure 6.20). The ascending portion of the midgut continues dorsally then turns and runs posteriorly

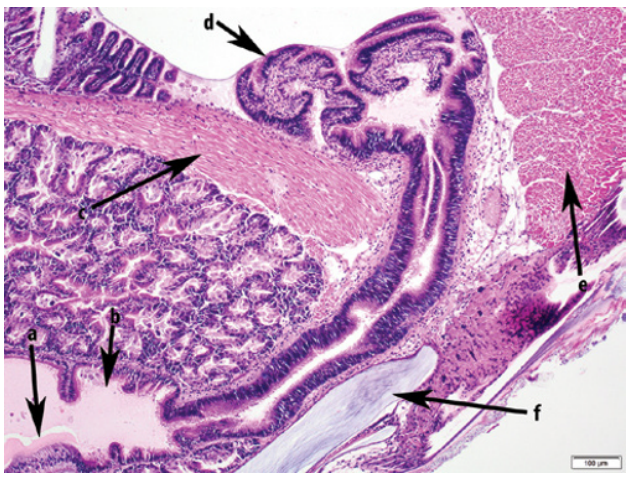


Figure 6.18 Sagittal section through a juvenile hard clam. a, gastric shield; b, stomach lumen; c, retractor muscle; d, aboral surface of a palp; e, adductor muscle; f, shell ligament (resilium).

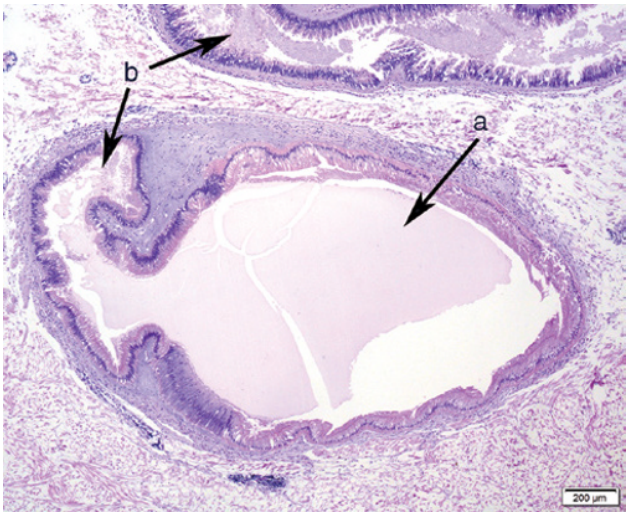


Figure 6.19 Section of a hard clam. a, style in the style sac; b, intestinal lumen.

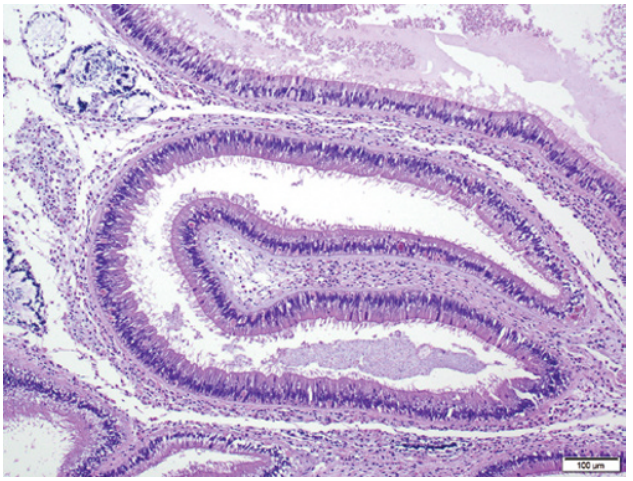


Figure 6.20 Section of a hard clam showing the typhlosole in the descending intestine.

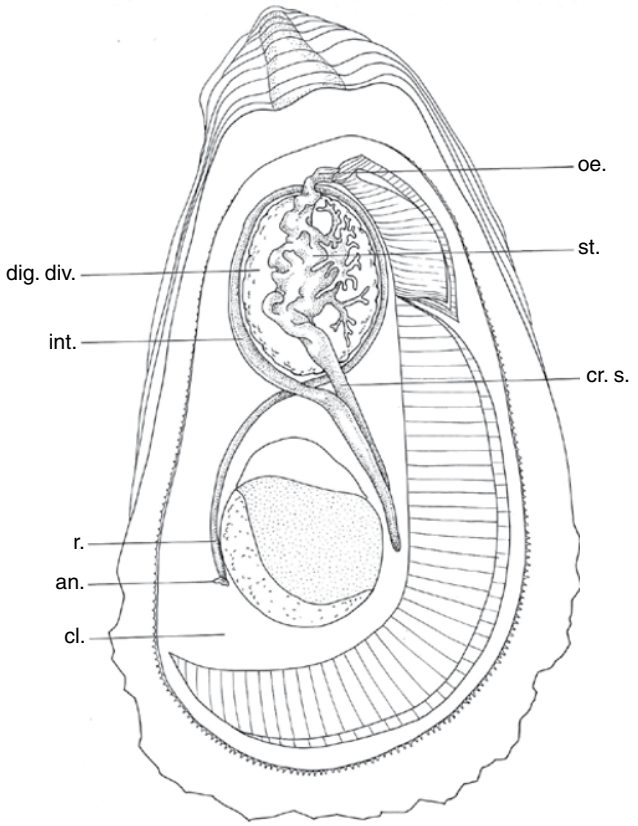


Figure 6.21 Diagram of the digestive system of an eastern oyster. an, anus; cl, cloaca; cr.s, crystalline style sac; dig. div, digestive diverticula; int., intestine (also termed midgut); oe, esophagus, r; rectum, st, stomach. *Source:* Galtsoff (1964).

through the heart to eventually form the rectum. In oysters, because of the directional changes in tissues that occur during metamorphosis, the midgut is more complicated. Using embrologically derived directions (ligament as dorsal, mouth as anterior) (Figure 6.21), the midgut is divided into a descending portion that leaves the ventral surface of the stomach and extends to the ventral/anterior portion of the adductor muscle. The midgut then makes an abrupt turn and ascends to the dorsal-posterior side of the stomach, which it then circles. From there, it descends again and courses through the pericardial sac and posterior to the adductor muscle. The rectum empties in the epibranchial chamber. Unlike in the clam, oysters contains a typhlosole along their entire length.

The intestine is lined by ciliated pseudostratified columnar epithelium and contains many mucous cells. The intestine is supported along its length by varying amounts of connective tissue but lacks any muscle. As the rectum enters the pericardial sac and becomes associated with the ventricle of the heart (Figure 6.22), the epithelium is supported by a thin, loose layer of sinusoidal connective tissue

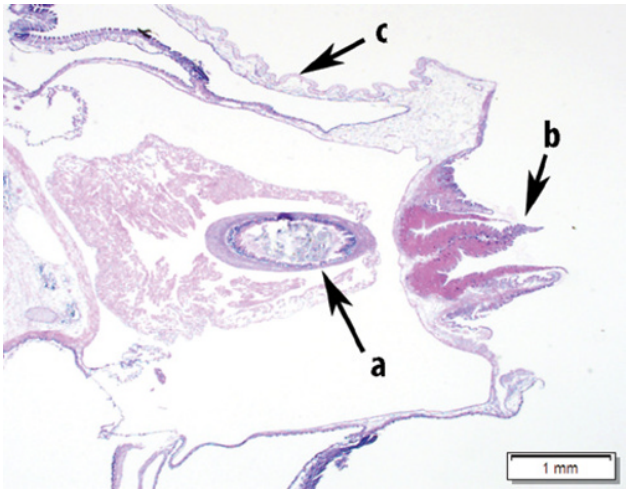


Figure 6.22 Section of a hard clam. a, rectum surrounded by the ventricle of the heart; b, ligament producing mantle epithelium; c, central zone of the membranous (pallial) mantle.

surrounded by a dense layer of collagenous tissue and circular and longitudinal layers of smooth muscle Numerous brown cells (presumed to be spent hemocytes) are noted between epithelial cells and are thought to be traversing the epithelium to exit in the feces. In clams, the descending and ascending loops of the midgut intestine extend into the foot, ventral to the digestive gland. In scallops, the loops of the midgut intestine extend ventrally into the gonadal tissue.

The stomach is connected to a large digestive gland (also termed digestive diverticula) by ducts. The digestive glands are arborizing tubular glands with primary, secondary, and tertiary ducts that lead into blind-ended tubules in which final digestion and absorption occur. Food particles that are not digested and absorbed by the tubules are moved back up the ducts into the stomach lumen. Primary ducts lead from the left and right sides of the anterior stomach to the secondary ducts and are lined by ciliated pseudostratified columnar epithelium. Secondary duct cells are divided into two types of epithelium within each duct (Figure 6.23). On one side of the tubule are type I cells characterized by long cilia projected from short simple columnar cells. Type II cells are noted on the other side of the tubule and are composed of tall, simple columnar cells with short cilia and apocrine secretion vesicles that bud into the duct. The two sides are partially separated by a typhlosole projecting into the lumen of the duct. Type II cells are responsible for moving partially digested food from the stomach into the digestive gland tubules and type I cells move undigested material out of the digestive gland back to the stomach (Langdon and Newell 1996). Connective tissues supporting the ducts thin as the ducts decrease in size and connect to the blind-ended tubules. The tubules are surrounded by the sinusoidal connective tissues. Tubules consist of two

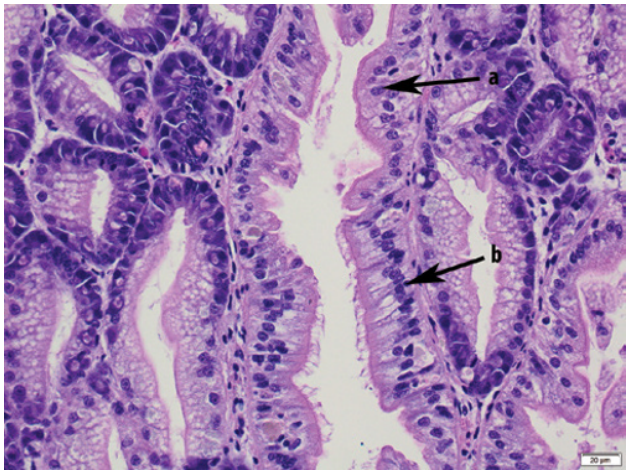


Figure 6.23 Section of a hard clam. a, type I cells of the digestive gland duct; b, type II cells of the digestive gland duct.

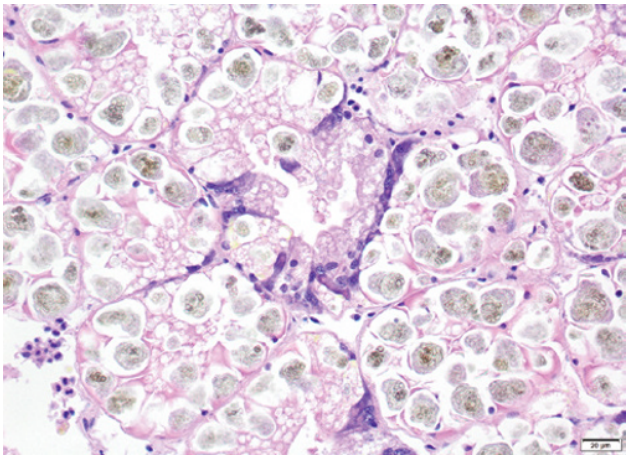


Figure 6.24 Section of the digestive gland from an Atlantic sea scallop (*Placopecten magellanicus*). Numerous residual bodies are noted in the absorptive tubular epithelium.

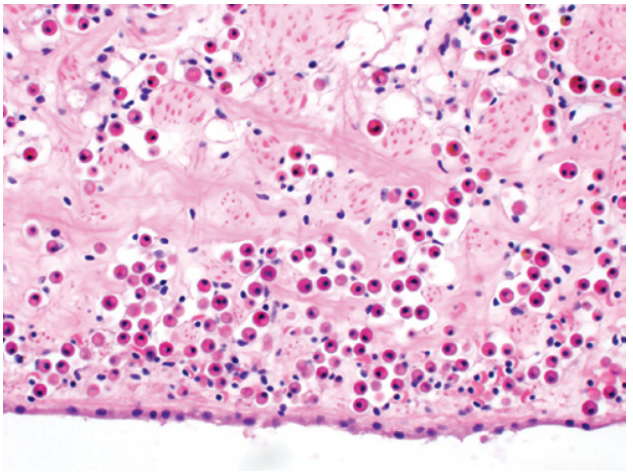


Figure 6.25 Section through the body wall of a giant clam with numerous zooxanthellae. *Source:* Photo credit: Elise LaDouceur.

cell types: the absorptive cells, which are larger and often vacuolated, and the smaller dense pyramidal regenerative cells (basophilic in HE stains). The lumina of tubules undergoing active digestion sometimes take on a four-pointed star appearance with basophilic regenerative cells occurring at the points of the luminal star. The height of the absorptive cells varies greatly with the stage of digestion, especially in animals undergoing tidal cycles.

The tubules in scallops have been described as more acinar-like. The absorptive cells in histologic sections often appear enlarged, are highly vacuolated and have been described as adipocyte-like (containing clear vacuoles). Alternately, depending on the stage of digestion, normal absorptive cells of scallops can be filled with residual bodies (small to large, brown/black inclusions) (Figure 6.24).

Giant clams (*Tridacna* spp.) develop a symbiotic relationship with zooxanthellae of the genus *Symbiodinium*. Epithelial lined tubes, extending from the stomach, branch and proliferate in the mantle, especially toward the mantle edges where sunlight is most likely to contact the mantle (Hirose et al. 2006). These tubes are filled to varying extents with zooxanthellae that provide nutrients to the clam (Figure 6.25).

6.3.4 Respiratory System

Larval bivalves contain two sheet-like gills (holobranchs). Each sheet is lined on both sides by epithelia that are attached to each other by connective tissues consisting of lacunar cells and hemolymph-filled sinusoidal spaces. During premetamorphic development, holobranchs are folded into a W shape with all three dorsal edges of the W attaching to the visceral mass. Thus in the postmetamorphic animal, the holobranch is divided into two, V-shaped structures, demibranchs, that together form the W shape (holobranch). Each V of the W is composed of one ascending and one descending section (lamella) of the holobranch. Each side of the V is connected by bridge-like tissue termed interlamellar junctions. The interlamellar junctions also contain sinusoidal connective tissue, thus connecting the vascular system of the two sides of the demibranch (Figures 6.26 and 6.27). The surfaces of the demibranch that line the inner sides of the V form the water tubules. Water tubular epithelium consists of ciliated, pseudostratified columnar cells along the interlamellar junctions and low cuboidal cells along the rest of the tubule. Mucous cells are prominent in the epithelium lining the interlamellar junctions but are also present within the rest of the epithelium lining the water tubules.

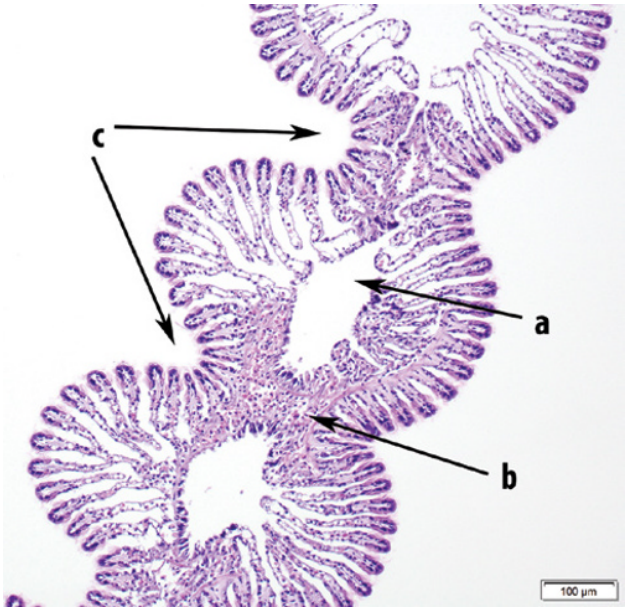


Figure 6.26 Section of a demibranch from a hard clam. a, water tubule; b, interlamellar junction; c, depressions on either side of a section of gill termed a plica.

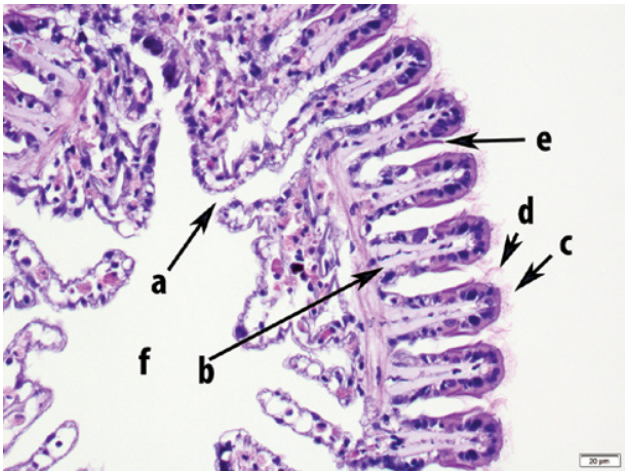


Figure 6.27 Section of a plica from a hard clam. a, ostia; b, collagenous rods supporting a filament; c, frontal ciliated cells of a filament; d, laterofrontal ciliated cells; e, paralateralofrontal ciliated cells; f, water tubule.

The outer surfaces of the demibranchs (i.e., the side exposed to the mantle cavity) develop plica which can be subdivided into both principal and ordinary filaments. Each plica projects into the mantle cavity and is delineated by deep (and shallow, depending on the species), depressions in the demibranch which help in channeling food to the ventral food groove. Ostia (holes) regularly spaced between the filaments of the plica allow water to pass through the outer surface of the demibranch from the mantle cavity into the water tubules within the V. From

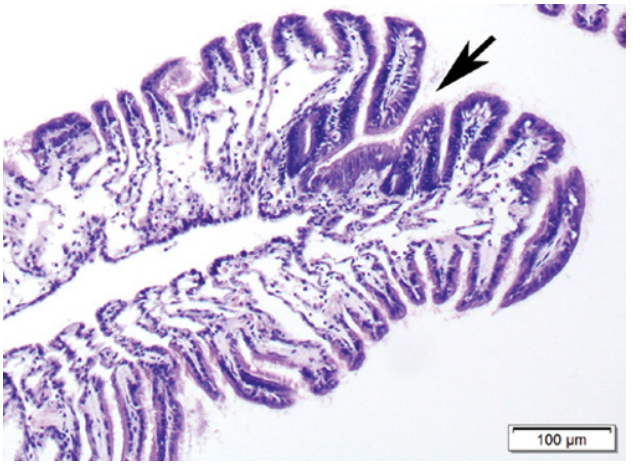


Figure 6.28 Section of a demibranch from a hard clam. Note the food groove (*arrow*) at the base of the demibranch.

there, water flows to the dorsal water tubule in clams, or to the epibranchial chamber in oysters, where water is deposited back in the mantle cavity.

Each filament of the plica contains two supportive collagen rods. At intervals, the rods elongate and are connected by muscle that can regulate the size of the ostia. Cells lining the pallial side of the filaments are specialized for collecting food particles and are named differently in different species. But most commonly, the cells are named as follows: frontal ciliated low columnar cells on the tip of the filaments, followed sequentially and bilaterally along the sides by anterior laterofrontal ciliated, then paralateralofrontal ciliated cells. Mucous cells are present between ciliated cell tufts. Food captured on the filament's cilia is mixed with mucus and moved by ciliary action to the ventral food groove (terminal groove) of each of the demibranchs (Figure 6.28).

6.3.5 Circulatory System

Much work has been done to identify the circulating cell types in clams and other bivalves (Cheng 1996; Eble 1996). However, most work still describes two basic types of hemocytes: agranulocytes (hyalinocyte) and granulocytes (Figure 6.29). These two basic types may or may not be subcategorized by different authors. The area/tissue from which hemocytes originate has never been determined, but probable progenitor cells of hemocytes have been noted in larval sinusoids. The agranulocyte is between 4 and 10 µm in diameter, small, round with clear to lightly blue to pink cytoplasm (HE or Giemsa stain). It contains a nucleus that occupies approximately half of the cell volume and, when the cell spreads on a glass slide, produces lobose pseudopodia. Granulocytes range between 10 and 30 µm in diameter

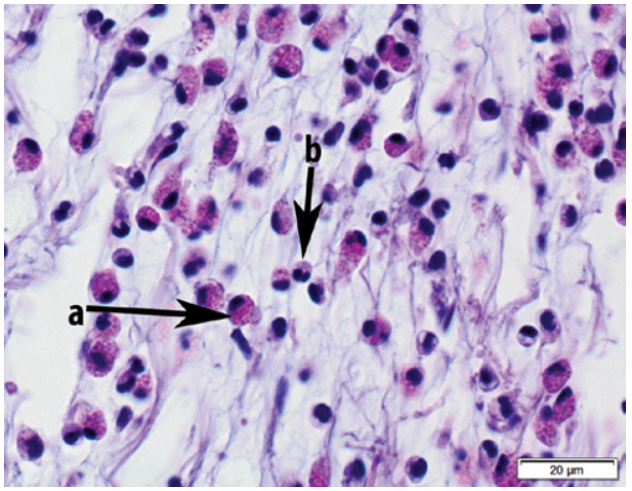


Figure 6.29 Section showing hemocytes in the connective tissues of a hard clam. a, granulocyte; b, agranulocyte.

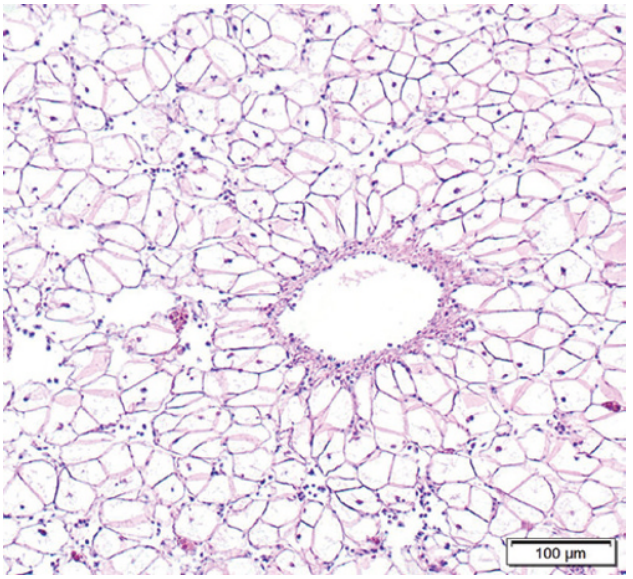


Figure 6.30 Section of an eastern oyster showing an artery surrounded by vesicular cells permeated by sinusoids.

and often contain granules in the cytoplasm that stain variable pink to blue. Granulocytes produce spiky filopodia when spread on a glass slide. Both are active in phagocytosis and both migrate to areas of infection and wounds and form aggregates or clumps, but granulocytes are considered more active than agranulocytes in these processes. Granulocytes degranulate at the site of infection or tissue disruption releasing digestive enzymes and, if unable to phagocytize the inciting cause, transform into fibrocyte-like cells that surround or "wall off" the inciting agent. These foci appear similar to mammalian granulomas. Lectin receptors have been identified on the surfaces of hemocytes and are hypothesized to be the primary method of recognizing nonself. Hemocytes phagocytize each other and in some disease situations, sequential phagocytosis can be seen histologically. Multinucleated giant cells are unusual, but have been noted in some disease conditions (Smolowitz 2018).

Hemocytes are an important part of the digestive system of bivalves. They are capable of diapedesis over epithelial surfaces and of returning to reenter the hemolymph with pinocytosed materials which are digested and then distribute nutrients to deeper tissues of the animal. Additionally, spent hemocytes containing brown, residual granules exit the body through epithelial surfaces, especially the gastric and rectal epithelium. Spent hemocytes can also be seen to cluster around vessels in the mantle, turning the mantle yellow in older clams. Hemocytes, with the exception of some unusual species (*Anadara* sp., for example), do not contain hemoglobin. Hemoglobin has been identified in the hemolymph in some bivalves, but noncell-associated hemocyanin is the most common oxygen-carrying pigment in bivalve hemolymph (Morse and Zardus 1997; Nagel 1985).

The atria of the heart are composed of scattered bundles of muscle cells held together with thin connective tissues. Atrioventricular valves separate the atria from the single ventricle. Endocardial cells do not line the inner surface of the atria and ventricle. The muscle bundles in the ventricle are thicker than those noted in the atria and the cells store glycogen, producing a bubbly appearance to the outer surface of the fibers in histologic sections. Myocardial muscles are classified as smooth by some authors and striated by others. But findings may depend on fixation methods and the occurrence of obliquely striated muscle (Morse and Zardus 1997). Low cuboidal cells form the epicardium of both the atria and the ventricle.

Large arteries in bivalves have relatively well-developed walls with layers of both circular and longitudinal muscle layers that are reduced as the vessels branch and decrease in size within the body (Figure 6.30). While the arteries are lined by simple squamous endothelium, the sinusoids and the aortic bulb (if present) are not. The large veins, into which sinusoids empty, are similar to arteries but have irregular lumens and relatively insubstantial walls.

6.3.6 Excretory System

Auricular glands (green glands) (Figure 6.31) are composed of a type of excretory cell and line portions of the exterior surface of the atria or the internal surface of the pericardial sac surrounding the atria. These cells, also sometimes termed podocytes, produce an ultrafiltrate (urine) that is deposited into the pericardial coelom. The urine exits the pericardial coelom through bilateral

renopericardial ducts into the left and right kidney which are fused to form a common lumen. The kidney is a convoluted tubular structure divided into a proximal part, considered the site of reabsorption, and a distal portion, considered an area of excretion (Figure 6.32). The kidney lies in a large hemosinus and modifies the urine in the kidney lumen/reservoir. The urine reservoir empties through two bilateral renopores (nephrostomes) between the gill and body into the mantle cavity.

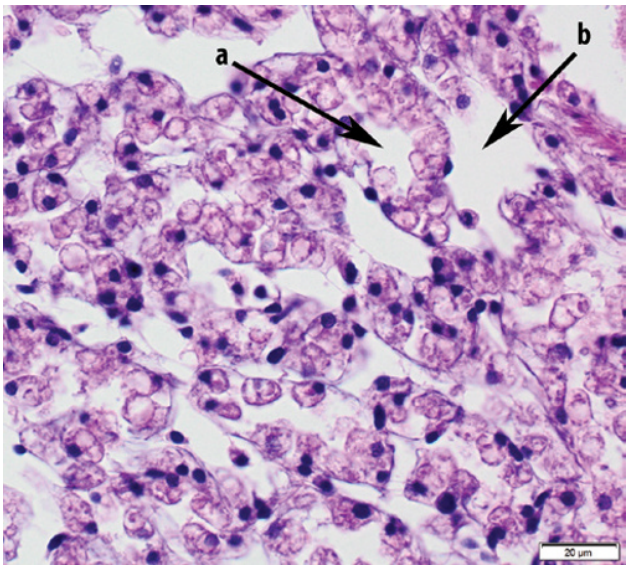


Figure 6.31 Section of a juvenile hard clam. a, lumen of the auricular (green) gland; b, sinusoid within the auricular gland.

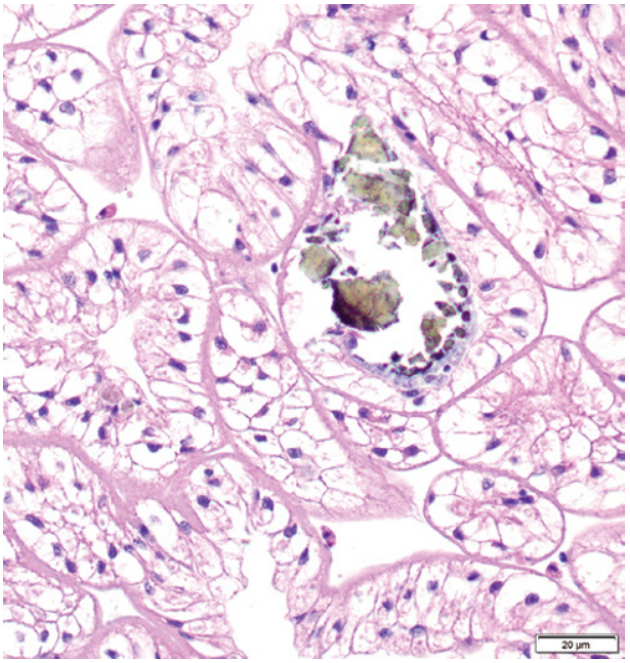


Figure 6.32 Section of a hard clam showing kidney tubules surrounded by sinusoids. Within one tubule is a crystalline concretion, a relatively normal finding in kidneys of several species.

6.3.7 Reproductive System

Cyclic production of eggs and sperm is noted in all bivalves. Several good studies have described the appearance of the gonadal tubules as the reproductive cycles occur (Barber and Blake 2016; Eversole 2001). When quiescent, both male and female gonadal tubules are composed of thin layers of connective tissue that separates the tubule from the surrounding intertubular connective tissues and sinusoids. Sparse to many flattened to cuboidal cells may be noted lining the follicular walls in the quiescent tubules. Oogonia begin to appear just before oocyte production begins (Figure 6.33). Oocytes remain attached to the follicular wall by a thin stalk until spawning occurs. Follicular cells are noted within the tubules and provide nutrients to the developing oocytes in some clams (such as *Mya* sp.). In other bivalves, nutrients are hypothesized to originate from either nutritive cells (similar to brown cells) or dissolved substances, that are present in the surrounding sinusoidal connective tissues. In males, the tubules give rise to spermatogonia which differentiate into spermatocytes and finally spermatids. In ripe female and male tubules, the lumina are filled with mature eggs and sperm. Centrally within the tubules of ripe males, abundant spermatids are oriented with the eosinophilic tails in the tubular centers. In oysters, the gonadal tubules lying just under the body wall differentiate into ducts. The outer epithelium (body wall side) of these ducts is composed of ciliated

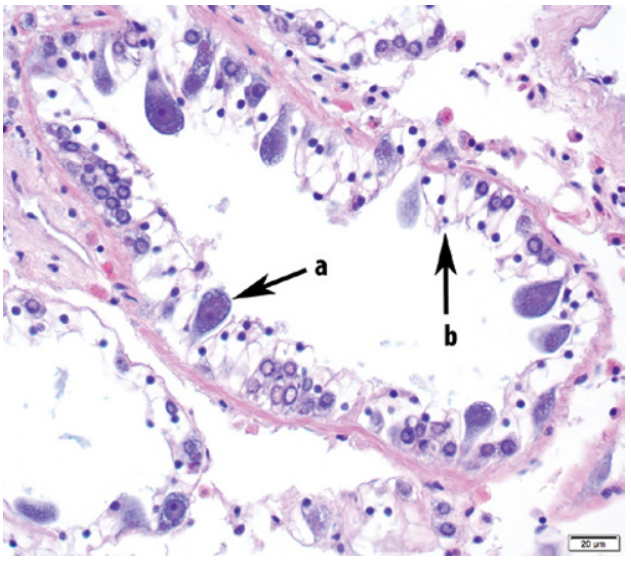


Figure 6.33 Section of a hard clam's gonadal tissue. a, developing oocyte; b, follicular cells.

cuboidal to low columnar epithelium and when about to spawn, the cells develop to become mucous cells containing abundant orange-red granules in HE-stained sections. The inner epithelium of the duct is similar to the gamete-producing epithelium of the tubules.

Sexes can be dioecious from metamorphosis (such as scallops). Many species are hermaphroditic, although most of these are protandric hermaphrodites, with many species of clams and oyster starting out as male during their first year of life. After the first spawning event, variable numbers of individuals within these populations become female for the second spawn (a transitional period forms a true, but temporary, hermaphrodite that can be noted histologically).

6.3.8 Sensory System

6.3.8.1 Nerves

Ganglia are similar histologically in all bivalves and are characterized by an outer layer of ganglionic cell bodies that are usually several cells thick and an inner core of nerve fibers forming a neuropile region (Figure 6.34). Large nerves leave the ganglia and communicate with other ganglia as well as other parts of the body. Hemoglobin is found in ganglia of several species of clams and, when present, provides a reddish-orange color to the ganglia and nerves. In addition to the three pairs of ganglia and numerous sensory tentacles on the siphons and mantle edges, two other important types of sensory structures exist in adult bivalves – the osphradium and eyes. The statocyst may also be retained in adults and was discussed in the section on larvae.

6.3.8.2 Eyes

Cephalic eyes consist of a cup of ciliated, pigmented cells with interspersed photosensory cells connected to the cerebral ganglion by nerve fibers (Morton 2008). The occurrence of a lens is debated (Rosen et al. 1978) but not readily seen. Pallial (tentacular) eyes vary greatly in structure from the simple structures noted in some clams, which are similar to cephalic eyes, to the more complex pallial eyes of scallops (Morton 2008).

The pallial eyes of scallops (Figures 6.35 and 6.36) sit atop a muscular stalk on the division of the middle lobe of the mantle and can be withdrawn into the shell quickly. The eyes are composed of a ciliated columnar cell cornea, circumferentially surrounded by pigmented cuboidal cells. Underlying the cornea is a thin layer of collective tissue attached to the cellular lens. Dividing the lens from the two-layered retina is a septum. The proximal layer of retinal receptors is classified as rhabdomeric and the distal as ciliary. Axons leave the retinal cells and course around the two layers, finally forming an optic nerve that runs through the eye stalk and joins the circumpallial nerve of the mantle. Underlying the retina (proximal to the eye stalk) are two additional layers. First is a tapetum-like structure consisting of cells that contain abundant refractile guanine crystals. The innermost layer of the eye consists of pigmented cells that are attached to underlying connective tissues of the stalk (Speiser and Wilkens 2016).

6.3.8.3 Osphradium

The osphradium is a specialized area of the epithelium overlying the area of the visceral ganglia (Haszprunar 1987). The epithelium in the osphradium consists of a focus of sensory cells with processes that extend to the visceral ganglia and supporting cells (Morse and Zardus 1997). Little is known of its function but it has been suggested that the osphradium is important in detecting spawning cues (Benninger and Le Pennec 2016).

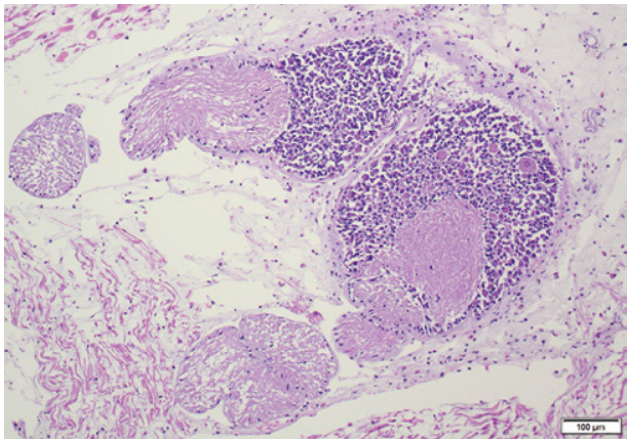


Figure 6.34 Section of a hard clam's pedal ganglia.

6.3.9 Hydromuscular System and Viseral Connective Tissues

6.3.9.1 Adductor Muscle

The adductor muscle of clams is divided into two parts. The smaller, often crescent-shaped catch (opaque/white) muscle is slow reacting and is used to maintain the animal in a feeding position (with inner mantle lobes forming a mantle cavity as discussed previously). The larger, usually rounder portion of the adductor muscle contains the "quick" fibers and is composed of smooth muscle in clams (Eble 2001). It is used to quickly close the valves of the shell. In clams, this portion is often pigmented pink due to the inclusion of hemoglobin in muscle cells. In scallops and oysters, the "quick" portion of the adductor muscle is termed the translucent portion of the adductor muscle and is not pigmented. Interestingly, the type of muscle making up the two parts of the adductor muscle varies greatly by species (ranging from

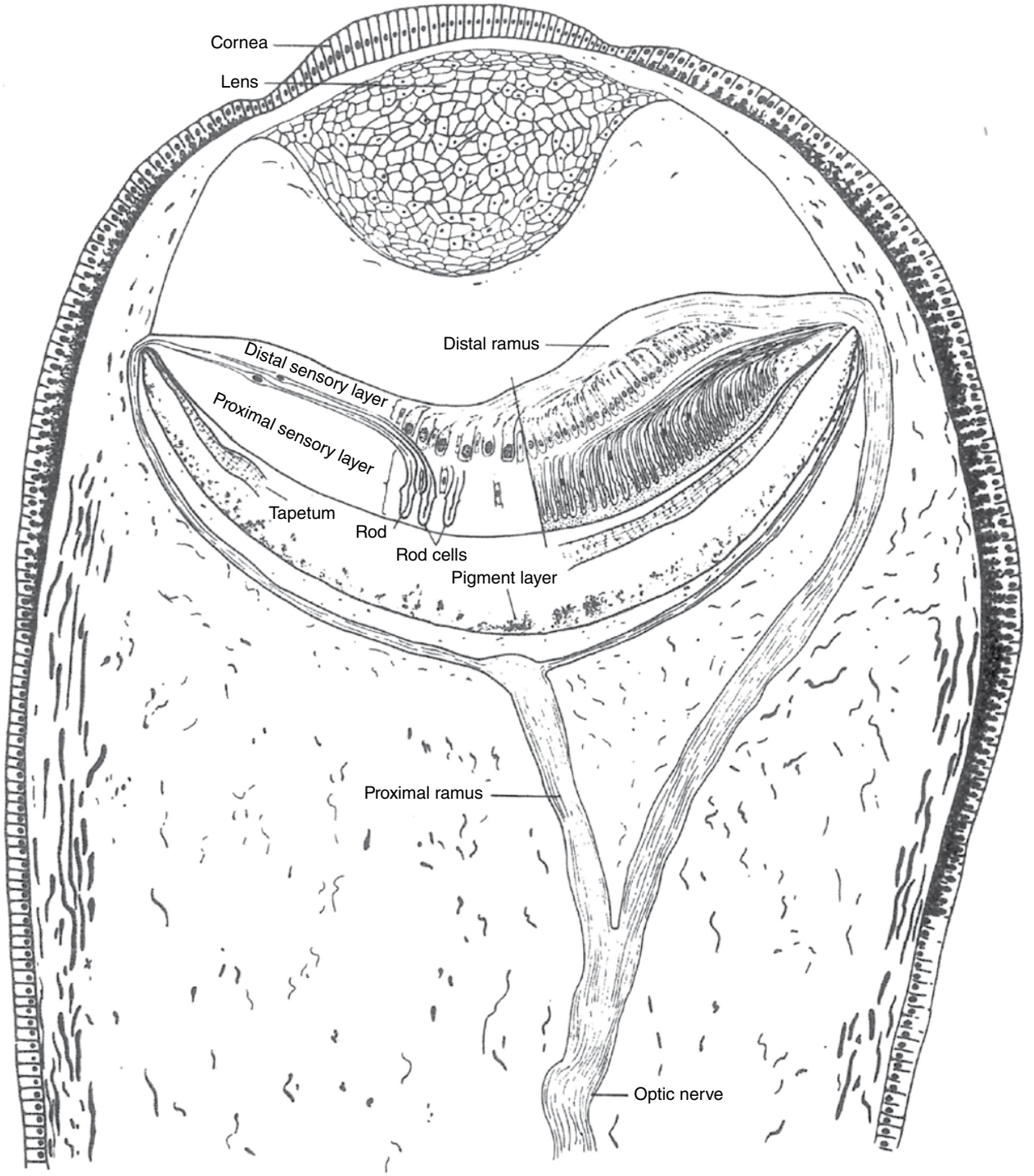


Figure 6.35 Diagram of a scallop mantle eye. *Source:* Speiser and Wilkens (2016). © 2016 Elsevier.

transversely striated to obliquely striated to smooth) (Chantler 2001), so there is no interspecies consistency. Muscle fibers are surrounded by delicate endomysium and muscle bundles by perimysium. Hemolymph sinuses and vessels permeate the tissue.

6.3.9.2 Foot

Clams, cockles, and mussels all have a foot, which is used to bury themselves in the sediment and to move across the sediment surface. Ciliated highly folded simple columnar epithelium covers the foot when it is contracted. Dense

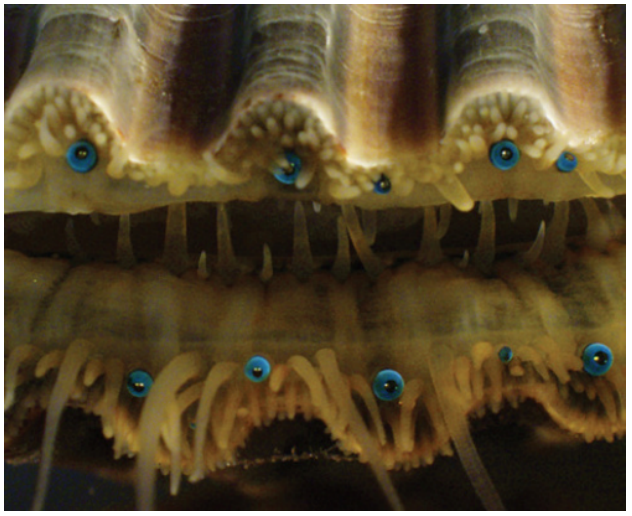


Figure 6.36 Mantle eyes of a bay scallop (*Argopecten irradians*). *Source:* Photo by Kathryn Markey Lundgren, Roger Williams University, Aquatic Diagnostic Laboratory.

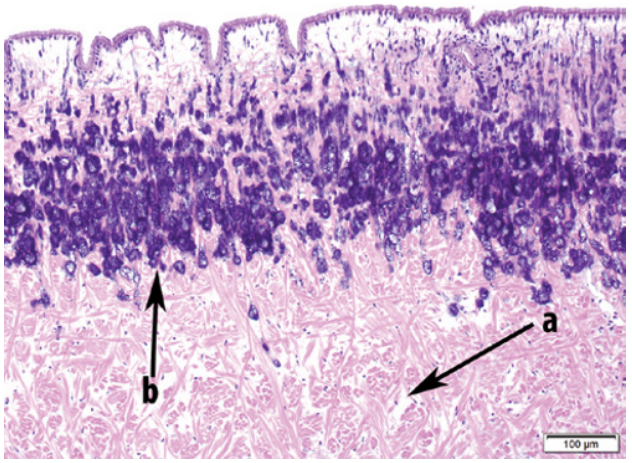


Figure 6.37 Section of a hard clam's foot. a, sinusoids of the foot; b, mucous gland underlying the epithelium of the foot.

connective tissue containing muscle fibers underlies the epithelium and connects to the substance of the foot, which is composed of numerous bundles of muscles, running in circular, longitudinal and transverse directions. Internal to the dense connective tissue of the outer surface of the foot are abundant, prominent, unicellular mucous glands with ducts that lead to the surface of the foot (Figure 6.37). Sinusoids permeate the muscular tissues of the foot, some of which are large enough to be seen grossly when the foot is dissected. Within the foot ventral to the pedal ganglia is a large byssal (pedal) gland that is connected to the posterior "heel" of the foot by a prominent duct (Figure 6.38). The gland is made of several different

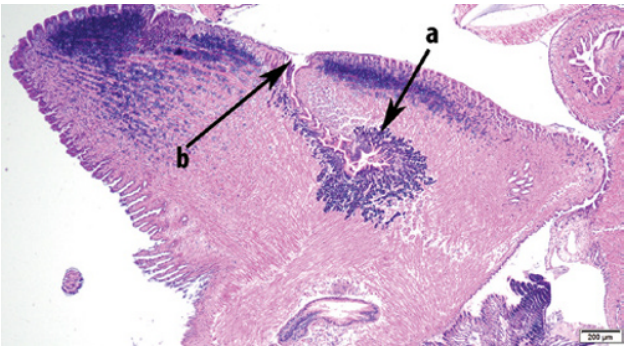


Figure 6.38 Section of the foot of a juvenile hard clam. a, byssal gland; b, duct.

types of glandular cells each producing substances associated with crawling, production of and attachment of byssal threads. The main product of the gland is byssal fibers which are responsible for attachment of postmetamorphic bivalves (as well as metamorphic larvae) to a substrate. In motile animals such as clams and scallops, the byssal strands break down in the early juvenile period and the animals are no longer attached to the sediment.

In scallops, the foot is much smaller than that of clams. After metamorphosis, in motile scallops, the foot degenerates and becomes very small, although the byssal gland does remain active. Some rare scallop species, with a more sedentary lifestyle, retain a larger foot (although not as big as clams) that can extend through a byssal notch between the valves of the shell and produce byssal thread that attaches the scallop to the sediment. In general, the structure of the scallop foot, although substantially smaller and located ventral to the mouth, is similar in complexity and cell types to the clam's foot.

6.3.9.3 Siphon

Siphons are found in clams, cockles, and mussels but are probably best developed in clams where they can be very large and can be dramatically extended and contracted. The incurrent and excurrent siphon channels are lined internally by simple cuboidal to low columnar epithelium. A prominent circular layer of muscle is noted just below this epithelium and internally to that are numerous unicellular glands as noted in the foot. The substance of the siphons is composed of muscle bundles and sinusoids as noted in the foot. In some clam species, valves are noted in the excurrent siphon pathway. The outer surface of the siphon is similar to the inner surface but in some clams a thickened periostracum (skin or sheath) is attached to the underling epithelium. Sensory tentacles line the lips of the siphons, especially the incurrent siphon. In some clam species, the incurrent and excurrent portions of the siphon are split from each other along portions of their length.

6.3.9.4 Vesicular Cells

Vesicular cells are connective tissue cells surrounding and connecting most organs in the visceral mass of bivalves. They are similar to fat cells in vertebrates and store lipid and glycogen. In histologic sections, they appear hollow due to loss of contents during processing. They often have a central to eccentric nucleus. In well-fed animals or animals about to produce gametes, the vesicular cells are as described above. In animals that have starved, undergone gamete production or are sick, the vesicular cells shrink and are smaller and more trabecular-like.

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7

Annelida

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7.1 Introduction

7.1.1 Taxonomy

An annelid, phylum name Annelida, also called segmented worm, is any member of a phylum of invertebrate animals characterized by the possession of a body cavity (or coelom), movable bristles (or setae), and a body divided into segments by transverse rings, or annulations, from which they take their name. They are found worldwide from the deepest marine sediments to the soils in our city parks and yards. As a major invertebrate phylum of the animal kingdom, the annelids number more than 9000 species distributed among three classes: the marine worms (Polychaeta), which are divided into free-moving and sedentary, or tube-dwelling, forms; the earthworms (Oligochaeta); and the leeches (Hirudinea). Earthworms and leeches are the familiar annelids for most people, but polychaetes make up the bulk of the diversity of Annelida of which most people are completely unaware. They are found in nearly every marine habitat, from intertidal algal mats downwards. There are even pelagic polychaetes that swim or drift, preying on other plankton, and a few groups occurring in fresh water and moist terrestrial surroundings (Rouse 2002).

A recent cladistic analysis of Annelida and other groups has resulted in a new classification of polychaetes (Rouse and Fauchald 1997). The Annelida is found to be monophyletic, though weakly supported, and comprises the Clitellata and Polychaeta. The group Polychaeta is split into two main clades: Scolecida and Palpata. Scolecida is a small group of less than 1000 named species, and these worms are all burrowers of one form or another, with bodies reminiscent of earthworms. Palpata makes up the vast majority of polychaetes and is divided into Aciculata and Canalipalpata. Aciculata contains about half of the polychaete species and largely encompasses the old taxonomic group Errantia. Representatives of this lineage are characterized by having internal supporting chaetae, or aciculae, in the parapodia. It includes major groups such as Phyllodocida and Eunicida, which tend to be mobile forms with well-developed eyes and parapodia for rapid locomotion. Canalipalpata, a group with more than 5000 named species, is distinguished by having long grooved palp structures that are used for feeding. Canalipalpata is divided into Sabellida, Spionida, and Terebellida. Most of these groups' members live in tubes and use their palps to feed in various ways.

7.1.2 Life History

7.1.2.1 Life Expectancy

The life span differs greatly among annelids. An earthworm can live for 4–8 years. Living a prolonged life span can be challenging because they are food sources for birds, toads, and rodents, and often are used for home composting, as well as commercial and recreational fishing.

Since both the polychaetes and oligochaetes are able to regrow lost parts (i.e., regenerate), it may appear that they are essentially ageless. Few longevity studies have been carried out with polychaetes, however. Most of the adults of species studied have a characteristic number of segments, which form rapidly during early life and prior to the appearance of gametes. Many polychaetes, especially among the nereids, reproduce only once and then die. In nature, these worms, usually quite sluggish after spawning, are eaten by fish and other animals. Species of polychaetes are known to live from one month (*Dinophilus*) to three years (*Perinereis*). Species that form stolons (stems), such as the syllids, or whose posterior end breaks off, such as the

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palolo, are capable of repeating the process but the number of times and the length of time for which they are able to do so have not been established. Most sedentary polychaetes survive following spawning, but, again, it is not yet known how often this process can be repeated.

The life span of oligochaetes is better established because they are frequently used in laboratory experiments. Some earthworms are believed to live as long as 10 years. Senescence, or aging, is known to occur in oligochaetes; *Eisenia*, for example, lives beyond a reproductive period with a progressive loss of weight. Aging oligochaetes darken in color, largely because of an increase in pigment deposition. In addition, the metabolic rates decrease and their physiologic processes slow.

Little is known about the life span of leeches. One species of *Erpobdella* requires a year to reach sexual maturity, after which it lays cocoons once and dies. Another species breeds once a year for two years and dies during the third.

7.1.2.2 Reproduction

Polychaetes can reproduce asexually, by dividing into two or more pieces or by budding off a new individual, while the parent remains a complete organism. The lifecycles of most living polychaetes, which are almost all marine animals, are unknown. Only a quarter of the studied species have two separate sexes, which release ova and sperm into the water via their nephridia. The fertilized eggs develop into *trochophore larvae*, which live as plankton. Later they sink to the sea floor and metamorphose into miniature adults. While some polychaetes remain of one sex all their lives, a significant percentage of species are full hermaphrodites or change sex during their lives (i.e., sequential hermaphrodites). Most polychaetes whose reproduction has been studied lack permanent gonads, and it is uncertain how they produce ova and sperm. In a few species the rear of the body splits off and becomes a separate individual that lives just long enough to swim to a suitable environment, usually near the surface, and spawn (Rouse 1998).

Reproduction in oligochaetes is primarily hermaphroditic; the number, arrangement, and location of the male and female gonads and their pores vary considerably among the various species. Lower oligochaetes (Microdrili) have one pair of testes and one pair of ovaries in successive segments. Higher oligochaetes (Megadrili) retain the two pairs of testes in segments 10 and 11 and one pair of ovaries in segment 13. Developing sperm are frequently stored in seminal vesicles before transfer to female seminal receptacles (spermatheca). Sperm ducts lead from the seminal vesicles to male pores located one or more segments behind the testes. The ovaries are simple outpouchings (ovisacs), with oviducts leading to female pores in the next posterior segment.

Though these crawlers have male and female reproductive organs, they require mating partners. Copulation in oligochaetes is reciprocal – that is, only sperm is exchanged, but mutually – and takes place in a head to tail position, with the two ventral surfaces in contact. In lower oligochaetes, the male pores of one worm and the female pores of another (openings of seminal receptacles) are opposite each other, and sperm pass directly from the male pores into the seminal receptacle of the partner. In higher oligochaetes genital pores do not lie opposite each other. In this case the seminal fluid is conducted from the male genital pores to the seminal receptacles via paired seminal grooves formed by attaching body surfaces of mating animals. Cells associated with the brain secrete a hormone that stimulates gamete development. The worms separate after the mutual sperm exchange.

The clitellum of the earthworm secretes a case, or cocoon, into which is secreted a material that serves as nourishment for the young and a mucous substance that aids in copulation. The cocoon slips forward and receives eggs as it passes the female pores and sperm as it passes the pores of spermathecae. Fertilization, therefore, takes place within the cocoon. The cocoon slides over the peristome, becoming completely sealed as it does so, and then it is buried. It takes between two and four weeks for baby earthworms to emerge from their cocoon.

7.1.2.3 Diet

The feeding methods of polychaetes are closely correlated with the varous modes of existence the members of the class display. Raptorial feeders include members of many families of surface-dwelling species, many pelagic groups, tubuculous eunicids, and other gallery dwellers. The prey consists of various small invertebrates, including other polychaetes, which are usually captured by means of an eversible pharynx (proboscis). Not all errant polychaetes that possess jaws are necessarily carnivores. A scavenger or omnivorous habit has evolved in many poychaetes. The jaws may be used, for example, to tear off pieces of algae. Some deposit-feeding polychaetes consume sand or mud directly when the mouth is applied against the substratum and are generally not selective. Selective deposit feeders lack a proboscis. Special head structures extend out over or into the substratum. Deposit material adheres to mucous secretions on the surface of these feeding structures and is then conveyed to the mouth along ciliated tracts or grooves. These polychaetes thus select organic deposit material from between sand particles. Many of the sedentary burrowers and tubiculous polychaetes are filter feeders. The head is usually equipped with special feeding processes, used to collect detritus and plankton from the surrounding water.

The majority of oligochaete species, both aquatic and terrestrial, are scavengers and feed on dead organic matter, particularly vegetation. The earthworm is an example of a foraging herbivorous annelid, obtaining food by eating its way through the soil and extracting nutrients from the soil as it passes through the digestive tract. Leeches are primarily bloodsuckers. The medicinal leech *Hirudo* feeds principally on mammalian blood, but it also sucks blood from snakes, tortoises, frogs, and fish; when young, it may eat oligochaetes. The secretion of hirudin facilitates feeding. The leech detaches after becoming engorged with blood and it may not attempt to feed again for up to 18 months. Marine leeches attach to, and feed directly from, the gills of fish. Other leeches are carnivorous and feed on oligochaetes and snails.

7.1.3 Relevance

Marine annelids may account for over one-third of bottom-dwelling animal species around coral reefs and in tidal zones. Burrowing species increase the penetration of water and oxygen into the sea-floor sediment, which encourages the growth of populations of aerobic bacteria and small animals alongside their burrows. The terrestrial burrowers loosen the soil so that oxygen and water can penetrate it. Both surface and burrowing worms help to produce soil by mixing organic and mineral matter, by accelerating the decomposition of organic matter and thus making it more quickly available to other organisms, and by concentrating minerals and converting them to forms that plants can use more easily. Earthworms are also important prey for birds ranging in size from robins to storks, and for mammals ranging from shrews to badgers. Large earthworms (*Lumbricus terrestris*) are cultivated and sold as bait for freshwater fishes and as humus builders in gardens. The sludge worm *Tubifex*, abundant near sewer outlets and thus an indicator of water pollution, is collected and sold as food for tropical fish. Polychaetes play an important role in turning over sediment on the ocean bottom. The medicinal use of leeches, which dates from antiquity, reached its peak in the first half of the nineteenth century. The European species *Hirudo medicinalis* formerly was exported throughout the world, and native species were used. Hirudin, an extract from leeches, is used as a blood anticoagulant. The estuarine flats of Maine and Nova Scotia are the principal sources of the bloodworm (*Glycera dibranchiata*), which is used as bait for saltwater fishes. Reproductive parts of the palolo (*Palola siciliensis*), which break off and are found in great numbers during the reproductive period, are used as food in Samoa in the south Pacific.

7.2 Gross Anatomy

Place the earthworm in the dissecting tray after rinsing off the mucus (Figure 7.1). Identify the dorsal side, which is the worm's rounded top, and the ventral side, which is its flattened bottom. Locate the conspicuous clitellum, a saddle-like swelling on the dorsal surface (Figure 7.1). The clitellum produces a mucus sheath used to surround the worms during mating and is responsible for making the cocoon within which fertilized eggs are deposited. The anterior of the animal is more cylindrical than the flattened posterior and is the closest to the clitellum. The ventral surface of the earthworm is usually a lighter color than the dorsal surface. The mouth is located on the ventral surface of the first segment while the anus is found at the end of the last segment. Find the anterior end by locating the prostomium (lip), which is a fleshy lobe that extends over the mouth (Figure 7.1). The other end of the worm's body is the posterior end, where the anus is located. Look for the worm's setae, which are the minute bristle-like spines located on every segment except the first and last. Bristle-like setae are used for locomotion. Find the pair of sperm grooves that extend from the clitellum to about segment 15, where one pair of male genital pores is located. Look also for one pair of female genital pores on segment 14. Try to find the two pairs of openings of the seminal receptacles on segment 10.

Using a pair of scissors, make a shallow incision in the dorsal side of the clitellum at segment 33 (Figure 7.2a). Using a pair of forceps, spread the incision open, little by little. Separate each septum from the central tube using a dissecting needle, and pin down each loosened bit of skin (Figure 7.2b). Continue the incision forward to segment 1. Starting at the anterior end, locate the muscular pharynx (food ingestion) (Figure 7.3). This is followed by a tube-like



Figure 7.1 *Lumbricus terrestris* in anesthetized state. A, anus; CL, clitellum; CS, contracted segments; DBV, dorsal blood vessel; ES, elongated segments; Per, peristomium; Pro, prostomium.

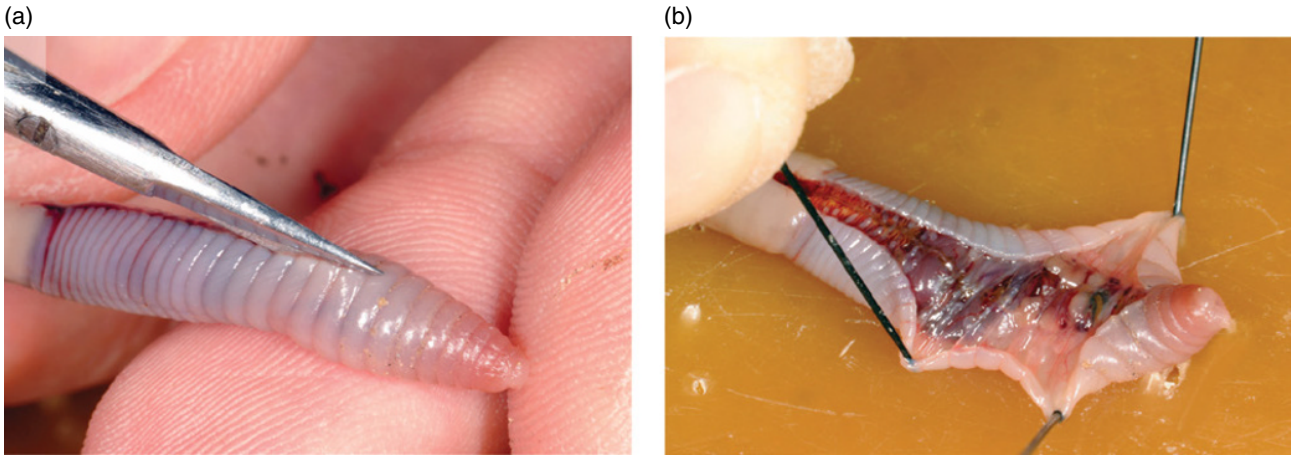


Figure 7.2 (a) One dorsal cut is enough to reveal the internal organs of the earthworm. (b) Pins in pairs are used to keep the body wall open. The intersegmental septa have to be torn to free the internal organs.

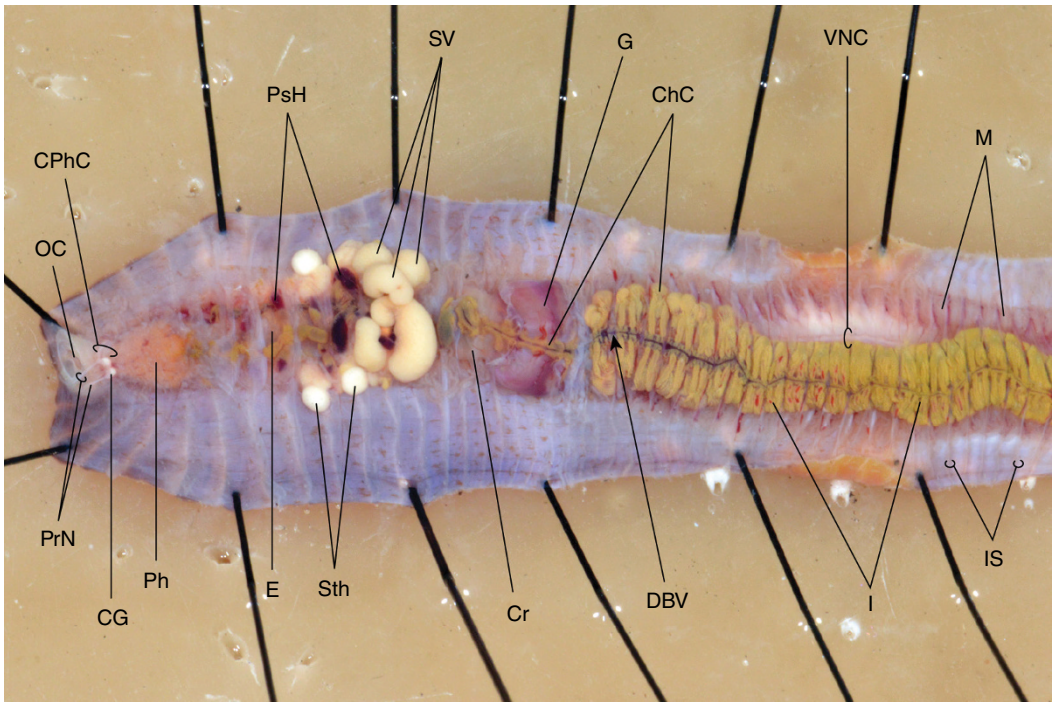


Figure 7.3 Dissected *Lumbricus* in water cover displaying the internal organs. CG, cerebral ganglion; ChC, chloragogen cells; CPhC, circumpharyngeal connective; Cr, crop; DBV, dorsal blood vessel; E, esophagus; G, gizzard; I, intestine; IS, intersegmental septa; M, metanephridia; Ph, pharynx; PrN, prostomial nerves; PsH, pseudohearts; OC, oral cavity; Sth, spermathecae; SV, lobes of seminal vesicle; VNC, ventral nerve cord.

esophagus, which terminates in a crop, which serves as a storage organ. Posterior to the crop, the gizzard can be found. While the crop is soft and thin, the gizzard is muscular; soil is ground up and churned within the gizzard. The gizzard is followed by a long intestine in which both digestion and absorption occur. Undigested material is voided through the anus. Identify the five pairs of pseudohearts and find the dorsal blood vessel (Figure 7.3). Look for smaller blood vessels that branch from the dorsal blood vessel. To find organs of the nervous system, push aside the digestive and circulatory system organs and locate the ventral nerve cord. Trace the nerve cord forward to the circumpharyngeal nerve ring, which circles the pharynx. Find one pair of ganglia under the pharynx and another pair of ganglia above the pharynx. The ganglia above the pharynx serve as the brain of the earthworm. The worm's excretory

organs are tiny nephridia. There are two in every segment. The earthworm is hermaphrodite; there are testes and ovaries in every individual. Most parts of the reproductive system are too tiny to locate even with a loupe. The two pairs of spermathecae are observable as small white spheres and the yellowish large lobes of seminal vesicle in the anterior part of the body (Figure 7.3).

7.3 Histology

7.3.1 Body Wall

7.3.1.1 Integument and Muscle Layers

A list of organs for histologic examination is provided in Table 7.1. The body wall consists of the epidermis, connective tissue that underlies it, and muscle layers (Figure 7.4a,b). The epidermis is the thinnest layer within the body wall, except for the clitellar region, where it is thick (Figure 7.4d). Large numbers of unmyelinated axons richly innervate the epidermis. Capillaries enmesh even the epidermis (Figure 7.4d). A fibrous layer beneath the basal lamina separates the epidermal epithelium from the body wall musculature. The connective tissue of the earthworm body wall is largely acellular, but at extremely rare intervals, cells are found embedded among the collagen fibers. Some of these scattered cells are filled with large numbers of phagocytosed bacteria; the rest are characterized by large vacuoles, which contain material that is interpreted as partially digested foreign matter. No cell which appears morphologically akin to the vertebrate fibroblast can be found. The same absence of connective tissue cells has been noted in the connective tissue of the gut and nervous system. Fibroblasts are either absent or extremely sparse in *Lumbricus* (Coggeshall 1966).

Under the connective tissue, there is a layer of circular muscles and below this is a much thicker layer of longitudinal muscles. The circular muscles contract to make the worm longer and thinner, the longitudinal to become short and stout (Tzetlin and Filippova 2005). The segment walls subdivide the circular muscle layer, while the longitudinal muscle layer is continuous throughout the segments of the animal (Figure 7.4a,b).

The intersegmental septa or dissepiments consist of the extracellular matrix (ECM) situated between the adjoining coelomic epithelia or muscle cells. In addition, blood vessels are formed by gaps within this ECM. The orientation of the muscles can be dorsoventral, oblique, or transverse, or they radiate from the intestine to the body wall (Tzetlin and Filippova 2005). There are radial muscle bundles on the anterior surface of dissepiments and circular ones on the posterior (Figure 7.4a upper right and Figure 7.4b upper and lower right panels) (Pilato 1981).

7.3.1.2 Cuticle and Epidermis

The cuticle of the earthworm consists of collagenous fibers embedded in an amorphous matrix (Figure 7.5b–d). As previous investigators have noted, the fibers are arranged in layers, each layer consisting of a single row of parallel fibers, and the main fiber axis of each layer making an angle of approximately 90° with respect to the fiber axis in the layers above and below (Coggeshall 1966). The outer surface of the cuticle, called the epicuticle, consists of a layer of homogenous, electronopaque material in which are embedded myriad small ellipsoidal bodies. The cuticle of Annelida consists of proteins, which are reinforced by a special type of collagen fiber; in contrast to the cuticle of arthropods, it does not contain chitin. Chitin is present only in the chaetae, which are bristles on the body wall (Peters and Walldorf 1986).

The epidermis of *L. terrestris* consists of a simple epithelium in the cephalic and terminal regions, and a pseudostratified epithelium in the clitellar region (Figure 7.5a,d). In all epidermal regions are present supporting, basal, sensory, and mucous gland cells. Supporting cells are columnar epithelial cells and

Table 7.1 Organs for histologic evaluation in Annelida.

Organ system		Organs
Body wall/musculoskeletal		Epidermis, muscle layers, setae, clitellum
Digestive	Alimentary canal	Oral cavity, pharynx, esophagus and calciferous (Morren's) glands, crop, gizzard, midgut and typhlosole, hindgut
Excretory		Metanephridium
Circulatory		Main blood vessels, pseudohearts, hemocytes
Nervous		Ganglia, giant nerves
Reproductive		Testis, seminal vesicles, vas deferens, seminal receptacles
Special senses/organs		–

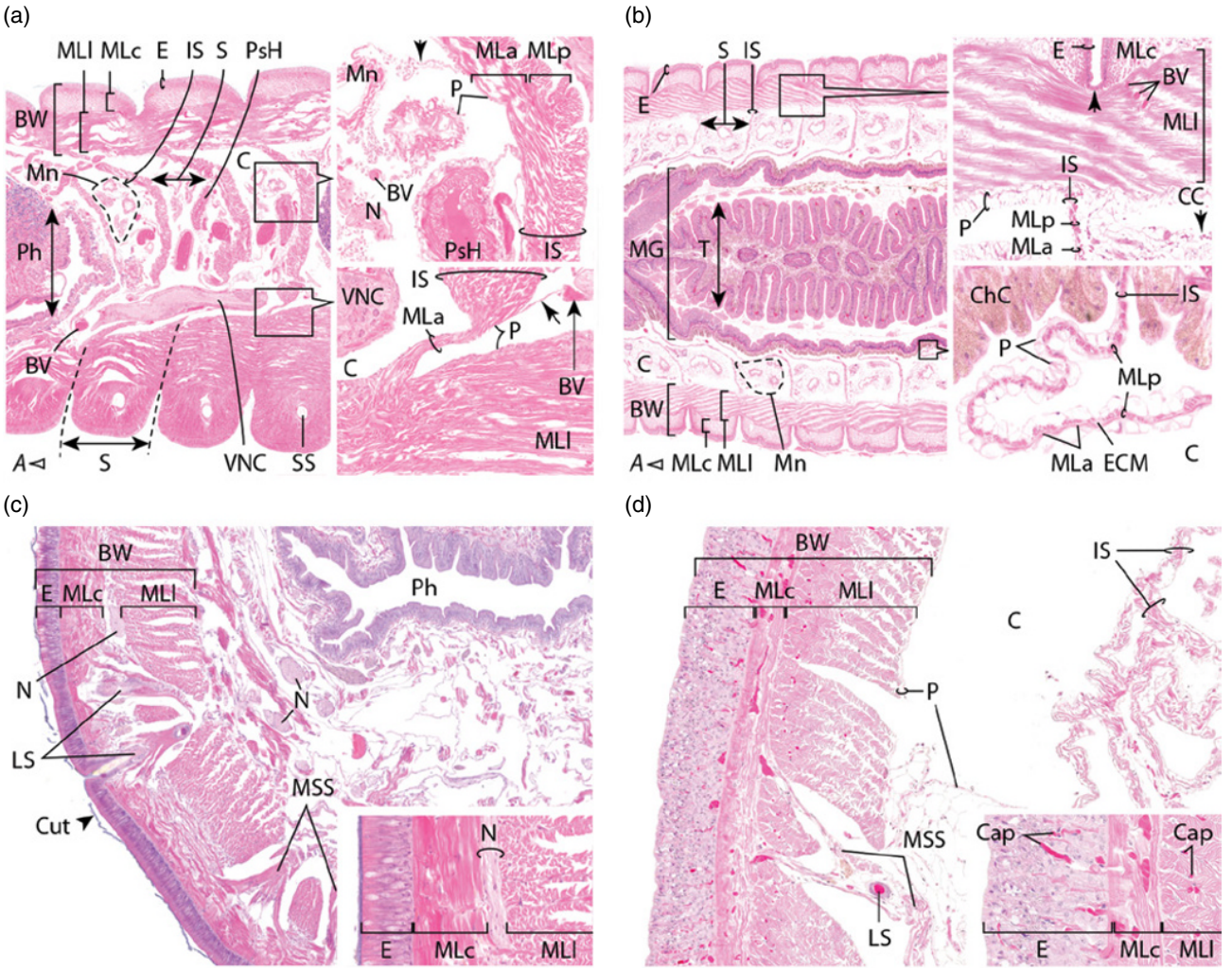


Figure 7.4 Structure of body wall layers in different regions of *Lumbricus*. (a) Layers of the body wall and dissepiments at low magnification (median sagittal longitudinal sections, Bouin's fixation), left panel: 15×; upper right panel: 100×, lower right panel: 150×; (b) layers of the body wall behind the clitellum (horizontal section plane, longitudinal section, Bouin's fixation), left panel: 15×, upper right panel: 150×, lower right panel: 200×; (c) Layers of the body wall at the setae (anterior end, Bouin fixation), 50× and 300× (inset); (d) Layers of the body wall at the clitellum, (formalin fixation), 100× and 300× (inset). A, anterior; BV, blood vessel; BW, body wall; C, coelom (body cavity); Cap, capillary; CC, coelomocyte; ChC, chloragogen cell; Cut, cuticle; E, epidermis; ECM, connective tissue layer of dissepiment; IS, intersegmental septum (dissepiment); LS, lateral seta; MG, midgut; MLa, muscle layer of intersegmental septum, anterior; MLc, muscle layer of body wall, circular; MLl, muscle layer of body wall, longitudinal; MLp, muscle layer of intersegmental septum, posterior; Mn, metanephridium; MSS, muscles of setal sac; N, nerve; P, peritoneum, Ph, pharynx; PsH, pseudoheart; S, segment; SS, setal sac; T, typhlosole; VNC, ventral nerve cord; arrows in panel (a), suspensory membranes.

they are not specialized as secretory or sensory cells (Figure 7.5a,b). They may contain pigment granules; the apical surface possesses microvilli, which penetrate the cuticle. The nuclei are prominent. Many dark, membrane-bounded granules, 100–200 nm in diameter, are found throughout the cytoplasm of the supporting cells, but they are most abundant in the perinuclear region. It is generally assumed that the cuticle is produced by these cells. Basal cells featured in the pseudostratified epithelium take part in regeneration (Figure 7.5d). The mucous gland cells (orthochromatic and metachromatic) are mainly present in the clitellar region, while neuroendocrine-like cells are more numerous in the cephalic and terminal regions (Licata et al. 2002).

In the epidermis of the oligochaete lumbricids, mucous and protein gland cells are commonly found. However, the characterization of the mucous secretion in the two (orthochromatic and metachromatic) cell types has been investigated only with conventional histochemical methods. The orthochromatic (or large granule) cells secrete a

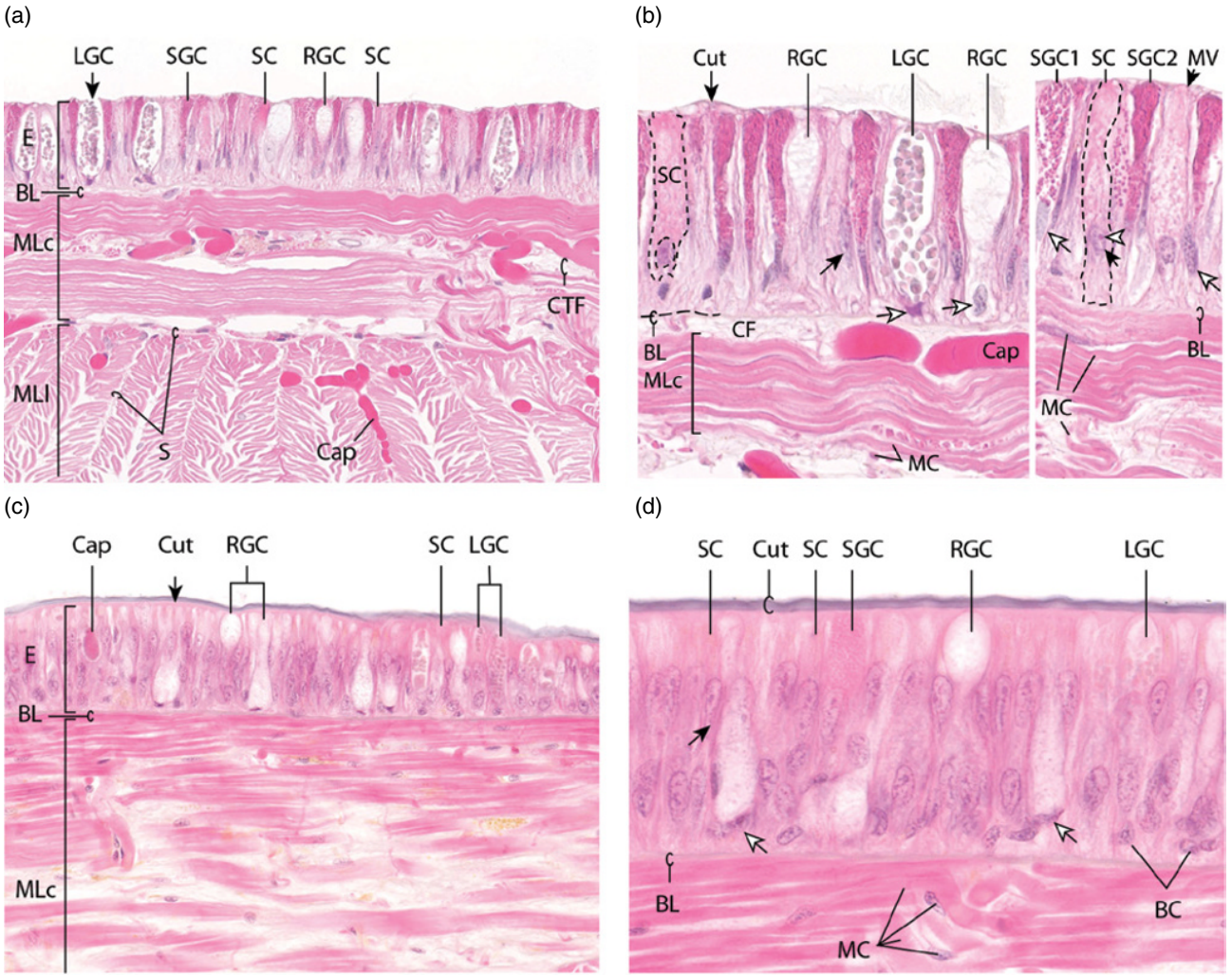


Figure 7.5 Morphology of epidermal cell types of *Lumbricus* after formalin and Bouin's fixation. (a) Epidermis and subepidermal layers (formalin fixation), 400×. (b) Epidermal cell types in high magnification (formalin fixation), 900×. (c) Epidermis and subepidermal layers (Bouin's fixation), 400×. (d) Epidermal cell types in high magnification (Bouin's fixation), 900×. BC, basal cell; BL, basal lamina; Cap, capillary; Cut, cuticle; CF, collagen fibers; CTF, collagen tissue fibers; E, epidermis; LGC, large granule cell; MC, muscle cell; MLc, muscle layer of body wall, circular; MLl, muscle layer of body wall, longitudinal; MV, microvilli; RGC, reticular gland cell; S, connective tissue septa; SC, supporting cell; SGC, small granule cell; SGC1, SGC2, small granule cell, type 1 or 2; black arrow, nucleus of supporting cell; white arrow, nucleus of gland cell; white arrowhead, nucleolus of supporting cell.

complex of neutral mucopolysaccharides, proteins, and lipids (sulfomucins and glycogen are absent) (Figure 7.5a–d). With the light microscope, they appear as a globular cavity filled with PAS-positive material. Moreover, the dense orthochromatic granules, to which has been attributed a lubricating action during locomotion, may also contain a pheromone important in mating. In contrast, the metachromatic (or reticular gland) cells produce a carboxylated slightly sulfated mucus, probably of low viscosity, which provides a respiratory film held to the surface of the cuticle by epicuticular projections (Figure 7.5a–d). The third type of gland cells, called "small granular cells," are rich in proteins and are scattered throughout the epidermis (Figure 7.5a,b,d). The secretion of these cells could aid water retention in the acidic mucous film or modify the viscosity of the lubricating mucus. A receptor-secretory function with paracrine action has also been attributed considering these cells as neuroendocrine-like, capable of producing bioactive substances (Licata et al. 2002).

7.3.1.3 Setae

Annelids possess chitinous setae (chaetae) that arise from a setal sac (chaetal sac) (Figure 7.6a,b). The setal sac is an ectodermal invagination that generally contains several setal follicles and is surrounded by the subepidermal ECM. Setae are closely paired (Figure 7.6a). Annelids possess segmental

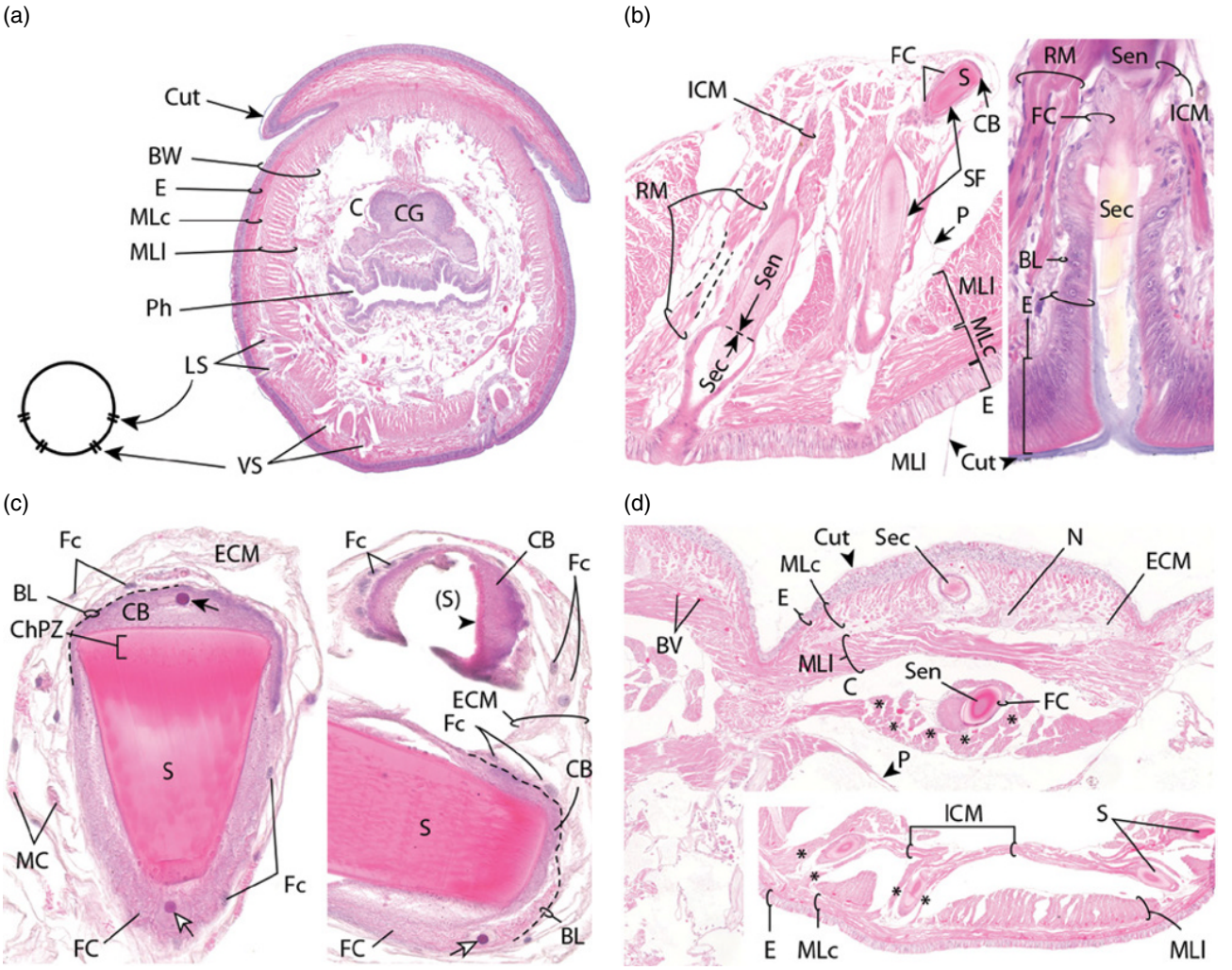


Figure 7.6 Setae and setal sac of *Lumbricus*. (a) Setal arrangement: closely paired (Bouin fixation), 20×. (b) Setal sac with two follicles (left side) and distal part of follicle (formalin [left] and Bouin [right] fixation), 120× (left) and 250× (right). (c) Chaetoblast, the deepest follicular cell and the ECM (formalin fixation), 530× (both panels), left: tangential plane, right: longitudinal plane. (d) Setal follicles and their muscles (formalin fixation), 100×, inset: 40×; horizontal plane, inset: cross-sectional plane. BV, blood vessel; BW, body wall; C, coelom (body cavity); CB, chaetoblast; ChPZ, chitin polymerizing zone; CG, cerebral ganglion; Cut, cuticle; E, epidermis; ECM, extracellular matrix; Fc, fibrocyte; FC, follicle cell; ICM, interconnecting or retractor muscle of setae; LS, lateral seta; MC, muscle cell; MLc, muscle layer of body wall, circular; MLl, muscle layer of body wall, longitudinal; P, peritoneum; Ph, pharynx; RM, radial or protractor muscle of setal sac; S, seta; (S), fallen out seta; Sec, seta, ectal part; Sen, seta, ental part; SF, setal follicle; VS, ventral seta; arrowhead, brush border; asterisk, radial or protractor muscle of setal sac; black arrow, nucleolus of chaetoblast; white arrow, nucleolus of follicle cell.

pairs of dorsal (notopodial) and ventral (neuropodial) setal sacs, each giving rise to a single row of setae (Tilic et al. 2015). Each seta is formed within one setal follicle, which consists of a basally located chaetoblast and several follicle cells (Figure 7.6b,d). All cells are epithelial, are interconnected by apical adherent junctions ("belt desmosomes"), face the seta and rest on the subepidermal ECM.

There are two types of setae. The general setae are sigmoid in shape with a sharply pointed tip. The other type of setae is associated with genital intumescences. They are called genital setae (or copulatory setae) because they take part in the process of copulation. A genital seta is straight with a grooved ectal part. Depending on the number of furrows, the ectal part of a genital seta could be trihedral or tetrahedral (Csuzdi and Zicsi 2003).

7.3.1.4 Clitellum

The clitellum is a saddle-like region of modified skin, prominently developed in sexually mature members of the Clitellata (Figure 7.7). The clitellar epithelium contains, in addition to nonspecialized supporting cells, a variety of secretory cells; the latter are responsible for a number of secretions associated with the reproductive processes of the Clitellata. These include the production of the egg

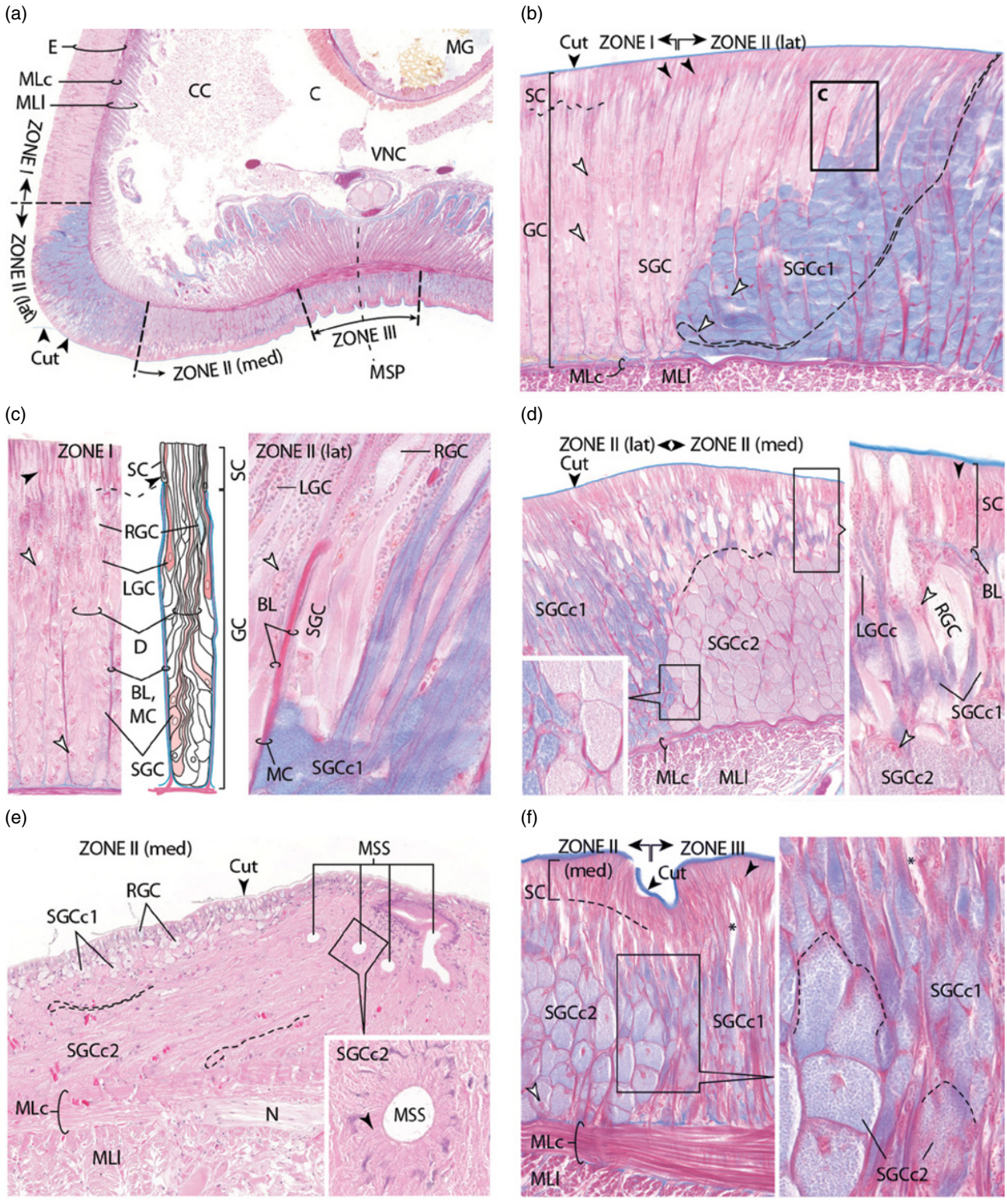


Figure 7.7 Organization of clitellar epidermis of *Lumbricus*. (a) Morphologically distinct zones of clitellum (Bouin fixation, Azan), 30×. (b) Boundary between zone I and zone II (Bouin fixation, Azan), 150×. (c) Organization of clitellar glands and gland cells in lateral part of zone II (Bouin fixation, Azan), 330× and 700×. (d) Glands in zone II (Bouin fixation, Azan), 150× and 550×. (e) Medial part of zone II (formalin fixation, HE), 150× and 700×. (f) Boundary between lateral part of zone II and zone III (Bouin fixation, Azan), 250× and 700×. BL, basal lamina; C, coelom (body cavity); CC, coelomocyte; Cut, cuticle; D, duct of gland cell; E, epidermis; GC, gland cell; LGC, large granule cell; LGCc, large granule cell, clitellar type; MC, muscle cell; MG, midgut; MLc, muscle layer of body wall, circular; MLl, muscle layer of body wall, longitudinal; MSS, middle setal sac diverticula; N, nerve; RGC, reticular gland cell; SC, supporting cell; SGC, small granule cell; SGCc1, small granule cell, clitellar type 1; SGCc2, small granule cell, clitellar type 2; VNC, ventral nerve cord; Zone I, II, III, morphologically distinct zones of epidermis; asterisk, sinus; black arrowhead in panel e, duct of SGCc2; black arrowhead in panel b, c and f, nucleus of supporting cells; dashed line in panel d, boundary between SGCc1 and SGCc2 glands; dashed line in panel b and e, contour of gland cells; white arrowhead, nucleus of gland cells.

capsule (cocoon), nutritive fluid, and the secretion of mucus and substances that aid in adhesion during copulation (Morris 1983).

The simple columnar epidermis of *L. terrestris* consists of supporting cells, large granular cells (LGC), and reticular cells, together with the small granular cells (SGC) typical of normal epidermis in the anterior and posterior transition zones. The proteinaceous nature of the granular clitellar cells, evidenced by their histochemistry and the pronase digestion of the granules in ultrasections, indicates that their secretion forms the cocoon wall. In the cocoon wall, parallel-oriented units are present similar in size to those present in the granules (Hess and Vena 1974). Since the mature granules show that the original secreted subunit (14 nm wide) is altered during maturation and storage, it is presumed that the stored form changes back into structural material at the time of secretion of the cocoon wall. The secretion of the metachromatic reticular cells has a finely fibrous nature and is reminiscent of that of the acid mucopolysaccharide secretion of normal lumbricid skin. The results suggest that the reticular cells secrete the cocoon wall and the globular cells the material that surrounds the developing embryos, the need for a nutritive-rich secretion in microdriles being obviated by the quantity of yolk present in their eggs (Richards 1977).

Three distinct regions (zones I–III) are distinguishable in the clitellum of the earthworm. This distinction is based largely upon a difference in the glandular elements found in them (Figure 7.7a).

- Zone I: on the dorsal and lateral surfaces with three kinds of glands. Reticular gland cells (RGC) are typical goblet cells with cytoplasmic mucus. LGC are long, slender, often twisted or convoluted cells extending much deeper into the thickness of the clitellum than the goblet cells and whose contents consist of large granules. SGC are deeper lying groups of gland cells enclosed within envelopes of connective tissue arranged in columns with finely granular contents. Each group is made up of a number of cells, each of which consists of an expanded portion which contains huge amounts of secretory granules and other cell organelles, and a slender elongated portion, which functions as a duct and extends upwards through the thickness of the clitellum until it opens on the surface. The cells are arranged around a central axis, the expanded portions lying in series one above another, and the ducts occupying the center of the group (Figure 7.7b,c).
- Zone II: the swellings along the sides of the clitellum (tubercula pubertatis). From the region of the lateral setae, the deeper-lying glands become gradually replaced by relatively larger cells more irregular in shape and less regularly arranged, until by the time the region of the ventral setal pores is reached, the former have become completely replaced by the latter. The contents of these cells are characteristic. The nearer the ventral setae are approached, however, the large-granule-containing glands (LGC) diminish in number, and this is often accompanied by a relative increase in the number of mucin-secreting glands. In the lateral lane of zone II, new types of mucus-secreting gland cells (stained by aniline blue) appear: the clitellar type of LGCc and SGCc. Two types of the latter can be distinguished on the basis of their Azan staining intensity. SGCc1 (type I) cells are dark blue, and open on the body surface. These cells are part of the lateral side of zone II (Figure 7.7a,b). SGCc1 gland cells are replaced by pale blue SGCc2 (type II) gland cells in the medial part of zone II (Figure 7.7d), which are in connection with the setal pore diverticula. The LGC cells have practically disappeared, and the superficial gland cells – chiefly mucus-secreting RGC and SGCc1 – are confined to a relatively thin layer simulating more nearly the ordinary epidermis, until it merges into the columnar epithelium of the setal pores (Figure 7.7e).
- Zone III: the ventral surface of the clitellum. The epidermis itself is less modified and consists of slender columnar supporting cells of the typical epidermis. The subepidermal layer is composed of thin, tall, aniline blue-positive cells with diffusely granular content. Despite their lighter blue staining, this morphology strongly resembles SGCc1 gland cells. The LGCs and RGC are completely missing from this region.

Between the groups of cells, large intercellular spaces or lacunae are present (Figure 7.7f, asterisk). This condition attains its maximum development in the median line, but at the outer margins approaching the region of the setal pores, the subepidermal tissue, with its lacunae, becomes increasingly replaced by the large cells with lamelliform contents associated with the diverticula of the ventral setal pores. The blood vessels are numerous and occupy the lacunae between the groups of cells (Figure 7.7c). The examination of sections of worms in both these stages shows that the papilla-like elevations are due to the development of the masses of glandular cells found in relation with diverticula of the ventral setal pores, and that these diverticula are already well established (Morris 1983).

7.3.2 Alimentary Canal

The alimentary canal of *Lumbricus* is straight and relatively simple. The annelid intestinal wall is composed of an inner layer of mucous membrane (tunica mucosa) consisting of a single layer of epithelium (lamina epithelialis)

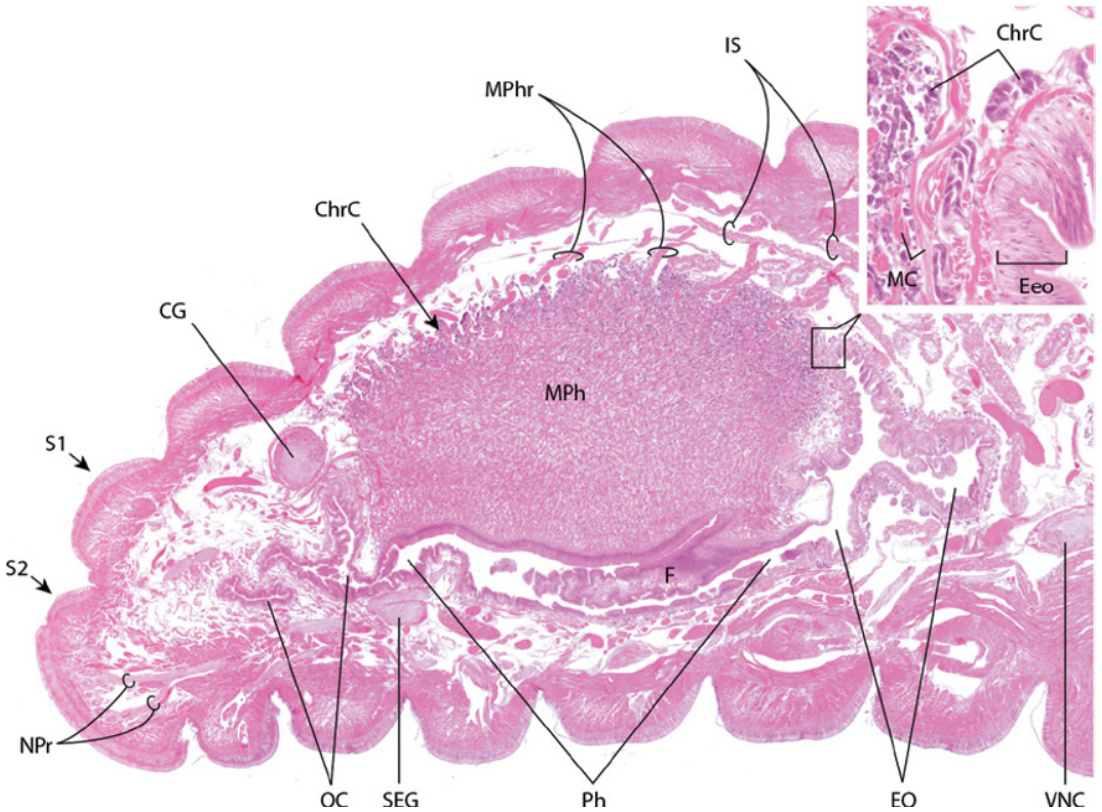


Figure 7.8 Foregut with chromophil cells in parasagittal section (Bouin fixation), 15× and 200× (inset). ChrC, chromophil cells; CG, cerebral ganglion; EO, esophagus; Eeo, epithelium of esophagus; F, fold of pharyngeal wall; IS, intersegmental septum; MC, muscle cell; MPh, muscles of pharynx; MPhr, radial muscles of pharynx; NPr, prostomial nerves; OC, oral cavity; Ph, pharynx; SEG, subesophageal ganglion; S1, first segment; S2, second segment (prostomium); VNC, ventral nerve cord.

resting on a delicate loose connective tissue layer (lamina propria) containing blood sinuses or capillaries. The lamina epithelialis always contains mucous gland cells and is typically ciliated. The tunica mucosa is followed by layers of inner circular and outer longitudinal muscle. The intestinal wall is covered by a layer of visceral peritoneum on the outer surface. The relatively extensive foregut, consisting of oral cavity, pharynx, and esophagus (Figure 7.8), is derived from the stomodeum and lined with epidermis, some of which secretes cuticle.

7.3.2.1 Oral Cavity

The mouth, located beneath the prostomium (see Figure 7.1), opens into a small oral cavity, which in turn opens into a more spacious pharynx. The oral cavity has a simple columnar epithelial lining in *Lumbricus*. A cuticle covers the epithelium (Figure 7.9). There is loose connective tissue with muscle cells and nerves under the epithelium. The circular and longitudinal muscle layers are very thin and discontinuous; they run immediately under the epidermis (Figure 7.9).

7.3.2.2 Pharynx

The dorsal wall of the pharynx is both very muscular and glandular and forms a pad, which is the principal ingestive organ. In earthworms the pharynx acts as a pump. This action of the pharynx is augmented by the action of strands of muscle fibers extending from the pharynx to the body wall (Figure 7.8). The pharyngeal lining is a pseudostratified columnar epithelium on the ventral side and a ciliated pseudostratified columnar epithelium on the dorsal side (Figure 7.10a,b). Under the thick layer of basement membrane there are chromophil cells, muscle cells, and nerves embedded in connective tissue (Figure 7.10a,b). The pharyngeal "gland cells" of earthworms are not gland cells in the usual sense, and do not communicate with the pharynx. The term *chromophil cells* is used for them because of their intense coloration by hematoxylin and similar stains. Chromophil cells are situated dorsally on the pharynx, and extend backwards as far as septum 5/6. The so-called septal glands of earthworms are aggregations of similar cells at a more posterior level (Keilin 1920) (Figure 7.8).

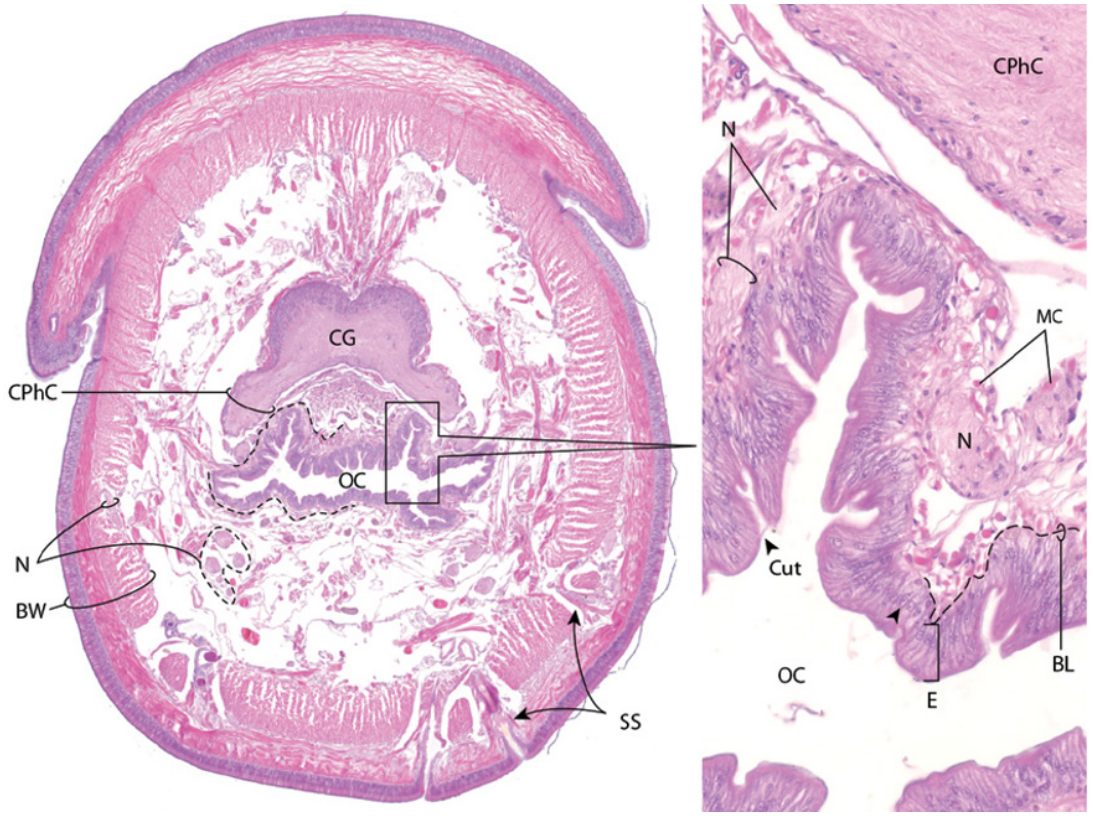


Figure 7.9 Histology of oral cavity. Oral cavity and its wall (Bouin fixation); 20× and 150× (inset). BL, basal lamina; BW, body wall; CG, cerebral ganglion; CPhC, circumpharyngeal connective; Cut, cuticle; E, epithelium of oral cavity; MC, muscle cell; N, nerve; OC, oral cavity; SS, setal sac; dashed line, run of basal lamina of the epithelium.

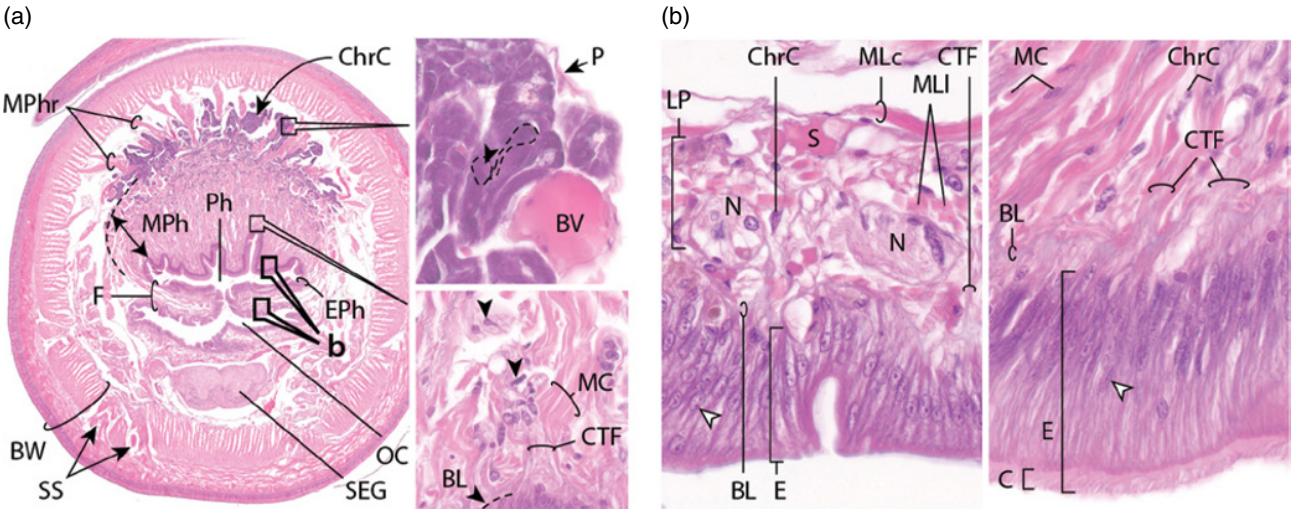


Figure 7.10 Histology of pharynx. (a) Pharyngeal wall and chromophil cells (Bouin fixation), 20× and 500× (insets). (b) Epithelium and subepithelial layer of pharynx (left panel: ventral side, right panel: dorsal side), 600×. BL, basal lamina; BV, blood vessel; BW, body wall; C, cilia; ChrC, chromophil cells; CTF, connective tissue fibers; EPh, epithelium of pharynx; F, fold of pharyngeal wall; LP, lamina propria; MC, muscle cell; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; MPh, muscles of pharynx; MPhr, radial muscles of pharynx; N, nerve; OC, oral cavity; P, peritoneal sheet (capsule); S, sinus; SEG, subesophageal ganglion; SS, setal sac; black arrowhead, nucleus of chromophil cell; white arrowhead, nucleus of epithelial cell.

Chromophil cells are oval or irregular in shape. They do not fit closely together; the interspaces are empty or contain an ECM. The nucleus is large and not conspicuous, spherical or ovoid, 4–6 μm in longest diameter, often peripherally situated. The cell body contains masses of deeply purple-staining material. The less deeply staining areas are more peripherally situated. The more densely colored portion usually encloses the nucleus, and on the whole is more central in position. The cell borders are indistinct and the cell body is prolonged as a fibril-like strand, and becomes progressively metamorphosed into fibrillar connective tissue. While most of the cells form a more or less compact aggregate on the surface of the pharyngeal mass, a number penetrate inwards toward the pharyngeal epithelium. The main function of the cells is probably metabolic.

In *L. terrestris* the wall of the pharynx is composed of obliquely striated longitudinal and circular muscle layers. On the ventral side, the circular muscle layer is a single layer while the longitudinal muscle layer is multilayered (Figure 7.10b). On the dorsal side there are radial muscle bundles, which are the most abundant (Figures 7.8 and 7.10a). They suspend the pharynx and help to dilate it.

7.3.2.3 Esophagus and Calciferous Glands

The esophagus is a straight tube lined with a simple columnar epithelium, which becomes ciliated toward its posterior part. There are well-developed esophageal pouches in segment X and lateral esophageal enlargements in segments XI and XII that are called calciferous glands in *Lumbricus* species (Figure 7.11). Calciferous glands are composed of epithelial plates or tubes in intimate contact with blood sinuses. They are typical of this genus. The location and structure of calciferous or *Morren's glands* are among the most important characters in earthworm taxonomy (Briones and Piearce 2011). The true function of the calciferous glands is excretion of calcium carbonate being passed into the gut as calcite crystals, which are chemically inactive in the gut. In *L. terrestris* in segment X the calciferous subspherical sacs open into the esophagus ventrally via dilation and just in front of septum 10/11. The pouches in segment X are elongated and often contain aggregates of calcite crystals of 0.5–2.5 mm size (Csuzdi and Zicsi 2003). In segments XI and XII they are conspicuous paired swellings (Figure 7.11). Their glandular portions consist of lamellae disposed radially to the esophagus. In addition, it is not unusual to find extracellular formations of variable size in between lamellae as well as calcite crystals (Méndez et al. 2009).

A common feature of the calciferous glands is that their secretory cells produce a concentrated suspension of calcium carbonate (CaCO3). In certain species, this calcareous secretion passes forward to the esophageal pouches where it precipitates as concretions of $CaCO_3$, which are then released to the gut and finally into the soil. These glands provide a mechanism of CO_2 regulation in their tissues; both environmental and metabolic CO_2 can be fixed in this way (Briones et al. 2008). Several functions have been attributed to this secretion. First, and most notably, the calciferous gland was considered by Darwin (Darwin 1881) to be an excretory organ that eliminates excess dietary calcium by fixing carbon dioxide (CO2) to form $CaCO_3$. This excretion, in turn, could act to neutralize humic acids in the ingested litter, making it more nutritious to the worm. On the other hand, the possibility that the $CaCO_3$ secretion might also act, secondarily, in neutralizing the acid content of the gut cannot be discarded. It has been suggested that the secretion of $CaCO_3$ increases with ingestion of acid food, but no decrease in the gland activity of worms living in alkaline soils has been observed. This production and release of $CaCO_3$ concretions could potentially have an important role to play in soil-carbon dynamics (Robertson 1936).

7.3.2.4 Calciferous Gland in Posterior Part of Segment X

This portion of the esophagus is covered by a mucous membrane, which forms folds (Figure 7.12a). This consist of a nonciliated simple columnar epithelium with a cuticle and a lamina propria under it containing chromophil cells and capillaries (Figure 7.12b). The circular and longitudinal muscle layers are thin; the longitudinal muscles are composed of discrete bundles (Figure 7.12b). Chromophil cells attach to the coelomic surface of the organ.

In this segment the calciferous glands make up paired outpocketings of the esophagus called calciferous sacs (Figure 7.12a). Their wall contains thin circular and longitudinal muscle layers like the esophagus itself (Figure 7.12c,d). In the glandular portions, the secretory epithelium folds up and forms lamellae. Each lamella is composed of two layers of a secretory epithelium with a connective tissue layer and blood sinus between them. Secretory chambers are tubuloalveolar type. The epithelium in the bottom of pouches bears very long cilia and club-shaped cytoplasmic extensions (Figure 7.12c,d). Nonciliated epithelium covers the border of calciferous gland sacs next to the esophageal wall (Figure 7.12b). Calcium spherulites can be identified in some of the secretory pouches (Figure 7.12c,d). Degrading cells (foamy cells) are in the epithelial lining and have foamy cytoplasm and a pyknotic nucleus (Figure 7.12d, inset).

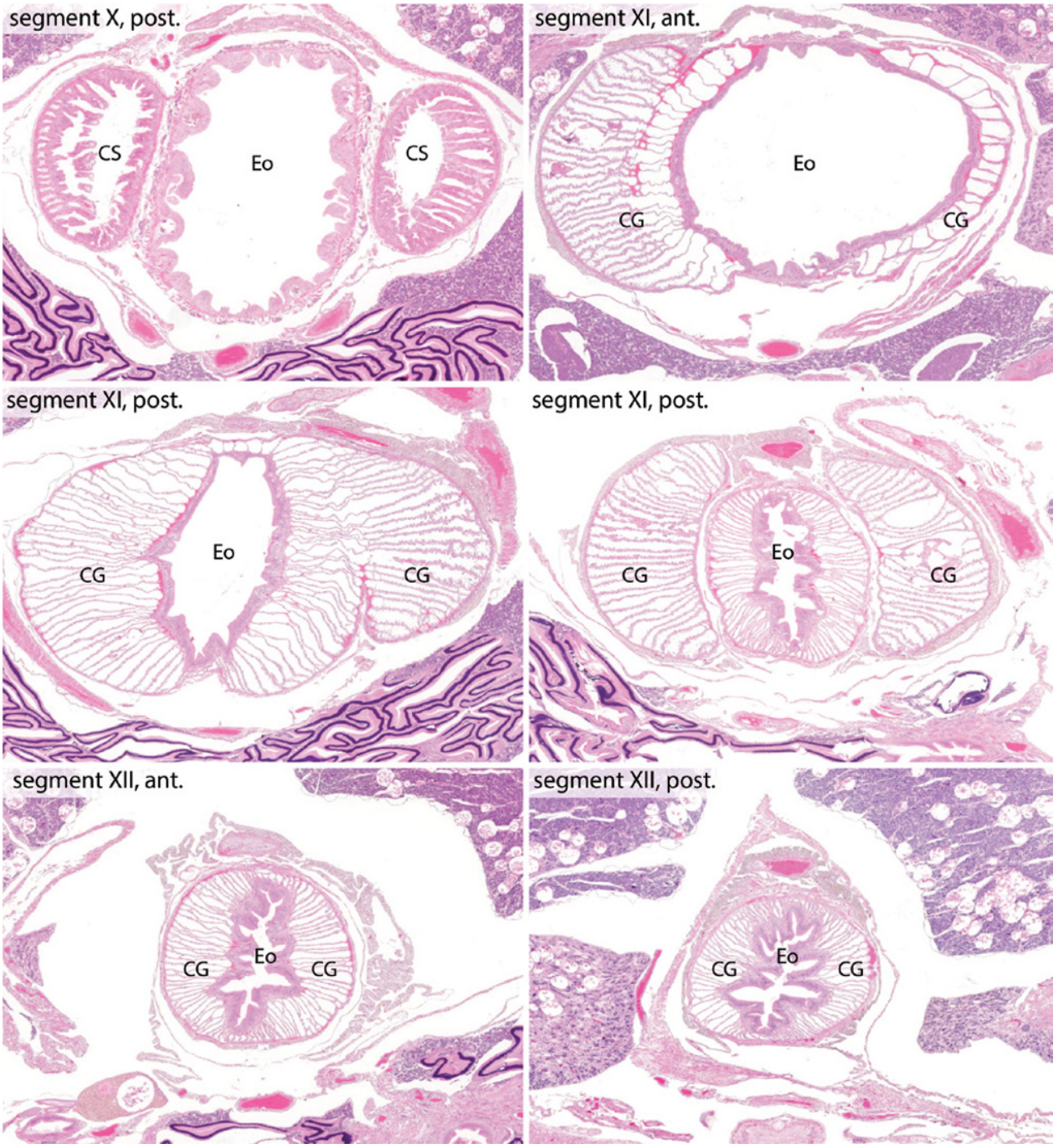


Figure 7.11 Morphology and position of esophagus and calciferous glands in segments X–XII (formalin fixation), 20× (all micrographs). CG, calciferous gland; CS, calciferous sac; Eo, esophagus.

7.3.2.5 Calciferous Gland in Anterior Part of Segment XI

The lateral wall of this portion of the esophagus is covered by a ciliated simple columnar epithelium, while the dorsal and ventral surfaces are nonciliated as in the previous segment (Figure 7.13a, inset). There are signs of secretion in the nonciliated epithelium (Figure 7.13a, inset). The visible thickening of the esophageal wall is caused by the lamina propria, which contains the glandular pouches disposed radially to the esophagus (Figure 7.13a). In this segment the calciferous glands are embedded in the wall of the esophagus; there are no separate calciferous sacs

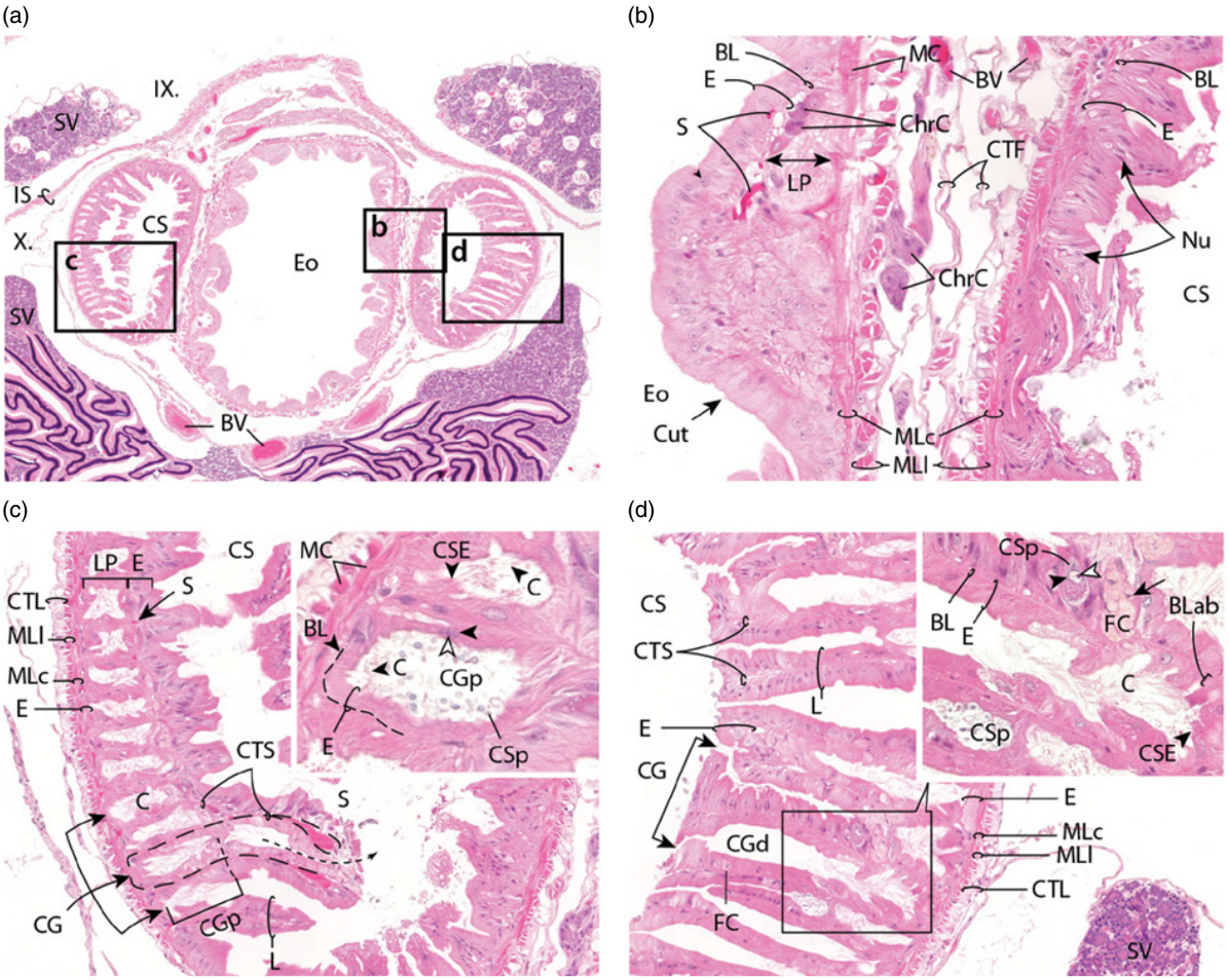


Figure 7.12 Esophagus and calciferous gland of segment X. (a) Esophagus and calciferous gland sacs in the caudal part of the Xth segment (formalin fixation), 35×. (b) Wall of esophagus and medial portion of calciferous gland sac (formalin fixation), 300×. (c) Tubulo-alveolar glands of calciferous gland sac (formalin fixation), 200× and 600×. (d) Tubulo-alveolar glands of calciferous gland sac (formalin fixation), 200× and 600×. BL, basal lamina; BLab, basal labyrinth; BV, blood vessel; C, cilia; CG, calciferous gland; CGd, calciferous gland, distal part; ChrC, chromophil cells; CS, calciferous sac; CSp, calcium spherulite; CSE, club-shaped extension; CTF, connective tissue fibers; CTL, connective tissue layer; CTS, connective tissue septum; Cut, cuticle; E, epithelium; Eo, esophagus; FC, foamy cell; IS, intersegmental septum; L, lamella; LP, lamina propria; MC, muscle cell; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; Nu, nucleus of epithelial cell; S, sinus; SV, seminal vesicle lobe; IX, IXth segment, coelomic cavity; X, Xth segment, coelomic cavity; black arrow, pyknotic nucleus of foamy cell; dotted arrow, secretion; black arrowhead, nucleus of gland cell; white arrowhead, nucleolus of gland cell.

(Figure 7.13a). The secretory epithelium is low-columnar and ciliated in the center of the glandular portions, and tall-columnar with abundant secretion at the periphery (Figure 7.13a,b). The epithelium in the bottom of pouches has basal striation and apically club-shaped cytoplasmic extensions (Figure 7.13b, inset). The secretory chambers are separated by lamellae, which are composed of two layers of a secretory epithelium with a connective tissue layer and blood sinus between them. There is a conspicuous blood sinus network at the border of the central and peripheral portion of the glandular pouches (Figure 7.13a, arrow). Calcium spherulites can be identified in most of the secretory pouches (Figure 7.13b).

7.3.2.6 Calciferous Gland in Anterior Part of Segment XII

This portion of the esophagus is covered by a mucous membrane, which forms folds (Figure 7.14a). This consists of a high ciliated simple columnar epithelium and an enlarged lamina propria layer under it containing glandular pouches and degrading foamy cells and capillaries (Figure 7.14b). In this segment the calciferous glands are embedded in the

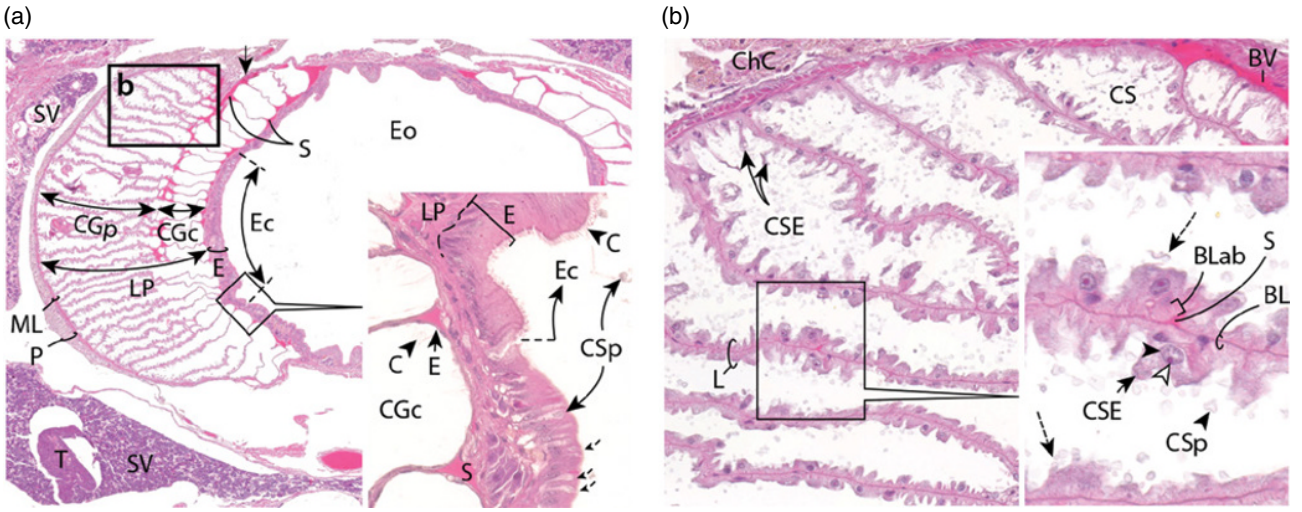


Figure 7.13 Esophagus and calciferous glands of segment XI. (a) Esophagus with calciferous glands in the anterior part of XI segment (formalin fixation), 50× and 500× (inset). (b) Peripheral part of calciferous glands with secretory epithelium (formalin fixation), 300× and 1000× (inset). BL, basal lamina; BV, blood vessel; C, cilia; CGc, calciferous gland, central part; CGp, calciferous gland, peripheral part; ChC, chloragogen cell; CSp, calcium spherulite; CSE, club-shaped extension; E, epithelium; Ec, ciliated epithelium; Eo, esophagus; L, lamella; LP, lamina propria; ML, muscle layer; P, peritoneum; S, sinus; SV, seminal vesicle lobe; T, testis; black arrow, sinusoid system; dotted arrow, secretion; black arrowhead, nucleus of gland cell; white arrowhead, nucleolus of gland cell.

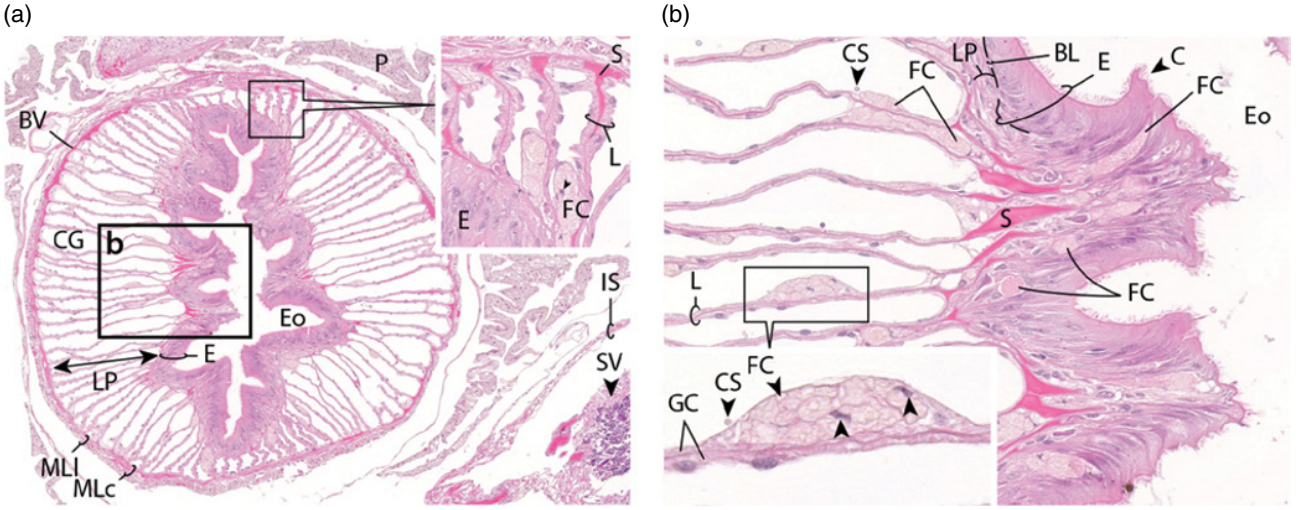


Figure 7.14 Esophagus and calciferous glands of segment XII. (a) Esophagus and calciferous glands in the anterior part of the segment XII (formalin fixation), 85× and 700× (inset). (b) Esophagus epithelium and lamellae of the calciferous glands (formalin fixation), 350× and 1000× (inset). BL, basal lamina; BV, blood vessel; C, cilia; CG, calciferous gland; CS, calcium spherulite; E, epithelium; Eo, esophagus; FC, foamy cell; GC, gland cell; IS, intersegmental septum; L, lamella; LP, lamina propria; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; P, peritoneal cell; S, sinus; SV, seminal vesicle; black arrowhead, nucleus of foamy cell.

wall of the esophagus and the glandular pouches are arranged radially around the esophagus. The circular and longitudinal muscle layers appear to be thin (Figure 7.14a). In the glandular portions, the secretory epithelium folds up and forms lamellae. The secretory epithelium is low and nonsecreting. Each lamella is composed of two layers of epithelium with a connective tissue layer and blood sinus between them. In the epithelial lining of lamellae, degrading foamy cells are observable with foamy cytoplasm and pyknotic nucleus (Figure 7.14b). There are very few calcium spherulites in the secretory pouches (Figure 7.14b).

7.3.2.7 Crop

In *Lumbricus* the distal end of the esophagus dilates into a wide crop, which is thin-walled and acts as a storage chamber. The mucosal epithelium is composed of a high, simple

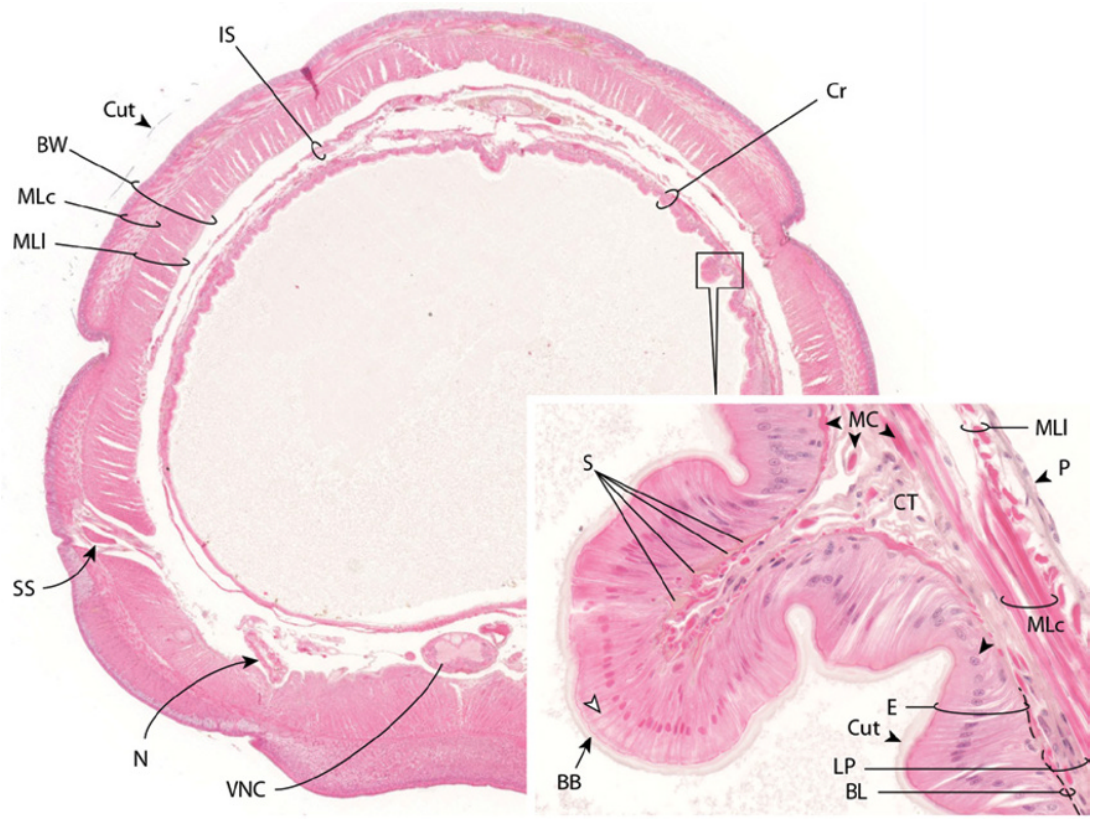


Figure 7.15 Histology of the crop (formalin fixation), 15× and 350× (inset). BB, brush border; BL, basal lamina; Cr, crop wall; CT, connective tissue; Cut, cuticle; E, epithelium; IS, intersegmental septum; LP, lamina propria; MC, muscle cell; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; N, nephridium; S, sinus; SS, setal sac; VNC, ventral nerve cord; black arrowhead, nucleus of epithelial cell; white arrowhead, supranuclear zone of epithelial cell.

columnar epithelium with a brush border (Figure 7.15). The brush border is embedded in the cuticle. Chitin is present in the cuticle of the crop and gizzard of Lumbricidae (Rudall 1955). The lamina propria makes folds and contains muscle cells and sinuses. The muscle layers (inner circular and outer longitudinal) are thicker than in the esophagus, but are continuous with the esophageal muscle layers. The outer surface is covered with a connective tissue sheath overlain by peritoneal cells (Figure 7.15). The endodermal origin of the crop and gizzard was demonstrated for *L. terrestris* by Menzi (Menzi 1919); the pharynx, however, is of ectodermal origin and is lined with a collagenous cuticle like the epidermis.

7.3.2.8 Gizzard

The gizzard, which is used for grinding food particles, is lined with cuticle and is highly muscular. The epithelium consists of narrow columnar cells; intimate connections between the cells are probably needed to resist mechanical stress, which is exerted on the epithelium when the underlying musculature works in order to triturate food particles or to transport food from the gizzard into the adjoining midgut. In the apical part, cell borders begin with septate junctions, whereas in basal parts adjoining the thick basement membrane, hemidesmosomes are found. In the lateral parts of the cells, the interdigitating parts may widen to form a labyrinth system of intercellular spaces (Figure 7.16). The apical part of the gizzard epithelium is covered with very long microvilli, usually measuring 4–6 µm. These are covered with a well-developed, filamentous surface coat. The basal parts of the epithelial cells show an intricate system of deep infoldings. Smaller cells are interspersed between the bases of the epithelial cells; these may be used during growth or regeneration of the epithelium (Figure 7.16). Numerous bundles of nerves are below these cells or at the base of normal epithelial cells.

A thick cuticle is secreted by and covers the gizzard epithelium. The nucleus of gizzard epithelial cells is irregular or oval and approximately in the middle of the cell. Vierhaus demonstrated enzymes, including protease, in the cuticle of crop and gizzard in *L. terrestris* (Peters and Walldorf 1986). The cuticle of the gizzard seems to be neither an aggregation of peritrophic membranes nor a protective cuticle, but it is a secretory product. The cuticle can

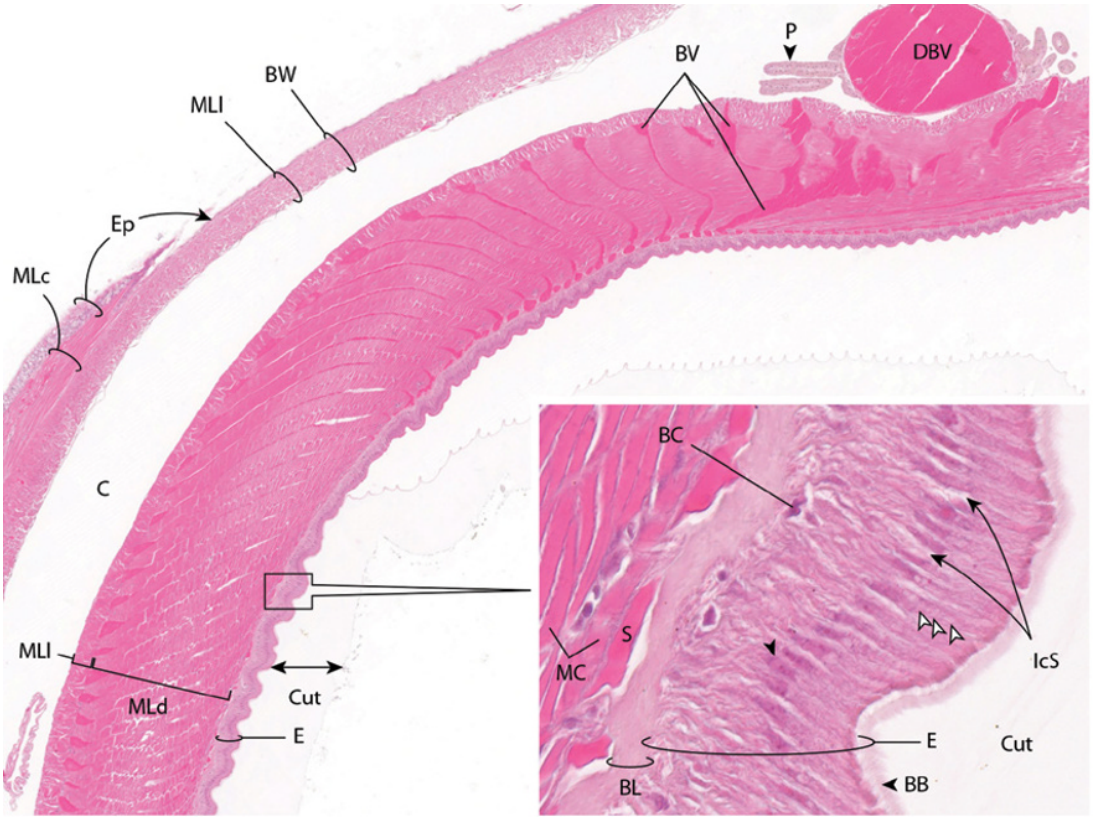


Figure 7.16 Wall of the gizzard (formalin fixation), 40× and 600× (inset). BB, brush border; BC, basal cell; BL, basal lamina; BV, blood vessel; BW, body wall; C, coelomic cavity; Cut, cuticle; DBV, dorsal blood vessel; E, epithelium of gizzard; Ep, epidermis; IcS, intercellular spaces; MLc, muscle layer, circular; MLd, muscle layer, diagonal; MLl, muscle layer, longitudinal; S, sinus; black arrowhead, nucleus of epithelial cell; white arrowhead, secretory granules in supranuclear zone.

be regarded as a carrier and reservoir for enzymes, at least for amylase. Proteases seem to be absorbed by the sponge-like cuticle.

The strong muscles of the gizzard may be used to force food into the midgut, and they may be used in collaboration with hard food particles to grind the food (Figure 7.16). In both cases, the epithelium has to resist considerable mechanical wear. It has probably, therefore, an unusually thick basal lamina and an elaborate system of interdigitations and desmosomes on the lateral parts of its cells. Other conspicuous properties seem to be connected with its high secretory activity. The blood sinus between the muscle layer and basal lamina, and the basal infoldings of the epithelium should be important for the supply of precursors for the synthesis of the cuticle. The nerve bundles at the base of the epithelial cells resemble those in the midgut epithelium. They appear to be neurohemal organs as they are synaptoid (i.e., without a postsynaptic counterpart), and they show an accumulation of smaller and larger types of vesicles ultrastructurally. They may control either the secretory activity of the epithelium and/or the movements of the muscle layer.

7.3.2.9 Midgut and Typhlosole

The intestine extends as a straight tube through the posterior two-thirds of the body. The anterior half of the intestine is the midgut, the principal site of secretion and digestion, and the posterior half, the hindgut, is absorptive. The midgut epithelium is composed of ciliated (absorptive) and glandular (secretory) cells (Figure 7.17a). Glandular cells are markedly varied in size, form, relative prominence, and number of inclusions – zymogen granules. The inclusions (described as granules from their appearance in fixed material) are enclosed in vacuoles. In addition to the usual classes of digestive enzymes, the earthworms secrete cellulase and chitinase (Millott 1948). The absorbed food materials are passed to blood sinuses that lie between the mucosal epithelium and the intestinal muscle layers. In many annelids, chitin is a component of peritrophic membranes, which are secreted by the midgut epithelium.

A remarkable feature of the epithelium is the investment of the glands by a sheath of four or five ciliated cells. It is subject to phasic change, especially at the free border, undergoing alteration under varying physiologic conditions. In certain phases, the free tips of the gland cells are

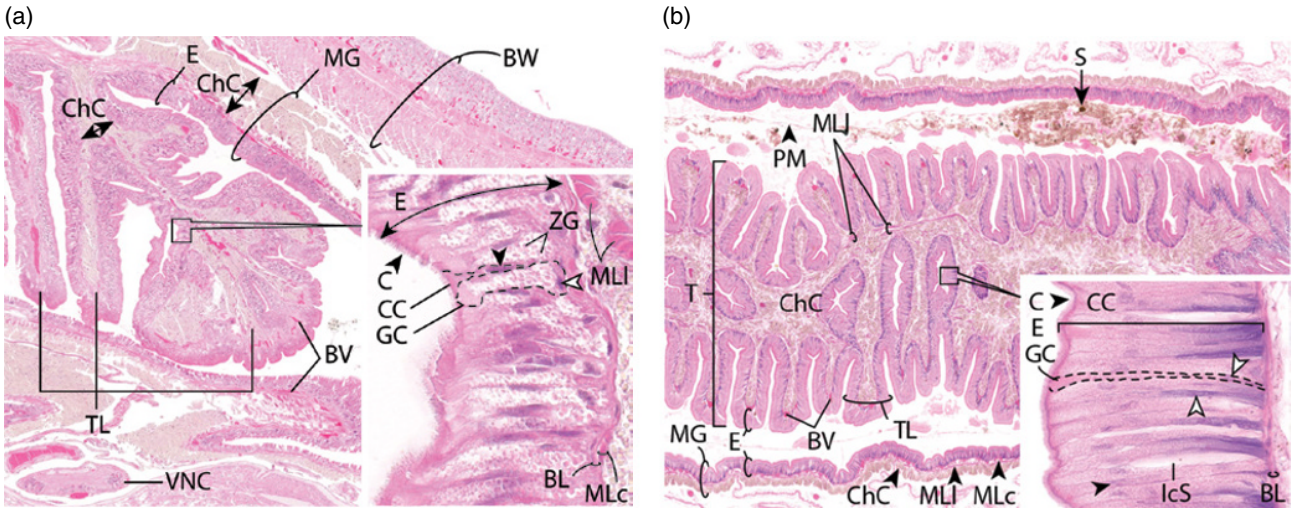


Figure 7.17 Histology of the midgut wall. (a) Midgut and typhlosole in cross-section (formalin fixation), 45× and 900× (inset). (b) Midgut and typhlosole in horizontal section (Bouin fixation), 30× and 900× (inset). BL, basal lamina; BV, blood vessel; BW, body wall; C, cilia; CC, ciliated cell; ChC, chloragogen cell; E, epithelium; GC, gland cell; IcS, intercellular space; MG, midgut wall; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; S, soil; T, typhlosole; TL, typhlosole, lobe; ZG, zymogen granules; VNC, ventral nerve cord; black arrowhead, nucleus of ciliated cell; white arrowhead, nucleus of gland cell.

completely overarched by the free ends of the ciliated cells, which thus form a continuous cover over them (Figure 7.17a). But frequently the end of the gland nearest the free border is drawn out into a long neck or papilla (Figure 7.17a), which becomes intercalated between the free ends of the ciliated cells and may even reach the lumen. There is no lamina propria under the lamina epithelialis. The midgut wall has two muscle layers: an inner circular and an outer longitudinal. The outer midgut wall is covered by chloragogen cells (Figure 7.17a,b).

A ridge or fold, called the typhlosole, projects internally from the middorsal wall of the midgut of *Lumbricus*. It is characteristic in many terrestrial oligochaetes, but its the size varies; it may consist of a simple fold or of multiple folds. In *Lumbricus* it is well developed and contains many folds in cross-section and in horizontal section as well (Figure 7.17a,b). The use of the shape of typhlosoles in earthworm taxonomy has long been debated. Early authors assigned evolutionary importance to this character, while subsequent researchers regarded the shape of typhlosoles as ecological adaptations with limited taxonomic value (Csuzdi and Zicsi 2003).

The midgut of the animal in Figure 7.17a does not contain any soil remnants as it was starved before dissection. Soil remnant and a thin peritrophic membrane around it are observable in the midgut of the animal in Figure 7.17b, which was not starved.

7.3.2.10 Hindgut

The hindgut is short and lined with epithelium from the proctodeum that is low simple columnar epithelium covered with cuticle (Figure 7.18). The basal lamina is undulant. On the other side of the basal lamina, chloragogen cells with chloragosomes can be observed. These cells form little nests under the waves of the epithelium. Beneath the mucous membrane, the circular muscle layer is quite thick; the longitudinal muscle layer is thinner and is composed of discrete bundles (Figure 7.18, inset).

7.3.3 Excretory System

The excretory system of *Lumbricus* is composed of two different and spatially separated substructures: the podocytes and the metanephridium. In annelids the podocytes generally rest on the coelomic side of the perivascular ECM (Bartolomaeus and Quast 2005). The metanephridium is typically attached to a septum, with a preseptal ciliated funnel, a coiled postseptal canal in two lobes, and a third lobe containing a terminal swollen muscular bladder which empties periodically through the nephridiopore (Goodrich 1945).

7.3.3.1 Podocytes

Podocytes consist ultrastructurally of a perikaryon and a flat peripheral part that is perforated by meandering slits. If there are two or more podocytes, they will interdigitate and will be connected by adherent junctions to each other and to adjacent peritoneal cells or epitheliomuscular cells. The meandering slits are bridged by additional extracellular material, which represents the actual site of filtration. Thus, the slashed periphery of the podocytes is the supporting structure of the filtration barrier and is functionally comparable to the filter of the terminal cell. However,

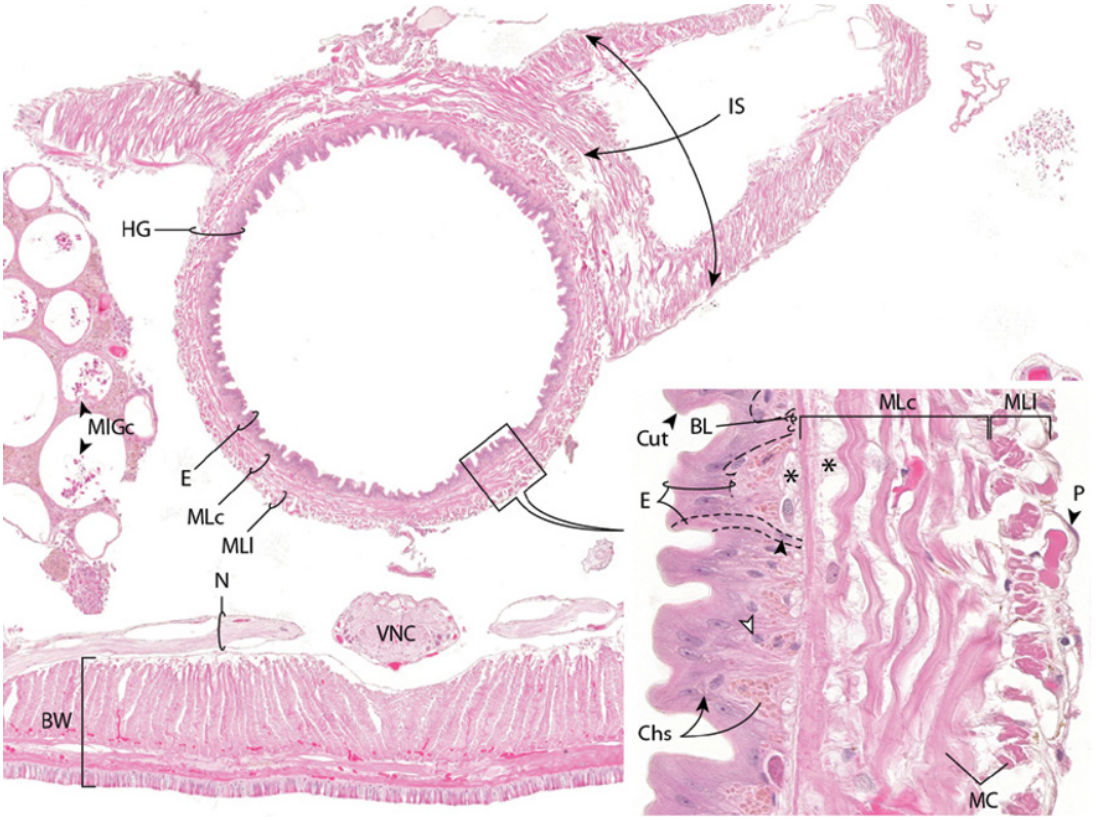


Figure 7.18 Histology of the hindgut. Formalin fixation, 40× and 550× (inset). BL, basal lamina; BW, body wall; Chs, chloragosome; Cut, cuticle; E, epithelium; HG, hindgut; IS, intersegmental septum; MlGc, *Monocystis lumbrici* encapsulated gametocysts with sporozoites; MLc, muscle layer, circular; MLl, muscle layer, longitudinal; N, nerve; P, peritoneum; VNC, ventral nerve cord; asterisk, extracellular matrix; black arrowhead, nucleus of epithelial cell; dashed line, run of basal lamina; white arrowhead, nucleus of chloragogen cell.

in contrast to the latter, it is never connected to duct cells. This is an important criterion to distinguish the podocyte from terminal cells ultrastructurally (Goodrich 1945).

7.3.3.2 Metanephridium

The metanephridium is divisible into two portions: a preseptal part and a postseptal duct. The preseptal part consists of the ciliated funnel or nephrostome and a short ciliated tube. The postseptal portion is a more extensive nephridial duct (nephrotubule). Metanephridia in Clitellata are mesodermal, which has been shown by cell lineage studies.

7.3.3.2.1 Ciliated Funnel Starting with the ciliated funnel in one segment, the metanephridia pierce the septum and open to the exterior in the following segment (Figures 7.19a and 7.20a). The internal opening is enlarged and consists of a wide funnel that rests on the septum close to the ventral nerve cord. The cells of the funnel are generally multiciliated and rest on the septal matrix. They are composed of three different substructures: the perikaryon, microvilli, and a ciliary element. Adjacent to the funnel epitheliomuscular cells or peritoneal cells are found. These are connected to the funnel cells by adherent junctions and also rest on the septal matrix.

The nephridiostome, small and simple in the lower Oligochaeta, becomes progressively larger and of more elaborate structure in the higher lumbricids. A prominent expanded upper lip is established, consisting of many ciliated marginal cells surrounding a ciliated central cell first discovered by Benham (Benham 1891) in *Lumbricus*. Here the lower lip is not ciliated, contains many nuclei, and usually has many ameboid coelomic corpuscles attached to it. Between the upper and lower lip there is a gutter, where the gutter cells surround the entrance of the nephrotubule. The ciliated funnel consists of a number of marginal cells, which are very long, narrow, and somewhat wedge-shaped arranged around a central point. The nucleus in each is placed near the outer end of the cell, which is rounded off peripherally, and ciliated entirely on one surface (Figure 7.19a,b).

As a rule, delicate sifting cilia on the upper lip of the nephridiostome tend to prevent any but the finest particles from passing into the nephridium, and it is well known

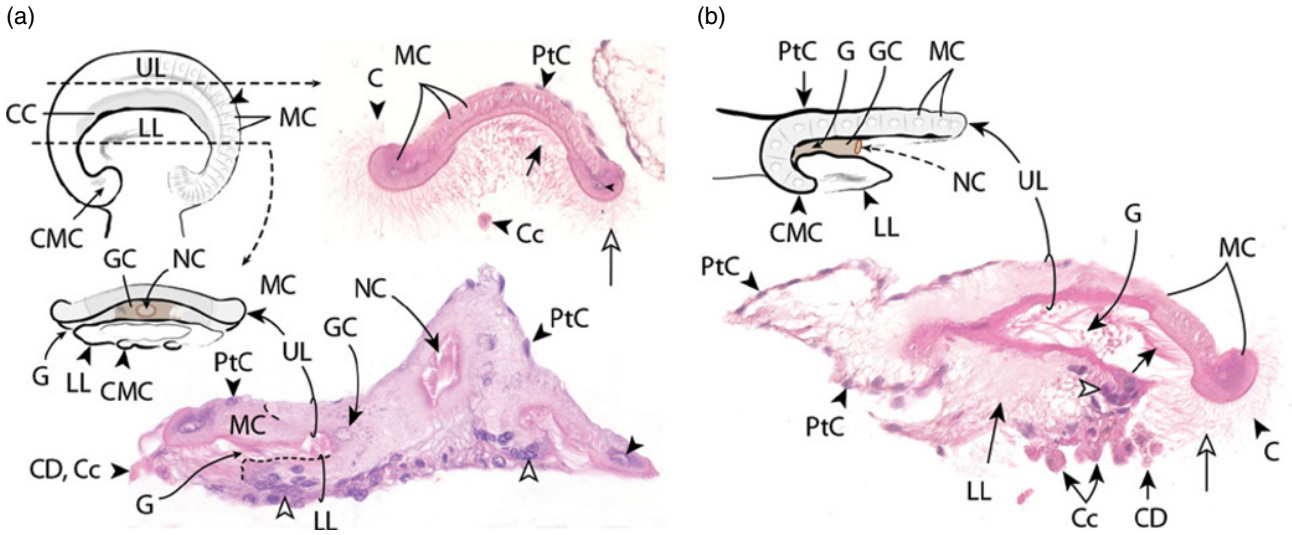


Figure 7.19 Microstructure of the ciliated funnel/nephrostome (drawings after Goodrich [1945]; for a better overview, cilia are not represented). (a) Cross-sections of nephrostome (*top*: Bouin fixation, *bottom*: formalin fixation), 500×. (b) Parasagittal section of nephrostome (formalin fixation), 500×. C, cilia; Cc, coelomocyte; CC, central cell in upper lip; CD, cell debris; CMC, centripetal marginal cell; G, gutter between upper and lower lips; GC, gutter cell; LL, lower lip; MC, marginal cell; NC, nephridial canal; PtC, peritoneal cell; UL, upper lip; black arrow, shorter cilia on inner surface of marginal cell; white arrow, long cilia on rim of marginal cell; black arrowhead, nucleus of marginal cell; white arrowhead, nucleus of lower lip.

that phagocytic leukocytes accumulate on the lower lip in lumbricids. These facts may be related to the necessity of preventing the long, narrow, and tortuous nephrotubule from being blocked by waste material (Benham 1891).

7.3.3.2.2 Nephrotubule The nephridiostome is followed by the narrow (about 30 µm in diameter), thin-walled tube (i.e., nephrotubule), which is ciliated over certain regions. It traverses the first loop, then the second loop, then traverses the first loop a second time in the reverse direction (Figure 7.20a,b). At the base of the first loop it is succeeded by the middle tube lumen, which suddenly enlarges to about 60 µm or more; the walls become much thicker, bear cilia, and have brown granules in the cytoplasm (Figure 7.20a,b3,d1).

The middle tube runs from the base of the second loop almost to its tip, gradually decreasing in diameter to about 20 µm, and then opens into the wide tube. In the wide tube four regions can be distinguished. At its commencement it shows a wide dilation, up to 100 µm or more in diameter, and bends around the tip of the second loop; this is known as the ampulla. It is not histologically distinguishable from the following region, known as the proximal segment, both of which have thick walls with radial striations in the basal portion of the cytoplasm and a brush border adjacent to the lumen (Figure 7.20d1). The wide tube traverses the second loop from tip to base. This part is the middle segment; it decreases in diameter, its walls become thinner, and in histologic section, it shows a gradual reversion toward the narrow tube (Figure 7.20b2). It leaves the second loop and traverses the first loop (Figure 7.20a). At the base of the first loop, there is a sudden transition to the distal segment, about 70 µm in diameter, with thicker walls showing the same basal striations as the proximal segment but lacking the brush border. The distal segment describes a wide sweep around the ventral part of the nephridium and enters the third loop (Figure 7.20a). About halfway toward the tip of the third loop, it opens into the bladder, but not at the point where the diameter of the nephridial duct appears to enlarge (Benham 1891). The wall of the bladder has a very thin epithelial lining surrounded by muscle fibers; it is also called the muscular duct (Figure 7.20a,c,d2). The bladder completes the third loop and enters the body wall under the ventral part of the nephridium to open eventually at the nephridiopore, just above the ventrolateral setae. The whole organ is covered with a layer of peritoneal epithelium, which forms a membrane uniting the three loops. The whole of the nephrotubule with the exception of the bladder is intracellular (Ramsay 1949).

7.3.4 Circulatory System

Like most triploblastic animals, the earthworm has a well-developed coelom and a blood vascular system. The coelom is highly compartmentalized, with distinct left and right cavities housed in each body segment. The coelomic fluid provides an important hydrostatic skeleton that enables locomotion. The blood vascular system is closed with

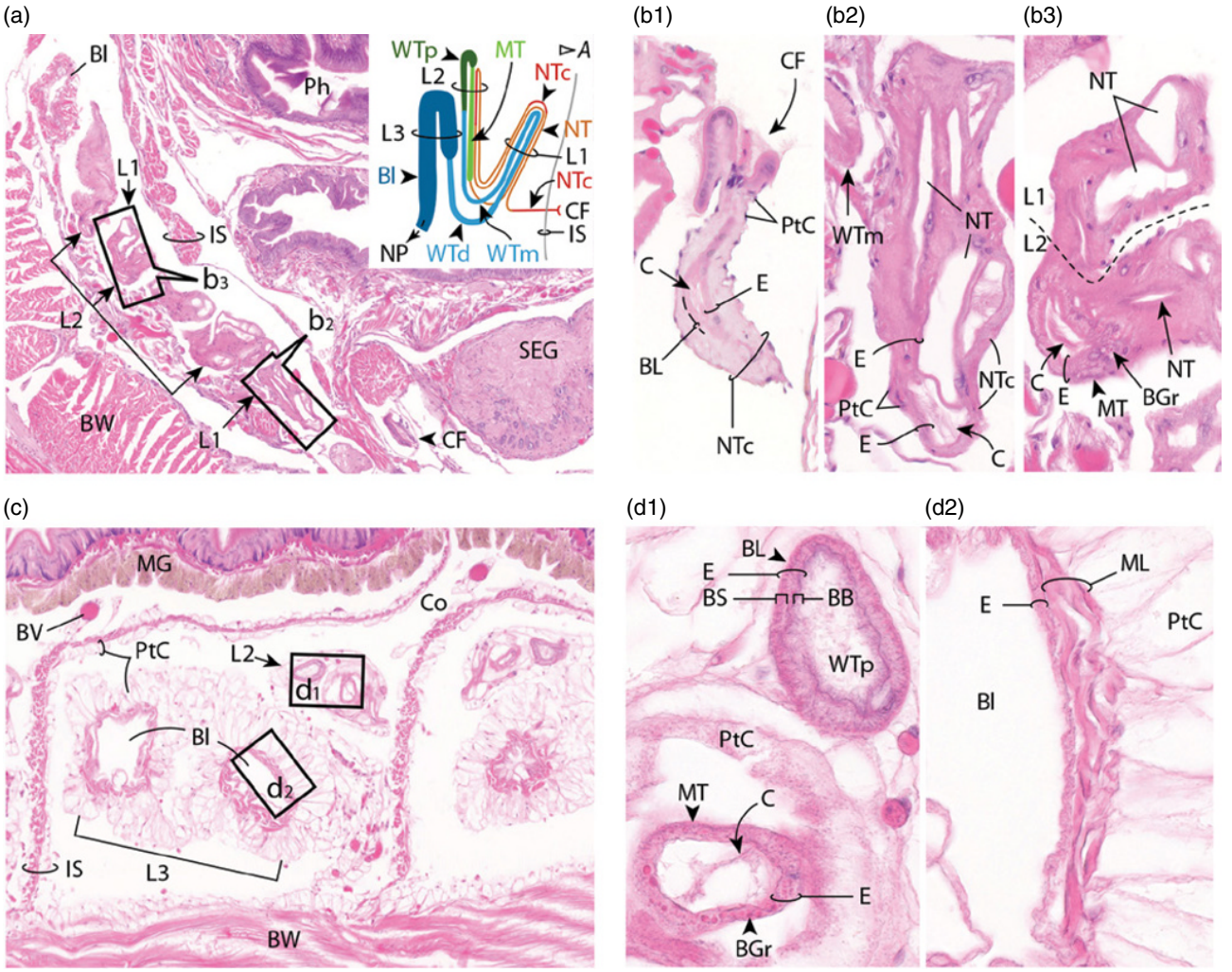


Figure 7.20 Histology of the nephridial duct. (a) Overview of metanephridium in cross-section and its reconstruction (Bouin fixation), 70× (drawing based on Ramsay [1949], anterior end toward right). (b) Preseptal part (b1) and segments of first and second loops (b2, b3) (Bouin fixation), 550×. (c) Second and third loop in horizontal section (Bouin fixation), 100×. (d) Segments of second (d1) and third (d2) loop in higher magnification (Bouin fixation), 600×. A, anterior direction; BB, brush border; BGr, brown granules; Bl, bladder; BL, basal lamina; BS, basal striation; BV, blood vessel; BW, body wall; C, cilia; CF, ciliated funnel; Co, coelom; E, epithelium; IS, intersegmental septum (dissepiment); L1, first loop; L2, second loop; L3, third loop of nephridial duct; MG, midgut; ML, muscle layer; MT, middle tube; NP, nephridiopore; NT, narrow tube, nonciliated portion, NTc, narrow tube, ciliated portion; Ph, pharynx; PtC, peritoneal cell; SEG, subesophageal ganglion; WTd, wide tube, distal segment; WTm, wide tube, middle segment; WTp, wide tube, proximal segment.

an extensive capillary bed. There is no heart; rather, circulation is carried out by contractile blood vessels. In annelids, blood is propelled by the pumping action of the blood vessels, which in turn is mediated by the contraction of specialized, differentiated coelomic mesothelial cells (myoepithelial cells).

7.3.4.1 Blood Vessels

There are three main longitudinal vessels: the dorsal vessel, the ventral vessel, and the subneural vessel (Figure 7.21a,b). The dorsal vessel collects blood from other vessels and drives it forwards through contractile peristaltic waves that originate at the posterior end of the animal and move forward. Anteriorly, the dorsal vessel ceases to be a collecting vessel and gives rise to five pairs of large commissures that connect to the ventral vessel. These commissures contain lateral "hearts" (pseudohearts), whose contractions are asynchronous with those of the dorsal vessel. The ventral vessel, which is suspended in the mesentery beneath the gut, is the main distributing channel, sending blood posteriorly behind the hearts. The ventral vessel has no peristaltic activity. In each segment the ventral vessel gives rise to segmental vessels and capillaries.

Invertebrate vessels are always lined by ECM. An extracellular layer of mesodermal cells, the basal lamina or basement membrane (Figure 7.21) outlines the vessels.

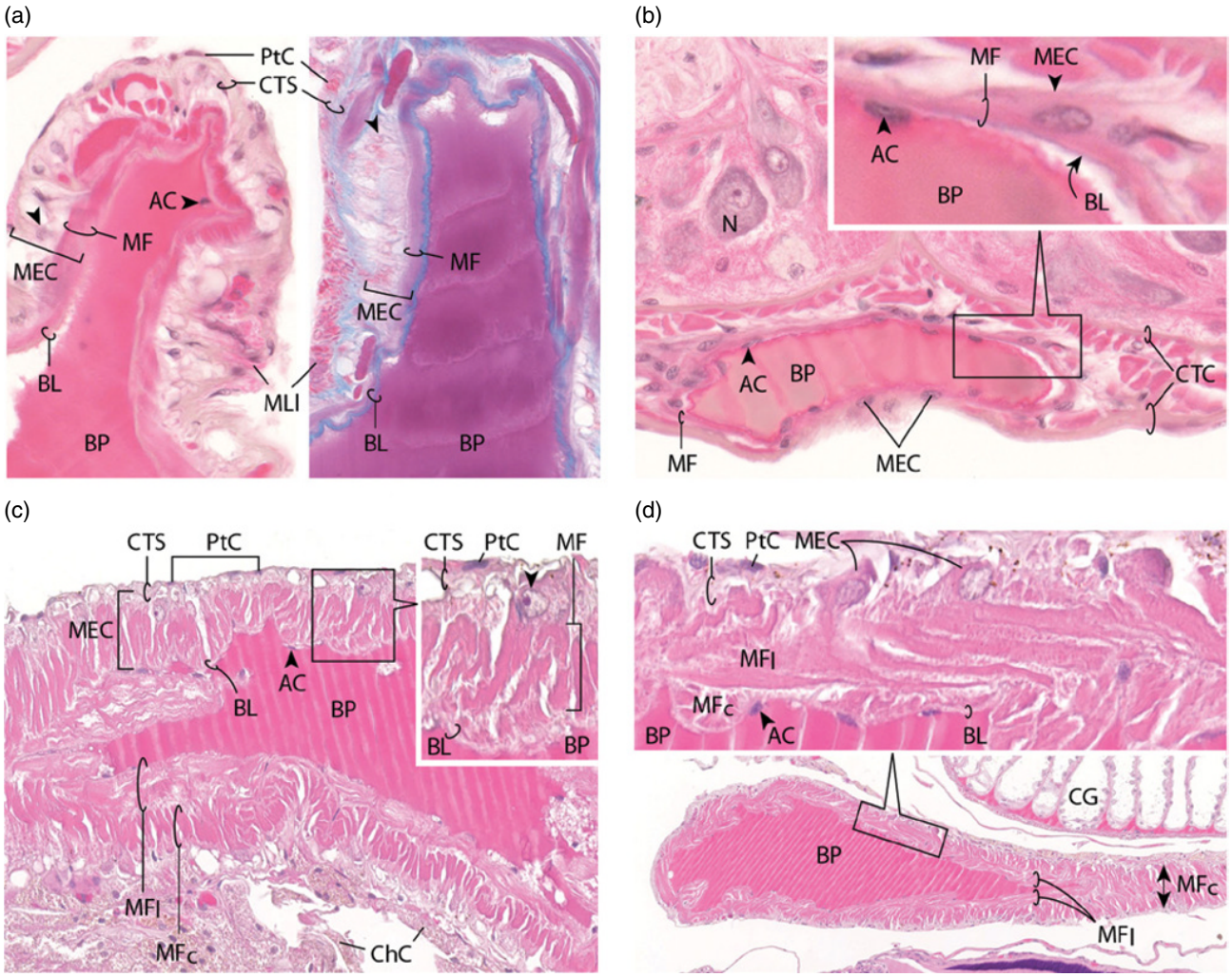


Figure 7.21 Histomorphology of blood vessels. (a) Ventral blood vessel; 600×; formalin fixation: HE and Bouin fixation: Azan. (b) Subneural blood vessel; 600× and 1000×. (c) Wall of a pseudoheart (segment XI); 400× and 1000×. (d) Wall of a pseudoheart (segment X); 150× and 1000×. AC, amebocyte; BL, basal lamina; BP, blood plasma; CG, calciferous gland; ChC, chloragogen cell; CTC, connective tissue capsule of ventral nerve cord; CTS, connective tissue sheath; MEC, myoepithelial cells; MF, myofibrils; MF_c, myofibrils, circular; MF_l, myofibrils, longitudinal; MLl, muscle layer, longitudinal; N, neuron; PtC, peritoneal cell; black arrowhead, nucleus of myoepithelial cell.

The thickness of this basement membrane varies across the vascular tree (0.03–1 µm). Only vertebrates possess a true endothelial lining, defined as a layer of epithelial cells expressing basoapical polarity (with the apical surface facing the lumen), intercellular junctions and anchoring to basement membrane. Blood vessels of the earthworm are lined only by a basement membrane and lack an endothelial layer (Figure 7.21). Small vessels and capillaries can be considered sinuses.

The dorsal vessel of the earthworm is embedded in the dorsal surface of the alimentary tract and traverses the length of the animal (see Figure 7.3). The dorsal vessel is a pulsating channel with very well-developed musculature in the wall. The innermost continuous layer of the vessel wall is a basement membrane about 1 µm in thickness. Outside this layer lie highly specialized myoendothelial cells corresponding to the inner muscular layer. The myofilaments in these myoendothelial cells are oriented longitudinally. Basement membrane material often extends between the different processes of the longitudinal myoendothelial cells. The myofilaments in these cells are grouped into myofibrils with tubular endoplasmic reticulum occupying the interfibrillar sarcoplasm. A row of circular profiles of endoplasmic reticulum can be traced from the interfibrillar endoplasmic reticulum to the surface cell membrane. Outside the longitudinal myoendothelial cells,

there is a well-developed circular muscle layer in which the myofibrils and interfibrillar endoplasmic reticulum are well organized.

The ventral vessel is formed by well-developed myoepithelial cells. Their basal (myofibrillary) parts form two layers; myofibrils running in both directions (circular and longitudinal) can be seen in the same cell. In the lateral part of the vessel wall, well-developed longitudinal myoepithelial cells can be observed. These also lie in contact with the internal basement membrane lining the lumen. On the peritoneal side of the lining cell layer there is a connective tissue sheath resembling that lining the lumen (Figure 7.21a).

The subneural vessel is embedded in the capsule of ventral nerve cord (Figure 7.21b). Myoendothelial cells surrounding the subneural vessel belong to this capsule, thus the outer surface of the myoendothelial cells is encompassed directly by connective tissue space. In the wall of the subneural vessel under the basal lamina, one layer of myoepithelial cells can be observed, in which myofibrils seem to be arranged circularly (Figure 7.21b).

The dorsal vessel is contractile and is the principal means by which the blood is propelled. Five pairs of anterior commissural vessels surrounding the esophagus are also contractile. They are referred to as pseudohearts (see Figure 7.3). The dorsal vessel and five pairs of pseudohearts are lined with a continuous complete layer of cells displaying myofilaments and hence qualifying for the designation of myoepithelial cells (myoendothelial cells) (Figure 7.21c,d). These myofilament-rich mesodermal cells (myoepithelial cells) have a contractile function. Myoepithelial cells have varying numbers of microfilaments, ranging from a delicate endowment of thin myofilaments in the capillary to highly specialized myoendothelial cells in the main pulsating dorsal blood trunk, which serves as the worm's pseudoheart or propulsive "aorta." The myoepithelial cells most specialized for contraction display well-organized sarcoplasmic reticulum and myofibrils with thick and thin myofilaments resembling those of the earthworm body wall musculature. In the ventral circulatory trunk, circular and longitudinal myofilaments are found in each myoepithelial cell. In the dorsal trunk, the lining myoepithelial cells contain longitudinal myofilaments. Outside these cells are circular muscle cells. The lateral parts of the dorsal vessels have an additional outer longitudinal muscle layer (Hama 1960).

The blood vessels of some invertebrates, including annelids, have cells lining the luminal surface, internal to the basement membrane. These cells have sometimes been referred to as endothelial cells. However, they form an incomplete lining, they lack intercellular junctional complexes typical of vertebrate endothelial cells and they rarely appear attached to the underlying basal lamina. A more appropriate term for this cell type is an amebocyte (Figure 7.21a–d). It likely represents a type of circulating hemocyte, which is distinct from endothelial cells. These lie against the inner lining of the basement membrane, covering only a small fraction of its surface. They have long, attenuated branching cell processes. All the vessels except the capillaries display scattered stellate amebocytes with intricate tenuous processes (Monahan-Earley et al. 2013).

The circulatory system has co-evolved with gas exchange mechanisms. Some invertebrates obtain their oxygen by simple diffusion through the skin. Integumentary gas exchange is characteristic of small soft-bodied animals with high surface:volume ratios. The earthworm has an extensive intradermal capillary network, which provides a high surface area for such exchange (Figure 7.4d). The blood plasma contains suspended particles of the iron-containing, hemoglobin-like, oxygen-carrying blood pigment erythrocruorin. Erythrocruorin of the earthworm is a naturally occurring high molecular weight (3.6 MDa) protein assembly of 144 hemoglobin subunits and 36 linker proteins that is extremely stable, resistant to oxidation, and transports oxygen similarly to vertebrate whole blood (Royer et al. 2006).

7.3.5 Immune System

7.3.5.1 Hemocytes

All cell types, with the exception of chloragogen cells, produce pseudopodia and are capable of phagocytosis, a vital component of the earthworm's immune response (Stein et al. 1977). Coelomocytes exhibit adoptive transfer, a process by which coelomocytes taken from previously grafted worms and injected into naive, ungrafted worms can confer the capacity for accelerated graft rejection to the new host. Since Metchnikoff described the "ameboid cells" of earthworms in 1891 and remarked on their phagocytic properties, a number of investigators have described and classified earthworm coelomocytes. The coelomocytes of *L. terrestris* have been classified into five major categories.

- Basophils (lymphocytic coelomocytes)
- Acidophils (inclusion-containing coelomocytes, two subgroups)
- Neutrophils (type I granulocytes)
- Granulocytes: exhibit heterogeneity with respect to staining properties of granules, including acidophilic granules, basophilic granules, or both types
- Chloragogen cells (eleocytes, two subgroups)

7.3.5.1.1 Basophils Basophils are the most numerous of the coelomocytes and vary in size between 5 and 30 µm (Figure 7.22a). The nucleus of basophils is compact, 4–8 µm

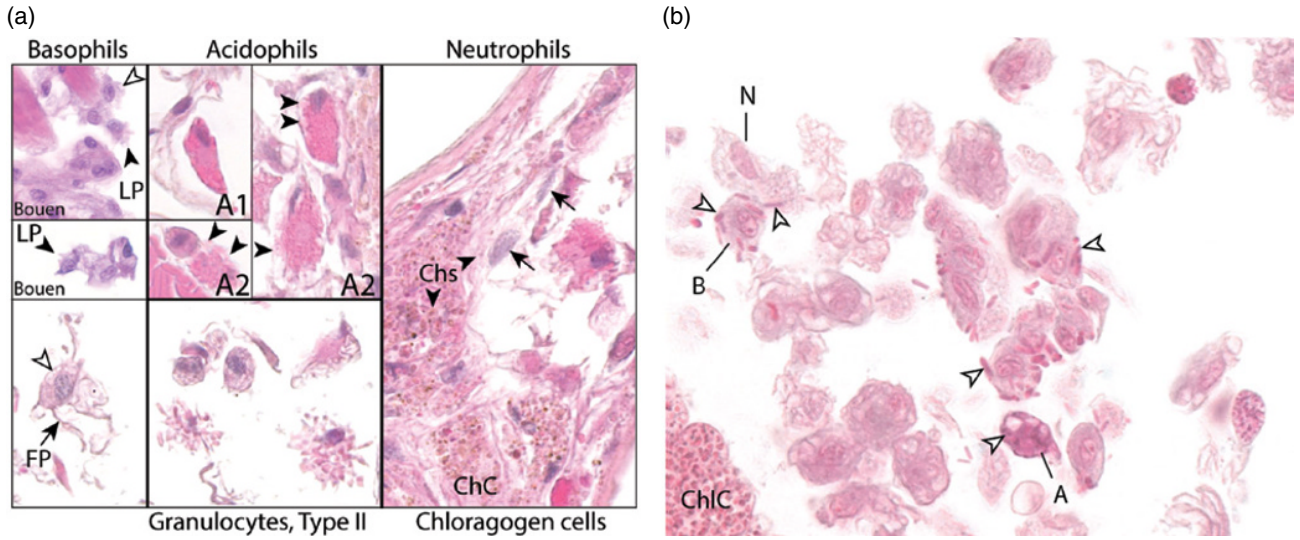


Figure 7.22 Coelomocytes. (a) Morphology of coelomocytes after Bouin fixation and Azan staining (panel (a), upper left photographs) and formalin fixation and HE staining (not indicated); 1000×. (b) Coelomocytes phagocytose bacteria; 1000×. A, acidophil; A1, acidophil, type I; A2, acidophil, type II; B, basophil; ChC, chloragogen cell; Chs, chloragosome; FP, filopodium; LP, lobopodium; N, neutrophil; black arrow, nucleus of neutrophil; black arrowhead, exocytosis; white arrowhead, phagocytosis of bacterial pathogens.

in diameter, and located either centrally or peripherally. Chromatin is condensed and stains dark blue violet. No nucleolus is visible in stained preparations. In stained preparations, the cytoplasm is strongly basophilic, occasionally contains small (approximately 0.5 µm) dark blue granules, and often has a slightly filamentous or "wispy" appearance. The numerous inclusion vacuoles contain a granular or flocculent material. These vacuoles may be secondary phagosomes that contain foreign debris resulting from phagocytosis and fusion with primary lysosomes (Figure 7.22b). Empty vesicles are scattered throughout the cytosol. The presence of numerous pseudopodia and vacuoles strongly suggests that these cells are mobile and phagocytic. In smaller cells, the cytoplasm is scanty and forms a thin shell around the nucleus. In larger cells, the more abundant cytoplasm is lighter blue and clear vacuoles are frequently present. Basophils show a strong tendency to adhere to one another and frequently form sheets or clumps. Single cells and the free edges of clumped cells may have small filopodia or larger petaloid pseudopodia extending from the cell surface (Linthicum et al. 1977).

7.3.5.1.2 Acidophils In acidophils, small (1–2 µm) clear vacuoles are frequently present which seem to penetrate the cells completely, producing an unusual perforated appearance (Figure 7.22a,b). The nucleus tends to be eccentric and is markedly so in larger cells of 20–30 µm. In the latter case, it is flattened, approximately 5–9 µm, and stains a moderately dark red-violet. The cell outline is usually quite distinct and may even appear thickened. Occasionally acidophils may be found with blebs projecting from the surface, some partially or completely separated from the cell. The material in these blebs is acidophilic and appears to be the same as that of the granules. Larger cells often lack the pseudopodial projections characteristic of basophils, while smaller acidophils more nearly resemble basophils in outline. Some acidophils contain larger granules or vesicles (2–4 µm) which also fill the cells. Outlines of the vesicles are often indistinct.

The inclusions are packaged in the convex portion of the Golgi complex and stored in the cytoplasm. Later they appear to undergo morphologic transformations (larger and lighter staining) as they reach the cell periphery. The presence of large empty vacuoles and inclusion bodies against the plasma membrane suggests that some may undergo exocytosis (Linthicum et al. 1977).

7.3.5.1.3 Neutrophils Neutrophils (type I granulocytes) are highly variable in size, ranging from 12 µm to as large as 40 or 50 µm (Figure 7.22a). The cell outline is usually quite irregular and difficult to distinguish. The nucleus is large (8–10 µm) and rose-colored. Relatively little heterochromatin is visible and clear, nonstaining areas intersperse the rather homogenous chromatin. At the edges of these clear areas, the chromatin sometimes stains more darkly. A blue-staining nucleolus is frequently present. The cytoplasm usually stains faintly blue, lavender or, rarely, pink. Small to medium-sized vacuoles are frequently present as well as ingested material such as bacteria or chloragogen granules. The cytoplasm is sometimes homogenous but at other times contains small

(approximately 1 μm) lightly staining granules, which are similar in color to the cytoplasm. These cells are probably highly mobile as suggested by their extensive microfilaments and blunt pseudopodia seen using electron microscopy. By light microscopy, these cells have neutrophilic staining properties. The numerous electron-opaque granules resembling lysosomes or specific and azurophilic granules of vertebrate neutrophils are prominent features of type I granulocytes (Linthicum et al. 1977).

7.3.5.1.4 Granulocytes The single most prominent characteristic of granulocytes (type II granulocytes) is the profusion of granules scattered throughout the cytoplasm (Figure 7.22a). Granules are easily observable, in contrast to those in acidophils, and are discrete so that the pale-blue cytoplasmic background is more visible. Granules show considerable variety in both staining properties and size. The nucleus is usually eccentric, 6–9 μm in size, stains a medium to dark purple and displays rather condensed chromatin. As in the case of acidophils, small surface blebs are occasionally present. Type II granulocytic coelomocytes are stellate in appearance due to their numerous pseudopodia. The extensive pseudopodial projections are unique to these cells. Empty vesicles appear in the peripheral cytoplasm. Two types of granules are present: dense and light staining.

7.3.5.1.5 Chloragogen Cells Chloragogenous tissue of earthworms surrounds mainly the midgut and the chloragocytes are attached to the periintestinal blood vessels and capillaries. Chloragocytes reserve high amounts of nutrients like glycogen and lipids, and they synthesize hemoglobin, heavy metal-capturing metallothionein-like proteins, and certain bacteriostatic proteins such as lysenin. They are thought to be progenitor cells of certain free coelomocytes such as eleocytes and probably certain granulocytes (Figure 7.22a). An earlier view was that the function of chloragogenous tissue was similar to the hepatopancreas of molluscs and arthropods. Chloragogen tissue is active in the metabolism. A second theory is that these cells are excretory in function, gathering waste products, which are presumably circulating freely in the blood, or coelomic fluid, concentrating them, transforming them into urea or ammonia and then the cells detach from the gut wall and autolyze in the coelom from whence the fluid is voided via the nephridia (Molnar et al. 2012).

There are two types of chloragocytes. Both are relatively large cells and both are packed with granules, as is characteristic of chloragogen cells. The first type is an oblong cell ranging in size from 10–25 μm wide by 30–60 μm long. The granules are spheroid or ovoid, regular in outline, and approximately 1–2 μm in diameter. They are frequently found in the coelomic fluid in a state of partial disintegration. The second type is smaller, more spherical, and 12–20 μm in diameter. It is found with less frequency than type I. The granules are of irregular shape, 0.5–2 μm diameter, and dark blue-violet when stained. They are usually arranged in clusters of 4–6 cells or more and the nuclei are often in a peripheral position. Within these major categories, several distinct cell types differ and may represent developmental stages (Laverack 1963).

The most characteristic structures of chloragocytes are the refractive, pigmented granules named chloragosomes. Electron microscopic examinations show that they are membrane-bound granules and possess a dense matrix, which varies from homogenous dense with no evident banding or structuring to lamellar or tubular-vesicular appearances. Morphologic data support an autophagosomal function of the chloragosomes (Cancio et al. 1995). Chloragosomes are modified lysosomes and their function is mainly nutritive under metabolic stress (Peeters-Joris 2000). The typical chloragosomes appear as spherical granules containing a matrix with a rather coarse network. Chloragosomes can fuse with the plasma membrane and discharge their contents extracellularly. Free chloragosomes can often be seen in the coelom and chloragosome-like structures in the coelomocytes.

7.3.5.2 Parasites

Monocystis lumbrici (Henle) (1845) is one of the most common and best known parasites in *Lumbricus*. *Monocystis* is a highly host-specific gregarian sporozoan. Its body is nonseptate, the mature trophozoites are large and extracellular, and it is a protozoan whose immature stage develops a resistant wall that encloses the sporozoites. The genera of Monocystidae are identified partly by the shape and structure of the trophozoites and partly by the shape and arrangement of the spores. There are no intermediate hosts, and all members of the family are parasitic in invertebrates (Sheridan 1986).

The minute sporozoites in the seminal vesicle of earthworm penetrate the sperm morulae, or mother sperm cells, and transform into trophozoites which grow at the expense of the germ cells (Figure 7.23a). Trophozoites live in the sperm morulae floating in the seminal vesicles. As trophozoites grow, they consume all but the tails of the sperms, which adhere to them, giving the appearance of a covering of cilia. By the time the trophozoites have matured, the sperm morulae have been absorbed (Olsen 1986). Mature trophozoites are sporonts or sporadins. These change to gamonts. Two of them come together and they secrete a double-layered cyst around themselves, which is considered as oocyst (Figure 7.23a). They unite to form a zygote, then the zygote forms 2-4-8 nucleated spores and they grow

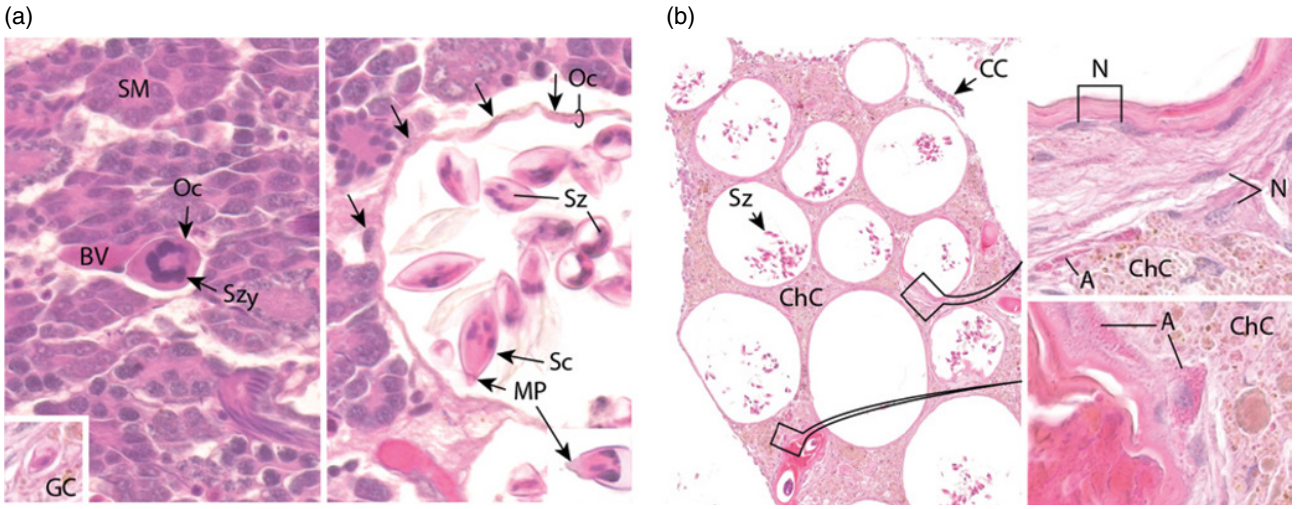


Figure 7.23 *Monocystis* infection and encapsulation. (a) *Monocystis* oocytes and sporozoites in vesicula seminalis; 1000× and 1800× (panel, inset left, bottom). (b) Encapsulated oocysts in coelomic cavity; 100× and 1000×. A, acidophil; BV, blood vessel; CC, coelomocyte; ChC, chloragogen cell; GC, gametocyte in oocyst; MP, mucoid plug; N, neutrophil; Oc, oocyst; Sc, sporocyst; SM, sperm morula; Sz, sporozoite; Sz_y, sporozoite, young; arrows, cells on external surface of gametocyst.

into independent cells with sporozoites within their capsule. They are released through the vas deferens into the cocoon or straight into the soil. Sporocysts are consumed by other earthworms and they get infected (Figure 7.23b). Predators feeding on infected earthworms can be considered as vectors, as the capsule of sporocysts open in their gut and they spread the sporozoites with their feces.

We identified sporozoites not only in seminal vesicles but in the coelomic cavity, where they were encapsulated by coelomocytes (Figures 7.18 and 7.23b).

7.3.6 Nervous System

7.3.6.1 Ganglia

The central nervous system is composed of the cerebral ganglion, the subesophageal ganglion, and the ventral nerve cord (Figure 7.24a). The ventral nerve cord consists of a chain of segmental ganglion pairs and the connectives, the longitudinal fiber bundles connecting these ganglia (Figure 7.24f). There is a trilaminar protective case around the central nervous system. The outer layer is the peritoneal (coelomic) lining, the middle one is a longitudinal muscle layer, and the innermost is a fibrous sheath consisting of a collagen-rich, fibrous tissue (Figure 7.24b). The fibrous tissue also penetrates the nervous system and forms an important part of the supportive glial zone (Figure 7.24b,d). Neuropile is surrounded by the supportive glial zone but no barrier, such as a fibrous septum, is interposed between these two regions. Perikaryons of neurons are pear-shaped or polygonal. Most of the neurons are unipolar; bipolar and multipolar neurons are rare. Interneurons are also primarily unipolar cells; motoneurons are uni-, bi-, or multipolar. Ganglion cells are flask-shaped unipolar neurons whose cell bodies are located in the supportive glial zone and whose axons are directed centrally into the neuropile (Figure 7.24b,c). Finally, it should be restated that neither blood vessels nor extensions of the fibrous capsule are found within the neuropile.

The nerve cells of cerebral ganglion are immediately beneath the capsule of connective tissue while the neuropile, the aggregate of fibers issuing from the cells, lies in the middle (Figure 7.24a,b). The main cell types of cerebral ganglion are the following: ganglion cells have large perikaryon and basophilic cytoplasm. Neurosecretory cells have small perikaryon and eosinophilic cytoplasm; they are situated above the large, light cells, immediately under the capsule of the ganglion (Figure 7.24c). Neurosecretory cells are in close proximity to capillaries in a zone of accumulated secretion just beneath the dorsal neuron group. The nerve cells are arranged in groups (Figure 7.24b). The cerebral ganglion contains dorsal, lateral, and ventral cell groups, which can also be divided into anterior and posterior ones (Aros and Vigh 1961) (Figure 7.24e). In frontal sections, mostly large and light cells are found in the anterior segments, while small cells are predominant in the posterior part of the ganglion (Otremba 1961). The small cells are situated above the large, light cells, immediately under the capsule of the ganglion. It has been noted that there are many large light cells in the anterior portion of the cerebral ganglion.

The subesophageal ganglion corresponds to two united ventral ganglia (Figure 7.24f). Two transverse bundles of fibers called commissures connect the two sides of each

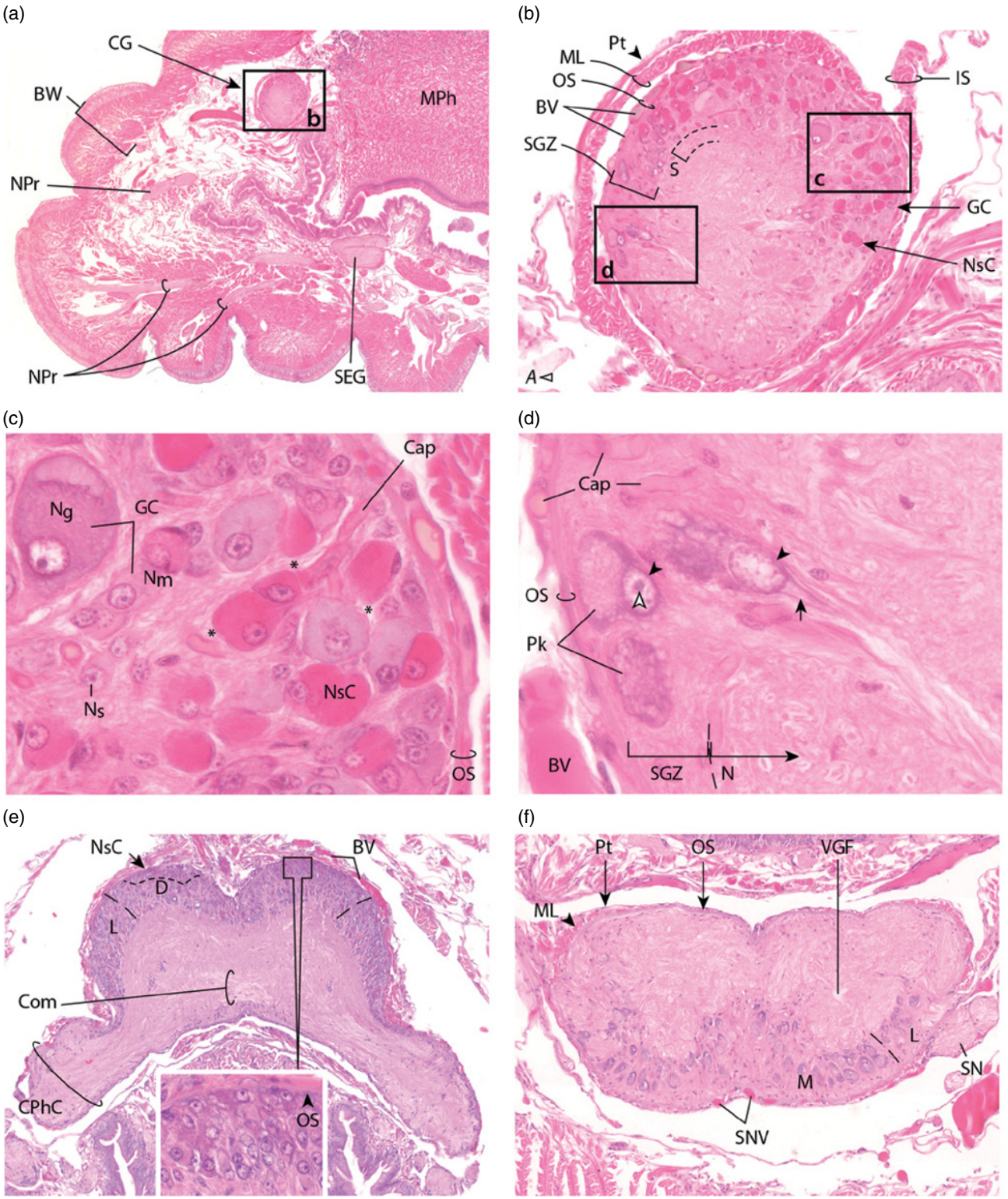


Figure 7.24 Histology of the cerebral and subesophageal ganglia. (a) Longitudinal section of the head with anterior organs of CNS (Bouin fixation), 35×. (b) Histology of the cerebral ganglion (Bouin fixation), 200×. (c) Neurosecretory and ganglion cells in the posterior part of cerebral ganglion; 1000×. (d) Ganglion cell morphology; 1000×. (e) Cross-section of the cerebral ganglia at the level of the circumpharyngeal connectives (Bouin fixation), 90× and 1000×. (f) Cross-section of the subesophageal ganglia at the level of the anterior part (Bouin fixation), 1000×. A, anterior; BV, blood vessel; BW, body wall; Cap, capillary; CG, cerebral ganglion; Com, commissure; CPhC, circumpharyngeal connective; GC, ganglion cell; IS, intersegmental septum (dissepiment); L, lateral cell group; M, medial cell group; ML, muscle layer; MPh, muscle of pharynx; N, neuropile; N_g, neuron, giant; N_m, neuron, medium; N_s, neuron, small; NsC, neurosecretory cell; NPr, prostomial nerve; OS, outer fibrous sheath; Pk, perikaryon of neuron; Pt, peritoneal sheath; SEG, subesophageal ganglion; SGZ, supportive glial zone; SN, segmental nerve; SNV, subneural vessel; VGF, ventral giant fiber; asterisk, close proximity of neurosecretory cells and capillary; black arrow, axon hillock; black arrowhead, nucleus; white arrowhead, nucleolus.

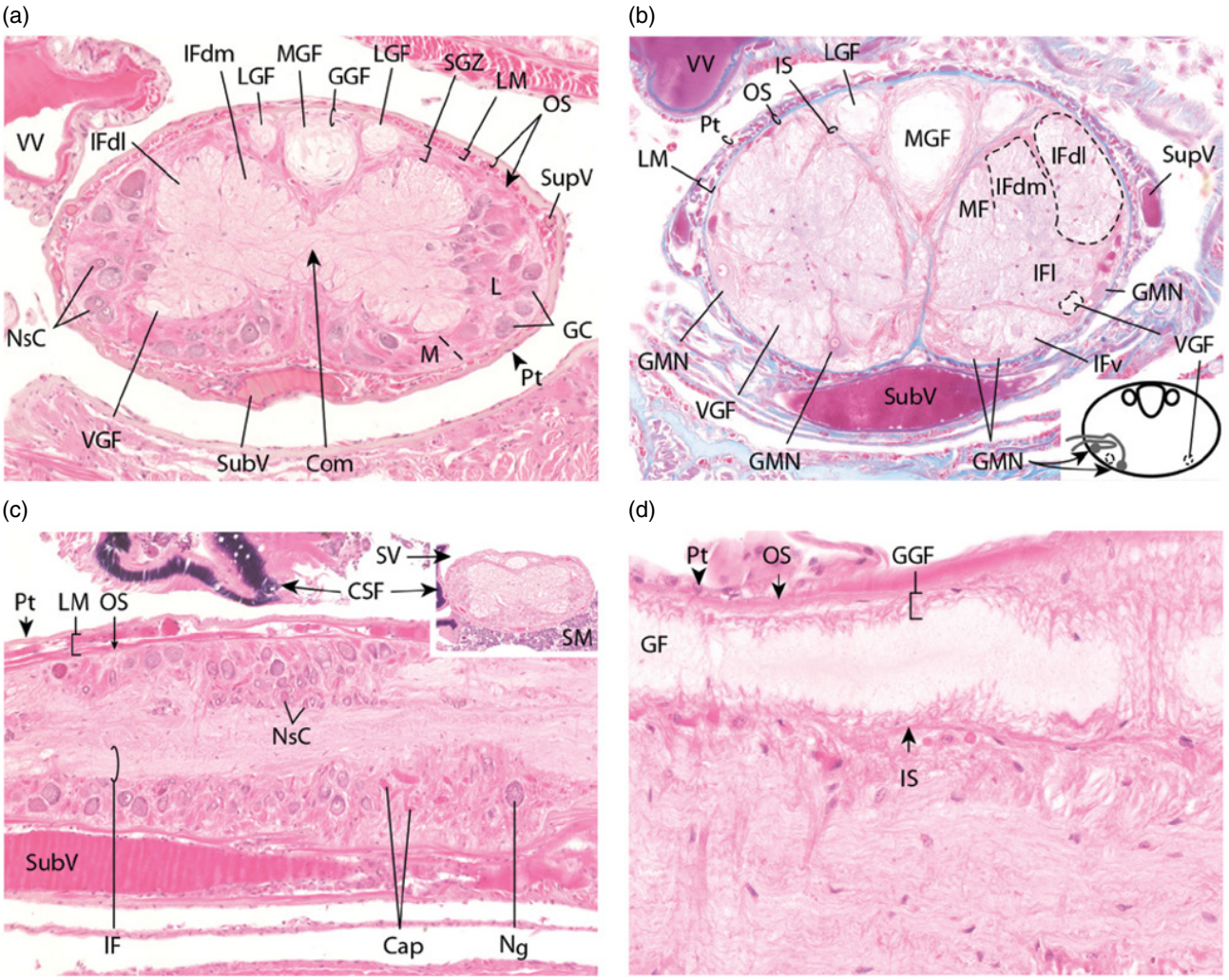


Figure 7.25 Some histologic features of the ventral nerve cord. (a) Overview of ventral nerve cord ganglia (formalin fixation), 180×. (b) Histology of ventral nerve cord connectives (Bouin fixation), Azan, 260×; inset is a drawing after Günther (1971). (c) Longitudinal section of ventral nerve cord ganglion (Bouin fixation), HE, 160×, cross-section (formalin fixation), 100×. (d) Longitudinal section of a dorsal giant fiber (Bouin fixation), HE, 470×. Cap, capillary; CSF, ciliated sperm funnel of vas deferens; GC, ganglion cell; GF, giant fiber; GGF, glial sheath of giant fiber; GMN, giant motoneuron; IF, interganglional fibers; IFdl, interganglional fibers, dorsolateral; IFdm, interganglional fibers, dorsomedial; IFl, interganglional fibers, lateral; IS, inner fibrous sheath; L, lateral cell group; LM, longitudinal muscle of nerve cord; LGF, lateral giant fiber; M, medial cell group; MF, medial fiber; MGF, medial giant fiber; N_g, neuron, giant; NsC, neurosecretory cell; OS, outer fibrous sheath; Pt, peritoneal sheath of nerve cord; SGZ, supportive glial zone; SM, sperm morula; SubV, subneural vessel; SupV, supraneural vessel; SV, seminal vesicle (cavity); VGF, ventral giant fiber; VV, ventral vessel.

ganglion. Nerve cells form medial and lateral groups (Aros and Vigh 1961). In addition to these cells, neurosecretory cells can be found in the posterior cell group of the anterior ganglion and in both cell groups of the posterior ganglion (Otremba 1961).

7.3.6.2 Giant Nerve Fibers

Earthworms possess three giant fibers within the dorsal side of their ventral nerve cord, one dorsomedial giant fiber and a pair of dorsolateral giant fibers, which run the length of the nerve cord (Figure 7.25a,b). These fibers are responsible for flight reflexes, twitches of the worm. Skin sensory cells of the worm front end are connected to the median giant fiber (MGF). The two lateral giant fibers (LGF) receive their input mainly from skin sensory cells of the hind end.

In the earthworm, two different escape responses are mediated by a MGF and by two synchronized LGF, depending on whether the animal is stimulated at its anterior or posterior end. These two fibers have segmental cross-connections and can be regarded as single functional. The median and lateral giant nerve fibers, which mediate startle responses in earthworms, are regularly connected with two groups of giant motor neurons in each ganglion of the

ventral cord. The sheath of giant fibers is loose myelin that consists of flattened cytoplasmic processes of small glial cells wrapped around the axon. No structures resembling the nodes of Ranvier in a vertebrate myelinated axon have been observed with either the light or electron microscope. Each giant axon connects to one cell body in each segment; the giant fibers are not fused axons. The septum across the giant axon, which appears as a single line in light micrographs, consists of two apposed plasma membranes separated by an intercellular gap (Coggeshall 1965). In each segment, the MGF has three branches and each lateral giant fiber has five branches. These branches are dendritic. No structural differences between the giant fibers in anterior and posterior regions of the worm seem to account for the functional polarity of the giant fiber system observed in behavioral studies (Mulloney 1970).

On light microscopic examination, the sheath of ventral giant fibers has a different texture and color than vertebrate myelin or the sheath of the dorsal giant axons (Figure 7.25a,b). Electron micrographs reveal that the sheath is not glial as in the dorsal axons but consists of innumerable tiny unmyelinated axons that surround the large axon and run parallel to it. It is not surprising, therefore, that its tinctorial properties differ from those of myelin (Coggeshall 1965).

Axons of motoneurons have two types of collaterals, one central and one peripheral. The central collateral bifurcates into descendant and ascendant projections, which connect neighboring ganglia. The peripheral collateral innnervates muscle fibers. Three motoneuron types have been described upon the size of perikarya: giant motoneurons (GMN) innnervate longitudinal muscles (Figure 7.25b). There are four pairs of them in each ganglion which act as specific efferent pathways of the giant fibers, innervating the longitudinal muscles. Middle-sized and small motoneurons innervate circular muscles (Günther 1971). Most of their axons are crossed, only a small number have ipsilateral axons.

Interneurons are either polysegmental or giant interneurons. The projections of polysegmental interneurons are called giant fibers or giant axons. Giant interneurons connect two adjacent ganglia, and make up the longitudinal interneuronal fiber bundles (Figure 7.25c).

7.3.7 Reproductive System

Reproduction in earthworms is hermaphroditic. In each specimen, there are two pairs of testes in segments 10 and 11 and one pair of ovaries in segment 13. Developing sperm are stored in seminal vesicles before transfer to female seminal receptacles (spermatheca). Sperm ducts lead from the seminal vesicles to male pores located one or more segments behind the testes. The ovaries are simple outpouchings (ovisacs), with oviducts leading to female pores in the next posterior segment.

7.3.7.1 Male Part

Two pairs of testes in segments 10 and 11 are enclosed in the suboesophageal testis sac (Csuzdi and Zicsi 2003). The testis of each side is free from its fellow; they are attached by a stalk to the anterior dissepiment of the segment, which they occupy. Vasa deferentia on both sides are connected to the dissepiment and penetrate through it; they wind up before reaching the male genital pores (Figure 7.26).

7.3.7.2 Testis and Seminal Vesicle – Spermatogenesis

In the earthworm, spermatogenesis begins in the testis (Figure 7.27a). Only the spermatogonial stages are found in the testes, whereas all the other stages of spermatogenesis are found in the seminal vesicles, which are located in segments 9, 11, and 12 (Figures 7.26 and 7.27a). The germline cells form syncytial cysts (clusters) at the onset of spermatogenesis in *Lumbricus*. The germ cells in the cysts are interconnected by wide cytoplasmic channels known as intercellular (cytoplasmic) bridges. These are modified contractile rings that do not close during late cytokinesis. These bridges ensure the cytoplasmic continuity between the interconnected cells and, as a result, the cytoplasm of the sister cells is common. The interconnections of germ cells into syncytial clusters regulate and synchronize germ cell development. At the early stages of spermatogenesis in *Lumbricus*, each germ cell is connected to the central and anuclear cytoplasmic mass, the cytophore via one cytoplasmic bridge (Świątek et al. 2016) (Figure 7.27a,b).

Primary spermatogonia are very large cells scattered singly or in small groups throughout the testis (Figure 7.27a). It is impossible to state how the first spermatogonial cell arises. There is no distinct layer of germinal epithelium in the testis of the earthworm. During the resting stage the chromatin consists of thick filaments, without granules, spread in a clear homogenous substance. In secondary spermatogonia, chromatin undergoes condensation into compact chromatic bodies. The secondary spermatogonia are smaller and arranged usually in groups of 8, 16, and 32 cells into a special chamber, the testes sacs. Secondary spermatogonia are generally pear-shaped and the nuclei are spherical. The nucleolus is usually spherical and centrally located with a clear margin. The cytoplasm surrounding the nucleus is a comparatively thin layer and contributes little to the size of the cell.

After the spermatogonia have been formed into groups of 16 and 32 cells, the first maturation division of the primary spermatocytes begins. Spermatocytes are smaller than the primary spermatogonia. They are also relatively

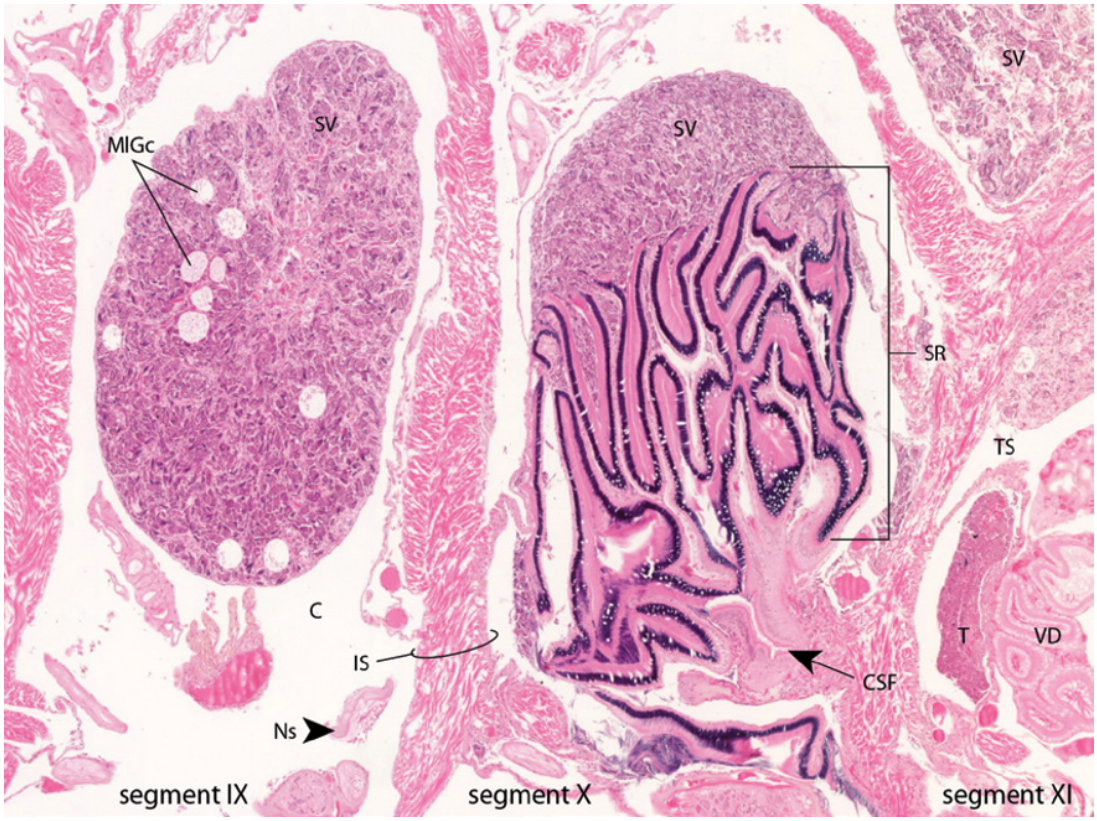


Figure 7.26 Overview of male genital organs in segments IX–XI (longitudinal section); Bouin fixation, 45×. C, coelom (body cavity); CSF, ciliated sperm funnel of vas deferens; IS, intersegmental septum (dissepiment); MlGc, *Monocystis lumbrici* encapsulated gametocysts with sporozoites; Ns, nephrostome; SR, sperm reservoir; SV, seminal vesicle, lobe; T, testis; TS, testis sac; VD, vas deferens.

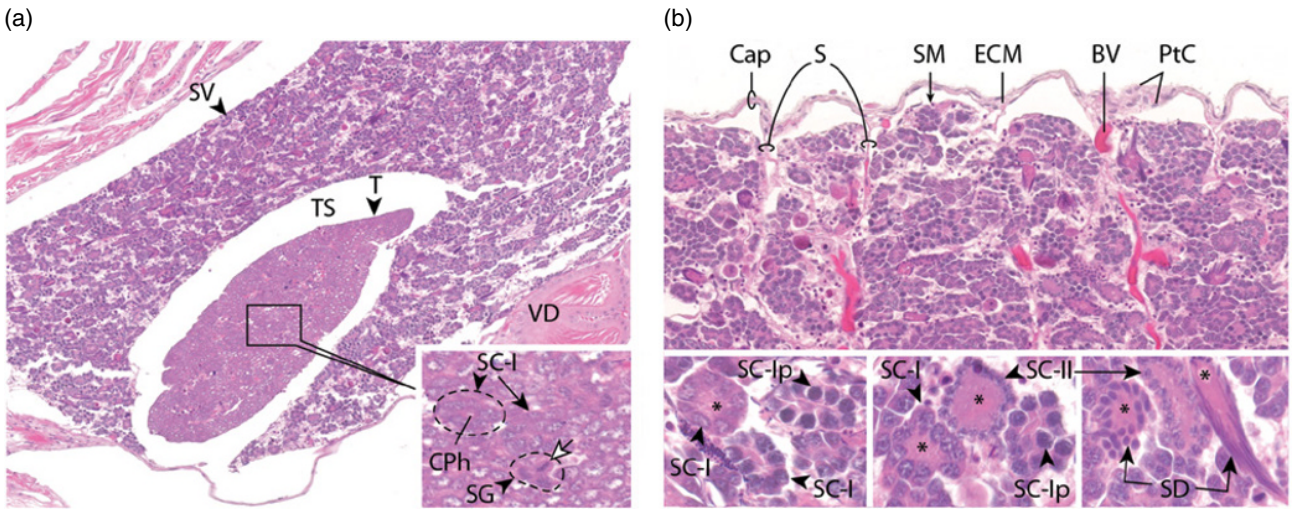


Figure 7.27 Testis and spermatogenic phases. (a) Testis in the lobe of seminal vesicle in segment X and spermatogonial divisions (formalin fixation), 150× and 1000× (inset). (b) Histologic architecture of seminal vesicle and maturation steps of spermatogenesis; 300× and 1000× (insets bottom). BV, blood vessel; Cap, capsule of seminal vesicle; CPh, cytophore; ECM, extracellular matrix; PtC, peritoneal cell; S, septum; SC-I, primary spermatocytes; SC-Ip, primary spermatocyte in pachytene stage; SC-II, secondary spermatocyte; SD, spermatid; SG, morula of spermatogonia; SM, sperm morula; SV, seminal vesicle; T, testis; TS, testis sac; VD, vas deferens; asterisk, cytophore; dashed line, sperm morula; white arrow, mitotic metaphase of primary spermatogonia.

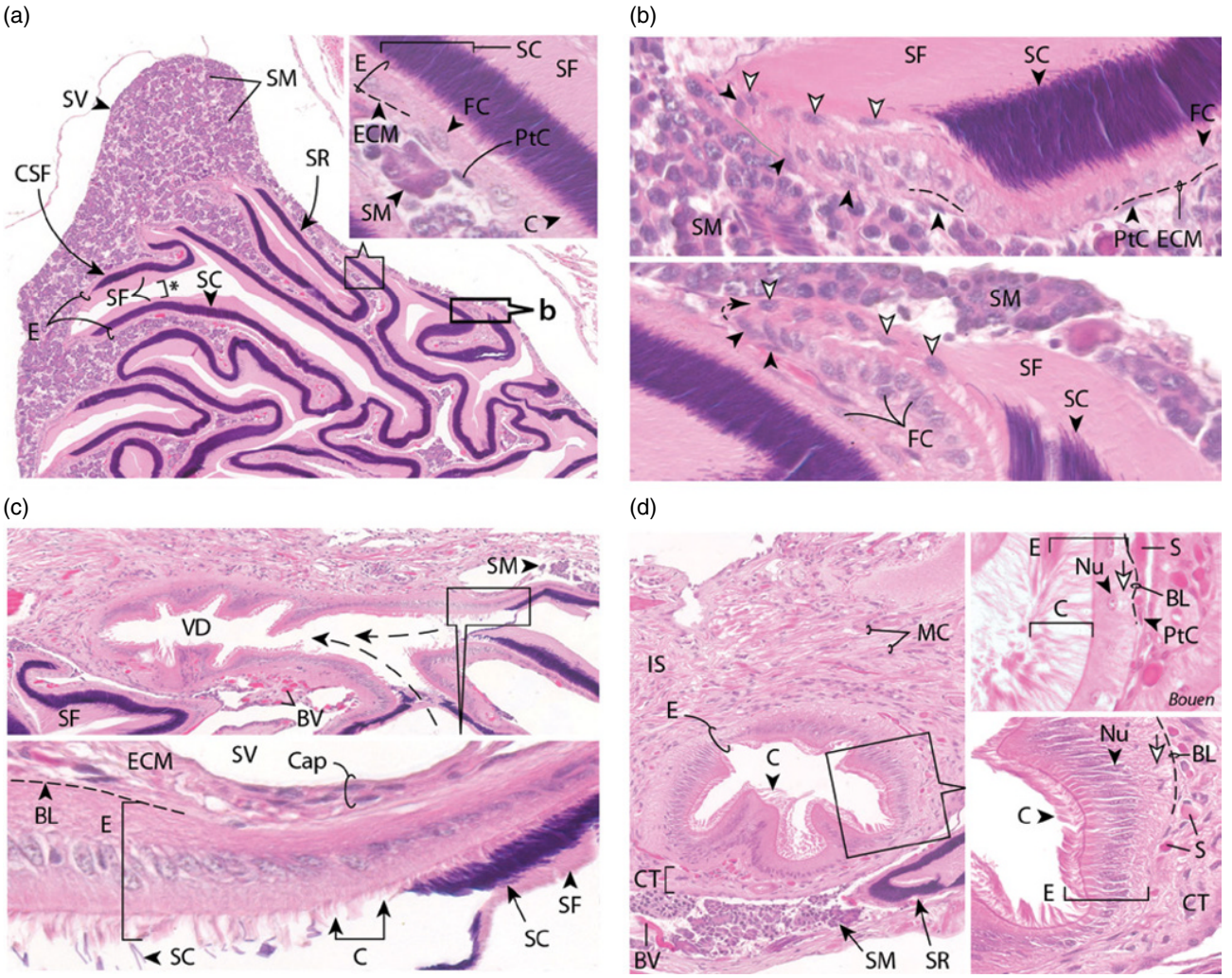


Figure 7.28 Sperm reservoir of seminal vesicle and vas deferens. (a) Overview and detail of sperm reservoir formed by folded ciliated sperm funnel (formalin fixation), 100× and 900× (inset). (b) High magnification of the rim of ciliated sperm funnel (formalin fixation), 850× and 900× (inset) (the bottom photograph is a magnified area of panel a). (c) Connection between sperm reservoir and vas deferens in segment XI (formalin fixation), 150× and 900× (bottom). (d) Vas deferens in cross-section in plane of dissepiment (left and right bottom: formalin fixation, right top: Bouin fixation); 200× and 600× (both right panels). BL, basal lamina; BV, blood vessel; C, cilia; Cap, capsule of the lobe of seminal vesicle; CSF, ciliated sperm funnel of vas deferens; CT, connective tissue; E, epithelium; ECM, extracellular matrix; FC, funnel cells; IS, intersegmental septum (dissepiment); MC, muscle cell; Nu, nucleus of epithelial cell; PtC, peritoneal cell; S, sinus; SC, sperm cell; SF, seminal fluid; SM, sperm morula; SR, sperm reservoir; SV, seminal vesicle, lobe; VD, vas deferens; asterisk, artificial fission; black arrowheads, nuclei of outer peritoneal cells; dashed arrow, path from sperm reservoir to vas deferens; white arrow, basal zone of epithelial cells; white arrowhead, nucleus of inner peritoneal cell.

the most numerous cells, which would imply that this stage has a very long duration. The nucleolus cannot be seen in the following stages of prophase of the primary spermatocyte. The primary spermatocytes then pass into the seminal vesicles where they continue their development and proliferate synchronously (Walsh 1954). Groups of 64 cells are secondary spermatocytes of the second maturation division (Figure 7.27b). The spermatocytes of the second maturation division are smaller than those of the first maturation division. Groups of 128 and very rarely 256 cells are the spermatids, which form morulae. By complex cytomorphogenetic processes, the spermatids, which are attached to a central cytophore, differentiate into motile elongate spermatozoa (Anderson et al. 1967).

7.3.7.3 Sperm Reservoir and Vas Deferens

In the tenth and eleventh segments are the ciliated sperm funnels of the vasa deferentia. The margins of the funnel are extremely folded and complicated (Grove 1925). The folds are so deep and extended that they form a sperm reservoir (Figures 7.26 and 7.28a,b). The pair of funnels of each segment are close together on either side of the

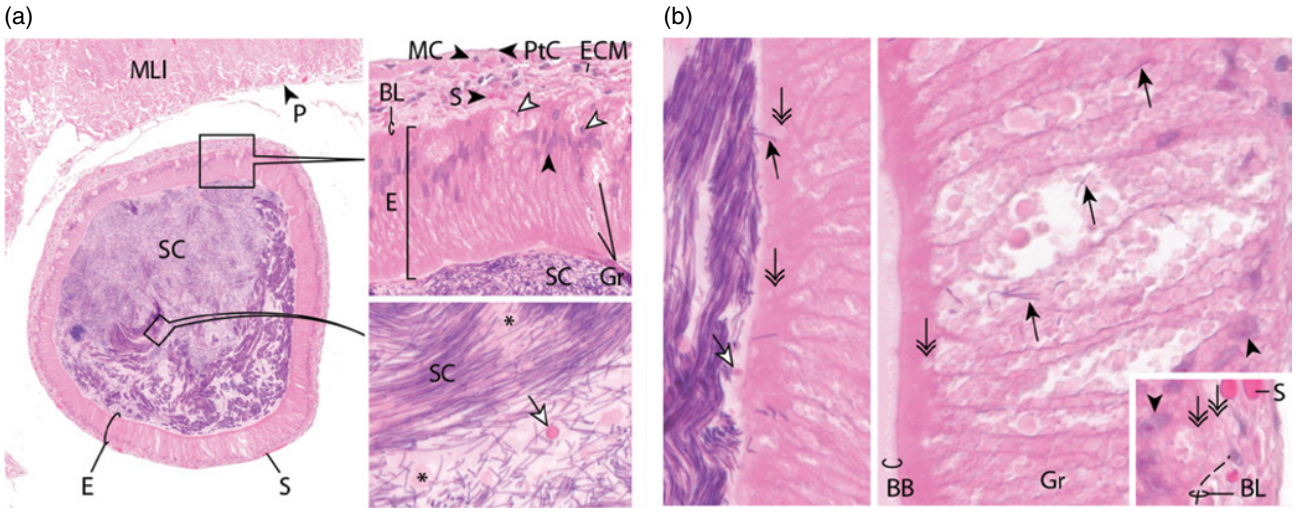


Figure 7.29 Seminal receptacle/spermatheca. (a) Seminal receptacle/spermatheca, overview, histology of the wall and the content (formalin fixation), 80×, 400× (*top right*) and 900× (*bottom right*). (b) Details of epithelial cells (referring to their transcytosis function) (formalin fixation), both micrographs: 1000×. BB, brush border; BL, basal lamina; E, epithelium; ECM, extracellular matrix; Gr, granules; MC, muscle cell; MLl, muscle layer, longitudinal; P, peritoneum; PtC, peritoneal cell; S, sinus; SC, sperm cell; asterisk, eosinophil substance; black arrow, sperm in epithelial cell; black arrowhead, nucleus of epithelial cell; double headed arrow, eosinophil granule in epithelial cell; white arrowhead, picnotic nuclei, white arrow, eosinophil granule.

median line and just above the ventral nerve cord. They are the wide beginning part of the vas deferens (sperm duct). The vas deferens has a characteristic ciliated epithelial lining (Figure 7.28c,d).

The sperm reservoir is lined by a ciliated epithelium containing funnel cells (Figure 7.28a,b). They develop from the same embryonic tissue as the ciliated funnel of metanephridia (Bartolomaeus and Quast 2005). The funnel cells rest on a thin ECM; peritoneal cells cover the coelomic side of this ECM. Seminal fluid fills up the reservoir, which is secreted by the funnel cells. The morphologically mature sperm cells insert themselves among the cilia (Figure 7.28a,b). The marginal opening of the funnel is very narrow in order to prevent the sperm morulae entering the vas deferens. The margin of the sperm funnel has three layers. The middle layer is formed by the funnel cells, which are ciliated. This layer continues in the epithelial lining of the reservoir. The outer layer of peritoneal cells folds up onto the funnel cells and ends where the row of mature sperm cells starts (Figure 7.28b). This arrangement suggests that the edge of the funnel is supported by this peritoneal fold.

The vas deferens is highly convoluted (Figure 7.26) and seems to be empty of seminal fluid (Figure 7.28d). Its lining is a simple, high columnar epithelium. Basal striation is observable in some epithelial cells.

7.3.7.4 Female Part: Seminal Receptacles

The inner lining of the seminal receptacles is a simple columnar epithelium with a well-developed brush border (microvilli) (Figure 7.29a,b). The nucleus lies close to the basal end. Between the nucleus and the apical end of the cell there is a relatively large extent of cytoplasm in which any secretion of the cell would accumulate (Figure 7.29b). Some evidence in support of the view that the secretion of the lining epithelium of the spermathecae provides a pabulum for the nourishment of the spermatozoa was observed in the sections of spermathecae stained with Ehrlich's hematoxylin. The epithelial cells secrete many eosinophilic granules containing mucin. Mucin is a protein, which spermatozoa may be able to utilize for nutritive purposes. Unmistakable traces of the tails of spermatozoa are observable in the epithelial cells (Grove 1925) (Figure 7.29b). The spermatozoa are found in the apical portion of the epithelial cells.

7.3.8 Histology Processing Techniques

The earthworms were collected during the rainy days of September, and kept in humid filter paper for two days to clear the intestines of soil particles (Salgado et al. 2014). Each earthworm was anesthetized by immersion in distilled water to which ethanol was gradually added to reach a 10% (v/v) ethanol solution. The procedure was finished when the worm did not react on pinching its tail. The earthworms were then rinsed off in anesthetic-free, dechlorinated tap water at room temperature. The tissue samples collected were immediately fixed in 10% cacodylate buffered formalin (pH 7.2–7.4), for 12 hours at 4 °C. It is also possible to use Bouin's solution for fixation, as tissue structure with a soft and delicate texture must be preserved. It is

composed of picric acid (4%, v/v), acetic acid (1%, v/v), and formaldehyde (15%, v/v) in an aqueous solution. Samples then should be processed routinely, embedded in paraffin (Paraplast®), sectioned at 1–2 µm with a Leica microtome, and stained with hematoxylin and eosin (H&E) or asocarmine, aniline blue and orange G (Azan trichrome stain) for examination by light microscopy. For photographic evaluation sections were scanned by a Pannoramic Midi (3DHistech) scanner (40× objective, number of focus levels: 3, focus step size: 3).

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8

Arthropoda

Arachnida

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8.1 Introduction

8.1.1 Taxonomy

Arachnids (class Arachnida) are arthropods that are largely terrestrial but a few, such as *Argyroneta aquatica* (the diving bell spider), are secondarily aquatic (Ruppert et al. 2004). The largest order of arachnids is the Araneae, the spiders, with over 40 000 described species (Ruppert et al. 2004).

The taxonomy of the arachnids has been somewhat contentious (Harvey 2002; Sharma et al. 2014). It is generally accepted that Arachnida is one of the three classes of Chelicerates, along with Merostomata (horseshoe crabs) and Pycnogonida (sea spiders) (Ruppert et al. 2004; www.itis.gov).

There are numerous orders of arachnids, including:

- Amblypygi – tailless whip scorpions
- Araneae – spiders
- Opiliones – harvestmen
- Palpigradi – micro whip scorpions
- Pseudoscorpiones – pseudoscorpions
- Ricinulei – hooded tick spiders
- Schizomida – short-tailed whip scorpions
- Scorpiones – scorpions
- Solifugae – sun spiders or camel spiders
- Uropygi – whip scorpions

In addition, there is a huge diversity of organisms within the subclass Acari (mites and ticks). Several schemes have been suggested in attempts to classify the Acari, many with complex layers of taxonomic ranks. In excess of 50 000 species have been described and by some estimates, more than a million species may exist. The salient reproductive features of arachnids include cleidoic eggs or ovoviparity. In scorpions, two types of embryonic development occur: apoikogenic (eggs containing yolk) and katoikogenic (yolkless – the embryos are nourished orally via diverticula in the female's oviuterus) (Cloudsley-Thompson 1993).

Scientific investigation of the morphology of arachnids began with the naturalist and physician Martin Lister (1639–1712), who published his *Tractatus de Araneis*, in Latin, in 1678. The father of microscopy, Antonie van Leeuwenhoek (1632–1723), described the dissection of spiders in one of his letters in 1673. There have been many important contributions to our knowledge of arachnids, including their histology in the latter part of the twentieth century. Legendre, writing in French, detailed the structure of the gastrointestinal tract and nervous system of spiders (Legendre 1958, 1953). Kerkut, Freeman, and Bracegirdle and Leake described the microscopic anatomy during presentations at the Arachnology International Conference, which included seminal studies in English, French, and German (Freeman and Bracegirdle 1971; Kerkut 1959; Leake 1975; Merrett 1978). Various texts during the same period discussed the responses of arachnids and other arthropods to various insults – for example, alcohols, heavy metals, and pesticides (Sparks 1972). Descriptions of pathologic examination of invertebrates have included histologic methods for examining insects; much of this is relevant to arachnids (Barbosa et al. 2015; Cooper and Cunningham 1991; Newton and Smolowitz 2018).

In this chapter, the emphasis is on Araneae with some discussion of other orders, predominantly Scorpiones, Ricinulei, and Solifugae. The bias is based on species frequently encountered and therefore studied, so although

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Opiliones, Pseudoscorpiones, and Acari are discussed, there are few data of other orders.

8.1.2 Life History

The life expectancy of arachnids ranges from a few weeks (some species of mites) to several decades, with one mygalomorph spider reported to have lived to 43 years (Mason et al. 2018). Females often outlive males. Arachnids typically lay eggs except for Scorpiones, which develop eggs that hatch internally. In oviparous species, sometimes eggs are merely dropped into the environment, but in many arachnids, there is parental care, in the form of protective webs (some spiders). Mothers may transport egg-cases or young (some spiders and scorpions). Arachnids undergo an "incomplete metamorphosis"; a juvenile that resembles the adult emerges from the egg and then, through a series of skin changes (ecdysis), grows to maturity. Arachnids vary greatly in their methods of feeding. Some mites feed on plant juices, others on organic debris. Most ticks ingest blood or hemolymph. The majority of spiders and scorpions are "carnivorous"; the former ingest prey through external digestion of internal organs and hemolymph.

8.1.3 Relevance for Conservation, Agriculture, Trade, Etc.

Some arachnids are threatened species, either at an international level (through Convention on Trade in Endangered Species [CITES] and the IUCN) or nationally (species and/or habitat protection). A substantial number of arachnids, notably ticks and mites, are a threat to agriculture (livestock/crops). Of the venomous arachnids (the majority of spiders, all scorpions, some pseudoscorpions), a few are hazardous to humans and other vertebrate animals, some are even lethal. Some species, such as harvestmen, are harmless. Trade in arachnids is restricted to species that are kept in zoos or by private arachnologists, and those that are used in biomedical research. The list of traded species is constantly growing with new species turning up in the pet trade before scientific description, as evidenced by taxonomic papers citing captive material as their source, such as for some *Tapinauchenius* (Hüsser 2018). Traded species are predominantly theraphosid spiders, but many scorpions and true spiders are also frequently traded in the international pet trade, although few are protected. As of January 2019, only 26 species are listed under the CITES all Appendix II, EU Annex B. These are species in the genus *Brachypelma* (family Theraphosidae), *Aphonopelma albiceps*, and *Aphonopelma pallidum* (family Theraphosidae, previously in genus *Brachypelma*), and four species of Scorpionidae of the genus *Pandinus* (*Pandinus dictator*, *Pandinus gambiensis*, *Pandinus imperator*, and *Pandinus roeseli*) (UNEP 2019). In total, 286 species have been evaluated by the IUCN redlist (www.IUCNredlist.org) with 52 species found to be critically endangered, thus conservation evaluation in the arachnid taxa to date has only evaluated a small percentage of the global species and these are heavily biased to the large spiders.

In biomedical research, peptides found in the venom of arachnids are becoming increasingly important in studying drug targets such as ion channels and others (Escoubas and Rash 2004). Although pain and neuroscience are the predominant indications (Trim and Trim 2013), other indications are also pursued including pesticides (Hardy et al. 2013). Alpha-latrotoxin (Tzeng and Siekevitz 1978) from the black widow spider (*Latrodectus mactans*) and imperatoxin from the emperor scorpion (*Pandinus imperator*) have been used to understand the biology of neurons.

Zoonosis is, strictly, an infection that can be acquired by humans from a *vertebrate* animal but it should be noted that certain arachnids can be a source of nematodes of the family Panagrolaimidae (Pizzi 2009; Pizzi et al. 2003), which can be of medical concern to humans.

8.2 Gross Anatomy

Although all arthropods have abundant segmentation embryonically, arachnids have reduced outward segmentation in adults. This is most pronounced in animals from the subclass Acari, which have no external segmentation. Most other arachnids are segmented into an anterior prosoma and a posterior opisthosoma with a narrow waist called a pedicel (the seventh embryonic segment) joining the two. The opisthosoma is segmented in eurypterids and scorpions; most other arachnids do not have opisthosomal segmentation. Some species also have a telson, or tail. In scorpions, the telson contains a venom gland and is connected to the aculeus (stinger), and in palpigrades, uropygids, and schizomids it acts as a sensory flagellum.

Arachnids have six paired appendages. The first are the chelicerae, the second are the pedipalps, and the last four are the walking legs. Each chelicera is attached to the cranial aspect of the prosoma via a fixed basal segment that is articulated with a movable fang. The fangs typically rest in a groove in the basal segment, from which they pivot outward to strike prey. Venom is delivered through a slit in the subterminal portion of the fang. The chelicerae are not only used for capturing prey, but also for defense and for grasping various objects. These are most well developed in the Solifugidae (Klann 2009). The second appendages, the pedipalps, have segmentation similar to the legs except

they lack one segment, the metatarsus. Pedipalps are not used for locomotion (except in Aranae), but for capturing and manipulating prey. The first segment of the pedipalps, the coxa, forms the preoral cavity and is frequently modified to macerate prey. In all but the Aranae, Solifugidae and Ricinulei, the pedipalps form grasping claws with a single mobile finger opposing a fixed finger. Additionally, male spiders produce a bulbous distension of the terminal aspect of the pedipalps, called the palpal bulb, upon sexual maturity, for copulation. Four pairs of walking legs attach to the prosoma caudal to the pedipalps. Walking legs have seven segments, including (from proximal to distal) the coxa, trochanter, femur, patella, tibia, metatarsus, and tarsus. The tip of the tarsus bears claws. The number of leg segments and number of claws may vary between species (Ruppert et al. 2004).

Paired eyes are on the craniodorsal aspect of the prosoma; in Araneidae, there are four pairs on an ocular tubercle. The cranioventral aspect of the opisthosoma has a number of book lung slits (in pulmonate species) that are adjacent to the epigastric furrow. The cloaca and spinnerets are located on the caudoventral aspect of the opisthosoma. The opisthosoma is typically covered by setae in Theraphosidae. Setae are detachable and urticating in most new-world theraphosids. There is a great deal of variation of the gross anatomy within Arachnida. Gross external anatomic features are provided for Theraphosidae in Figure 8.1, Scorpiones in Figures 8.2 and 8.3, and Acaridia in Figure 8.4.

8.2.1 Dissection

There are multiple dissection techniques that can be applied to arachnids. The method described here is the authors' preferred technique with respect to Araneae. The dissection method may need to be modified for other orders of Arachnida. Successful microdissection is greatly aided by stereo microscopy with 2×–5× magnification and through the use of size 5 microforceps and bow scissors.

The prosoma and opisthosoma are dissected separately starting with the prosoma. The prosomal carapace is cut at its periphery with shears and then gently lifted to expose the underlying muscle and nerves (Figure 8.5a,b). Muscular attachments from the carapace to the underlying structures will need to be cut for the carapace to be easily removed. The eyes and the optic stalk can be visualized and these can be followed down to the cranial part of the supraesophageal ganglion (sometimes referred to as the central ganglion, protocerebrum or "brain") (Figure 8.6).

Gentle dissection of dorsal musculature will expose a hard cartilaginous structure, the endosternite. In Araneae, the endosternite is centrally located and proximal to the pedicle originating from the dorsal opisthosoma. Dorsal to the endosternite is the esophagus and stomach which leads to the opisthosoma. Beneath the endosternite is the central ganglion. The central ganglion can also be easily identified by removing a leg and following the nerve branch to the central ganglion.

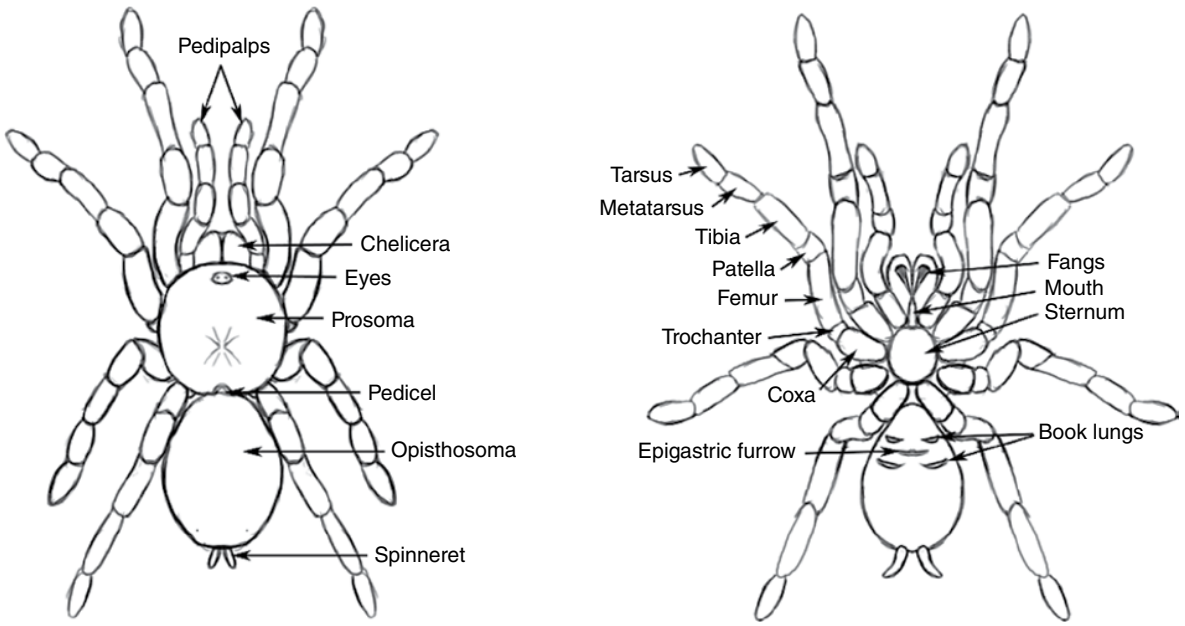


Figure 8.1 Gross anatomy. Dorsal (*left*) and ventral (*right*) view of theraphosid spider.

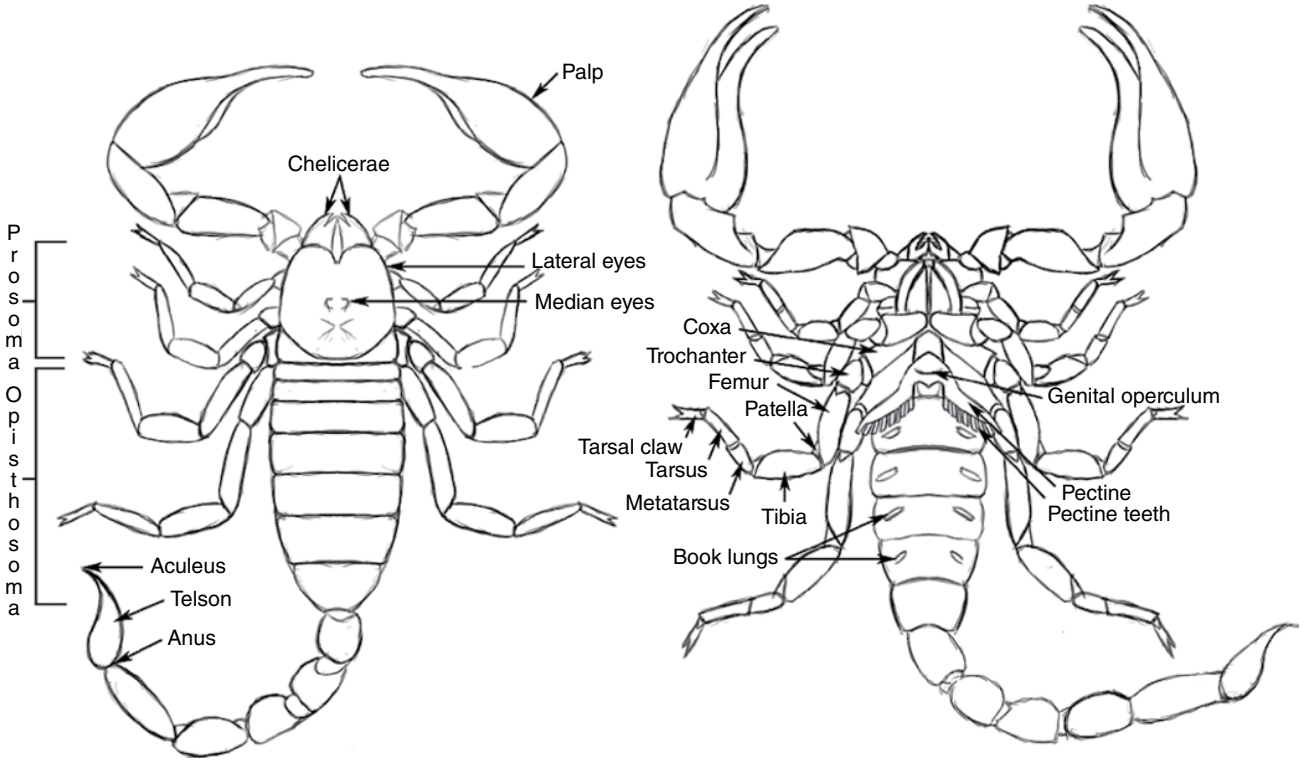


Figure 8.2 Gross anatomy. Dorsal (*left*) and ventral (*right*) view of scorpion.



Figure 8.3 Mesolense image of unidentified Pseudoscorpion from Peru. Source: Reproduced with permission from G. McConnell (University of Strathclyde, and R. Piper and D. Wilcockson (University of Aberystwyth).

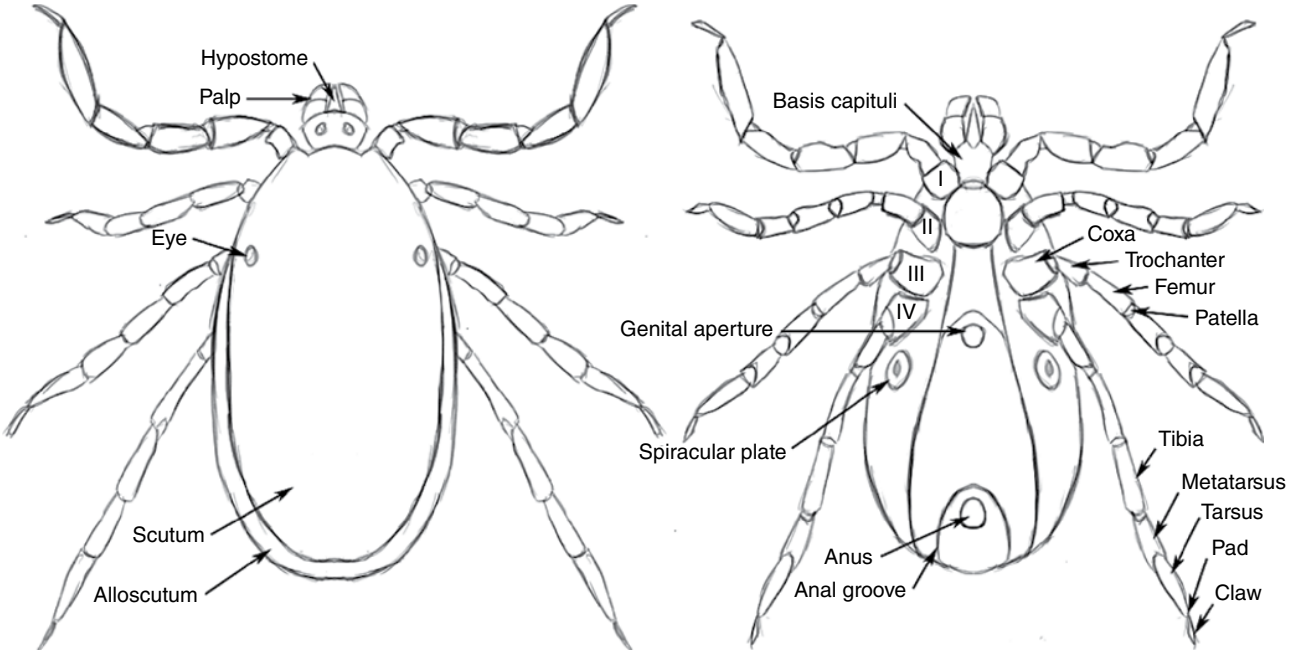


Figure 8.4 Gross anatomy. Dorsal (*left*) and ventral (*right*) view of ascarid mite. Roman numerals correspond to numbering of coxae.

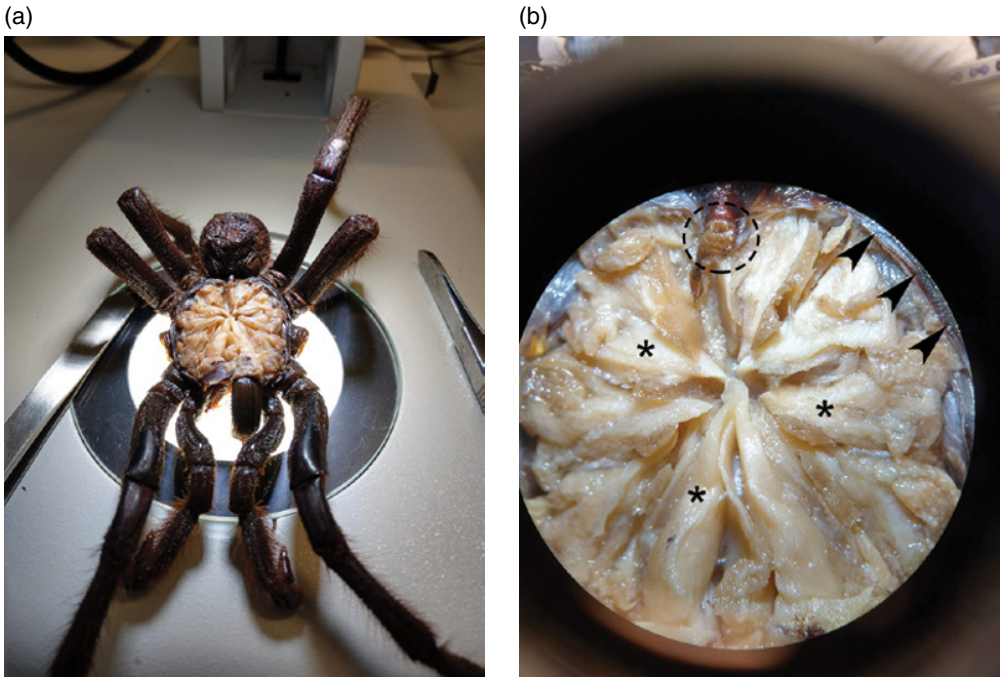


Figure 8.5 Dissection of a theraphosid spider (*Therophosa stirmi*). The prosomal carapace has been cut away (a). On higher magnification using a dissecting microscope (b), the edge of the prosomal carapace is at the margin of the image (*arrowheads*). Most of the exposed tissue is dorsal carapacial muscle (*asterisk*). The pedicel is also visible (*dashed circle*).

It is advisable to detach a leg for individual dissection; this is best achieved by applying firm traction upwards while holding the femur of the leg. Cutting the cuticle around the leg is often necessary to fully expose the nerve and muscles within the leg. Muscles surround the nerve and artery, which runs parallel to the nerve.

For the opisthosoma, careful removal of the exoskeleton in a similar way to the prosoma is recommended. Organs within the opisthostoma are tightly adhered together with connective tissue, so dissection of the internal organs is best done with an appreciation of the different textures of the organs, along with their gross locations.

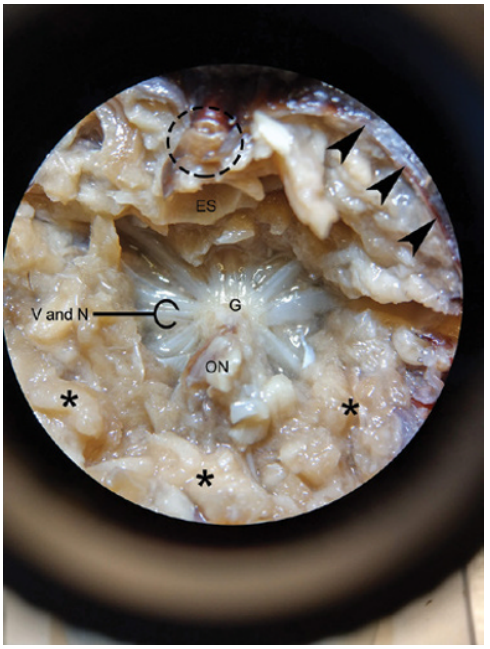


Figure 8.6 Dissection of a theraphosid spider (*Therophosa stirmi*) using a dissecting microscope, continued from Figure 8.5. The prosomal carapace has been removed, leaving only the edge of the prosomal carapace is at the margin of the image (*arrowheads*). The endosternite (ES) has also been cut away to expose the underlying tissues. Dorsal carapacial muscle (*asterisk*) and the pedicel (*dashed circle*) are visible at the periphery. The subesophageal ganglion (G) is at the center of the image, off of which branch the optic nerve (ON), and multiple small nerves, each with an accompanying vessel (V and N).



Figure 8.7 Dissection of a scorpion (*Parabuthus transvaalicus*). The prosomal and opisthosomal carapace has been removed. The inner aspect of the opisthosomal carapace has a groove (*arrows*) representing the recess in which the heart lies. The dissection reveals the eye (circle), midgut diverticula (MD), heart and neural ganglia (*arrowheads*), metasomal muscle (*asterisks*), and telson (T).

Silk glands (Araneae) are usually simple to identify as they have a distinctive spheroid appearance similar to a "bunch of grapes" and exist ventrocaudally. Silk glands in males are often smaller than in females. Ovaries and testes are cranial to the silk glands but can be difficult to differentiate from them. Some arachnid species have paired and unpaired reproductive tracts. The luminal alimentary tract can be dissected by navigating from the cloaca and stercoral sac and working through the tract cranially. Very gentle dissection is required. Malpighian glands are interspersed with the midgut diverticula and appear as spherical distinct organs. They terminate in a series of ducts into the luminal alimentary tract though this is often very difficult to see on gross dissection. Cardiac tissue can be challenging to identify, but when the opisthosomal cuticle is carefully dissected, it is possible, in some species, to see the indentation of the pericardial sac on the ventral cuticle and this can aid in dissection and identification (Figure 8.7).

Though dissection can be very useful when looking at specific organs and gross pathology, the authors find that histologic sectioning of the whole opisthosoma is more fruitful, especially when trying to determine the presence of pathology, as the overall tissue structure is maintained more successfully.

For dissection of Araneomorph spiders, a similar technique can be followed, but due to the fragile nature of the opisthosoma it needs to be gently peeled back from cranial to caudal, over the dorsal surface. The heart will be found attached to the ventral surface of the cuticle. Acari and Opiliones specimens can be investigated by inserting the microforceps laterally into the anterior portion of the opisthosoma and peeling the cuticle away in sections. The authors' preferred technique for Opiliones requires removal of legs from one side by breaking off at the trochanter and then the fused prosoma and opisthosoma can be opened by dissecting laterally to remove the dorsal cuticle.

8.3 Histology (Table 8.1)

8.3.1 Body Wall/Musculoskeletal

The overall body structure can be separated into the prosoma and opisthosoma, all of which is covered by a chitinous exoskeleton. The exoskeleton acts to protect the internal organs from the external environment and as a sensory organ through chitinous hair and slit sensilla. The limbs are required for movement, protection, and sensation. The pedipalps have sensory, feeding and locomotor roles, and the terminal bulb is the location of sperm storage prior to insemination in Araneae.

Table 8.1 Organs for histologic evaluation in Arachnida.[a]

Organ system		Organs
Body wall/musculoskeletal		Cuticle, epidermis, endosternite, skeletal muscle
Digestive		Oral cavity, pharynx, esophagus, sucking stomach, midgut tube, midgut diverticula, hindgut
Excretory		Malpighian glands (*kidneys*), coxal glands, stercoral sac
Circulatory		Heart, pericardial sac, arteries, sinuses
Immune		Hemocytes
Respiratory		Book lungs, tracheoles
Nervous		Supraesophogeal ganglia, subesophageal ganglia, optic lobe, peripheral nerves
Reproductive	Male	Testes, palpal bulb
	Female	Ovary, uterus
Special senses		Ocelli, cuticular sensilla
Special organs		Venom gland, silk gland

[a] Alternative names for organs are provided parenthetically, in italics.

8.3.1.1 Cuticle

The cuticle thickness varies across the body of an arachnid, often being thicker over the prosoma and thinner over the opisthosoma. The cuticle presents difficulties in the sectioning stage of processing. Fixation with AZF fixative or Davidson's fixative for 5–7 days can aid in softening the cuticle and thus make sectioning less disruptive to internal tissues.

The cuticle is divided into layers: epicuticle, exocuticle, endocuticle, and epidermis. The epicuticle is thin and acellular; it can often be lost in processing or not take up stain as readily. The exocuticle and endocuticle are much thicker and account for the majority of the cuticle thickness; these layers may be difficult to distinguish from each other or may have a clear bilayer. The endocuticle is eosinophilic and acellular with distinct chitinous layering. With the exception of the oral cuticle, there are frequent cuticular canals and junctions (Kennaugh 1968; Krishnan 1953) (Figures 8.8 and 8.9). The uptake of eosin can vary across the cuticle, with the innermost endocuticle taking on more stain and the outermost exocuticle taking up less. The thickness of and proportion of the different layers of the cuticle vary depending on function, anatomic location, and species. The prosomal cuticle has a thicker endocuticle layer resulting in an overall thicker cuticle whereas articular cuticle is thin and is often predominantly exocuticle and epicuticle (Hadley 1981).

Several different structures are within or attached to the cuticle. Hair sensilla and cuticular ducts pass through the cuticle (Schaber and Barth 2015). Striated skeletal muscle and other cartilaginous structures can be directly attached to the basilar aspect of the cuticle as a cuticular junction. Cuticular villi are present in some species.

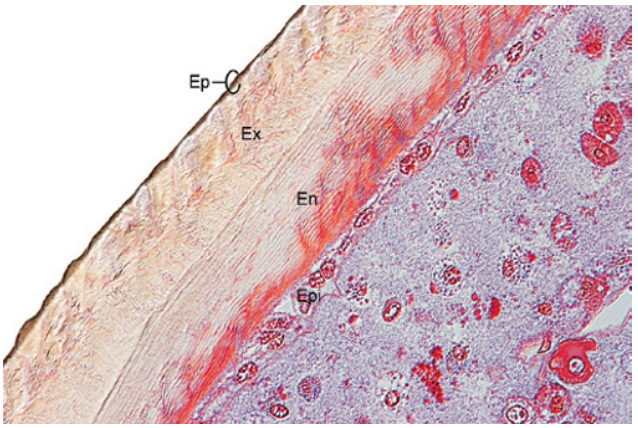


Figure 8.8 Transverse section of wolf spider cuticle showing a simple epidermal layer (Epi) overlain by the endocuticle (En), exocuticle (Ex), and epicuticle (Ep), 400×. Modified trichrome.

The dorsal opisthosoma of new-world Theraphosidae (except Ischnocoline subfamily) contain urticating setae not detected in any other arachnids. These were first described in 1972 as four classes with a further class subsequently discovered on the palps of *Ephebopus* spp. (Cooke et al. 1972; Foelix et al. 2009). Urticating setae are characterized by a weak attachment breakpoint at their base or sockets (types V and VI) and penetrating tips with barbs on the shaft (Bertani and Guadanucci 2013). These characteristics have evolved for defense and are irritating to mucous membranes with potential for penetration and thus should be considered as an occupational allergen (Castro et al. 1995).

The cuticle can line and extend into other internal structures within the body. For instance, the oral cavity and proximal gastrointestinal tract are often lined with cuticle

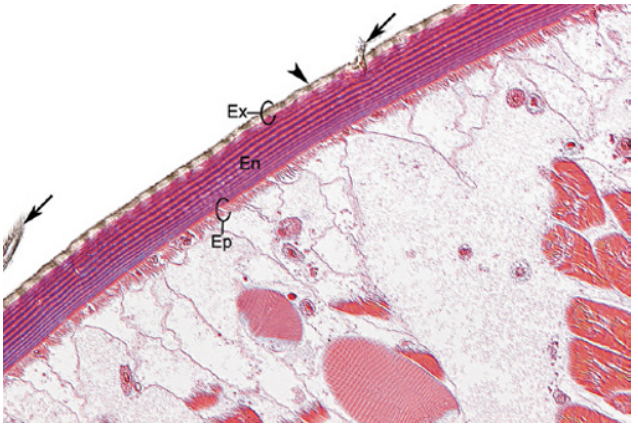


Figure 8.9 Transverse section of cuticle of an unspecified arachnid species. Hair sensilla (*arrows*) arise from the cuticle, which is composed of epicuticle (*arrowhead*), exocuticle (Ex), and endocuticle (En), and is subtended by epidermis (Ep). 200×. Trichrome.

and this cuticle can extend further into the surrounding organs (often adjacent to or involving ducts and sinuses). Further to this, chitin can serve as tendinous attachments from internal organs and structures to the cuticle. The epidermis is composed of a simple single cell epithelial layer directly below the cuticle (Hadley 1981). Collagen fibers and myofibers can extend directly from the basilar aspect of the epidermis to deeper structures within the limb or body.

8.3.1.2 Epidermis

The epidermis (also called hypodermis) is arranged as a single layer of columnar cells with basilar oval nuclei. Below the epidermis, there are occasional glands and ducts. Cells with ducts and glands have cytoplasmic vacuoles or granules. In pigmented species and in pigmented areas, there may be pigment deposits within or around the epidermis. White coloration in spiders is caused by the accumulation of guanocytes, which are cells that store guanine crystals. These cells are in the midgut and can form a contiguous layer below the epidermis due to the close proximity of the peripheral portions of the midgut to the epidermis. The guanine crystals in these cells reflect light. This light reflection produces white coloration in regions of the exoskeleton that are transparent. A well-known example of guanocytes causing white coloration is on the opisthosoma of the common garden spider (*Araneus diadematus*) (Foelix 2011).

Excessive melanin pigmentation within the exocuticle and endocuticle can also occur in response to inflammation. If inflammation is the cause of pigment deposits, bacteria, fungi, or inflammatory hemocytes may be present.

8.3.1.3 Connective Tissues

Within Araneae, there is an endosternite (also called the cartilage shelf) which is present dorsal to the central ganglion and ventral to the gastrointestinal tract (the mouth, esophagus, and proximal intestines) and dorsal muscle (Figure 8.10). The exact position and length of the endosternite vary within the Arachnida and this feature is also present in Alpigradida, Acarida, Opilionida, Ricinuleida, Scorpiones, Solifugae, and Pseudoscorpionida (Firstman 1973).

The endosternite is composed of an eosinophilic to chondroid matrix with lacunae. The lacunae contain vacuoles with peripheralized chondrocytes (Figure 8.11). Myofibers

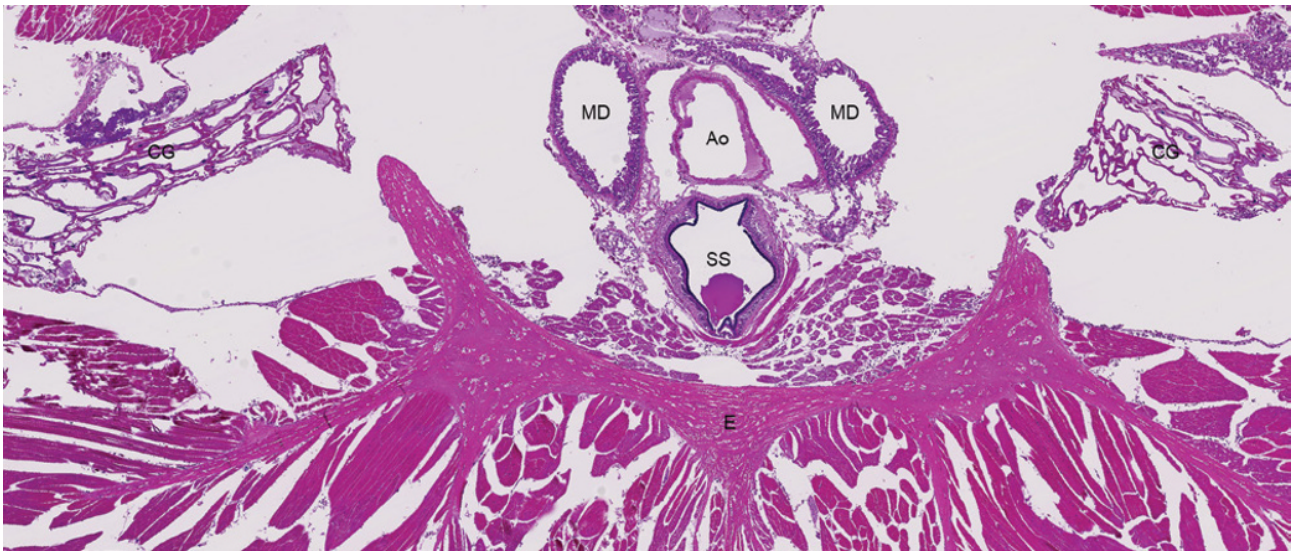


Figure 8.10 Prosoma of a birdeater tarantula (*Theraphosa blondi*). The endosternite (E) is a rigid shelf in the prosoma to which striated muscle attaches. The sucking stomach (SS) is dorsal to the endosternite, and the aorta (Ao) is dorsal to the sucking stomach. Midgut diverticula (MD) are lateral to the aorta. Coxal glands (CG) are lateral to the midgut diverticula. 9×. HE.

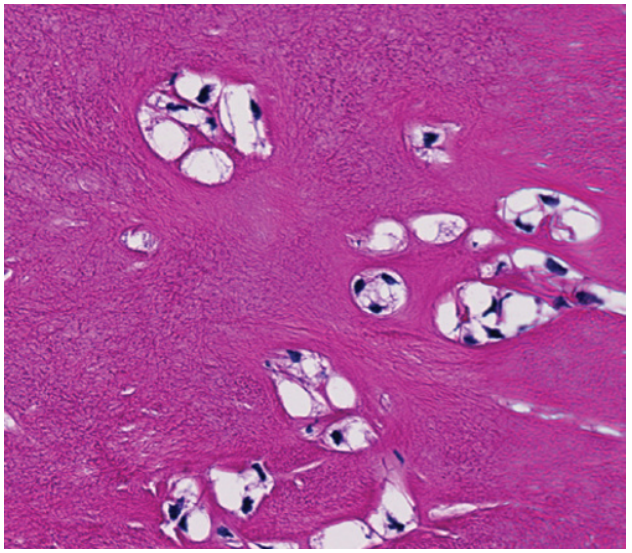


Figure 8.11 Endosternite of a birdeater tarantula (*Theraphosa blondi*). Cells of the endosternite are clustered in lacunae. 400×. HE.

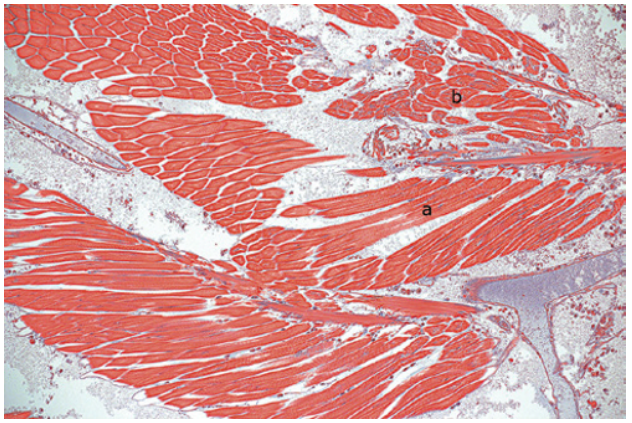


Figure 8.12 Cheliceral muscle in an unspecified wolf spider species. Longitudinal (a) and transverse sections (b) of muscle are present. 200×. Masson's trichrome.

can be attached directly to the cartilage shelf. Skeletal muscle is present dorsal to the endosternite. In some arachnid species, there can be some calcification present within the endosternite (Kovoor 1978).

The muscle in arachnids is predominantly striated skeletal muscle with nuclei both peripherally and centrally within the fibers (Figure 8.12). Muscle is predominantly found within the prosoma, the proximal limbs and the epidermal layer of the cuticle. Muscles vary in size depending on their location and function. The legs have extensor and flexor muscle at every joint except for the femur-patella and tibia-metatarsal joints, both of which lack extensors. The extension of these joints is caused by an increase in hemolymphatic pressure (i.e., hydraulic action). The muscles attached to the cuticle often elongate terminally and

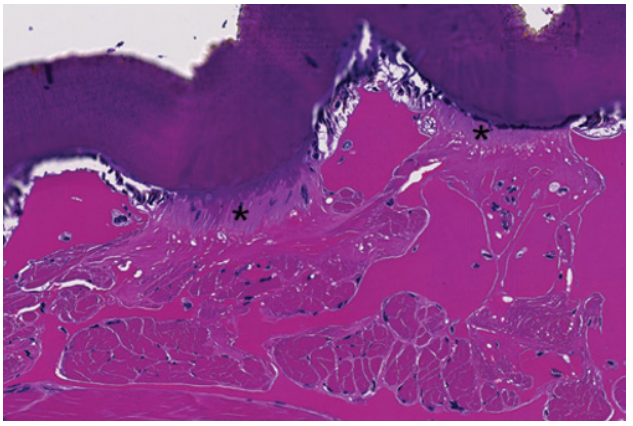


Figure 8.13 Skeletal muscle of a birdeater tarantula (*Theraphosa blondi*). Skeletal muscle attaches directly to the internal surface of the cuticle via tendinous extensions (*asterisks*). Adjacent to areas of attachment are pigmented epidermal cells. Skeletal muscle bundles are separated by hemolymph. 125×. HE.

are continuous with fine cuticular tubes surrounded by the epidermis (Figure 8.13); this portion of the muscle is referred to by some as a "tendon," and is prominent in the tarsal claws (Foelix 2011).

8.3.2 Digestive System

The digestive system is composed of the luminal alimentary tract and the digestive ceca (also called midgut diverticula). The luminal alimentary tract is composed of the preoral cavity (formed by the coxa of the pedipalps), pharynx, esophagus, stomach, midgut, and hindgut. The midgut is the only portion of the alimentary tract without an inner cuticle lining, and as such is the primary site of food absorption. The majority of arachnids are liquid feeders so they lack specific mouthparts; however, many use the chelicera, characteristic of the class, for prey manipulation and to extract fluid for digestion. Cheliceral manipulation ranges from piercing in Acari, to envenomation in Araneae, to crushing in Solifugae. The preoral cavity, typically located within the prosoma, encompasses the chelicarae and pedipalps, which manipulate food material into the oral cavity, which leads to the pharynx and esophagus. The esophagus is continuous with the sucking stomach, which leads to the midgut, which is composed of a midgut tube and numerous prosomal and opisthosomal diverticula (also called digestive ceca). Prosomal diverticula extend into the legs in some species. At the junction between the midgut and hindgut in arachnids (except for solifuges), there is a blind-ended pouch called the stercoral sac (also called cloaca). Malpighian tubules (excretory organ) terminate in the stercoral sac (when present) or at the midgut–hindgut junction.

8.3.2.1 Oral Cavity

The oral cavity within Arachnida is predominantly located between the chelicerae. It is proposed that hair sensilla lining the preoral cavity are associated with taste (Foelix 2011). The preoral cavity, oral cavity, and pharynx are lined by a thick sclerotized cuticle with a subjacent pseudostratified epithelium. Some species have a prominent labrum over the dorsal opening of the mouth; Ricinulei has a specialized cuticular plate dorsally over the oral cavity called the cucullus (Talarico et al. 2011). There are minimal or no pores within alimentary cuticles. On the ventral aspect of the preoral cavity and oral cavity are setae-like protrusions or "teeth," which help filter liquified ingesta (Figure 8.14).

8.3.2.2 Pharynx, Esophagus, and Sucking Stomach

The pharynx begins after the oral cavity. The pharynx has a variable shape on transverse section, with some species having a distinct "horseshoe" shape in Ricinulei (Talarico et al. 2011) or a "Y" shape in Solifugae (Klann and Alberti 2010). The cuticle luminal lining of the pharynx is thick and very sclerotized, with a thick epithelium. Additional setae-like protrusions can be seen on the ventral aspect. Dorsal to the luminal pharynx is a relatively large pharynx dilator striated muscle which is responsible for the pumping action that transports liquified ingesta through the esophagus.

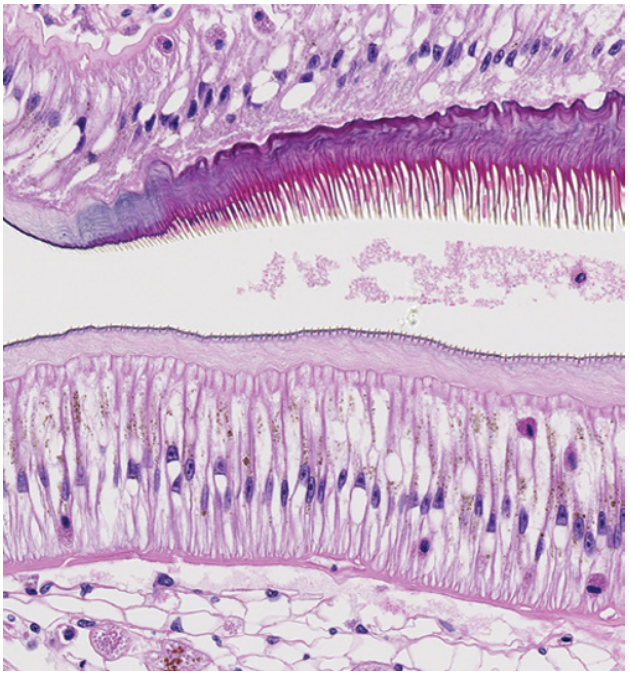


Figure 8.14 Oral cavity setae of a zebra tarantula (*Aphonopelma seemanni*). Setae-like protrusions over the epithelium help filter ingesta. 200×. HE.

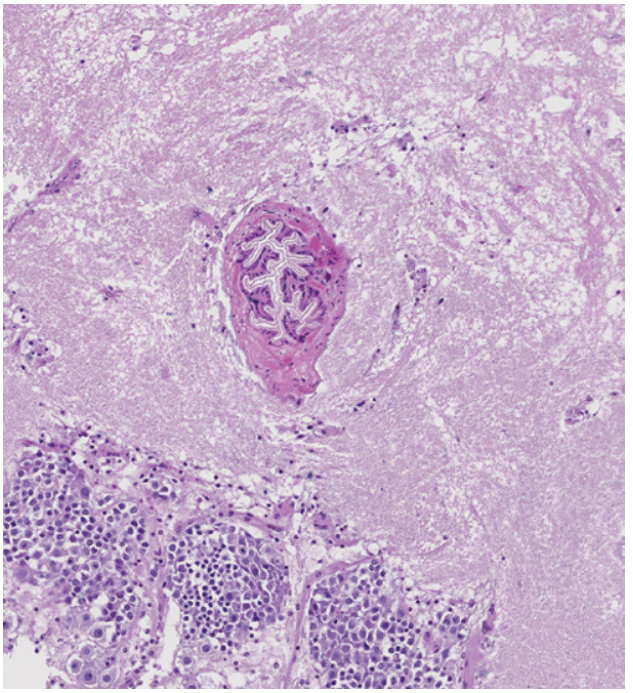


Figure 8.15 Esophagus of Asian jungle scorpion (*Heterometrus* sp.). The esophagus is a chitin-lined, luminal structure surrounded by neuropil that runs horizontally through the brain, dividing the brain into the supraesophageal ganglion (dorsal to the esophagus; not pictured) and the subesophageal ganglion (ventral to the esophagus; pictured). 20×. HE.

The esophagus is a narrow, chitin-lined luminal structure that leads to the sucking stomach and lies dorsal to the endosternite. It is lined by a circular striated muscle that is attached to the basilar aspect of the esophageal cuticle. The esophagus passes horizontally through the central nervous system (CNS), diving the CNS into the sub- and supraesophageal ganglia, such as described for Solifugae (Klann and Alberti 2010) (Figure 8.15).

The esophagus terminates in the sucking stomach, which is the main pump for food intake. The lumen of the sucking stomach has a larger diameter than the esophagus but is often collapsed in histologic section in a resting state (this resembles a "collapsed square"). The lateral and dorsal walls are attached to large muscle bundles; the muscular attachments to the lateral walls are attached to the endosternite (Figure 8.16). The sucking stomach is bordered dorsally by the aorta. When these muscles contract, the stomach luminal diameter expands greatly, acting as suction for liquefied ingesta (Foelix 2011).

8.3.2.3 Midgut Tube and Midgut Diverticula

The sucking stomach ends at, and empties into, the midgut tube at the caudal end of the prosoma. The midgut tube has numerous branches called midgut diverticula or digestive



Figure 8.16 Sucking stomach of an Arizona blond tarantula (*Aphonopelma chalcodes*). The sucking stomach is a chitin-lined cavity that forms a "collapsed box" shape when the animal is not actively ingesting food. The walls are attached to skeletal muscle that is anchored to the endosternite. 90×. HE.

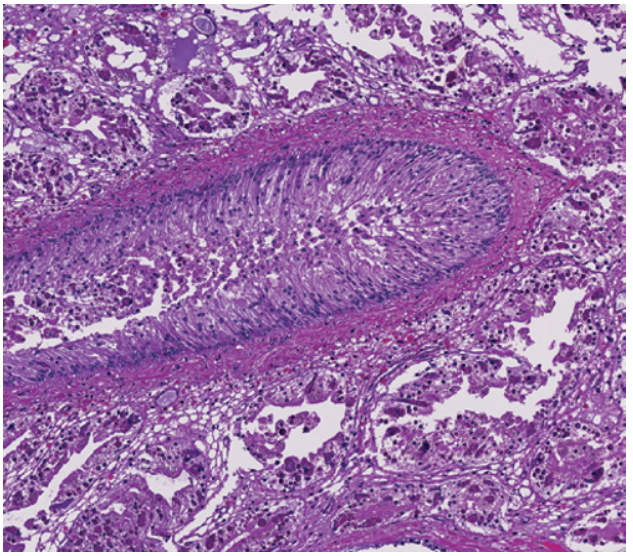


Figure 8.17 Midgut tube of a birdeater tarantula (*Theraphosa blondi*). The midgut tube is lined by tall columnar, simple epithelium and surrounded by a muscle layer. Unlike most other portions of the gastrointestinal tract, the epithelium is not lined internally by chitin to facilitate digestion. The midgut tube is surrounded by numerous midgut diverticula, which increase the absorptive surface area. 84×. HE.

ceca. These diverticula fill the majority of the opisthosoma and are present in smaller numbers in the prosoma (Ruppert et al. 2004).

The midgut tube epithelium is composed of a simple epithelial layer of tall columnar cells with apical microvilli resting on a basement membrane. Deep to the basement membrane is "intermediate tissue" composed of collagenous connective tissue or skeletal muscle. The epithelial layer is made up of secretory and digestive cells. Digestive cells contain lipid droplets and food vacuoles. They are significantly larger and more numerous than secretory cells. A specialized type of digestive cell is the guanocyte, which takes up metabolites and stores them as guanine crystals in the cytoplasm. More information on guanocytes is in the body wall and excretory sections. Secretory cells have a larger proportion of cytoplasm with smaller numbers of lipid droplets than digestive cells, with translucent poorly staining vacuoles (Figure 8.17). The appearance and size of a digestive cell are dependent on the nutritional state of the animal, with the ratio of digestive and secretory cells changing based on seasonal changes in nutritional state (Lipovšek et al. 2004, 2018). A third cell type has also been observed rarely which has an absence of vacuoles and spherocytes (Klann and Alberti 2010; Ludwig et al. 1994; Talarico et al. 2011).

Diverticula branch from the midgut tube in the prosoma and opisthosoma. The number and configuration of the diverticula branches are variable between the Arachnida orders. The prosomal diverticula can extend significantly down the limbs (Figure 8.18). The epithelium of the diverticula is similar to that lining the midgut tube except the diverticula cells have numerous conspicuous droplets of variable size (Figure 8.19) (Klann and Alberti 2010).

8.3.2.4 Stercoral Sac

The stercoral sac (also called cloaca) is a blind pocket extending from the caudal midgut tube in the opisthosoma which acts to store excretory (from Malpighian tubules) and digestive (from midgut diverticula) waste (Figure 8.20). Histologically, abundant crystals (largely guanine) can be observed in the stercoral sac lumen (Figure 8.21). The process of elimination takes place by egress of waste material from the stercoral sac into the anus and then through a short, cuticle-lined rectum. The stercoral sac epithelium is made up of a tall epithelial layer, a basal lamina, and thin muscle fibers. The epithelial cells are closely packed with nuclei either apically or basally positioned. In some species, the epithelial cells are similar to those of the Malpighian tubules and assist in excretion of nitrogenous waste (see below) (Figure 8.22) (Ruppert et al. 2004).

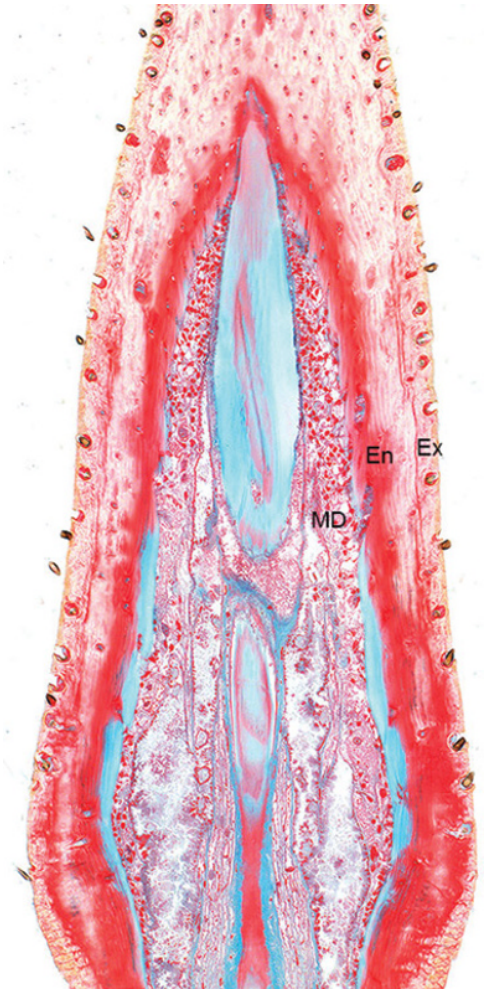


Figure 8.18 Longitudinal section of an unspecified arachnid limb demonstrating the microstructure of the femur. Midgut diverticula (MD) extend down the majority of the limb. Distinct endocuticle (En) and exocuticle (Ex) layers are present. Cuticular junctions can be seen within both the endocuticle and exocuticle. 150×. Modified trichrome.

8.3.3 Excretory System

Arachnids have variable strategies between taxa as to the regulation of water and salt as well as the excretion of nitrogen, with different anatomic structures taking on this role. Nitrogen is excreted in multiple different forms within arachnids, though within true arachnids it is predominantly excreted as guanine with variable amounts of uric acid and adenine (Anderson 1966; Butt and Taylor 1995; Ruppert et al. 2004). These substances are nearly insoluble in water and crystalize (Foelix 2011), allowing them to polarize in histologic sections. Arachnids have two types of dedicated excretory organs for the elimination of waste: Malpighian tubules and coxal glands (also called saccate nephridia). Arachnids may possess either of these organs or both. Scorpiones have two pairs of Malpighian tubules

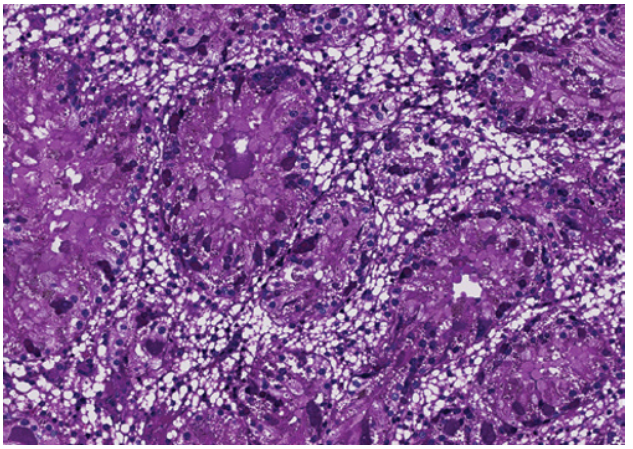


Figure 8.19 Midgut diverticula of birdeater tarantula (*Theraphosa blondi*). The midgut diverticula fill many spaces in the opisthosoma. They are organized into both discrete, tubular structures, and insterstitial tissue without evident tubular formation. Midgut diverticula are composed of large epithelial cells with numerous cytoplasmic droplets of variable size and staining properties. 170×. HE.

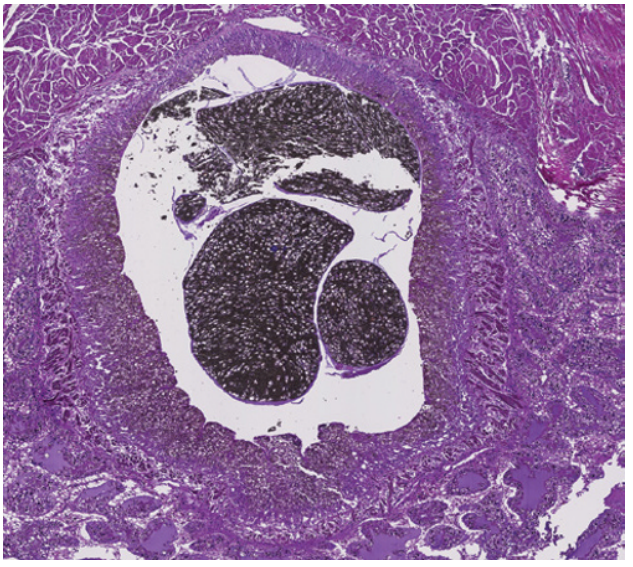


Figure 8.20 Stercoral sac of a birdeater tarantula (*Theraphosa blondi*). The stercoral sac forms a large tubular structure in the prosoma surrounded by abundant midgut diverticula. The stercoral sac lumen contains highly refractile excretory and digestive products, such as guanine. 25×. HE.

and one pair of coxal glands that empty into the third pair of walking legs. Araneae, Ricinulei, and Solifugae have Malpighian tubules and one (Araneomorphae, Ricinulei, and Solifugae) to two (Mesothelia and Mygalomorphae) pairs of coxal glands. Other species, including those from Pseudoscorpiones and Opiliones, have prosomal coxal glands but lack Malpighian tubules (Gilbert 1952). Acari vary in their excretory systems and may have one pair of

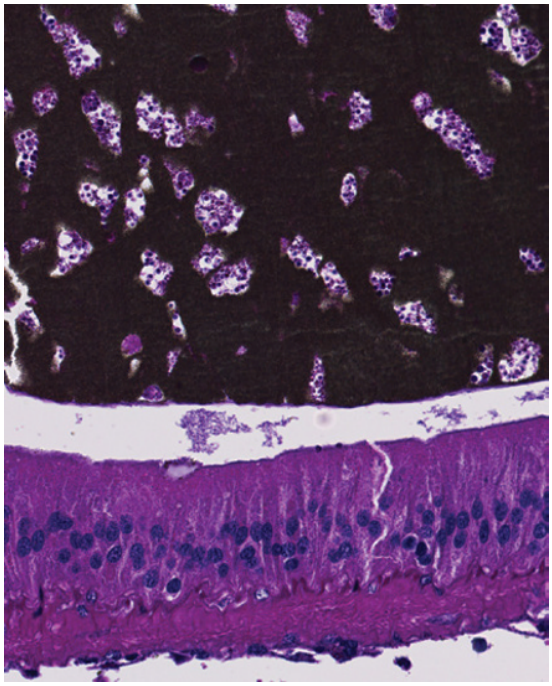


Figure 8.21 Stercoral sac of a birdeater tarantula (*Theraphosa blondi*). The stercoral sac mucosa is composed of simple, columnar epithelium with basl nuclei. Digestive and excretory products are in the lumen. 228×. HE.

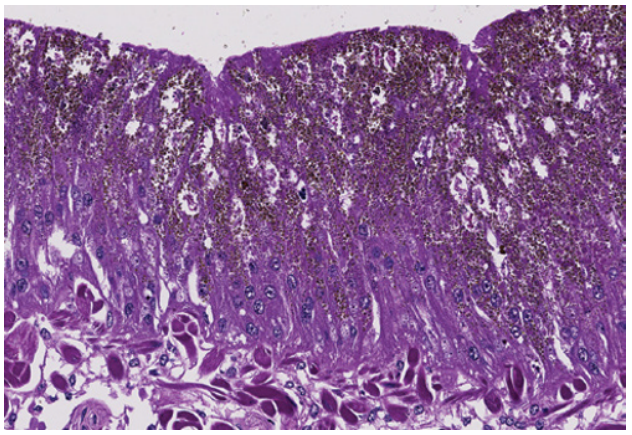


Figure 8.22 Stercoral sac of a birdeater tarantula (*Theraphosa blondi*). The stercoral sac mucosal epithelium is highly excretory in some species. The mucosal epithelial cells have abundant intracytoplasmic excretory products that are refractile with polarized light (not shown). 228×. HE.

coxal glands, one or two pairs of Malpighian tubules, or both (Ruppert et al. 2004).

In addition to dedicated excretory organs, many species also have other methods for excreting nitrogenous wastes. Nephrocytes are large cells (30–80 µm in diameter) free in the hemolymph that absorb nitrogenous waste and store or transfer it to excretory organs (Figure 8.23). The epithelium

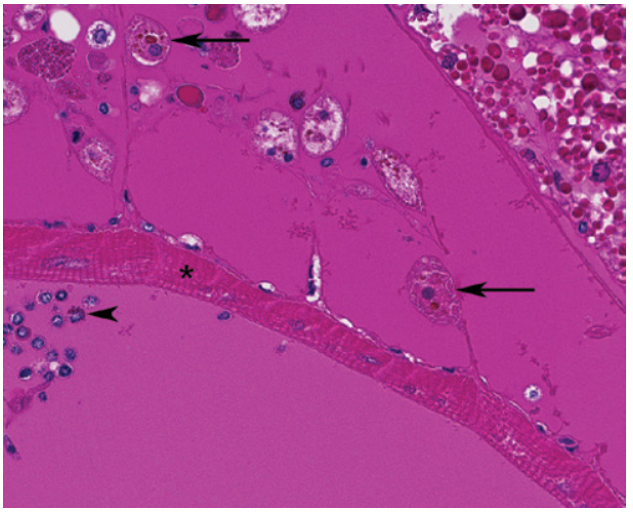


Figure 8.23 Nephrocytes of a vinegaroon (order Thelyphonida). Nephrocytes are in the hemolymph in the pericardial sac. They are large (up to 80 µm in diameter), polygonal cells with cytoplasmic excretory products (*arrow*). For size comparison, hemocytes are within the cardiac lumen in the lower left (*arrowhead*). The cardiac wall is striated (*asterisk*). Midgut diverticula surround the pericardial sac in the upper right of the image. 332×. HE.

of the intestine and stercoral sac may be similar to that of Malpighian tubules, capable of absorbing nitrogenous waste from hemolymph and transferring it to the lumen of the alimentary canal. Guanocytes and interstitial cells in the digestive ceca absorb guanine. Guanocytes are specialized cells that are present in the epidermis and intestinal epithelium (Foelix 2011).

8.3.3.1 Malpighian Tubules

Malpighian tubules are luminal organs surrounded by a thin basal lamina. These tubules absorb nitrogenous wastes from the hemolymph and deposit them into the tubular lumen that leads to a series of ducts, which eventually terminate into the stercoral sac for incorporation into the feces. They contain two cell types within the wall. Type 1 cells have large basilar nuclei and can contain palely eosinophilic guanine droplets. Type 2 cells are basophilic with small nuclei (Hazelton et al. 2002). Often Malpighian glands contain guanine pigments that are brown and lighter than melanin (Figure 8.24).

8.3.3.2 Coxal Glands

Coxal glands are present in pairs (one or two) in the prosoma of some arachnids. They are ventrally positioned and extend into the coxae of the walking legs, opening at the first and/or third coxae (Figure 8.25). These glands release fluid during feeding and play a role in ion and water balance (Foelix 2011). Coxal glands have variable components depending on the species, and there are inconsistencies in

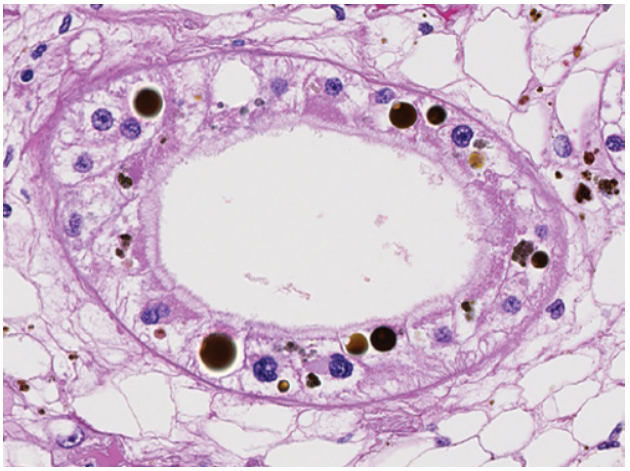


Figure 8.24 Malphighian tubule of a zebra tarantula (*Aphonopelma seemanni*). Malphighian tubules are small tubules lined by a single layer of cuboidal epithelial cells that frequently have intracytoplasmic brown inclusions representing excretory material. There are reduced granules in the surrounding midgut diverticula. 400×. HE.

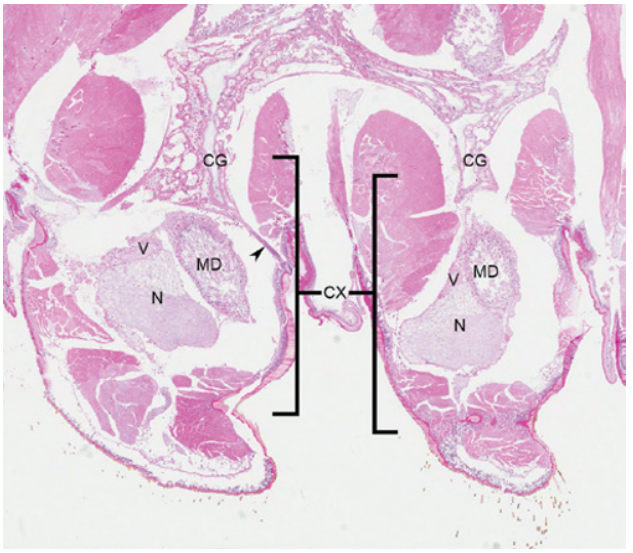


Figure 8.25 Coxae (CX) of two walking legs of a Trinidad chevron tarantula (*Psalmopoeus cambridgei*). The coxal glands (CG) extend into the coxae and empty via ducts (*arrowhead*). The midgut diverticula (MD), vessels (V), and nerves (N) occur in triads in the limbs. 16×. HE.

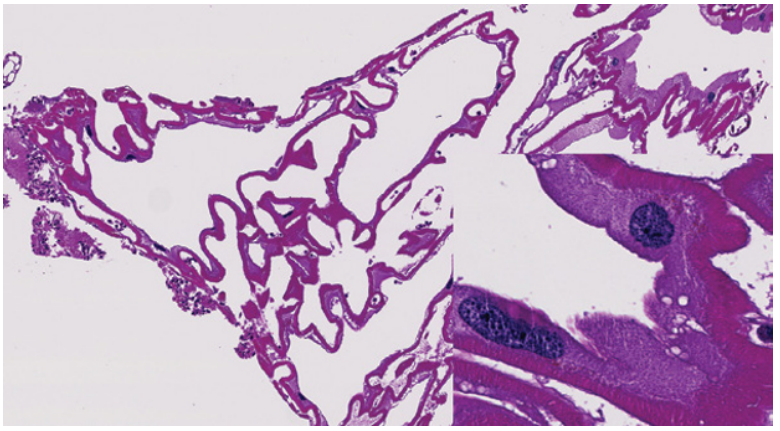


Figure 8.26 Coxal gland of a birdeater tarantula (*Theraphosa blondi*). The coxal gland is a highly convoluted tube lined by a single layer of cells that segmentally have abundant, basilar cytoplasmic striations (inset) and large oval nuclei. 80×; inset 400×. HE.

the literature regarding the terminology of the parts of the coxal glands (Klann 2009). Many references describe segments of the coxal gland to include a labyrinth (the largest portion; also called mucous segment), sacculus, and tubule. The sacculus and cranial aspect of the labyrinth are immediately lateral to the supraesophageal. The caudal end of the labyrinth is lateral to the anterior midgut ceca, and the outer surface of these two structures may be in direct apposition. The coxal gland is a highly convoluted tube lined by a single layer of epithelial cells with a thin, internal chitinous skeleton called the skeletal support. The skeletal support is very thin (often <1 μm diameter), and may not be within the resolution of the light microscope. The skeletal support is continuous with the coxal gland duct. The coxal gland epithelial cells have cytoplasmic striations over the majority of the length of the gland (Figure 8.26). The sacculus is apparently a small, thin-walled structure lined by cells that lack striations; the sacculus is sometimes omitted from studies on coxal glands as it is difficult to obtain in histologic section (Klann 2009; Woodring 1973).

Additional glands may be associated with the coxal gland that open into the same duct. These glands are adjacent to the coxal gland and form small tubules of cells with abundant cytoplasmic granules (Figure 8.27) (Woodring 1973).

8.3.4 Circulatory System

The circulatory system of arachnids is made up of the heart, the internal arteries, the limb arteries, and the venous sinuses, all of which transport hemolymph. Hemolymph

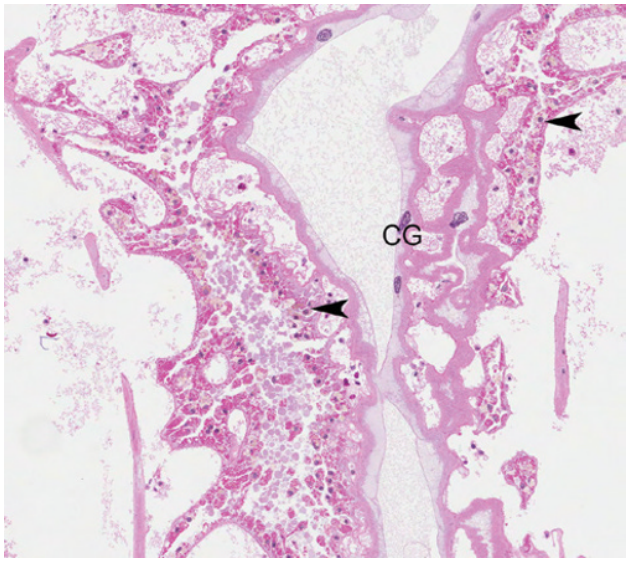


Figure 8.27 Coxal gland of a birdeater tarantula (*Theraphosa blondi*). The coxal glands (CG) are associated with smaller glands composed of highly pigmented epithelial cells. 50×. HE.

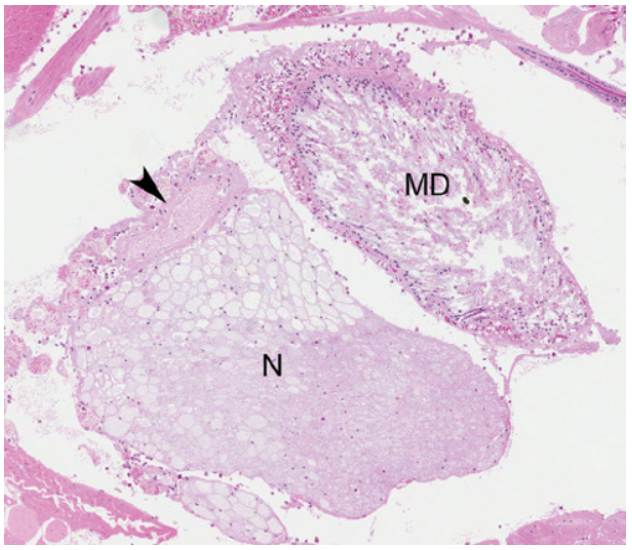


Figure 8.28 Coxa of a Trinidad chevron tarantula (*Psalmopoeus cambridgei*). The midgut diverticula (MD), vessel (*arrowhead*), and nerve (N) occur in a triad in the limb. 50×. HE.

is primarily made up of hemocyanin and hemocytes; there is no albumin present in hemolymph (Kennedy et al. 2019). Hemocyanin is a multifunctional protein with a role in oxygen and protein transport. The heart pumps hemolymph into the prosomal vessels and in turn into the limbs, thus maintaining the hemostatic pressure required for arachnids to move. Venous return occurs via sinuses which pass hemolymph through the book lung lamellae and eventually back into the heart. The vessels are often closely associated with nerves/ganglia and midgut diverticula (Figure 8.28).

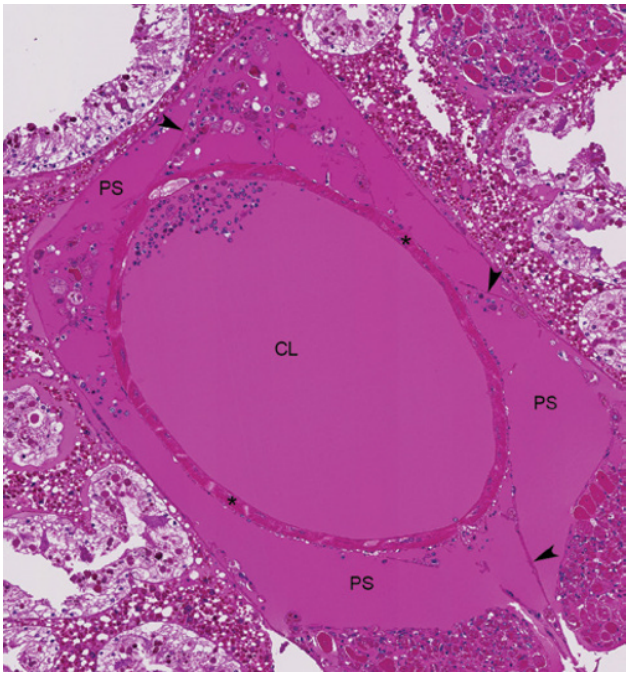


Figure 8.29 Heart of a vinegaroon (order Thelyphonida). Thin ligaments (*arrowheads*) suspend the heart in the pericardial sac (PS), which contains fluid, nephrocytes, and hemocytes. The cardiac lumen (CL) is surrounded by the myocardium (*asterisks*). The pericardial sac is surrounded by midgut diverticula. 70×. HE.

8.3.4.1 Heart

The heart is a long luminal tubular organ that extends along the dorsal midline of the anterior portion of the opisthosoma. It is suspended in a pericardial sinus by a series of ligaments (Figure 8.29). The pericardial sinus is composed of smooth muscle and collagen. Several holes (ostia) in the heart open into the pericardial sinus, allowing hemolymph to flow into the heart. When the heart contracts, backpressure causes the ostia to close, pumping hemolymph into the aorta. When the heart relaxes, the ligaments shorten due to inherent elasticity, which leads to cardiac expansion, opening of the ostia, and filling of the heart.

The adventitia of the heart is a thin layer of outer connective tissue. The myocardium is composed of striated musculature creating sinuses often containing hemocytes. The cardiac myofibers are orientated transversely with visible Z lines and central elongated nuclei (Sherman 1973). There is no endothelium but there is a simple basal membrane. The heart incorporates additional vessels within the opisthosoma that originate from the book lungs. A thin ganglion is on the dorsal side of the heart for neurogenic contraction.

8.3.4.2 Hemolymph Vessels

Arachnids have a semi-closed circulatory system with distinct arteries that transport hemolymph across the body and maintain the hydrostatic pressure required for movement.

Artery walls are composed of myofilaments that can change diameter. These vessels are within the prosoma, the limbs, and the opisthosoma. They do not have an endothelial layer but are composed of a simple basal lamina with a collagenous connective tissue layer of variable thickness (Shigei et al. 2001). In pulmonate arachnids, hemolymph vessels and respiratory tracheoles often run in parallel.

8.3.5 Immune System

Hemolymph accounts for 20% of the body weight of arachnids. It is essential not only for the immune system but also for hydraulics that allow the legs to function (Foelix 2011). For the majority of Arachnida, the primary component of hemolymph is the multimodal protein hemocyanin which accounts for approximately 80% of total protein (Paul et al. 1994). It has multiple functions beyond oxygen transport, including protein storage, transport, and buffering. Evidence suggests hemocyanin may also act as a reserve for cuticle proteins during ecdysis (Paul et al. 1994).

Hemocytes circulate freely in hemolymph and are broadly classified into five morphologic types: prohemocyte, granulocyte (with subtypes), spherulocyte, oenocytoid, and plasmatocyte (Soares et al. 2013). Differentiation of the types of hemocytes may only be possible ultrastructurally (transmission electron microscopy). There is variation in hemocytes between orders and use of nomenclature; oenocytoid cells are reportedly absent in scorpions (Ravindranath 1974) and Opiliones (Dannhorn and Seitz 1987). Microbial challenge in Theraphosidae has demonstrated that hemocytes utilize antimicrobial peptides and migrate to sites of infection (Fukuzawa et al. 2008). Plasmatocytes have been reported to phagocytose bacteria (Dannhorn and Seitz 1987). Hemocyanin has phenoloxidase activity and deposits melanin on microorganisms and foreign bodies (Figure 8.30); arachnids lack the prophenoloxidase gene found in insects (Bechsgaard et al. 2016). The clotting cascade in the majority of arachnids, except some mites, is similar from genomic studies to that of Limulus (Bechsgaard et al. 2016). All hemocytes are mononucleate and may differ by cytoplasmic volume and shape.

Hemocytes are formed by budding prehemocytes (or hemoblasts) from the heart on the luminal side of the myocardium. The hemoblasts then differentiate into the various types of hemocytes.

8.3.6 Respiratory System

Arachnida have two distinct respiratory organs. Depending on the species, arachnids can have book lungs, tracheoles, or both. Pulmonates respire through the use of "gill-like" book lungs which are present in Scorpiones,

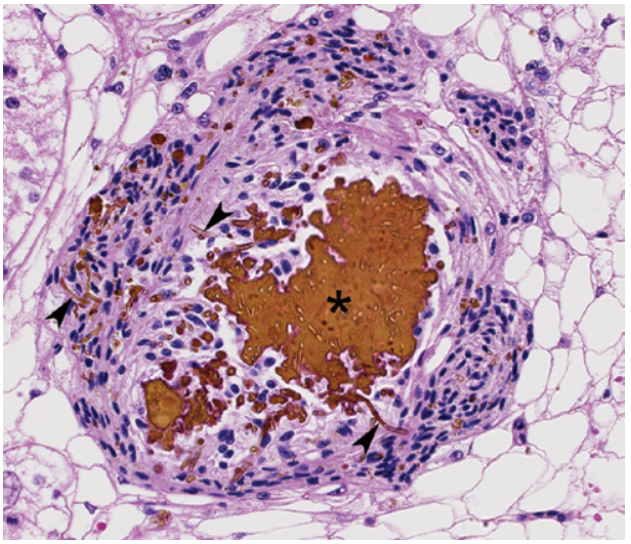


Figure 8.30 Melanin and hemocytes in the midgut diverticula of a zebra tarantula (*Aphonopelma seemanni*). Hemocytes form a nodule with central melanization (*asterisk*) associated with fungal infection. Fungal hyphae are highlighted with arrowheads. 300×. HE.

Araneae, Amblypygi, Uropygi, and Schizomida (Kamenz et al. 2005; Sharma 2017). Tracheates, for example the Solifugae and Opiliones, lack hemocyanin and respire through a distinct tracheal system with terminal branches of tracheoles that permeate the internal organs (Franz-Guess and Starck 2016). Most pulmonates have both book lungs and one to two pairs of tracheae (Foelix 2011).

8.3.6.1 Book Lungs

In pulmonate arachnids, the cranioventral aspect of the opisthosoma has several hairless regions centered on small slits in the cuticle that are called "lung slits." These lung slits are openings between the environment and an atrium in the inside of the opisthosoma that is the entrance to the respiratory tree. The atrium is a pocket within which the chitinous lamellae of the book lungs "float" (Figure 8.31). The lamellae are made up of two thin (0.02 μm) layers of epidermis that are barely visible except for their nuclei; these are lined by a thin (0.03 μm) layer of cuticle. Occasionally, epidermal cells are oriented perpendicular to the lamellae and form pillars holding the lamellae apart (Figure 8.32). The lamellae form a series of hemolymph channels surrounded by air, allowing oxygen to diffuse into the hemolymph and be absorbed by hemocyanin. After oxygenated hemolymph leaves the book lungs, it enters the lung vein and travels to the pericardium, where it enters the heart during diastole via opened ostia (Farley 2018; Foelix 2011; Paul et al. 1994; Scholtz and Kamenz 2006). Within the hemolymph channels of the book lungs there

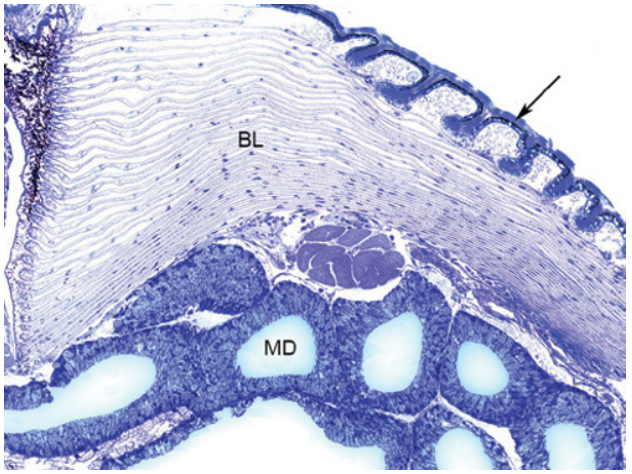


Figure 8.31 Book lungs of an unspecified arachnid. The book lungs (BL) are immediately deep to the opisthosomal body wall (*arrow*) and composed of numerous lamellae. Midgut diverticula (MD) are deep to the book lungs. 250×. Toluidine blue.

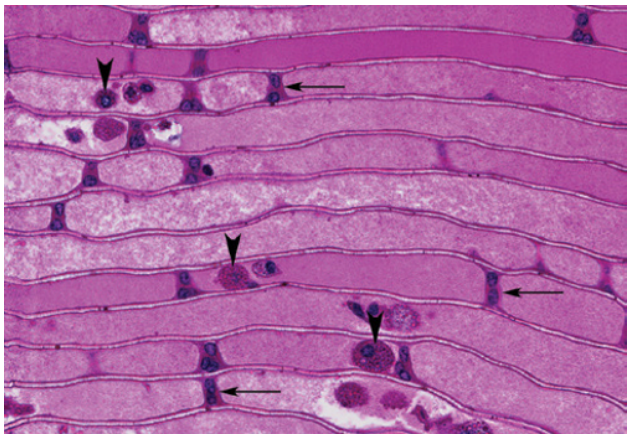


Figure 8.32 Book lungs of a vinegaroon (order Thelyphonida). Thin chitinous lamellae form parallel, air-filled tubes surrounded by hemolymph studded with hemocytes (*arrowheads*). Lamellae are evenly spaced by pillar cells (*arrows*) that hold lamellae apart. 332×. HE.

can be small numbers of hemocytes. The number of distinct book lungs is variable, with theraphosids and true arachnids typically having four distinct book lungs and scorpions having eight.

8.3.6.2 Tracheal Tube System

In tracheate arachnids, there are spiracle-like openings in the body wall caudal to the book lungs in the third abdominal segment that lead from the external environment into a cuticle-lined tracheal system. This system is composed of branching tracheoles, which are air-filled tubes with very thin (0.1–0.3 µm in diameter) walls that deliver oxygen to the hemolymph. The tracheoles are open ended and stop

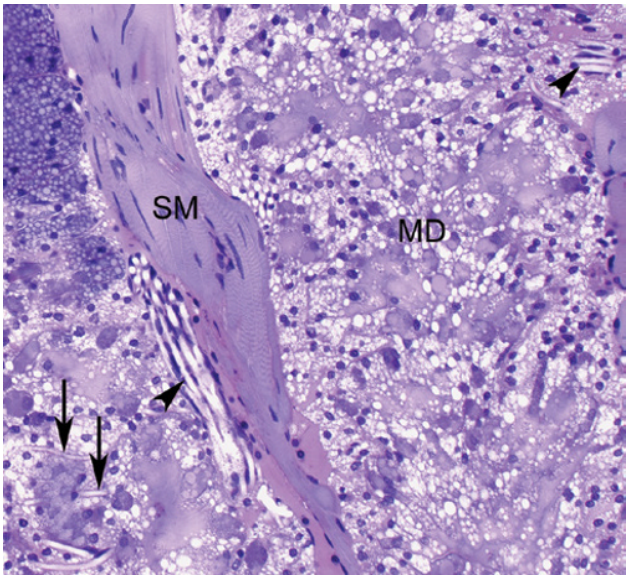


Figure 8.33 Limb of an unspecified arachnid. The tracheoles occur singly (*arrows*) and in clusters (*arrowheads*). The midgut diverticula (MD) extend down the limb. Striated muscle (SM) is also present. 150×. Modified trichrome.

just short of the tissues they supply (Figure 8.33). This results in oxygenation of the hemolymph, which makes the final transfer of oxygen to the tissues. This is in contrast to insects in which the tracheoles insert directly into cells. Tracheoles are composed of chitin that is continuous with the external cuticle (Foelix 2011).

8.3.7 Nervous System

The arachnid nervous system, like that of all arthropods, is divided into a series of ganglia that differ in the extent of fusion and division by order. There is a CNS in the anterior position. This topic was extensively reviewed in 2013 (Babu 2013) although the use of the term "brain" in arachnids is still under discussion. In spiders, the supraesophageal ganglion is most often referred to as the brain or protocerebrum, receives sensory input from the optic nerves, and communicates with the chelicera. The ventral subesophageal ganglia of the arachnid CNS consists of paired ganglia connected to each walking leg, chelicera, and pedipalp, with a posterior pair of ganglia serving the opisthosoma (Fabian-Fine et al. 2015). The cheliceral nerves innervate the anterodorsal region of the subesophageal ganglion (Loesel et al. 2011). The subesophageal and supraesophageal ganglia are separated by the esophagus, which traverses between the two horizontally (Figure 8.34).

Histologically, the CNS is organized into a marginal layer of neurons (the cortex) and an inner mass of nerve fibers (the neuropil). The outside of the CNS is covered by con-

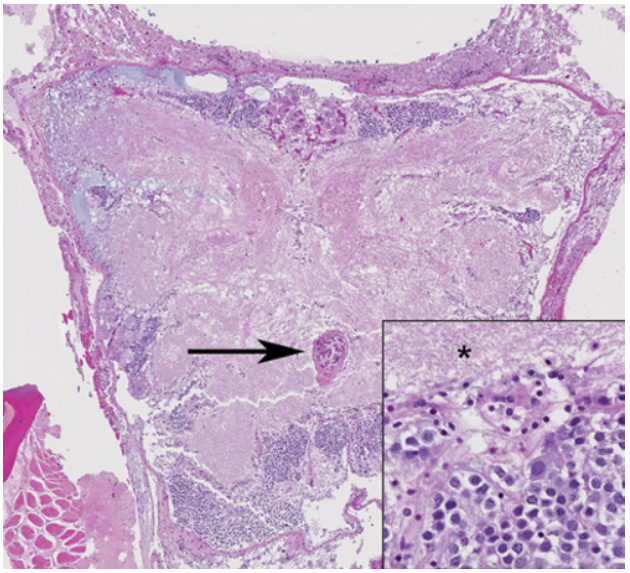


Figure 8.34 Brain of Asian jungle scorpion (*Heterometrus* sp.). The supra- (above) and sub- (below) esophageal ganglia are separated by the esophagus (*arrow*). The brain is divided into the cortex, composed of neurons, and the inner mass of nerve fibers called the neuropil (*asterisk*; inset). 25×; inset 150×. HE.

nective tissue that is particularly thick in Orthognatha. The neurons vary in size. Largest neurons are believed to represent motorneurons; smaller neurons are often interneurons connecting ganglia. Clusters of neuroendocrine cells are present in the supra- and subesophageal ganglia. Clusters of neuroendocrine cells can also be found immediately caudal to the supraesophageal ganglion and lateral to the sucking stomach (Foelix 2011).

In scorpions, the ganglia are paired throughout the prosoma and opisthosoma ventral nerve cord, whereas opisthosoma ganglia are mostly absent from the other orders. Solifugids such as *Galeodes* spp. and uropygids such as *Thelyphonus* spp. have single opisthosoma ganglia present (Babu 2013). Although at first glance harvestmen (order Opiliones) appear to have a single undivided soma, this is actually due to a broad pedicle connecting prosoma and opisthosoma. The Opiliones nervous system shares the structure common to most arachnids of supra- and subesophageal ganglia located in the prosoma, with no ganglia in the opisthosoma (Park et al. 2018). Innervation of the peripheral tissues serves both motor control and sensory input. In the walking legs these nerves are termed A, B, and C, with nerve B being the motor trunk and nerve C containing the majority of the sensory afferents from the slit sensilla and hair receptors. Nerve A is also sensory but is termed the "small leg nerve" (Seyfarth et al. 1985). The neuronal cell bodies reside within the ganglia and have large circular nuclei, whereas the surrounding glia has flattened nuclei (Fabian-Fine et al. 2015). Arachnid nervous tissue is poorly stained with eosin, but immunostaining for GABA (Fabian-Fine et al. 2015), orokinin (Roller et al. 2015), and cholinergic antigens (Fabian-Fine et al. 2017) has been proven to label subsets of neurons. Peripheral nerves are composed of axon bundles similar to other species.

8.3.8 Reproductive System

Arachnids are dioecious without exception. The females of most species are larger and longer-lived than the males. Some (Araneae and Opilliones) male arachnids have palpal bulbs (enlarged palpal tarsi) at the end of the pedipalps to transfer sperm to females. These palpal bulbs are present only in adults, are not present in other arthropods, and represent a sexually dimorphic character (Foelix 2011). The gonads of both male and female are internal as paired structures within the opisthosoma. Sperm and eggs are released to the outside in both sexes through a ventral opening called the epigastric furrow, and fertilization is internal. Some species, especially scorpions, which are live-bearers, and some spider families, rear their young after hatching.

8.3.8.1 Female Reproductive System

The female reproductive system has been defined in a limited number of arachnid species. In Araneae, ovaries are present ventrally within the opisthosoma and are attached to a luminal oviduct, which exits at the epigynum (or copulatory orifice) within the epigastric furrow. Ovaries are adjacent to the silk glands and ventral to the stercoral sac. The oviduct is paired or unpaired (Alberti and Palacios-Vargas 2015; Talarico et al. 2009). The majority of female Araneae possess spermathecae (also called seminal receptacles), paired or unpaired, where sperm are deposited during copulation (Figure 8.35). In species lacking spermathecae, sperm is

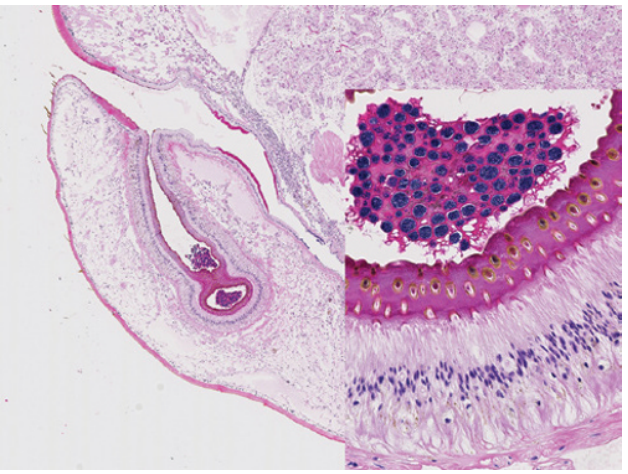


Figure 8.35 Spermatheca of a zebra tarantula (*Aphonopelma seemanni*). An invagination of the body wall stores sperm. On higher magnification (inset), the spermatheca is lined by tall, thin columnar cells lined apically by the cuticle, which forms an invagination to hold sperm. Sperm are organized into packets surrounded by an envelope (coenospermia). 17×; inset: 150×. HE.

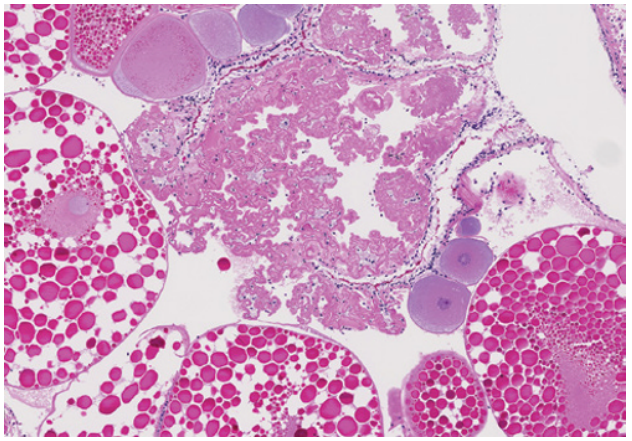


Figure 8.36 Ovary of a zebra tarantula (*Aphonopelma seemanni*). Ovarian follicles are in various stages of development, from mature follicles (large and filled with abundant, brightly eosinophilic granules), to developing oocytes (smaller and basophilic), to ovulated follicles with only a wrinkled outer membrane remaining. 67×. HE.

deposited into the uterus externus and oviducts (Bertani and da Silva 2002). Spermathecae are prominent within the epigastric furrow, which is an invagination of the cuticle into the opisthosoma.

Oocytes have distinct development phases that have been established in several arachnid species. These are the previtellogenic (phase 1), vitellogenic (phase 2) and postvitellogenic (phase 3). Often multiple different phases can be present in the same animal (Figure 8.36). In the first two phases, the oocytes protrude into the opisthosoma and are attached to the ovarian epithelium by a pedicel. In the last phase, the oocytes protrude into the uterus with the pedicel breaking down and the process of oviposition taking place (Ouriel 2016).

Previtellogenic oocytes are devoid of yolk granules and are lined with a thin basal lamina and vitelline membrane. Vitellogenic oocytes are still attached to a pedicel with a thickened basal lamina and vitelline membrane with some granular eosinophilic lipid granules. Postvitellogenic oocytes are much larger than vitellogenic oocytes and have a spherical shape; they are detached from the pedicel and contain copious lipid granules.

In live-bearers (e.g., scorpions), the gravid uterus distends the opisthosoma and is filled with multiple developing fetuses (Figure 8.37). The wall of the uterus is multilayered, including an inner polygonal to cuboidal epithelial layer, and an outer muscular layer.

8.3.8.2 Male Reproductive System

All male arachnids have internal testes in the opisthosoma. In Araneae, sperm and seminal fluid are not transferred directly to the female, but instead stored in bulbous distensions on the distal end of pedipalps called palpi, palpal bulbs, or palpal organs, before mating. Very few species of arachnids have a copulatory penis for direct transfer of sperm to the female. In sexually cannibalistic spiders, males only fill their palpal bulb (copulatory organ, see below) once and do not produce sperm after the final molt. In long-lived species with males that mate multiple times, sperm production is frequently continuous. In addition to sperm, males make seminal fluid that contains various proteins and peptides to increase reproductive success. Accessory sex glands are present in some species, although seminal fluid is likely produced in the testes (Michalik and Uhl 2005; Ruppert et al. 2004).

Most species possess two thick tubes of testes that are between the midgut diverticula; in scorpions, four tubes make up the testes. The testes are continuous with two thin, convoluted vasa deferentia. The vas deferens and much of the testes are bordered ventrally by the silk

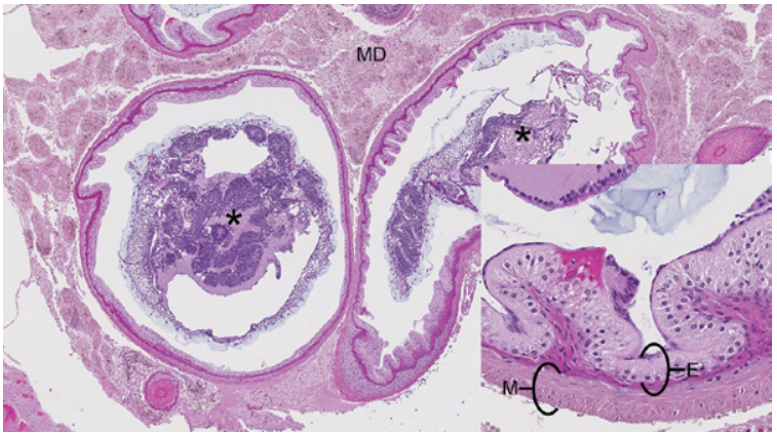


Figure 8.37 Uterus and fetuses of Asian jungle scorpion (*Heterometrus* sp.). Several cross-sections of the uterus are visible in the opisthosoma, and are surrounded by midgut diverticula (MD). The uterine wall has a thin, outer muscular layer (M), and a simple, inner mucosal epithelial layer (E) that are separated by thin, brightly eosinophilic, flattened cells (inset). Fetal tissues (*asterisks*) have an embryonic appearance (hypercellular with high nuclear:cytoplasmic ratio) and are surrounded by a small amount of myxomatous matrix. 40×; inest 200×. HE.

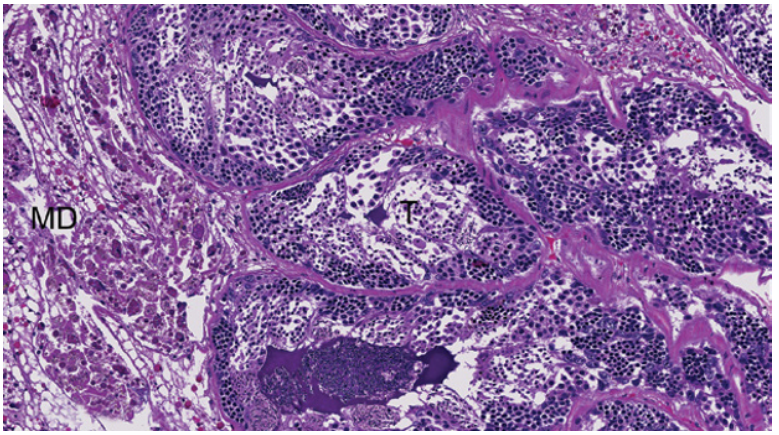


Figure 8.38 Testes of a birdeater tarantula (*Theraphosa blondi*). Multiple lobes of the testes (T) are in the opisthosoma, surrounded by midgut diverticula (MD). All stages of spermatogenesis are seen within an individual lobule. 125×. HE.

glands. The vasa deferentia fuse to form the ejaculatory duct, which opens into the genital opening in the epigastric furrow on the ventral aspect of the opisthosoma. This organization holds true for almost all families of spiders, except for theraphosid and mesothelid spiders, both of which have convoluted testes; theraphosids have no distinct region of demarcation between the testes and vas deferens (Klann 2009; Michalik and Uhl 2005; Ruppert et al. 2004).

The following is a general description of the microscopic appearance of the adult male genital tract of spiders from most families, though some variation in this organization may exist depending on species. The testes have numerous lobules showing all stages of spermatogenesis in animals with active sperm production (Figure 8.38). Testes have somatic cells with apical cilia at the periphery of lobules that are admixed with germ cells. Germ cells early in spermatogenesis are round with a large spherical nucleus. After this, the nucleus elongates until it becomes long and often coiled. The testicular lumen contains secretory fluid and spermatozoa. The junction of the testes with the vas deferens has a narrow, valve-like appearance. The vas deferens in some species is surrounded by a thick layer of striated muscle. The proximal vas deferens has a large diameter and contains spermatozoa and seminal fluid. As the vas deferens continues toward the genital opening, it narrows markedly in diameter (Figure 8.39). In the palpal organ, there are frequent glandular epithelial cells that are presumed to assist in the release, protection, and preservation of sperm (Klann 2009; Michalik and Uhl 2005). Spermatozoa are deposited as coenospermia (packets of spermatozoa surrounded by a flexible envelope) within the spermathecae or uterus externus (Bertani and da Silva 2002). In Scorpiones sperm is deposited in a sclerotized spermatophore on a stalk that is attached to a flat surface, to which the female is guided by the male (Vrech et al. 2018).

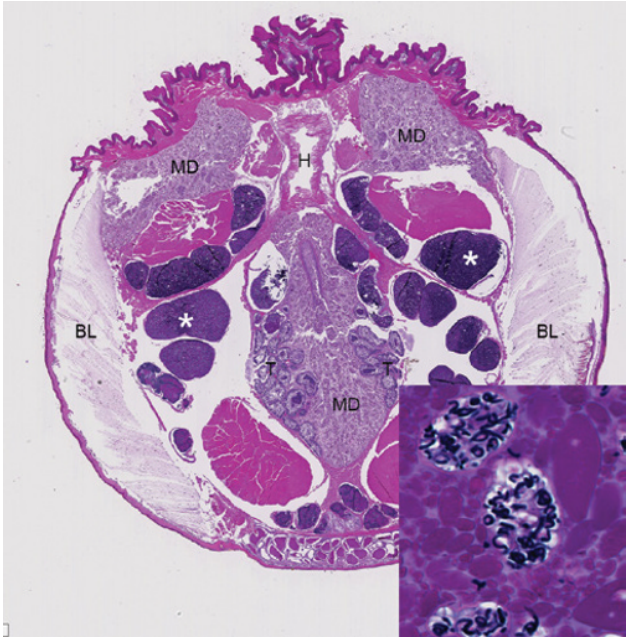


Figure 8.39 Cross-section of an opisthosoma of a birdeater tarantula (*Theraphosa blondi*). The vasa deferentia (*asterisks*) have multiple tubes that are medial to the book lungs (BL) and lateral to the testes (T). Ventrally, the vasa deferentia are narrowest as they approach the genital opening (not pictured). The heart (H) is in the dorsal opisthosoma. Midgut diverticula (MD) fill many spaces between other organs. The vasa deferentia are filled with seminal fluid and sperm, which are curved to spiral-shaped (inset). 5×; inset 400×. HE.

8.3.9 Special Senses

Sensory organs in arachnids include the eyes and cuticle sensilla, which function in proprioception and chemosensitivity.

8.3.9.1 Eyes

Unlike other arthropods, Arachnida has a series of ocelli, or simple eyes, rather than compound eyes. The number and arrangement of eyes vary between the different taxa of

Arachnida. Araneae has two median eyes (main eyes) on the optical tubercle and three pairs of lateral eyes (secondary eyes), which often share the same distinct ganglion (Miether and Dunlop 2016).

The cornea is a modified portion of the cuticle that is paler than the surrounding cuticle and may have eosinophilic transverse bands (Figure 8.40). The lens is a spherical, modified portion of the cuticle deep to the cornea. The basilar aspect of the lens is lined with a single layer of cells called the vitreous body. The retina is separated from the vitreous body by a thin preretinal membrane. The retina consists of a layer of long columnar cells with dense pigment deposits and basal nuclei (also called the lentigen layer) (Klann 2009). Beneath the retina is the optic nerve, whose ganglia reside in the optic lobe of the protocerebrum. Secondary eyes often have a tapetum layer between the retina and optic nerve; the shape of the tapetum can have marked variation based on the arachnid family. The main eyes lack a tapetum and always appear black.

8.3.9.2 Cuticular Receptors

Cuticular receptors (called cuticle sensilla) are present over much of the cuticle (see Figure 8.9). There is a small amount of variation between mechano-, chemo-, thermo-, and osmoreceptors. They are continuous with cuticular channels (approximately <2 μm diameter) that transverse through the cuticle (Willemart and Giribet 2010). There has been a debate about silk production from spider foot pads since first published (Gorb et al. 2006; Pérez-Miles et al. 2009; Rind et al. 2011); these are now understood to be chemoreceptor setae (Foelix et al. 2012).

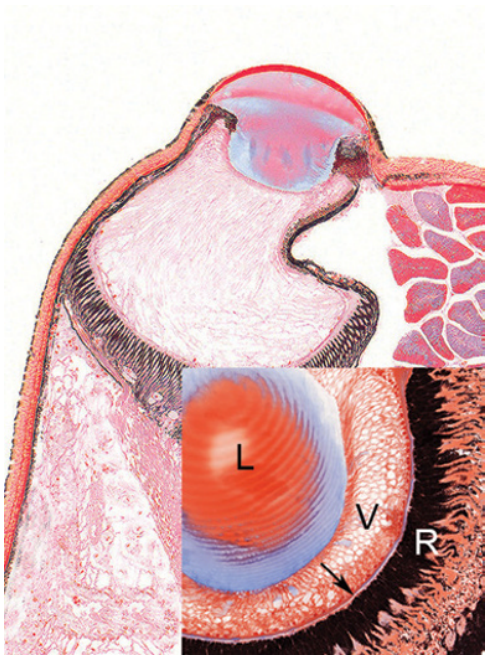


Figure 8.40 Longitudinal section of an eye of an orb-weaver spider (family Araneidae). The cornea and lens (L) are modified portions of the cuticle. The vitreous body (V) is deep to the lens and immediately below it is the retina (R). The retina is continuous with the optic nerve. In the inset (transverse section of an eye), the thin, preretinal membrane (*arrow*) is visible, which separates the vitreous from the retina. The retina is composed of long, pigmented cells. 150×; inset 400×. Modified trichrome.

8.3.10 Special Organs

8.3.10.1 Venom Gland

Three orders of Arachnida contain species with well-defined envenomation organ systems (i.e., venomous species): Araneae, Scorpiones, and Pseudoscorpiones. Although Opiliones and Uropygi have defensive secretory glands, these are not considered venomous due to lack of an injection apparatus (Eisner et al. 1978). There is growing evidence that the hematophagous species of Ixodida should be considered venomous as their salivary glands contain venom-like toxins and these are known to cause host intoxication such as tick paralysis (Cabezas-Cruz and Valdés 2014). The Ixodida salivary gland has a similar structure to that of other venomous Arachnida with luminal secretory cells surrounded by muscle (Walker et al. 1985).

The unrelated anatomic locations of the venom systems are evidence of divergent evolution within arachnids (Arbuckle 2017). Envenomation can aid in predator defense, prey incapacitation, and copulation, the latter occurring in the scorpion genus *Hadogenes* as venom sedates females prior to copulation (Leeming 2003). The envenomation system includes the venom gland, which is a tubular structure terminating in a conical point. The distal end of the venom gland contains a sphincter leading to the venom duct, which exits the injecting structure just proximal to the apex of the aculeus. In Mygalomorphae, venom glands are located on the dorsal midline of the chelicera and terminate within the chelicera (Rocha-e-Silva et al. 2009). In Araneomorphae, the venom glands penetrate deep into the prosoma (Santos et al. 2000). In both groups, the injecting structures are a pair of hinged fangs attached to the ventral side of the chelicera (Figure 8.41). Opposing fangs in Araneomorpha are parallel in a cranial direction in theraphosidae. In Scorpiones, venom glands are located in the telson, distal to the anus, and drain into a shared duct exiting the aculeus in a single appature (Taib and Jarrar 1993; Yigit and Benli 2008).

Venom glands of Araneae and Scorpiones are luminal, oval to cylindrical structures with a single layer of secretory epithelium. In Araneae, the secretory epithelium is surrounded by a dual layer of muscle cells (Santos et al. 2000). In Scorpiones, the paired venom glands are connected directly to the external aspect of the cuticle with a thick

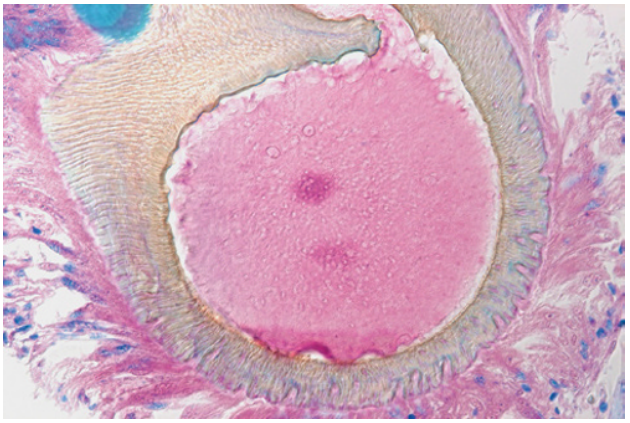


Figure 8.41 Basal segment of fang within an unspecified arachnid species. 200×. Modified trichrome.



Figure 8.42 Venom gland of a birdeater tarantula (*Theraphosa blondi*). Subjacent to the cuticle and epidermis, the venom gland is oval with a central lumen lined by cells with numerous cytoplasmic vacuoles and droplets. These cells have basal nuclei and are surrounded by a layer of striated muscle (SM; inset). 40×; inset 400×. HE.

muscle layer on the adluminal surface (Figure 8.42). Secretory epithelial cells are polar and mononucleate with basal nuclei and apical membranes containing secretory vesicles (Santos et al. 2000; Taib and Jarrar 1993). The venom glands of Scorpiones also have mucous-secreting cells (Taib and Jarrar 1993), making their venom viscous.

In the majority of Pseudoscorpiones species, the venom gland is located in the tarsus (mobile finger) of the palp and exits via ducts in the palp (see Figure 8.3). Some species have venom glands on the fixed finger, such as *Neobisium flexifemoratum,* or even on both, such as *Shravana laminata* (von Reumont et al. 2014). Some species lack venom apparatus (Chthoniidae) (Beccaloni 2009).

8.3.10.2 Silk Glands

Silk is a distinctive feature of spiders. Many aspects of spider biology and ecology are dependent on silk production. Silk is produced by several large glands located in the caudoventral region of the opisthosoma in Araneae and the chelicera of some Pseudoscorpiones (Beccaloni 2009). Silk is extruded from the silk glands by spigots on the tips of spinnerets. Spinnerets are processes arising from the legs on the abodomen. Most spiders have three pairs of spinnerets (anterior, middle, and posterior). Silk is in liquid form inside the silk glands and hardens when it is drawn out of the glands by spigots (Ruppert et al. 2004).

There are many discrepancies in the literature regarding silk gland classification. Generally, silk glands may be of several types: ampullate, aggregate, cylindrical, aciniform, piriform, lobed, and cribellate. Ampullate and aggregate glands are reported as the most prominent types in most species. Cylindrical glands enlarge in gravid females of some species as these glands produce the silk of the egg cocoon. In some species, males lack cylindrical glands entirely. Silk glands are generally highly convoluted, glandular structures that are in the opisthosoma, and frequently ventrally located (Figure 8.43). Determination of specific types of silk glands may not be possible without targeting specific glands via dissection prior to histology. An overview of the histologic appearance of some of the types of silk glands follows.

Ampullate glands are histologically composed of tubules lined by tall columnar cells with oval, basal nuclei. The cytoplasm has discrete droplets that are also present in the glandular lumen. Cytoplasmic droplets may vary in size along the length of the gland. The anterior, middle, and posterior pairs of ampullate glands may have different colors of luminal secretory material (pink, purple, and bluish, respectively). The ampullate ducts frequently occur in triads in histologic sections due to their convoluted nature. The columnar epithelium of the ducts is lined by an apical cuticle. Aggregate glands have thin walls and large lumina. They are lined by a simple columnar epithelium with round, basilar nuclei and apical cytoplasmic droplets. Aggregate ducts have a cuticle lining, similar to ampullate ducts, but are surrounded by few polygonal cells with a large amount of deeply basophilic cytoplasm and large, oval nuclei. Cylindrical glands have thick walls and narrow lumina. They are generally lateral to the aggregate glands. Cylindrical ducts are similar histologically to ampullate ducts but with a thicker cuticle. Aciniform glands are lined by low columnar cells. Their lumina contain globular

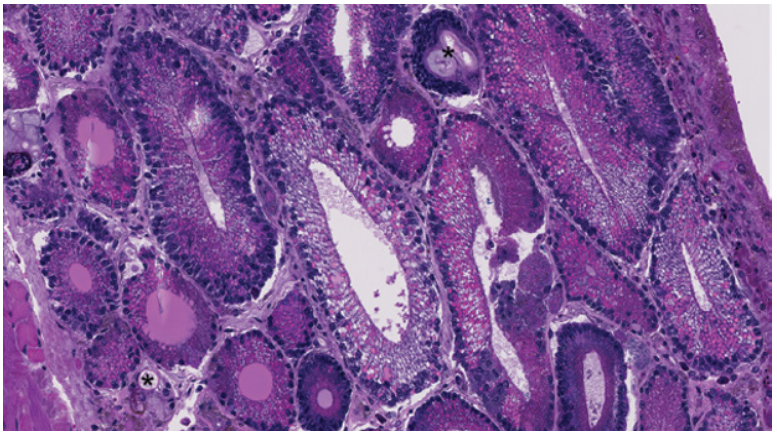


Figure 8.43 Silk glands of a Trinidad chevron tarantula (*Psalmopoeus cambridgei*). Both the silk glands and their respective ducts (*asterisks*), which are lined internally by chitin, are visible. 200×. HE.

protein droplets. Some aciniform ducts are not lined by epithelium. Piriform glands are pear-shaped (as suggested by their name) and lined by columnar epithelium. The anterior cells produce droplets of one color, while the posterior cells produce droplets of multiple colors (Kovoor and Muñoz-Cuevas 1998; Mullen 1969; Ruppert et al. 2004).

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Disclaimer

The views expressed in this chapter are those of the authors and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or US Government.

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9

Arthropoda

Merostomata

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[5] *Disney's Animals, Science and Environment, Orlando, FL, USA*

9.1 Introduction

This class of chelicerate arthropods has only four extant species: one from North America (*Limulus polyphemus*, American horseshoe crab) and three from Asia (*Tachypleus tridentatus*, tri-spine horseshoe crab, *Tachypleus gigas*, Indo-Pacific horseshoe crab, and *Carcinoscorpius rotundicauda*, mangrove horseshoe crab) (Smith et al. 2017). Although termed "crabs," these animals are more closely related to spiders and scorpions, which are also chelicerate arthropods, than true crabs, which are crustaceans. Horseshoe crabs (HSC) are often referred to as "living fossils" as current forms resemble fossils dating back to the Jurassic Period approximately 200 million years ago (Walls et al. 2002). HSC are marine invertebrates that feed on algae, marine worms, clams, other molluscs, and fish. They migrate to deep ocean waters in the winter, and move to shallow waters to spawn in the summer. *L. polyphemus* molts approximately 16–17 times in 9–10 years to reach maturity. *Tachypleus* spp. and *C. rotundicauda* both require relatively fewer molts (approximately 13) to reach maturity (Fahrenbach 1999). Juvenile HSC are morphologically similar to mature adults but have shorter telsons and are smaller. During spawning, HSC eggs serve as a major source of food for migratory bird populations.

Sources of mortality in wild populations of *L. polyphemus* include complications of hemolymph collection for biopharmaceutical production, natural predation, commercial harvest for bait, spawning habitat loss, pollution, bycatch, climate change, and collection of specimens for research, education, and aquaria (Smith et al. 2017). HSC are used heavily by the biopharmaceutical industry for an enzyme in their hemolymph called limulase amebocyte lysate (LAL), which detects small quantities of endotoxin. Due to this property, the Food and Drug Administration requires that nearly all pharmaceutical products are tested with LAL prior to market to screen for contamination (Anderson et al. 2013; Walls and Berkson 2003).

9.2 Gross Anatomy

The body of HSC may be divided into three parts: prosoma (cephalothorax), opisthosoma (abdomen), and telson (tail). Dorsally, on the prosoma, there are two medially located ocelli and two laterally located compound eyes (Figure 9.1). A hinge joint connects the prosoma and opisthosoma. The margin of the opisthosoma is lined bilaterally by six spines that are movable by a hinge joint, but are not under nervous control. A dorsal midline ridge extends most of the length of the prosoma and opisthosoma and is called the keel. An inhalant channel separates the opisthosoma and prosoma, and aids in water flow to the book gills. Ventrally HSC have 14 pairs of specialized appendages (Figure 9.2). The first six reside in the prosoma while the rest are opisthosomal appendages. The first pair, the chelicerae, consist of a small pair of pinchers used for collecting food. The second, the pedipalps, help define the sex of HSC. Males have a larger hook-shaped bulbous claw used to attach themselves onto females during mating whereas females' claws appear the same as the other walking legs. The walking legs comprise appendages three through five. The sixth appendage, also known as the pusher leg or swimmer leg, is used to propel the animal through water. Located centrally in the prosoma is the gnathobase or mouth, which is made up of the coxa leg segments.

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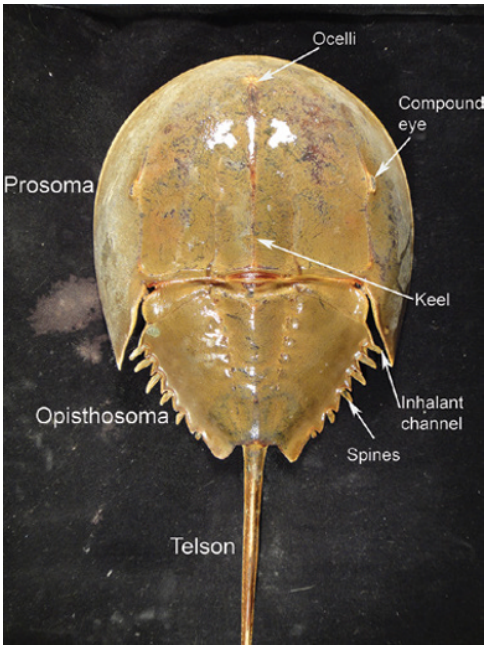


Figure 9.1 External photograph of the dorsum of a horseshoe crab, *Limulus polyphemus*. The body is divided into prosoma, opisthosoma, and telson.

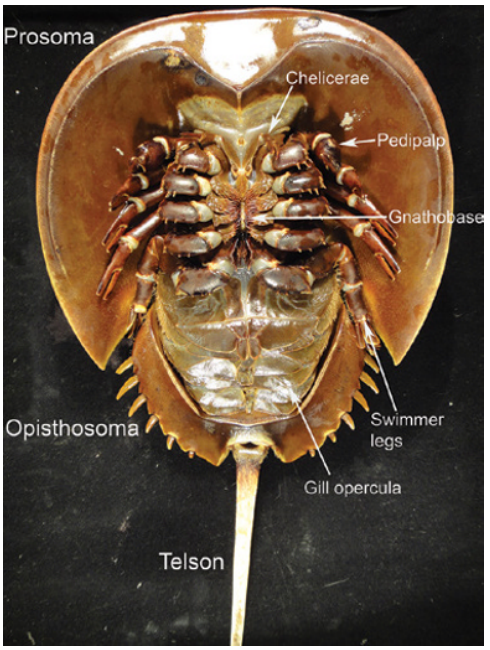


Figure 9.2 External photograph of the ventrum of *L. polyphemus*. Walking and swimmer legs are attached to the prosoma, and the gills are attached to the opisthosoma.

The opisthosomal appendages include the genital operculum, gill opercula, and telson. The genital operculum covers and protects the book gills (Figure 9.3). The eighth through thirteenth appendages are gill opercula which individually protect and contain the ventrolateral paired



Figure 9.3 External photograph of the ventrum of *L. polyphemus*. A gill operculum is reflected to reveal the subjacent book gills.

book gills. Finally, the last appendage is known as the telson, which functions to right the animal if it has been flipped upside down.

9.2.1 Dissection

An excellent tutorial on the anatomy and dissection of HSC is provided by Bergdale with images, an abridged version of which follows (Figure 9.4) (Bergdale 2017). The authors prefer a ventral approach as organs remain *in situ* during examination, and the internal viscera are more easily accessed ventrally.

For a ventral approach, place the animal in dorsal recumbency. Use pruning shears to cut around the entire edge of the prosoma, approximately ½ cm from the margin of the prosoma. Gently peel back the ventral, inner carapace to reveal the interdigitating hepatopancreas and gonads, which may be distinguishable in gravid females. Cut the muscular attachments that extend through the hepatopancreas and attach to the ventral carapace, as well as those that extend through the hepatopancreas and attach to the dorsal carapace; cut as close to the carapace as possible both dorsally and ventrally to avoid damaging the viscera. The hepatopancreas and gonads should be completely separable from the ventral carapace (Figure 9.5). Push the gnathobase laterally to expose the mouth. Make a circular cut around the mouth in the soft tissue. Observe the excretory system (i.e., coxal glands) if still intact; these are bright orange, paired structures with four lobes each on either

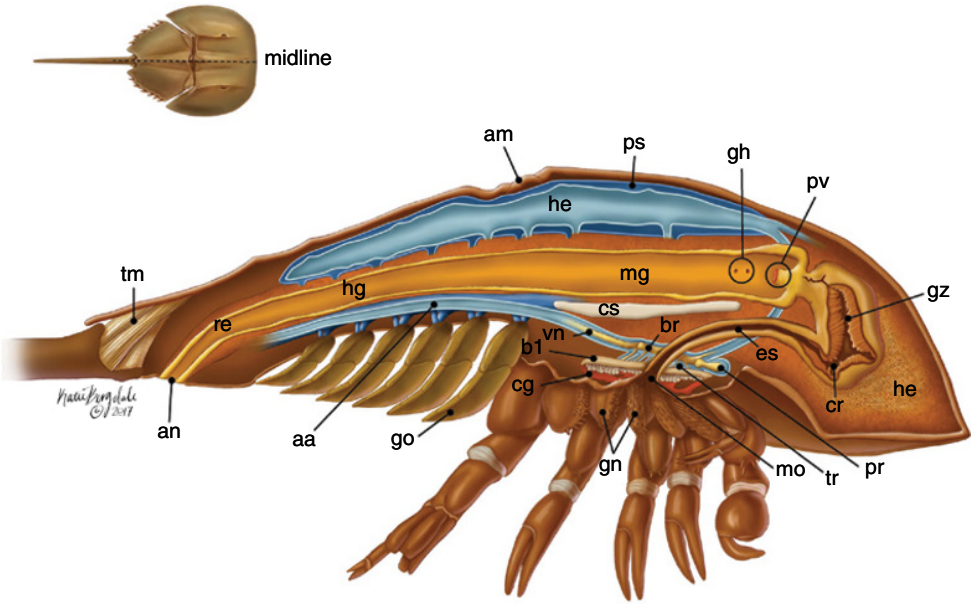


Figure 9.4 Illustrated sagittal section of *L. polyphemus*. am, arthrodial membrane; an, anus; bl, bladder; br, brain; ca, coelomic artery; cg, coxal gland; cr, crop; cs, cartilage shelf (endosternite); es, esophagus; gh, glandular hepatic ceca; gl, gill operculum; gn, gnathobase; go, genital operculum; gz, gizzard; he, heart; hg, hepatopancreas and/or gonadal tissue; hi, hindgut; mg, midgut; mo, mouth; pr, protocerebrum; ps, pericardial sac; pv, pyloric valve; re, rectum; tm, telson muscles; tr, tritocerebrum, vn, ventral nerve cord. Source: Illustration by Katie J. Roorda. Bergdale (2017).

side of the arterial ring. Observe the arterial ring, located directly posterior, which completely houses the brain. Next cut through all the gill opercula to completely separate the internal anatomy from the carapace. Starting at the mouth, cut up through the esophagus to expose the gizzard. Flip the specimen to the dorsal side and continue opening the intestinal tract. Observe the heart and pericardial sac located anteriorly to the midgut.

9.3 Histology

9.3.1 Body Wall/Musculoskeletal

Table 9.1 outlines the organs for histologic examination horseshoe crabs. The body of a horseshoe crab is organized into three segments: prosoma, opisthosoma, and telson. The prosoma is the largest and most anterior body segment. The opisthosoma is smaller than the prosoma; it is the midbody, and houses the book gills. The telson is the tail-like structure attached to the caudal end of the opisthosoma. The entire body, except for joints, is overlain by the carapace, which provides a physical protective barrier for the internal body. Skeletal muscle attaches to the inner carapace and is under nervous control, permitting movement of body parts (e.g., legs, telson, etc.).

9.3.1.1 Cuticle

The cuticle ranges from thin and flexible to thick and stratified, such as over the dorsal prosoma. It is composed of protein and chitin. In thick regions, the entire cuticle is up to 1500 μm thick and is subdivided into three layers (Figure 9.6). The outermost epicuticle is thin (approximately 5–15 μm thick), acellular, and frequently does not stain well on routine HE, paraffin-embedded sections. The middle layer (exocuticle) is thick, chitinous, acellular, birefringent, and pale eosinophilic. This layer is laminated and approximately 450 μm thick. The inner endocuticle is deeply eosinophilic, and approximately 550 μm thick. The basilar aspect of the inner endocuticle has a variably present, 2–10 μm thick, granular, basophilic region at the junction with the epidermis. A thin (3–15 μm diameter), hard, cuticle is over the gills and lacks discrete layering. A thin, bilayered, relatively flexible cuticle covers parts of the ventral prosoma, arthrodial membranes, and the mucosa of the fore- and hindgut. It is composed of endocuticle and epicuticle, which are demarcated by a feathered borderline (Figure 9.7) (Fahrenbach 1999).

There are several adornments and structures that extend through and from the cuticle. Skeletal muscle attaches directly to the underside of the cuticle (i.e., myocuticular junction), resulting in a focal deviation at the site of attachment. Long, striated myofibers extend directly from the

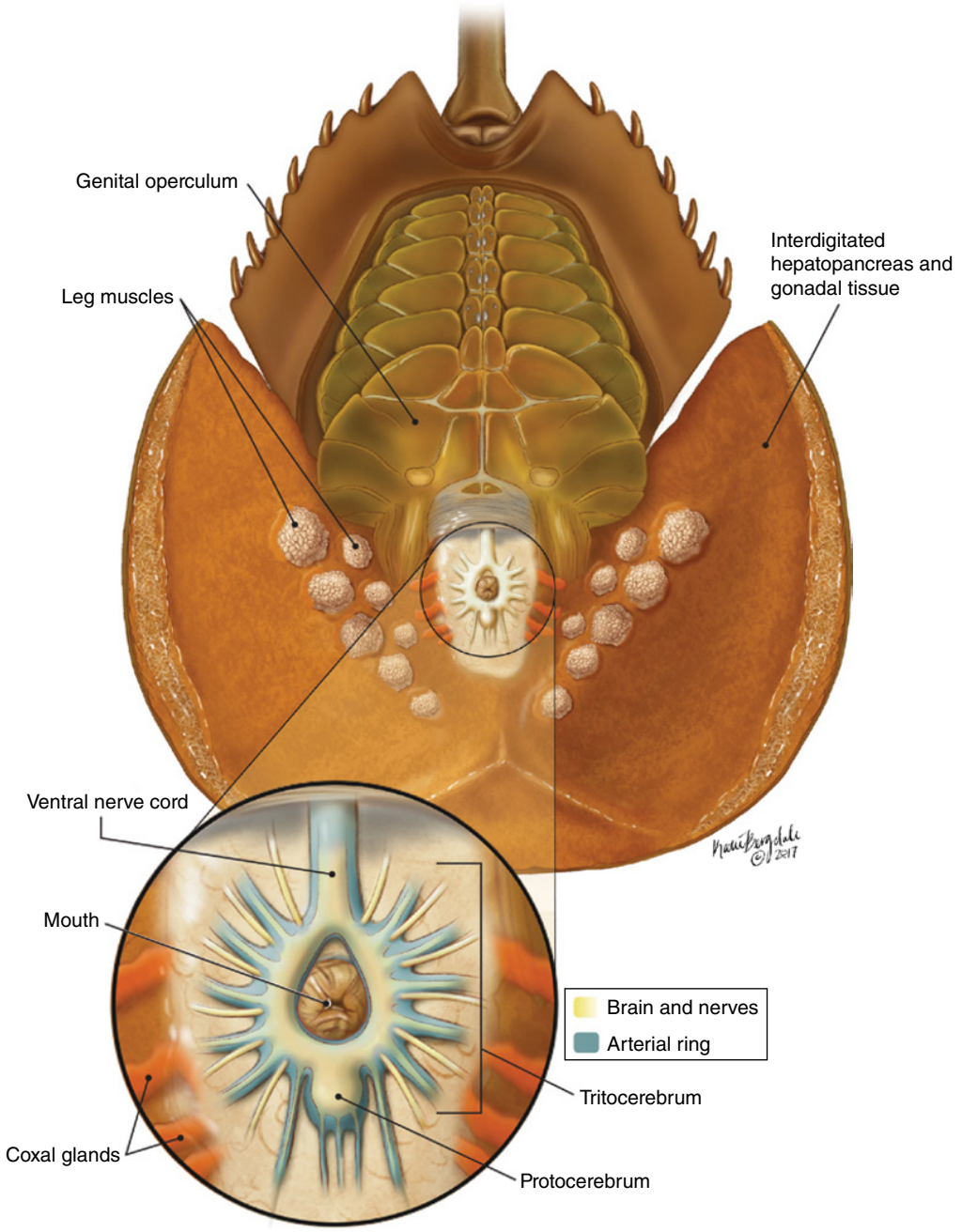


Figure 9.5 Illustrated ventral dissection approach of *L. polyphemus*. The ventral carapace has been removed by cutting the muscular attachments between the hepatopancreas and the carapace, and cutting around and removing the gnathobase (not pictured). Deep to the region of the gnathobase is the mouth surrounded by an arterial ring. The brain resides entirely within the arterial ring, and most nerves reside inside the arterial system. Lateral to the brain are four pairs of coxal glands, which are bright orange. After the nervous structures and coxal glands are removed, the genital opercula can be removed to expose the remainder of the digestive tract (not pictured). Source: Illustration by Katie J. Roorda. Bergdale (2017).

Table 9.1 Organs for histologic evaluation in Merostomata.[a]

Organ system		Organs
Body wall/musculoskeletal		Cuticle, epidermis/dermis, cartilage, arthrodial membrane, skeletal muscle
Digestive	Alimentary canal	Mouth, esophagus, proventriculus (crop and ventriculus [*gizzard*]), intestines (*midgut*), rectum (*hindgut*)
	Hepatopancreas	Interstitial cells, collecting tubules (*midgut diverticula*)
Excretory		Coxal glands (*kidneys*, *coxae*)
Circulatory		Heart, hemocoel, arteries
Immune		Hemocytes
Respiratory		Book gills
Nervous		Brain, circumesophageal ring, ventral nerve cord, peripheral nerves
Reproductive	Male	Testis
	Female	Ovary, oviduct
Special senses/organs		Compound eyes, ocelli (*median eyes*), rudimentary eyes, ventral (*endoparietal*) eye

[a] Alternative names for organs are provided parenthetically, in italics.

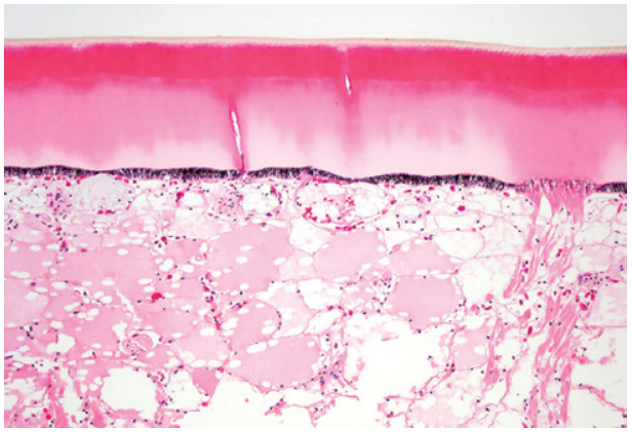


Figure 9.6 Body wall of *L. polyphemus*. The cuticle is organized into a poorly staining epicuticle, densely staining exocuticle, and inner endocuticle. Immediately deep to the cuticle are the pigmented epidermis, glands, and skeletal muscle, the latter of which attaches directly to the cuticular wall. 40×. HE.

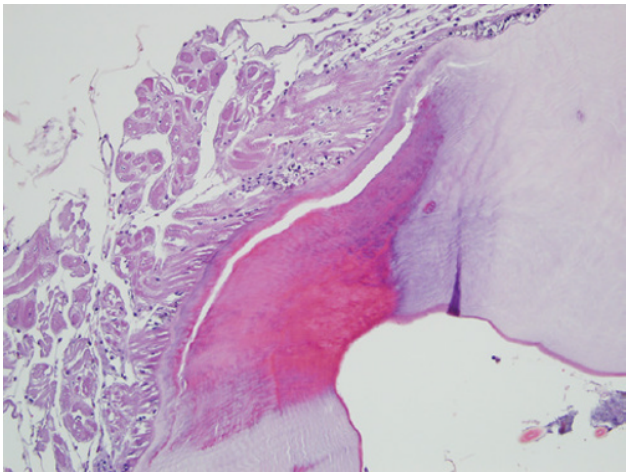


Figure 9.7 Arthrodial membrane of *L. polyphemus*. The cuticle is focally thin and flexible representing the arthrodial membrane, which is demarcated by a feathered margin from the flanking hard cuticle. 100×. HE.

basilar aspect of the epidermis deep into the body wall (Figure 9.6). Tendinous attachments, which are fewer than muscular attachments, appear similarly. Long, infolded pillars of hard cuticle extend into the body wall and serve as a muscular attachment for the gills and telson. Thin pores extend from the base of the cuticle through the epicuticle. These pores appear to originate at the epidermis. They can be straight or wavy and are generally thicker in the endocuticle with narrowing at the epicuticle. A variety of setae, bristles, and spines emanate from the surface of the cuticle multifocally, particularly on the ventral aspect of HSC. The cuticle overlying the eyes is altered into a lens or cuticular cones to facilitate vision (see section 9.3.9).

9.3.1.2 Epidermis and Dermis

The epidermis is composed of packets of columnar cells arranged in a single layer on a basal lamina. Nuclei are oval and basilar. Epidermal cells associated with the hard cuticle generally have apical melanization, while those associated with thin, flexible cuticle are generally nonpigmented. The dermis contains two types of glands. Mucous glands are oval, horizontally oriented, and directly underneath the epidermis. They are up to 250 µm in diameter and contain droplets that vary in size and color (Figure 9.8). Serous glands are smaller, pear-shaped, and surround a 10 µm diameter duct. Serous glands are most common under the

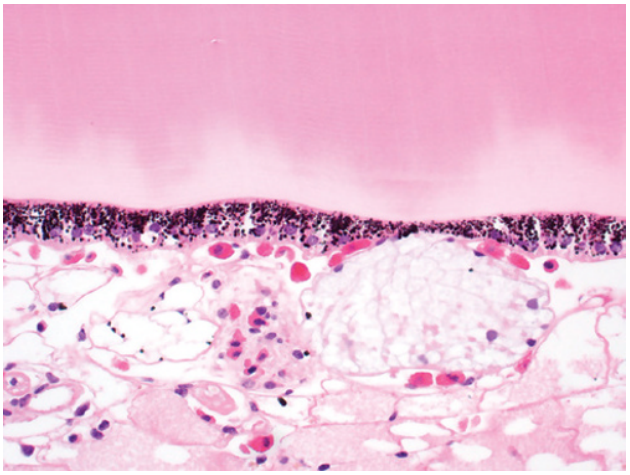


Figure 9.8 Epidermis of *L. polyphemus*. The epidermis is a simple layer of cells with basilar, round nuclei, and abundant pigment granules. A mucous gland and small vascular spaces containing hemocytes are also pictured. 400×. HE.

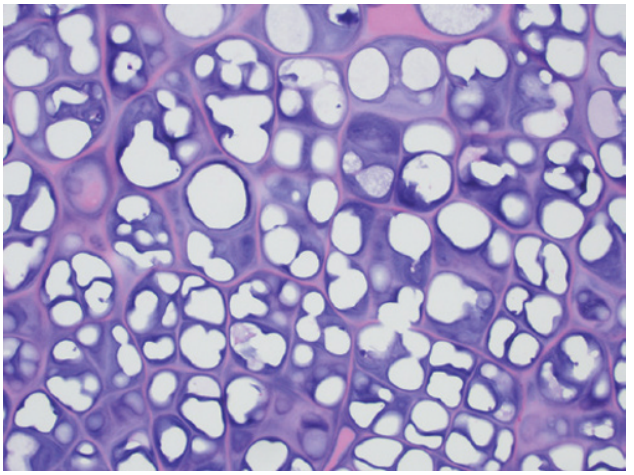


Figure 9.9 Cartilage of *L. polyphemus*. Numerous lacunae are surrounded by a deeply basophilic matrix. 400×. HE.

flexible cuticle (Fahrenbach 1999). Both glands are associated with acellular ducts that traverse the overlying cuticle. Ducts coalesce to form larger canals on the outer surface of the carapace. Approximately 10–12 ducts may empty into one large canal (Stagner and Redmond 1974). The remaining tissue of the dermis is composed of interwoven, small vascular channels, loose connective tissue, and reserve cells (Fahrenbach 1999).

9.3.1.3 Connective Tissues and Muscle

A cartilage shelf (also called endosternite) is present dorsal and lateral to the ventral nerve cord, and lateral and ventral to the intestines. The caudal portion of the shelf is adjacent to the anterior-most book gills. The cartilage is composed of pale basophilic chondroid matrix studded regularly with lacunae that contain a large clear vacuole and peripheralized chondrocytes. Larger lacunae may be separated by partitions (Figure 9.9). The cartilagenous matrix is immediately surrounded by fibrous connective tissue that is further surrounded by hepatopancreas, dermis, or skeletal muscle. All muscle in HSC, both visceral and structural, is striated, similar to other arthropods. Muscle fibers vary in size (ranging from 10 to 130 μm in diameter) and are surrounded by bundles of collagen, fibroblasts, and axons. Myocytes can have both peripheral and central nuclei.

9.3.2 Digestive System

The digestive system can be divided into the luminal alimentary tract and the hepatopancreas. The alimentary tract has multiple sections that make up a single tube and spans almost the entire length of the prosoma and opisthosoma. It is connected to the hepatopancreas by pairs of ducts that

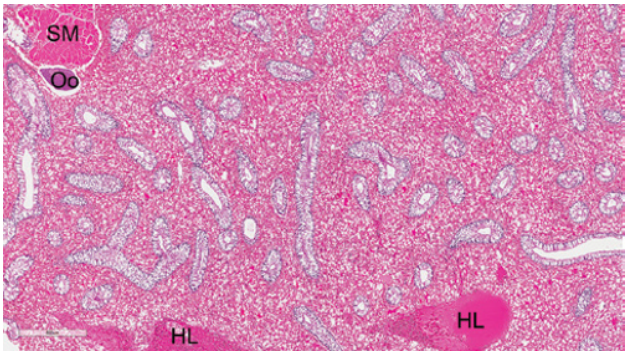


Figure 9.10 Hepatopancreas of *L. polyphemus*. The majority of the organ is composed of eosinophilic interstitial cells with regularly spaced collecting tubules. Cross-sections of skeletal muscle (SM), oocytes (Oo), and hemolymph sinuses (HL) are multifocally present. 36×. HE.

represent the termination of convoluted hepatopancreatic collecting tubules and empty into the midgut.

9.3.2.1 Hepatopancreas

The hepatopancreas is a large organ that occupies the majority of the prosoma. It fills the space around other organs in the prosoma and interdigitates with skeletal muscle of the legs and gonads. Additionally, the hepatopancreas surrounds multiple hemocoel sinuses containing hemocytes. The organ is composed mostly of interstitial cells interrupted regularly by collecting tubules (Figure 9.10).

Collecting tubules branch from the anterior midgut and branch extensively thereafter. Tubules vary from 50 to 500 μm in diameter. Collecting tubules have simple, tall, columnar epithelial cells, and are surrounded by a 1 μm thick basement membrane and a muscular network composed of

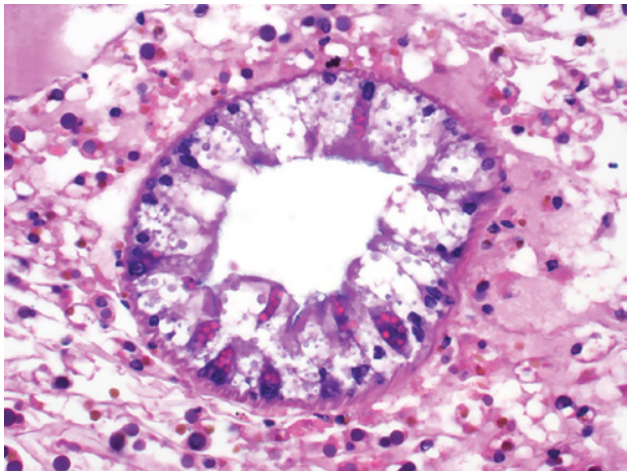


Figure 9.11 Collecting tubules of *L. polyphemus*. Light and dark cells are arranged in a single layer on a basal lamina. 400×. HE.

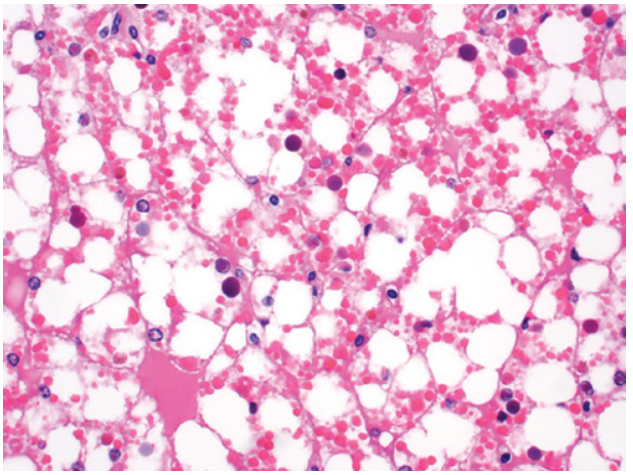


Figure 9.12 Interstitial cells of *L. polyphemus*. Large, polygonal, interstitial cells of the hepatopancreas have eccentric nuclei and abundant, eosinophilic, cytoplasmic granules. 400×. HE.

circular and longitudinal striated muscle (Herman and Preus 1972). The tubular epithelium is 30–75 μm tall with a thin brush border. There are two primary cell types: light cells (also called acidophils) and dark cells (also called basophils), named for their staining characteristics by light microscopy with HE staining. Dark cells have secretory granules that are discharged by merocrine secretion upon ingestion of food. They are analogous to the exocrine pancreas of mammals, and discharge enzymes such as lipase, amylase, and trypsin; as such, their granules may be referred to as "zymogen granules." Light cells are much more numerous (approximately four times) than dark cells. These are phagocytic cells that ingest fine food particles, digest the particles, and presumably pass on the contents to interstitial cells (see below). Light cells have abundant, basophilic, laminated, spherical concretions in the cytoplasm that are discharged into the lumen upon digestion (Figure 9.11). A third cell type, enteroendocrine cells, are scattered basally, and are roughly spherical with abundant eosinophilic granules; similar cells are found in the intestinal mucosa (Fahrenbach 1999).

Interstitial cells are storage cells. They are large with vacuolation and abundant eosinophilic, spherical granules and fewer, smaller basophilic granules that vary in size and shape (Figure 9.12). Eosinophilic granules are typically present in interstitial cells of well-fed animals, and are especially abundant around the heart, gut, and central nervous system. In starved HSC, interstitial cells have reduced cytoplasm that lack large protein granules, have reduced numbers of small protein granules, and lack glycogen (Herman and Preus 1972).

9.3.2.2 Alimentary Tract

The luminal alimentary tract can be divided into the oral cavity, esophagus, proventriculus (which can be further divided into the crop and ventriculus, also called gizzard), intestines (also called midgut), and rectum. A chitinous cuticle lines the mucosa of the oral cavity, esophagus, proventriculus, and rectum (see section 9.3.1.1). The oral cavity, also called the mouth, is between the third to fifth limbs and is bristly with multiple spiny gnathobases projecting into the walking legs that help grind and deliver food into the mouth.

The segments of the esophagus and proventriculus are difficult to differentiate using histology alone. The descriptions below hold true for an empty alimentary tract. However, the thicknesses of the various layers, as well as the mucosal folds, likely change with distension of the alimentary tract, and distinguishing different segments of the anterior alimentary tract may be impossible using histology alone. The esophageal mucosa is folded into approximately 6–8 deep, longitudinal folds (Fahrenbach 1999). The cuticle overlying the esophageal mucosa is approximately 250 μm thick (see section 9.3.1.1). The subjacent epithelium is 15–25 μm thick and columnar with basal nuclei. The submucosa is heavily vascularized with reserve cells, arterioles, and few muscle fibers. The subjacent tunica muscularis externa is 250 μm thick. The proventriculus is divided into an orad crop and an aborad ventriculus; in both, the mucosa is highly folded with similar layers as the esophagus. The tunica muscularis externa is relatively thick, and is thickest in the ventriculus (approximately 3–4 mm thick). The ventricular mucosa is folded into 13–15 longitudinal ridges with a 500 μm thick cuticle that has deep notches and rounded teeth (Figure 9.13). The mucosal epithelium is cuboidal (Fahrenbach 1999). Unlike the esophagus, the submucosa is relatively acellular with connective tissue and blood vessels (Figure 9.14). The pyloric valve marks the termination of the ventriculus and forms folds that directly oppose one another.



Figure 9.13 Ventriculus of *L. polyphemus*. The mucosa is overlain by a thick band of chitin resembling the cuticle. The mucosa has numerous small folds. The subepithelial connective tissue is composed of a loose network of blood vessels and striated myofibers. The muscularis is thick with striated muscle. 20×. HE.

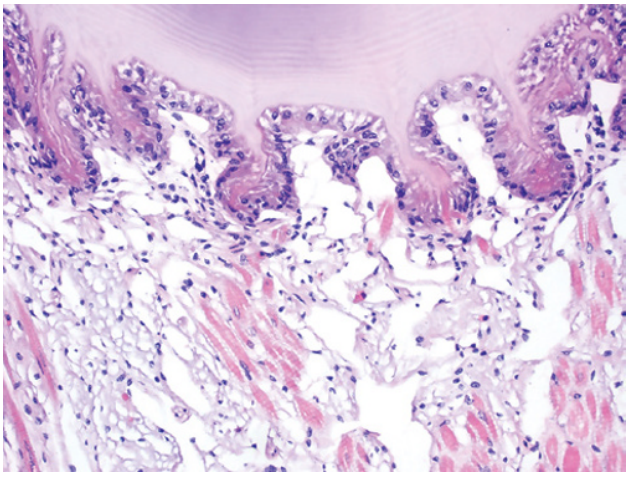


Figure 9.14 Ventriculus of *L. polyphemus*. Higher magnification of the ventricular mucosa shows a single layer of cuboidal to low columnar cells forming the mucosa. 200×. HE.

The intestines are relatively thin (approximately 0.5 mm) and can easily be distinguished from the other segments of the alimentary tract by the lack of a chitinous mucosal cuticle. The intestinal mucosa has low folds and is composed predominantly of "basophils," which are tall (40–100 µm tall) and narrow epithelial cells that extend the full height of the mucosa (Figure 9.15). A second cell type, termed "acidophils," are 5–15 µm tall and have abundant eosinophilic granules; these cells are suggestive of enteroendocrine-type morphology on electron microscopy and comprise less than 1% of intestinal mucosal epithelial cells. Acidophils are located basally to halfway up the epithelium. The intestinal epithelium is subtended by a 3–8 µm

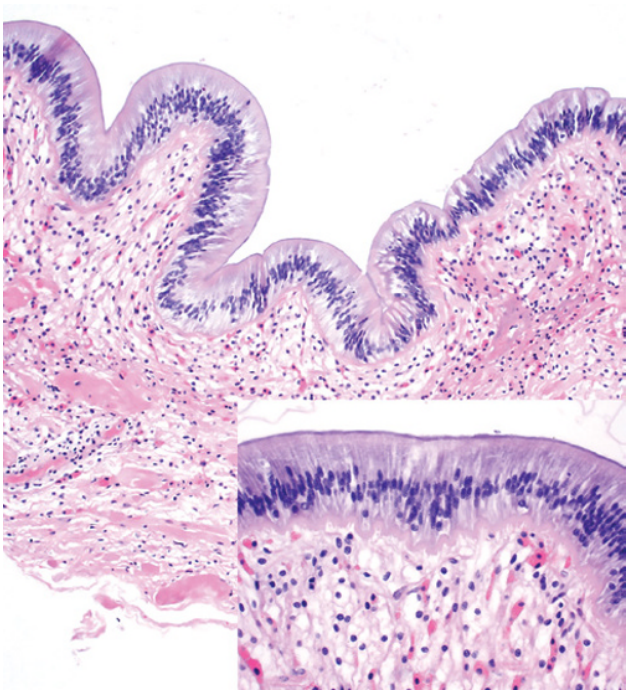


Figure 9.15 Intestine of *L. polyphemus*. A simple mucosa is subtended by reserve cells and vascular spaces. 100×. HE. Inset: Higher magnification revealing detail of mucosa, basal lamina, reserve cells and hemocytes. 400×. HE.

thick basal lamina that is undulating. The submucosa and tunica muscularis externa are relatively thin and loosely organized with interstitial spaces containing occasional hemocytes. Abundant diverticula extend from the intestines and interdigitate with the hepatopancreatic interstitium; these diverticula are the collecting tubules (see section 9.3.2.1). The intestines terminate in the rectum, also called the hindgut. This section of the alimentary tract has a larger diameter than the remainder of the intestines due to substantial internal rectal folds, and an approximately 40 µm thick cuticle lining the mucosa. The muscularis of the rectum is complex with an inner circular layer that is 150 µm thick, a middle longitudinal layer 200 µm thick, and an outer circular layer 200 µm thick. Feces contain abundant hepatopancreatic spherites and small fragments of digesta (e.g., bones, shells, etc.) (Fahrenbach 1999).

The tunica muscularis of all portions of the gut is composed of striated muscle interspersed regularly with vascular sinuses and nerves. Vascular sinuses have very low numbers of circulating hemocytes in healthy animals.

9.3.3 Excretory System

Coxal glands (also called coxae) are organs analogous to the kidneys of other species. Coxal glands are responsible for the regulation of sodium, chloride, calcium, hemolymph

pH, and osmolality. They are divided into four lobes, each of which is encased in connective tissue. Each lobe is further divided into four zones, which are defined by their location and cellular density. The outermost zone (zone 1) has the highest cellular density, while the innermost zone (zone 4) has the lowest cellular density and largest vascular channels (Figure 9.16). Zone 1 is approximately 150 μm wide. It is composed of numerous, small (10–25 μm wide) vascular channels that usually contain one or two granular hemocytes and are directly adjacent to small urinary tubules lined by one to few podocytes. Podocyte nuclei are generally round to oval with dense chromatin, and may slightly bulge into the urinary tubular lumen (Figure 9.17). Zone 1 has a small amount of interstitial stroma. Zones 2 and 3 have urinary tubules and fewer vascular spaces of increasing diameter that culminate in zone 4, in which a "labyrinth" is created by large dilated urinary tubules and slightly smaller and many fewer dilated vascular spaces. The amount of collagenous interstitial tissue also increases from zone 2 to 4. In zone 4, urinary tubules are up to 150 μm wide, and vascular spaces are up to 100 μm wide. In zones 2 and 3, urinary tubules are lined by low (approximately 2–5 μm high) renal tubular epithelial cells. In zone 4, urinary tubular cells are flattened, and similar in appearance to cells lining vascular channels; urinary tubules and vascular channels are distinguished in zone 4 by their size and the presence or absence of luminal granular hemocytes. Urine is excreted through the excretory duct, which is 500 μm wide and lined by 25–30 μm tall cuboidal epithelium. Urine and hemolymph are typically isoosmotic in salt water.

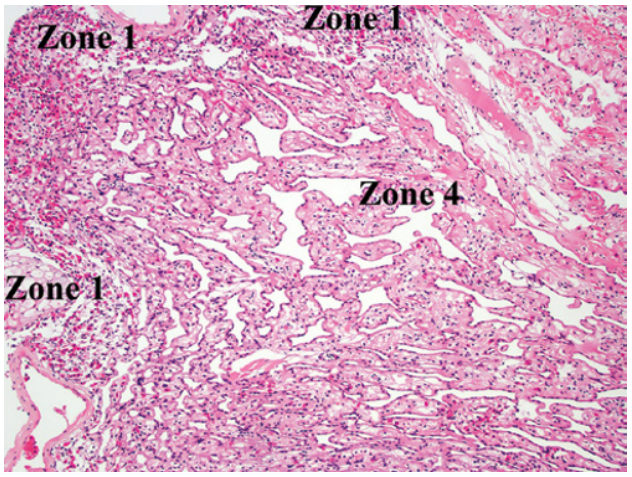


Figure 9.16 Coxal gland of *L. polyphemus*. Zones of the coxal gland are pictured. Zone 1 has small vascular spaces containing hemocytes interspersed with slit-like urinary spaces. Zone 4 has large urinary spaces separated by dense stroma. 40×. HE.

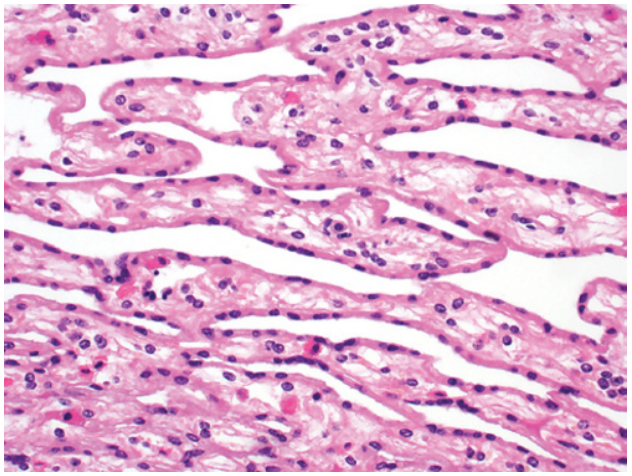


Figure 9.17 Coxal gland of *L. polyphemus*. Zones 2–3 of the coxal gland show urinary spaces lined by low cuboidal cells surrounded by dense stroma containing small vascular spaces with hemocytes. 400×. HE.

9.3.4 Circulatory System

The major components of the HSC circulatory system are the heart, arteries, and hemocoel sinuses, all of which contain hemolymph. The blood of HSC is called hemolymph as there are not separate lymphatic and vascular systems. Two types of cells are present: granulocytes (also called hemocytes), which are immune effector cells (see section 9.3.5), and cyanocytes, which produce hemocyanin. Hemocyanin is an oxygen-carrying molecule in the hemolymph that facilitates oxygenation of the hemolymph, and hence oxygen delivery to tissues. Cyanocytes are rare in comparison to hemocytes, as the latter make up the majority of circulating cells. The precursor cells to cyanocytes are cyanoblasts, which develop hemocyanin crystals in their cytoplasm. Intracellular hemocyanin crystals coalesce to form a single, giant hemocyanin crystal (up to approximately 140 μm in length) that peripheralizes the nucleus and eventually ruptures the cell, releasing hemocyanin into the hemolymph. Cyanocytes are rarely observed in tissue interstitium, particularly the eye and coxal gland. Cyanoblast hematopoiesis is believed to occur during embryogenesis and ecdysis (Fahrenbach 1999).

Hemolymph is pumped by the heart into various arteries that are named inconsistently in the literature (e.g., median aorta, paired ventral aorta, anterior and posterior lateral arteries hepatomarginal trunk, collateral artery, frontal artery, etc.) (Bergdale 2017; Fahrenbach 1999; Stagner and Redmond 1974). Hemolymph exits the arteries into small collateral branches and enters the hemocoel between the tissues, which are divided into sinuses. Hemolymph returns to the heart by small veins draining sinuses. These veins enter the gills, which oxygenate the hemolymph via

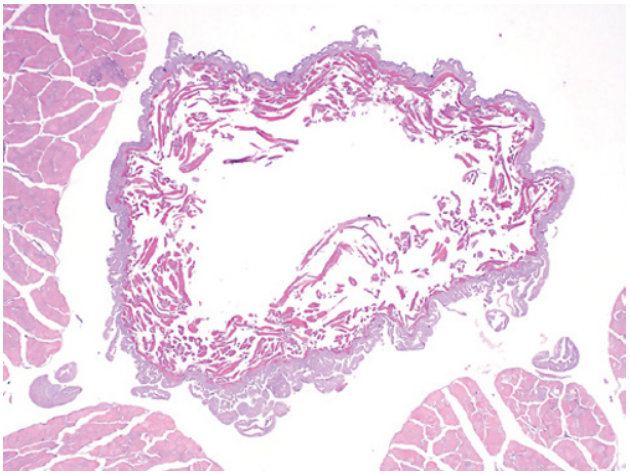


Figure 9.18 Heart of *L. polyphemus*. The heart has an outer epicardium and an inner myocardium composed of loose trabeculae of striated cardiomyoctes. Cellular preservation is poor due to autolysis. 40×. HE.

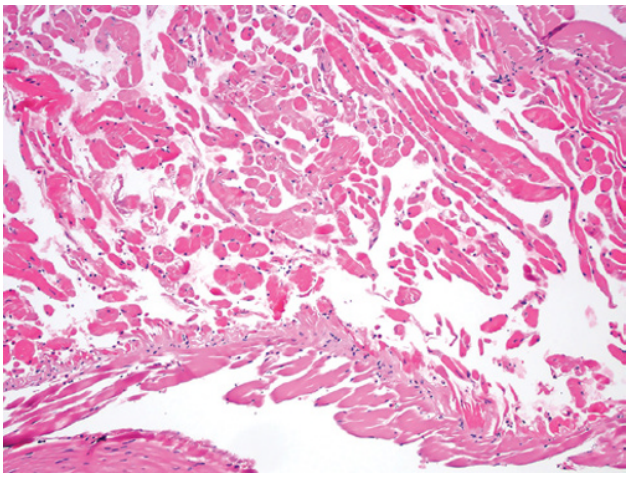


Figure 9.19 Heart of *L. polyphemus*. Higher magnification of the heart shows detail of the epicardium (bottom of image) and haphazardly arranged myocardiocytes. Cellular preservation is poor due to autolysis. 100×. HE.

hemocyanin, and return blood to the pericardium and then heart (Bergdale 2017; Stagner and Redmond 1974).

9.3.4.1 Heart

The heart is a long, tubular organ that extends along the dorsal body cavity. The heart is contained in a pericardial sac that immediately abuts hepatopancreatic tissue on the external surface. Cardiac musculature is organized into abundant sinuses created by individual and small clusters of striated cardiomyocytes with elongate nuclei. The cardiac myofibers are oriented in various directions, creating a seemingly haphazard arrangement. The cardiac lumen is not lined by endothelium, and is separated from the myocardium by a basal lamina. Obtaining well-preserved sections of heart can be challenging in diagnostic cases that were euthanized because euthanasia solution is typically injected directly into the heart, causing euthanasia artifact; alternatively, animals that die spontaneously frequently have artifact from autolysis (Figures 9.18 and 9.19).

9.3.4.2 Arteries

Blood vessels are not lined by endothelium and instead are separated from the hemolymph by only a basal lamina, which is relatively thick in large arteries (Stagner and Redmond 1974). The large arteries ensheath large peripheral nerves. Their wall comprises a compilation of fibroblasts, collagen, and striated muscle arranged circumferentially (Figure 9.20) (Fahrenbach 1999). Some smaller diameter arteries (called arterioles in some texts) ramify directly onto organs, while other organs are bathed in hemolymph sinuses/hemocoels, as seen in other arthropods (Fahrenbach 1999).

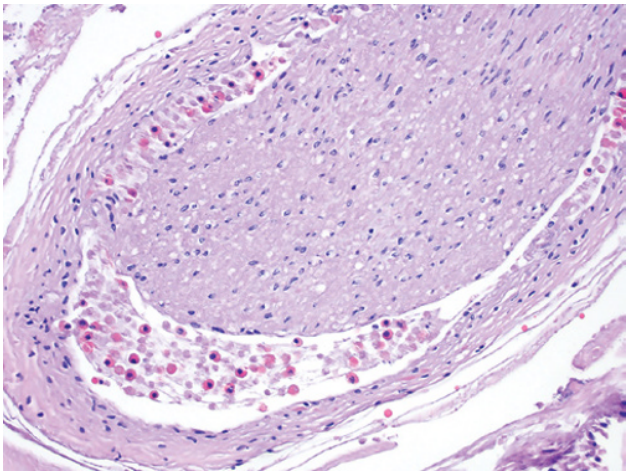


Figure 9.20 Artery and nerve of *L. polyphemus*. Nerve is contained in an arterial sheath. Between the nerve and sheath is a vascular space with hemolymph. 200×. HE.

9.3.5 Immune System

Hemocytes, also called "granulocytes," are the immune effector cells of HSC. The location of hemocyte production is believed to be pillar cells of the gills (see section 9.3.6) and possibly the periphery of the heart (Fahrenbach 1999). Hemocytes are 15–20 μm diameter round cells with numerous 1–2 μm diameter, eosinophilic, cytoplasmic granules (Figure 9.21). In pathologic conditions, hemocytes become elongate to ameboid and adhesive, and discharge their cytoplasmic granules (i.e., become "agranular hemocytes" or "degranulated hemocytes") (Fahrenbach 1999). They aggregate around wounds, infectious agents, and foreign material to aid in the immune response (Figure 9.22) (Bursey 1977;

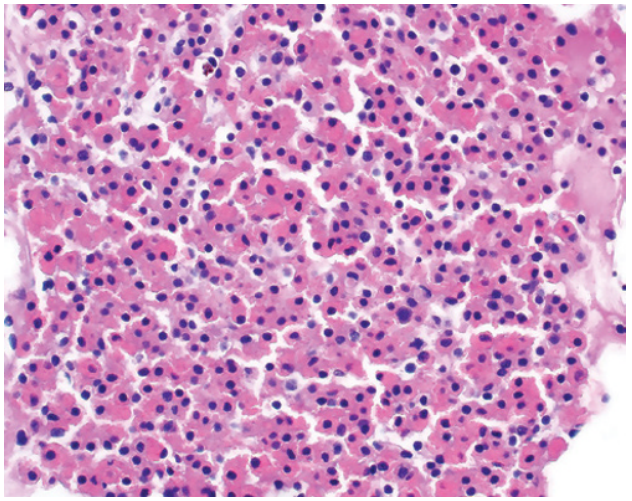


Figure 9.21 Vascular sinus of *L. polyphemus*. Hemolymph is composed of eosinophilic fluid and numerous round cells with granular, eosinophilic cytoplasm. 400×. HE.

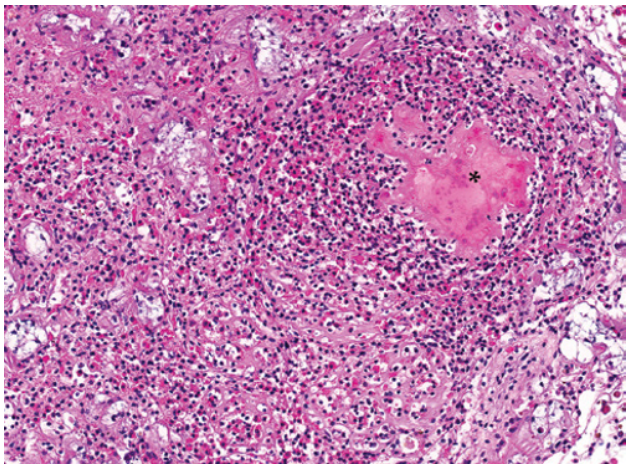


Figure 9.22 Inflammatory coagulum of *L. polyphemus*. A central coagulum (*asterisk*) of extracellular, eosinophilic material encasing bacteria is surrounded by abundant granular and agranular hemocytes. 200×. HE.

Stagner and Redmond 1974). Hemocyte granules contain a number of recognized antimicrobial substances, agglutinins, and factors to activate clotting. Clotting factors activate coagulen, leading to the formation of an insoluble gel called a coagulum. This coagulum occludes wounds and engulfs and immobilizes microbes, which are subsequently killed by antimicrobial substances released from entrapped hemocyte granules (Muta and Iwanaga 1996).

9.3.6 Respiratory System

The book gills (also referred to as "book lungs") are specialized appendages specific for respiration. HSC pulsate their book gills to facilitate water washing over them, allowing

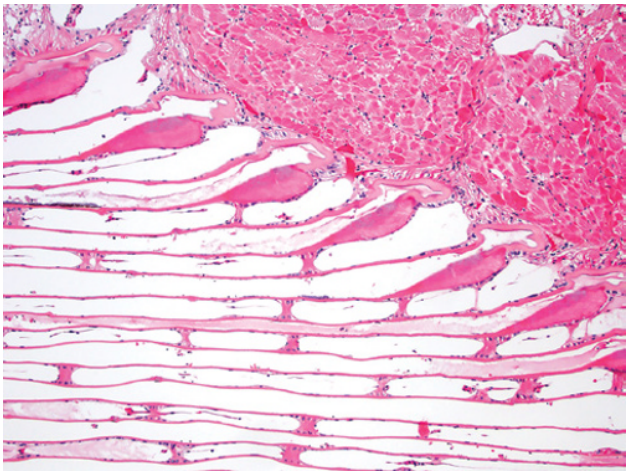


Figure 9.23 Book gills of *L. polyphemus*. The bases of the gills have large muscle bundles. 100×. HE.

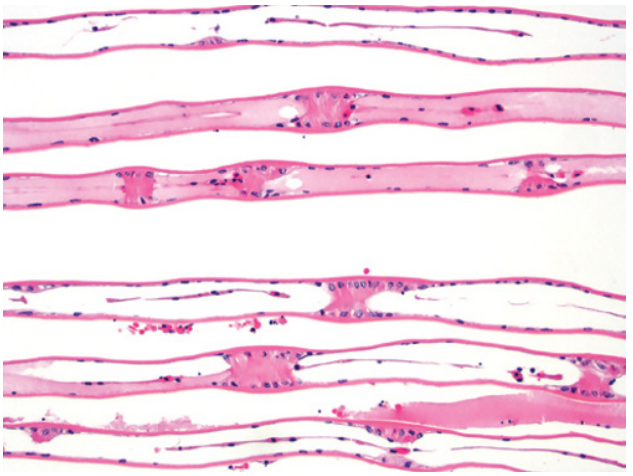


Figure 9.24 Book gills of *L. polyphemus*. The gills are composed of two parallel layers of chitin that encloses the hemolymph space. Chitin layers are held apart by clusters of pillar cells. 200×. HE.

oxygen transfer to the hemolymph, which is carried via copper in hemocyanin. Pulsation of the gills, in addition to the muscular function of the heart, also serves to help circulate hemolymph. The gills also have some role in regulation of sodium and chloride, though this is largely controlled by the coxal glands (see section 9.3.3). HSC have five gill opercula, each of which has five, paired book gills. The opercula have basilar skeletal muscle that facilitates pulsing of the book gills (Figure 9.23). Each book gill has 150–200 gill lamellae overlain by a 3–5 μm thick cuticle (see section 9.3.1.1). Gill lamellae are arranged in two, thin epithelial layers that enclose a vascular lumen that contains hemolymph; hemocytes are often visible in the branchial lumen. The epithelial layers are held apart by pillar cells (Bergdale 2017). Pillar cells occur in clusters and interdigitate with thin collagen bundles (Figure 9.24).

9.3.7 Nervous System

The central nervous system in HSC is divided into an anterior portion that is also called the brain, or supraesophageal ganglion, and a caudal portion that forms a ring around the mouth/proximal esophagus, ventral to the endosternite, called the circumesophageal ring. The supraesophageal ganglion receives input from the eyes. The ventral portion of the supraesophageal ganglion is called the corpora pedunculata, which forms the bulk of this tissue, and the dorsal portion is called the central neuropil (or protocerebrum), which is loosely "H-shaped." The circumesophageal ring has nervous connections to all appendages, the heart, and the intestines. A large, bilobed ventral nerve cord extends caudally from the circumesophageal ring, ventral to the intestine. Multiple large ganglia are present throughout the body, including abdominal ganglia, optic nerve ganglia, and a cardiac ganglion. The cardiac ganglion serves a pacemaker function to the heart, and consists of pigmented and nonpigmented neurons (Fahrenbach 1999; Chamberlain and Wyse 1986).

The brain and peripheral nerves are surrounded by a thick fibrous capsule. There is no blood–brain barrier, and multiple small vessels lined by glial cells (i.e., "vascular neuroglial") penetrate the neural parenchyma. The brain has multiple large ganglia surrounded by large bundles of white matter. Neurons vary greatly in size. Some neurons, called "Kenyon cells," are small with a 5–10 μm diameter nucleus and minimal cytoplasm; these are present in the corpora pedunculata (Figure 9.25). Large neurons (up to 100 μm in diameter) with a moderate amount of eosinophilic cytoplasm occur in ganglia throughout the brain, as well as in the periphery (Figure 9.26). Large neurons are generally associated with supporting glial cells. Axons in the brain are large and can be up to 15 μm in diameter. As previously mentioned, large peripheral nerves are within large arteries (see section 9.3.4), and surrounded by a few glial cells. Small peripheral nerves may or may not have evident supporting glial cells (Fahrenbach 1999).

Glial cells have been separated into three types ultrastructurally. Stellate astrocytes and velate astrocytes support and secrete extracellular matrix in the nervous system. Velate astrocytes also support Kenyon cells in the corpora pedunculata and central body. Vascular neuroglia occupy the arterial circulatory space between sheaths of arteries and nervous tissue (see section 9.3.4) (Fahrenbach 1999).

9.3.8 Reproductive System

Horseshoe crabs are dioecious, and male and female gonads occur in separate individuals. Both ovarian and testicular reproductive tissue is interspersed with the interstitium of the hepatopancreas (Figure 9.27). *Limulus* sp. reach sexual maturity in approximately 10 years. Fertilization is external. The female lays approximately 75 000 eggs in one breeding season that are subsequently covered with mature spermatozoa.

9.3.8.1 Female Reproductive System

The ovary is composed of anastomosing tubules lined by a 20–30 μm thick, vesiculated epithelium. Oogonesis occurs in the wall of the tubules and oocytes then herniate through the tubular lumen. Oocytes are generally large and any connection with ovarian tubules is difficult to appreciate using light microscopy. After ovulation, the oocyte returns to the tubule via "reverse herniation," leaving a wrinkled outer chorion in its place (Figure 9.28). The oviducts are

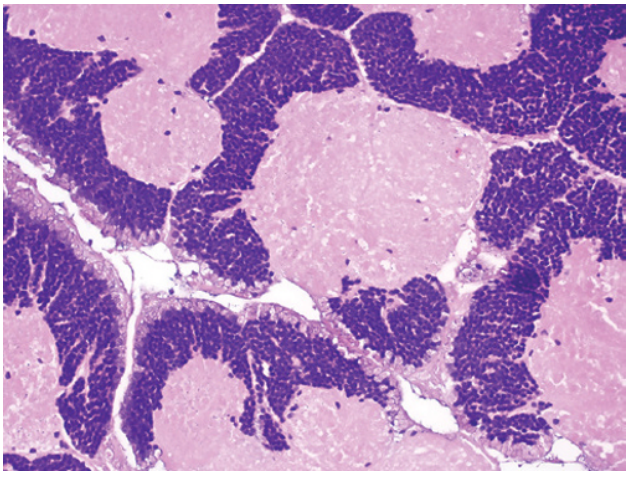


Figure 9.25 Corpora pedunculata of *L. polyphemus*. Numerous bands of Kenyon cells are separated by white matter.

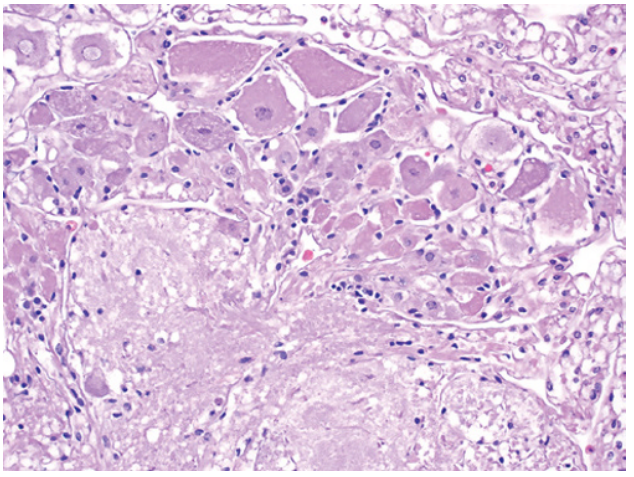


Figure 9.26 Brain of *L. polyphemus*. Large neurons occur in clusters and are surrounded by glial cells.

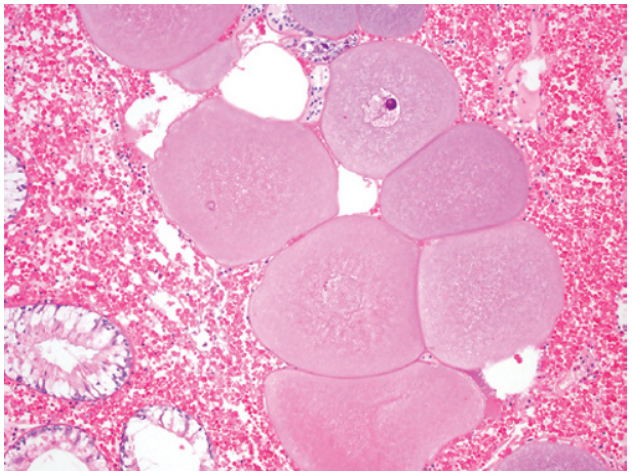


Figure 9.27 Hepatopancreas and ovary of *L. polyphemus*. Ovarian tissue is admixed with the hepatopancreas. Several collecting tubules are seen at the bottom left of the image. Several ovarian tubules are seen between the oocytes in the upper portion of the image. There are numerous interstitial cells filled with abundant, brightly eosinophilic, cytoplasmic granules. 200×. HE.

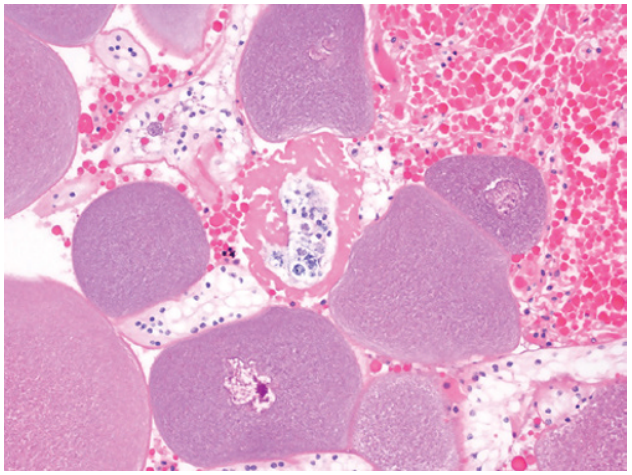


Figure 9.28 Ovary of *L. polyphemus*. Large oocytes, surrounded by a thin membrane, are interspersed with multiple ovarian tubules lined by a single layer of heavily vesiculated epithelial cells. Centrally, there is a site of previous oocyte ovulation, leaving behind a folded, shriveled outer chorion. 200×. HE.

lined by thick, pseudostratified epithelium and contain remnants of oocytes. They are surrounded by a 500 μm thick muscular layer with longitudinal, and fewer radial and circular, muscles (Fahrenbach 1999).

9.3.8.2 Male Reproductive System

Sperm ducts are similar in histomorphology to the oviducts. Seminiferous tubules are 30–500 μm in diameter and lined by few spermatogonia or developing spermatocytes.

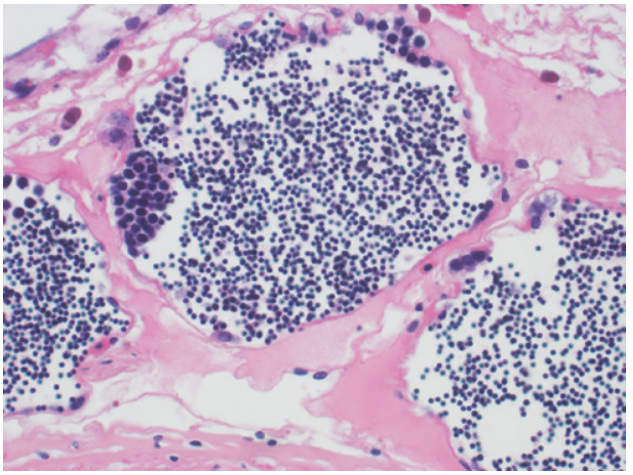


Figure 9.29 Testis of *L. polyphemus*. Multiple seminiferous tubules are pictured. At the margin of the tubules, there are occasional clusters of spermatogonia. The lumina contain numerous, spherical, deeply basophilic, mature spermatozoa.

The lumen contains numerous mature spermatozoa that are 2–3 μm in diameter (Figure 9.29).

9.3.9 Special Senses

Sensory organs in HSC are limited to eyes and various cuticular receptors. HSC have multiple sets of eyes. The most conspicuous are the compound eyes, which are located laterally on the prosoma, and will be the focus of ocular discussion. Median eyes (also called ocelli) are also present. Additionally, there is a set of rudimentary eyes present next to both the median and compound eyes (Bergdale 2017).

9.3.9.1 Compound Eyes

Compound eyes are visible grossly as bean-shaped organs ventral to the lateral spines of the prosoma. Histologically, the cuticle elevates overlying the eyes and is modified from the typical layering to form multiple cuticular cones that bulge toward and have processes extending into ommatidia, which are analogous to the retina (Figure 9.30). Several types of pigment cells are present in the compound eye and are virtually identical by light microscopy, including cells that surround cuticular cones (i.e., distal pigment cells), form the epidermis between cuticular cones (i.e., guanophores), and surround retinular cells (i.e., intraommatidial pigment cells). Pigment within these cells shifts intracytoplasmic location (e.g., basilar vs apical) in response to different light intensities. Retinular cells are the primary photoreceptors and are radially oriented around a central dendrite to form

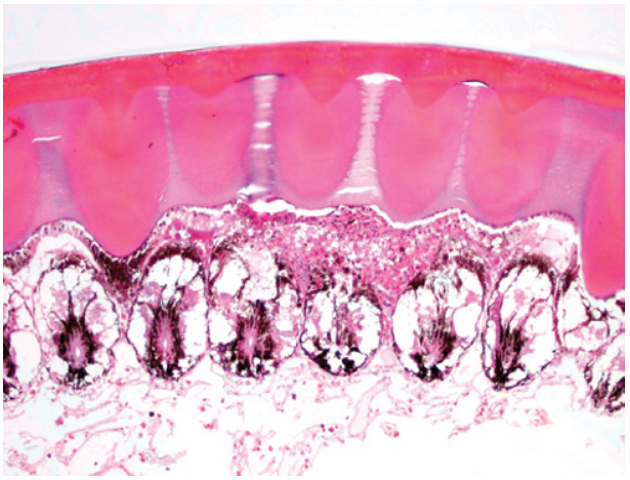


Figure 9.30 Compound eye of *L. polyphemus*. The cuticle is bowed outward and modified to form multiple cuticular cones, each of which corresponds to a subjacent ommatidium. 100×. HE.

an ommatidium. Each ommatidium is approximately 100 µm in diameter (Figure 9.31) (Fahrenbach 1999).

9.3.9.2 Cuticular Receptors

Morphologically, there is little variation in cuticular receptors. These receptors (or "sensilla") serve mechano-, chemo-, thermo-, and osmoreception functions. Most receptors are papillary cuticular cones with a thin (approximately <1 µm diameter) central channel that extends through the entire thickness of the cuticle into subjacent neural plexi (Figure 9.32) (Fahrenbach 1999).

Disclaimer

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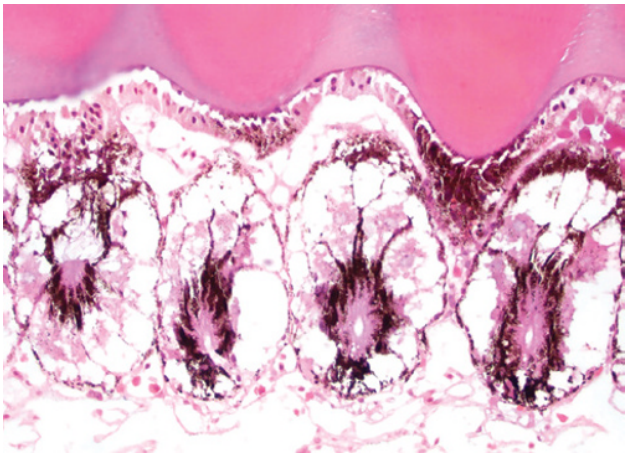


Figure 9.31 Compound eye of *L. polyphemus*. Four ommatidia are pictured, each of which has radially arranged, pigmented, retinular cells surrounding a central dendrite. 400×. HE.

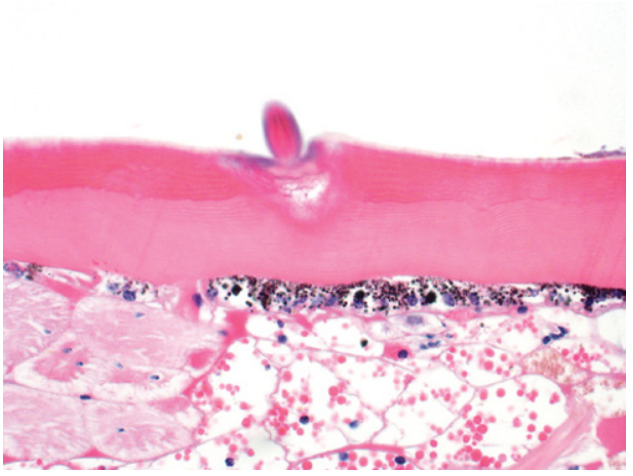


Figure 9.32 Sensilla of *L. polyphemus*. The cuticle is focally expanded by sensillum, which is composed of proteins and chitin (similar to the rest of the cuticle) and centrally contains a narrow pore. This pore penetrates the thickness of the cuticle (not pictured) to transmit sensory material from the external environment to internal receptors. 200×. HE.

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10

Arthropoda

Myriapoda

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10.1 Introduction

Phylum Myriapoda consists of 13 000 species divided into four classes: Chilopoda (centipedes), Diplopoda (millipedes), Symphyla (garden centipede, pseudocentipede), and Pauropoda (millipede-like arthropods). All four classes are terrestrial. Their range varies from temperate to tropical, moist forest floor to grasslands, and semi-arid to arid habitats. Symphylans and pauropodans are typically microscopic and live in the soil. Most chilopods are nocturnal and some are intertidal (Ruppert et al. 2004). All are dioecious and fertilization is internal with sperm transfer indirectly via spermatophores.

Diet varies among phyla. Chilopods are predatory and venomous, consuming predominantly small arthropods but also earthworms, snails, and nematodes. Larger scolopendropmorphs can consume small vertebrates including amphibians, snakes, lizards, birds, and mice. Diplopods, symphylans, and pauropods live primarily on leaf litter and soil debris and are important environmental detritivores, enhancing decomposition of dead plant material. A few do consume live vegetation and one family (siphonophoridae) have a modified gnathochilarium to pierce live plants and feed on juices. One species (Nematophora) has a carnivorous/omnivorous diet and feeds on harvestmen, hexapods, centipedes, and earthworms. Chilopods have defensive adaptations in addition to their poison forcipules (fangs). These include anal legs that can be used to pinch. They may also autotomize limbs to escape predators and replace lost legs with the next molt. Most scolopendromorphs and geophilomorphs have repugnatorial glands on the ventral aspect of each segment and some have coxal organs, both of which make defensive secretions.

Myriapods are used as experimental models of research in the genetic control of segmentation. Conservation risks are similar to other terrestrial species and include habitat destruction and collection of species for the pet trade. Like most terrestrial invertebrates, conservation programs and environmental impact assessments often pay little to no attention to the subterranean habitat. Millipedes live from one to 10 years depending on the species (Ruppert et al. 2004).

10.2 Gross Anatomy

Myriapods share a similar body plan. They have a head and an elongate body (trunk) composed of numerous individual segments and lack the defined thoracic or abdominal divisions present in hexapods. Each segment is composed of a dorsal tergite and ventral sternite that are connected laterally by paired pleurites. The cuticle is heavily calcified and typically each segment is either cylindrical or slightly flattened due to lateral projections from the tergites (paranotum). Segments bear one (Chilopoda, Symphyla, Pauropoda) or two (Diplopoda) pairs of legs depending on class. Each diplopod segment is derived from the fusion of two segments (diplosegment), hence the paired legs on each ring. Chilopods range from 4 to 6 cm in length, though they can be as small as 1 cm to as long as 30 cm (*Scolopendra gigantea*). They have between 15 and 191 pairs of legs and the pairs are always odd; no "centipede" truly has 100 legs. Diplopods range from 2 mm to 30 cm in length and have up to 710 legs. Symphylans are small, typically 2–10 mm long, with the exception of *Hanseniella magna* which reaches up to 30 mm in length. They typically have 15–24 segments, of which only 10 or 12 segments bear one set of legs. The last

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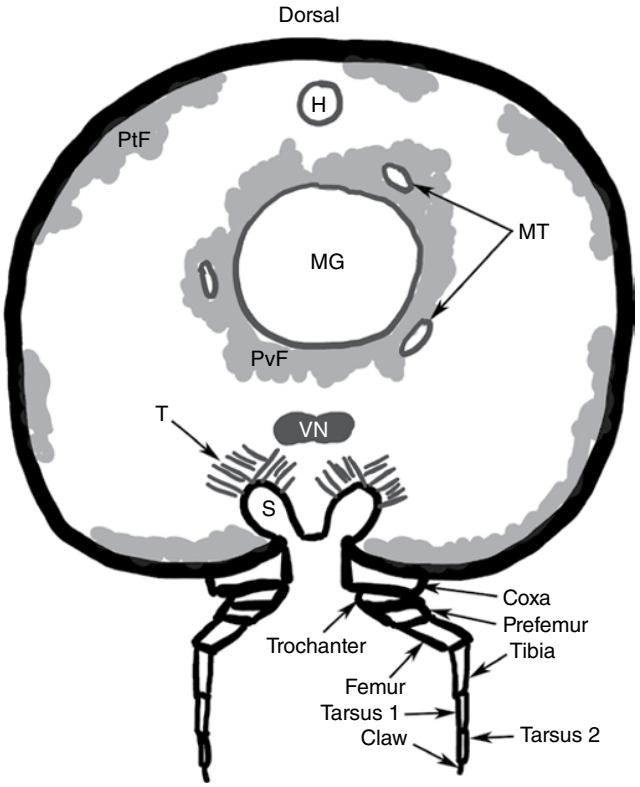


Figure 10.1 Schematic of a transverse section of a diplopod with the segments of the appendages labeled. H, heart; MG, midgut; MT, Malpighian tubules; PtF, parietal fat body; PvF, perivisceral fat body; S, spiracle; T, tracheae; VN, ventral nerve cord.

segment lacks legs but has a pair of spinerettes (cerci) (Minelli and Golovatch 2001). Most pauropods are microscopic, measuring only 0.5–0.7 mm long. The largest are only 1.9 mm long. The trunk typically bears 5–6 segments and only 8–11 pairs of legs on each (Minelli 2011).

Each walking leg has eight identifiable segments; from anterior to posterior these are the coxa, trochanter, prefemur, femur, tibia, tarsus 1, tarsus 2, and claw (Figure 10.1). The appendages of the first segment are enlarged forcipules (maxillipeds, poison fangs) in centipedes. They are curved toward the ventral midline and have a terminal pointed fang that connects via a duct to the poison gland which is typically in the telopodite. The terminal pair of legs on the last trunk segment are also modified and not used in locomotion. These can have sensory, defensive, or aggressive adaptations, including pincers.

A feature of all myriapods is the genital opening. In symphylans, it is between the coxa of the fourth segment. In pauropods, the opening is between the second pair of legs (Minelli 2011). In centipedes, the last two segments are the pregenital and genital segments. The genital segment bears a telson. Either segment may have a pair of gonopods, which in female lithobiomorphs and scutigeromorphs are used to manipulate the eggs after laying. In diplopods, the first segment is legless and referred to as the collum. The second through fourth segments have only a single pair of appendages and are not considered diplosegments. In most taxa, one or both legs of the seventh ring are gonopods, modified to transfer sperm from the male to the female. The body terminates in the telson with a ventral anus present. Some of the posterior segments are similarly limbless.

The head has one pair of antennae. In millipedes, antenna are divided into eight segments with four chemoreceptive cones at the tip of the eighth segment. Centipedes and millipedes have simple lateral eyes. Symphylans and pauropods have no eyes. All myriapods lack median ocelli. The head is composed of multiple complex segments. In chilopods, this includes a dorsal cephalic shield, labrum (upper lip), paired mandibles, and a lower lip formed from either the first or second maxillae. The coxa and sternite of the forcipule form a plate-like coxosternite that covers the ventral surface of the head. In diplopods, the head consists of a domed dorsal shield and ventrally two pairs of mandibles and the gnathochilarium, which may represent the fused first and second maxillae. The broad flattened plate is attached to the posterior ventral surface of the head. It has distal sensory palps that form the floor and posterior wall of the preoral cavity. Six short sensory structures (apical pegs covered with chemoreceptive sensilla) are also present. Along the medial edges of the mandibles there are teeth and a rasping surface.

The digestive system begins with a preoral cavity formed by the various maxillary and mandibular plates. This connects to a soft hypopharynx and extends as a single long tube along the middle of the coelom. Segments of the intestinal tract consist of a foregut (pharynx and esophagus), midgut, and hindgut (Figure 10.2). The foregut makes up approximately two-thirds of the gut. The hindgut is extremely short and the midgut forms the majority of the remaining tract. The heart lies on the midline dorsally within the coelomic cavity (Figure 10.3). It extends the length of the body immediately dorsal to the intestinal tract. There are pairs of ostia present at each segment and in some species arteriole branches are present. The nervous system is not strongly cephalized. The syncerebrum, circumenteric connectives and small subesophageal ganglion are present and visible. A ventral nerve cord is apparent along the ventral coelom. There are paired ganglia in each segment. The openings to the respiratory tract (spiracles) are typically paired and are present in each segment, though the distribution pattern varies with each taxon and some segments may be lacking. In all but scutigeromorphs, the spiracles are present in the pleural membrane immediately anterior to the coxa of the walking legs.

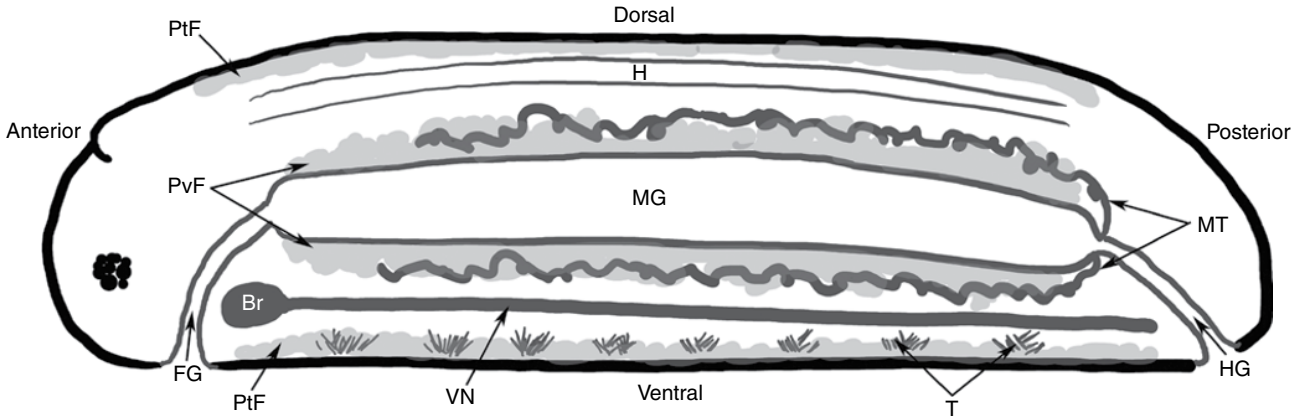


Figure 10.2 Schematic of a longitudinal section of a myriapod. Br, brain; FG, foregut; H, heart; HG, hindgut; MG, midgut; MT, Malpighian tubules; PtF, parietal fat body; PvF, perivisceral fat body; T, tracheae; VN, ventral nerve cord.

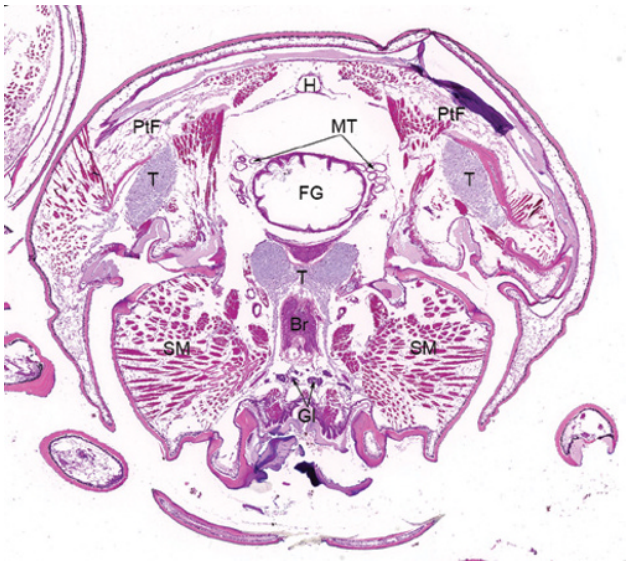


Figure 10.3 Transverse histologic section of a giant African millipede (*Archispirostreptus gigas*) at the level of the head. Br, brain; FG, foregut; Gl, glands; H, heart; MT, Malpighian tubules; PtF, parietal fat body; SM, skeletal muscle; T, tracheae.

10.3 Histology

10.3.1 Body Wall/Musculoskeletal System

Table 10.1 provides a list of organs for histologic examination in myriapods. Myriapods require a humid environment due to the permeability of their cuticle which is not waterproof and lacks the lipid/wax characteristics of spiders and insects. The lipids present appear to repel water from the outside, rather than preventing water loss internally. The integument, particularly in the tergites, is heavily mineralized and impregnated with calcium salts. The surface is generally smooth but some taxa have ridges, tubercles, spines, or isolated bristles.

Repugnatorial glands are present in all diplopod taxa other than bristly millipedes, usually one pair of glands per diplosegment. Openings are located on the sides of the tergites or the margins of the paranota. Glands consist of a secretory sac that empties to a duct leading to an external pore. Secretory composition depends on species but may include aldehydes, quinones, phenols, iodine, chlorine, and hydrogen cyanine (Ruppert et al. 2004).

10.3.2 Integument

The cuticle rests on an epidermis (also called hypodermis), which is subtended by a basement membrane. It is generally composed of three layers: the epicuticle, exocuticle, and endocuticle (Figure 10.4). The outer epicuticle is very thin (approximately 1 μm). Internal to this is the exocuticle, which is hard and golden to brown colored. The thickness of the exocuticle varies; it is typically much thicker than the epicuticle but much thinner than the endocuticle. The thickest layer is the internal endocuticle, which is layered and eosinophilic. Multiple thin canals may extend through the cuticle to the surface (e.g., for secretions of epidermal glands) (Figure 10.5) (Minelli 1990). Flexible portions of the cuticle called arthrodial membranes are present at articulations of adjacent cuticle plates. The cuticle in these regions has loss of distinct layering and is thin (Figure 10.6).

10.3.3 Parietal Fat Body

There are two fat bodies of myriapods: one surrounds the midgut and regional viscera and is called the perivisceral fat body, and the other is immediately below the epidermis and is called the parietal fat body. Fat body cells (sometimes called adipocytes) are arranged in cords or aggregates. These cells are polygonal and have moderate anisocytosis.

Table 10.1 Organs for histologic evaluation in Merostomata.[a]

Organ system		Organs
Body wall/musculoskeletal		Cuticle, epidermis/dermis, arthrodial membrane, skeletal muscle, repugnatorial glands
Digestive	Alimentary canal	Preoral cavity, foregut (pharynx, esophagus), midgut, hindgut, rectum
	Storage and exocrine glands	Salivary glands, fat body
Excretory		Malpighian tubules, maxillary nephridia (*saccate nephridia*)
Circulatory		Heart, hemocoel, ostia, cephalic aorta
Immune		Hemocytes
Respiratory		Spiracle, atrium (*tracheal pouch*), tracheae
Nervous		Brain, periesophageal ring, subesophageal ganglion, ventral nerve cord
Reproductive	Male	Testis, gonopore (paired)
	Female	Ovary, oviduct, gonopore (single)
Special senses/organs		Eye, Tömösváry organ, antennae

[a] Alternative names for organs are provided parenthetically, in italics.

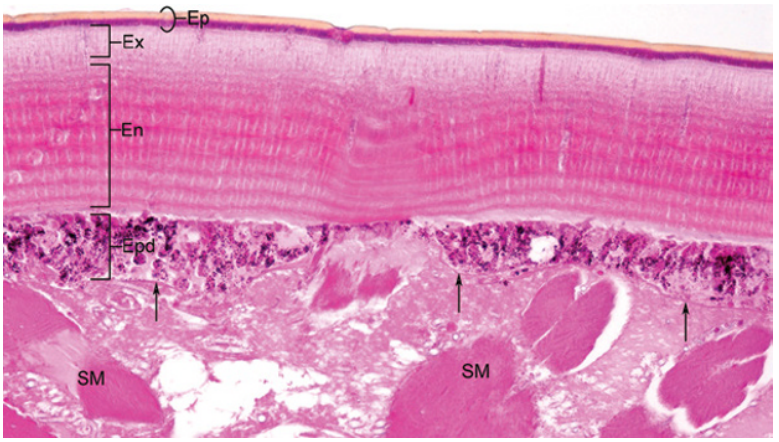


Figure 10.4 Detail of the layers of the cuticle of a giant African millipede (*Archispirostreptus gigas*) including the thin epicuticle (Ep), exocuticle (Ex), and distinct layering of the thick endocuticle (En). The epidermis (Epd) is below the endocuticle and is subtended by a basement membrane (arrows). SM, skeletal muscle. 400×. HE.

The nuclei are round. The cytoplasm is highly vacuolated and may contain granules or spherules (Figure 10.7). Sudan III stain has been used to demonstrate fat in the cytoplasm of these cells (Fontanetti et al. 2004).

10.3.4 Skeletal Muscle

Myriapods have numerous skeletal muscles in the body and limbs. Skeletal muscle is typical as seen in other groups and is striated. Skeletal muscles can attach directly to the internal surface of the cuticle, or via tendons that attach to the connective tissue deep to the cuticle (Figure 10.8). The cuticle has multiple apodemes (inward growths or projections of the cuticle) to which skeletal muscles also attach. Similar to arachnids, extension of some joints is facilitated through hydrostatic pressure of the hemolymph, rather than specific extensor muscles. This hydrostatic pressure is antagonized by skeletal muscle contraction of flexor muscles (Minelli 1990).

10.3.5 Digestive System

The digestive system is composed of a foregut (pharynx and esophagus), midgut, and hindgut. Digestive enzymes are provided by buccal, mandibular, maxillary, and vesicular glands associated with the preoral cavity and pharynx (Figure 10.9). Salivary glands open into the preoral cavity in millipedes.

In chilopods, prey items are held by the second maxillae and the forcipules and are manipulated by the mandibles and first maxillae. Some centipedes masticate prey, others pull the prey into the gut using the pharynx. Geophilomorphs have small teeth and partially digest their prey in the preoral cavity prior to ingestion.

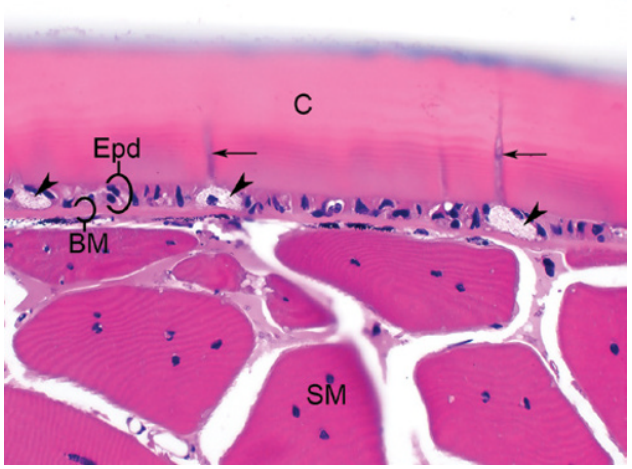


Figure 10.5 Body wall of a giant desert centipede (*Scolopendra heros*). The epidermis (Epd) is a simple layer of columnar cells interspersed with occasional glandular, polygonal cells (arrowheads). The epidermis rests on a basement membrane (BM) and is covered by a multilayered cuticle (C). Multiple channels (*arrows*) traverse the cuticle representing ducts for secretions of the glandular cells. SM, skeletal muscle. 400×. HE.

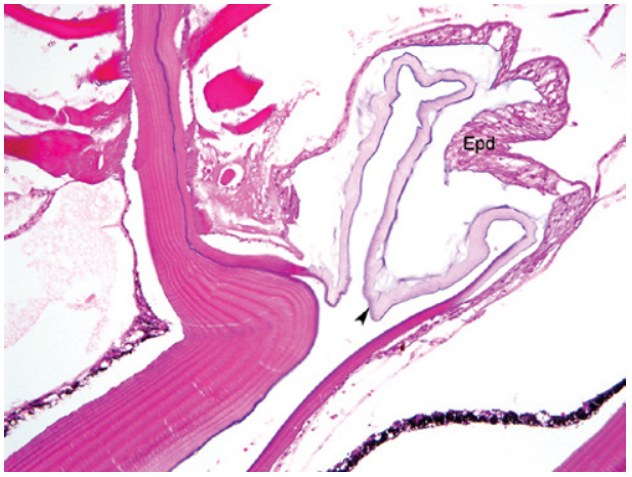


Figure 10.6 The arthrodial membrane between two tergites in a giant African millipede (*Archispirostreptus gigas*). The arthrodial membrane (*arrowhead*) is thinner than the surrounding cuticle with loss of distinct layering. The epidermis (Epd) is artifactually separated from the cuticle. 200×. HE.

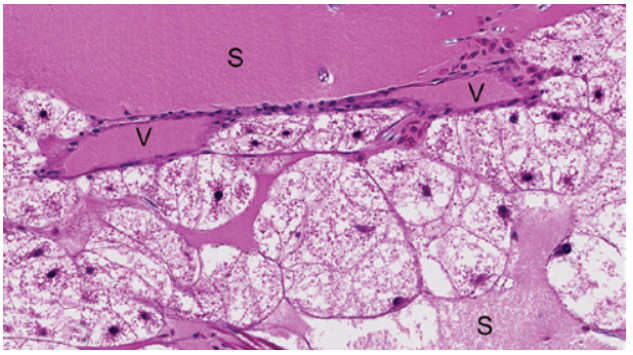


Figure 10.7 The parietal fat body in a giant African millipede (*Archispirostreptus gigas*). Large cells with a central or an eccentric, small, round nucleus and heavily vacuolated cytoplasm are separated into lobules by a fine fibrovascular stroma. Two hemolymphatic vessels (V) and sinuses (S) are also visible. 280×. HE.

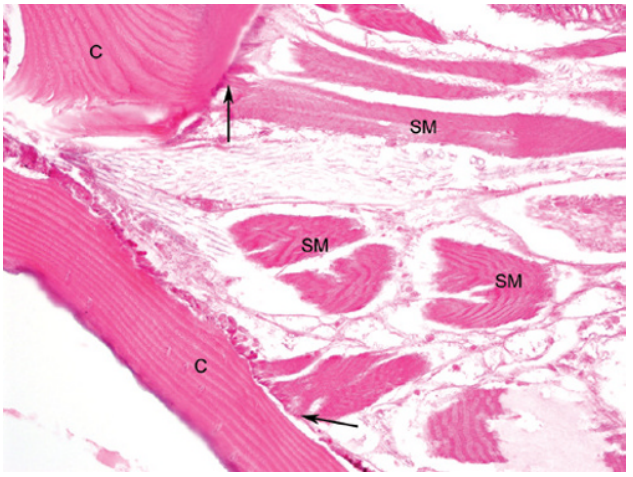


Figure 10.8 Longitudinal and cross-sections of skeletal muscle fibers (SM) in a giant African millipede (*Archispirostreptus gigas*) demonstrating regular striation and multiple direct attachment sites (*arrows*) to the cuticle (C). 300×. HE.

Digestion and absorption occur in the midgut. The midgut creates a peritrophic membrane to surround food, similar to hexapods and crustaceans. Surrounding the midgut is tissue referred to as the perivisceral fat body that appears similar to chloragogen tissue of annelids. The cells store glycogen and may play a role in excretion by storing/sequestering toxins. This fat body also stores lipids, protein, and uric acid.

In pauropods, the digestive tract consists of a preoral cavity with paired salivary glands. This transitions to the ectodermal foregut which extends to the third segment. The midgut is entodermal and amuscular with a diameter typically twice of the foregut. It begins at the fourth segment, extends to the 10th segment and may be convoluted or straight. At the 10th segment it narrows and becomes a short winding tube before the rectum. The rectum is characterized as ovoid, with an outer circumferential muscle layer and inner longitudinal muscle layer (Minelli 2011).

Many organisms can be found in the luminal alimentary tract of myriapods. Nematodes, gregarines, and other protozoa have all been found in the intestine of millipedes, some of which are associated with significant inflammatory response (A. Newton, pers. obs.).

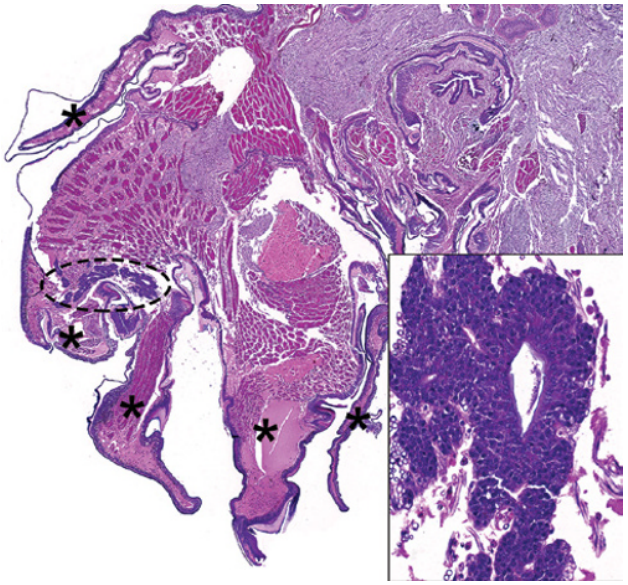


Figure 10.9 Head of a giant African millipede (*Archispirostreptus gigas*) showing the appendages comprising the mouthparts (*asterisks*). Glands (*ellipse*) are located below the cuticle. On higher magnification (inset), glands are arranged around central ducts with a chitinous lining. 27×; inset 320×. HE.

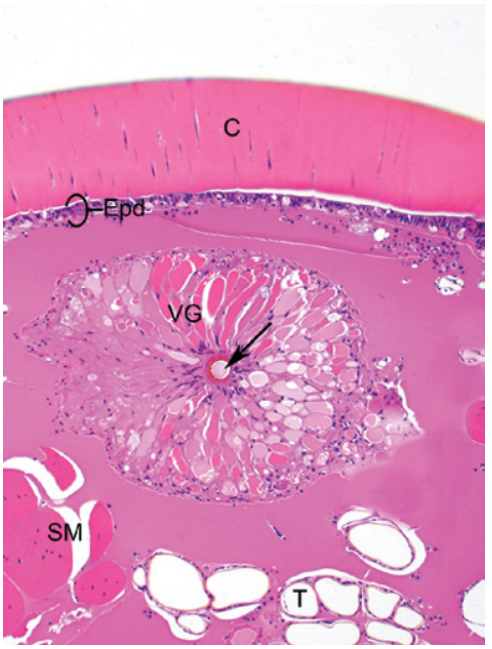


Figure 10.10 Venom gland of giant desert centipede (*Scolopendra heros*). The venom gland (VG) has numerous glandular cells with eosinophilic droplets in the cytoplasm. These cells are organized around a central duct (*arrow*). Tracheae (T) supply oxygen to the gland. C, cuticle; Epd, epidermis; SM, skeletal muscle. 100×. HE.

10.3.5.1 Glands

The cephalic glands (also called salivary glands) include the buccal, mandibular, maxillary, and vesicular glands. These glands are likely related to feeding and grooming. Their structure and function have not been fully elucidated (Minelli 1990).

Poison glands (also called venom glands) are present in all centipedes. In most centipedes these glands open into a subterminal duct in the forcipules. The poison glands are innervated by a nerve from the subesophageal ganglion, and supplied by numerous tracheoles. Poison glands are arranged in tubules composed of a single layer of tall, piriform epithelial cells with basal nuclei that radiate around a central excretory canal, which is lined by a cuticle (Figure 10.10). Poison gland cells have holocrine secretion, and their size varies by the amount of secretory product in the cytoplasm.

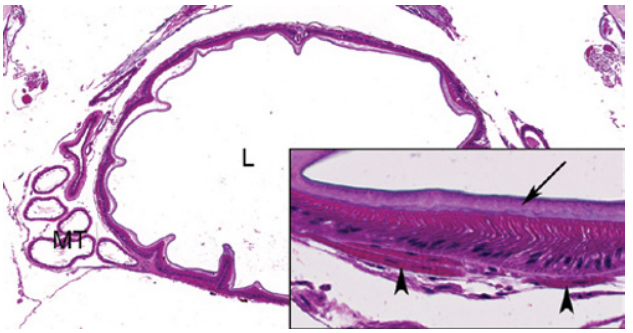


Figure 10.11 Longitudinal section through the foregut (esophagus) of a giant African millipede (*Archispirostreptus gigas*). On higher magnification (inset), the mucosal epithelium is composed of columnar cells with basilar nuclei and is lined internally by cuticle (*arrow*). Muscular layers (*arrowheads*) composed of striated muscle are outside the mucosa. L, lumen; MT, Malpighian tubules. 50×; inset 400×. HE.

10.3.5.2 Alimentary Canal

The preoral cavity is bordered anteriorly by the labrum, laterally by the mandibles, and ventrally by the hypopharynx.

The foregut is lined throughout its length by a chitinous cuticle that is uniform in thickness. The mucosal epithelium has longitudinal folds that are more pronounced aborad. The mucosal epithelium is simple with columnar cells that have basilar nuclei. The mucosa is surrounded by two layers of striated muscle: an inner longitudinal layer and an outer circular layer (Figure 10.11). The longitudinal muscle is relatively thin, and the circular muscle is 2–3 layers thick. These muscles frequently have evident tracheole ramifications. The foregut terminates at the esophageal valve, which has a deep fold.

The midgut lumen is larger than in the foregut, is uniform in diameter throughout its length, and lacks folds. At the start of the midgut, the epithelial cells are cuboidal

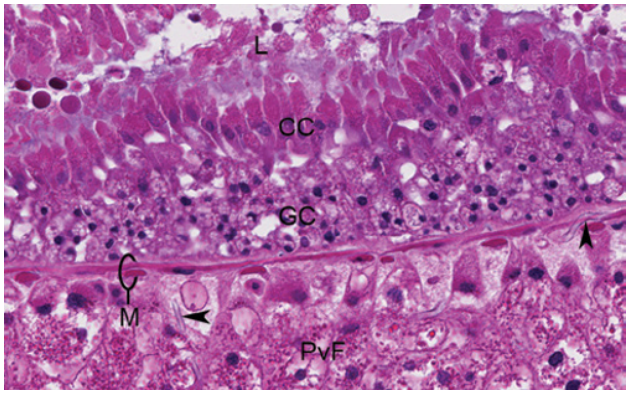


Figure 10.12 Cross-section of the midgut of a giant African millipede (*Archispirostreptus gigas*). The mucosal epithelium is composed of an outer columnar cell layer (CC) with tall columnar cells that have basal oval nuclei and an inner goblet cell (GC) layer. The muscle layers (M) are composed of a continuous inner muscle layer and a discontinuous outer muscle layer. The midgut is surrounded by the perivisceral fat body (PvF). Tracheae (*arrowheads*) penetrate the fat body and mucosa. L, midgut lumen. 300×. HE.

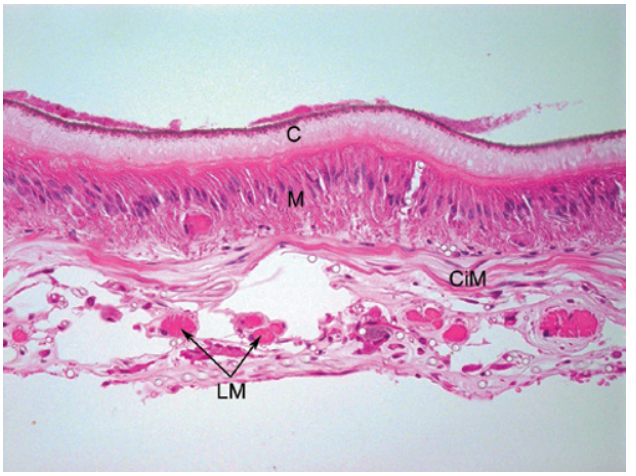


Figure 10.13 Cross-section of the hindgut of a giant African millipede (*Archispirostreptus gigas*). The hindgut mucosa (M) is covered internally by a chitinous cuticle (C). The outer longitudinal muscles (LM) are thin and appear incomplete compared to the circular muscle layer (CiM). 400×. HE.

(though they elongate to become columnar distally in the midgut) and the circular muscles are regionally less pronounced (Miley 1930). The epithelium is simple and surrounded by two muscular layers, similar to the esophagus; the outer longitudinal muscles are thinner and sometimes appear discontinuous. The outer muscular layer is surrounded by the perivisceral fat body (see below). The midgut lacks a chitinous cuticle, likely due to the absorptive function of this segment (Miley 1930). The midgut epithelium has two cell types. Some of the epithelial cells are columnar (approximately 140 µm in height in some species) with basal oval nuclei. The remaining cells are goblet cells (Figure 10.12).

There is a mucosal fold at the junction of the midgut and hindgut that is immediately anterior to the insertion of the Malpighian tubules. The mucosal epithelial cells transition from high columnar in the posterior midgut to slightly lower columnar in the proximal hindgut. The hindgut mucosa is covered by a chitinous cuticle. The longitudinal muscles are generally thin and sometimes appear incomplete (this is true throughout the length of the hindgut except at the junction with the midgut, where they are thicker). The circular muscles gradually become thicker over the length of the hindgut (Figure 10.13) (Miley 1930).

The rectum has a thick band of circular muscles, but lacks longitudinal muscles. The mucosa has many folds which cause marked narrowing (or closure) of the lumen (Miley 1930). Mucosal epithelial cells become shorter as they near the anus (Minelli 1990).

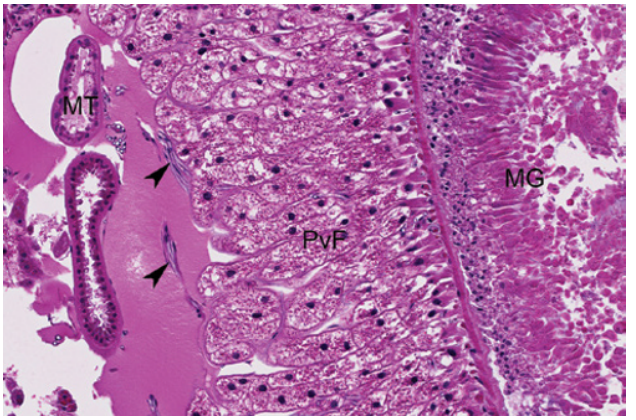


Figure 10.14 The perivisceral fat body (PvF) of a giant African millipede (*Archispirostreptus gigas*) surrounds the midgut (MG) and demonstrates similar characteristics to the parietal fat body (Figure 10.7), including large cells with abundant heavily vacuolated cytoplasm, a central or eccentric round nucleus and lobular architecture. Tracheae (*arrowheads*) are associated with oval nuclei. Malpighian tubules (MT) are also pictured. 200×. HE.

10.3.5.3 Perivisceral Fat Body

There are two fat bodies of myriapods: one surrounds the midgut and regional viscera and is called the perivisceral fat body, and the other is immediately below the epidermis and is called the parietal fat body. The perivisceral fat body extends from around the midgut to incorporate portions of the gonads and Malpighian tubules (Figure 10.14). Histologically, the appearance is identical to the parietal fat body (described in section 10.3.1) (Fontanetti et al. 2004).

10.3.6 Excretory System

The primary excretory organs in myriapods are Malpighian tubules, which produce uric acid and ammonia (Minelli 1990). In species confined to moist habitats and nocturnal activity, the excretory wastes are predominantly ammonia (Brusca and Brusca 2003). Maxillary nephridia (also called saccate nephridia) are present in some species but whether they are functional in excretion is not clear. These glands each have two ducts to the exterior.

10.3.6.1 Malpighian Tubules

Centipedes and millipedes have one and two pairs of Malphigian tubules, respectively, that arise just posterior to the midgut–hindgut junction (Brusca and Brusca 2003). These tubules extend toward the head, and are often long and looped. They are blind-ended, and their ends are attached to tracheae. The tubules are lined by a simple layer of cuboidal epithelial cells with a moderate nuclear:cytoplasmic ratio resting on a thick basement membrane. The orad portion of the Malpighian tubules is frequently widened into ampullae, which have wider lumina and are lined by columnar epithelium, with occasional apical cytoplasmic vacuolation and granules. Generally, the number of epithelial cells in cross-section per tubule increases in large animals and in orad portions of the Malpighian tubules (i.e., toward the ampullae). For example, larger species (e.g., *Lithobius forficatus*) may have 20 cells in cross-section of a Malpighian tubule, while smaller species (e.g., *Lithobius tricuspis*) may have only 8–12 cells in cross-section. No muscle layers are present (Figure 10.15) (Miley 1930; Minelli 1990).

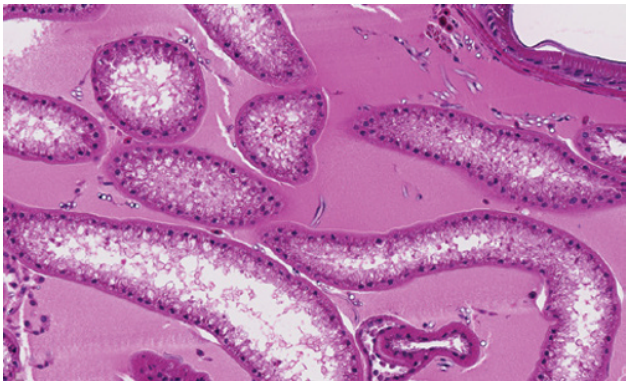


Figure 10.15 Cross-sections of Malpighian tubules in a giant African millipede (*Archispirostreptus gigas*) demonstrating the simple cuboidal epithelium, prominent lumen, and lack of muscular wall. 300×. HE.

10.3.6.2 Maxillary Nephridia

This organ, which is not present in all myriapods, is located in the head near other cephalic glands. Nephridia are paired, and each nephridium has two openings to the outside of the body, one medial to the first maxillae and the other medial to the second maxillae. Each nephridium has a utriculus, labyrinth, and sacculus. The utriculus, also called urinary bladder, lies between the ventral nerve cord and body wall, and opens to the external environment via a cuticle-lined duct in the first maxilla. The utriculus is lined by a simple epithelium that is highly folded anteriorly. The utriculus opens via a small ostium into the labyrinth, which is lined by secretory epithelium. The labyrinth opens to the external environment via a cuticle-lined duct in the second maxilla. The labyrinth connects to the sacculus, which is dorsal to the other chambers. The sacculus is composed of numerous lacunar spaces overlain by small hemolyphatic vessels. Analogies have been made between the sacculus and vertebrate glomeruli; although similar morphologically, any functional similarities between these two structures are unknown (Minelli 1990). For detailed images on arthropod nephridia (also called coxal glands), see Chapter 8 (Arachnida).

10.3.7 Circulatory System

The body cavity is a hemocoel divided by two horizontal diaphragms into pericardial (dorsal), perivisceral, and perineural (ventral) sinuses. The hemocoel is full of hemolymph. The hemolymph pumps from the heart to the aorta to the sinuses and is returned to the pericardial sinus and then back to the heart via paired ostia. The heart (dorsal vessel) is a muscular tube. In diplopods, the heart is perforated by two pairs of ostia per diplosegment, but anterior segments have one pair of ostia each. In chilopods, one pair of ostia is present per segment (Brusca and Brusca 2003). The heart ends blindly posteriorly in the trunk. Anteriorly there is a short cephalic aorta that extends into the head.

10.3.7.1 Heart

The heart is a muscular tube that is in the dorsal coelom. It ends blindly posteriorly, but is continuous with a short aorta anteriorly. The ostia perforate the heart and may be continuous with short vessels. The cardiac wall is composed of striated, circular muscle fibers lined externally by an adventitia, which is surrounded by a pericardial sac (Figure 10.16) (Miley 1930). Beating of the heart (systole and diastole) is regulated by the "heart nerve," which is a visceral ganglion dorsal to the heart (Minelli 1990). A heart is absent in pauropods, likely due to their small size (Ruppert et al. 2004).



Figure 10.16 Heart of a giant African millipede (*Archispirostreptus gigas*). The heart is composed of a striated muscular wall surrounding a lumen (L) that contains hemolymph with small numbers of hemocytes. The heart lies in a pericardial sac (PS), in which it is suspended by thin ligaments (*arrowheads*). The heart is innervated by a "heart nerve" (*arrow*), which is dorsal to the heart. C, cuticle; Epd, epidermis; PtF, parietal fat body. 200×. HE.

10.3.7.2 Hemolymph Vessels

The Vasculature is limited to arteries (i.e., vessels pumping blood from the heart). Arteries lack endothelia and do not anastomose (Figure 10.7). They vary in diameter, with some ranging from 0.2 to 0.3 mm (Minelli 1990).

10.3.8 Immune System

The primary immune effector cell in myriapods, similar to other arthropods, is the hemocyte. Hemocytes serve numerous functions, including phagocytosis, wound healing, and hemolymph clotting. They also discharge numerous antimicrobial substances from their granules to aid in pathogen defense. The literature on myriapod hemocytes is highly convoluted with up to seven different types of hemocytes described. A more recent review of hemocytes in myriapods describes three primary types of hemocytes across all myriapod taxa: prohemocytes, plasmatocytes, and granular hemocytes (Xylander 2009). Descriptions of hemocytes are frequently at an ultrastructural level, and discerning these types of hemocytes may not be possible at the resolution of the light microscope.

Prohemocytes are the smallest at <9 µm in diameter, spherical, and have a large nucleus:cytoplasmic ratio. Plasmatocytes and granulocytes are both substantially larger than prohemocytes. Granulocytes are the most numerous hemocytes in myriapods and have numerous cytoplasmic granules (Figure 10.17). Other types of hemocytes (e.g., spherulocytes) may exist in some taxa (Minelli 1990; Xylander 2009).

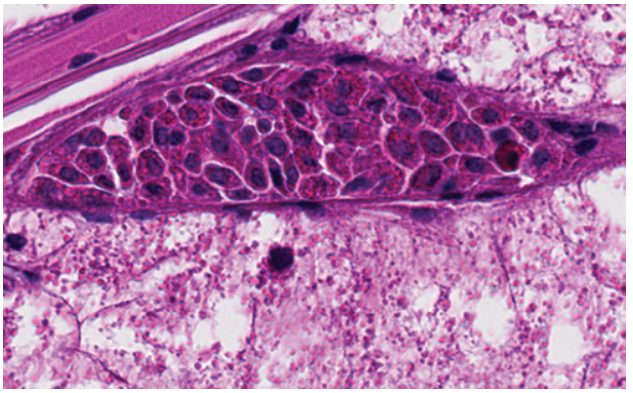


Figure 10.17 Hemolymphatic vessel of a giant African millipede (*Archispirostreptus gigas*) is filled with granular hemocytes. Hemocytes are round with a slightly eccentric ovoid nucleus and a moderate amount of cytoplasm containing eosinophilic granules. 400×. HE.

10.3.9 Respiratory System

Myriapods use a tracheal system for gas exchange, except for pauropods, which lack tracheae likely due to their small size. Spiracles do not have muscles and cannot be closed, therefore water loss to the environment is continuous. In many myriapods, oxygen exchange occurs directly with the tissues, without a transport medium (hemolymph); less commonly, hemolymph contains respiratory pigment and facilitates gas exchange, such as in Scutigeromorpha. Individual spiracles are typically present within each segment as pairs. In chilopods, spiracles are present in the pleural membrane near the coxae anterior and lateral to the walking legs. In diplopods, there are two pairs of spiracles per diplosegment located on the sterna. The spiracle opens into an atrium lined by cuticular hairs (trichomes), which function to reduce water loss and prevent particulate entry. The atria are hollow rigid apodemes that also function as sites of muscle attachment. At the base of the atrium, the tracheae arise. The tracheal system varies by taxon. It may be a series of longitudinal trunks, a network of interconnected tubes, or unconnected tubes. Tracheae terminate into fine blind-ended tracheoles in the tissues.

The exception to the above plan is in scutigeromorph centipedes, which have unpaired spiracles located on the dorsal midline near the posterior margin of the tergites of the leg-bearing segments. They have a tracheal system more similar to arachnids. Spiracles open into an atrium which contains two large tufts of tubular trachea that are in contact with hemolymph of the pericardial sinus. The hemolymph is oxygenated as it passes over the tracheae immediately prior to entering the heart. Glomeris diplopods are unique in that they can close their tracheae to the environment.

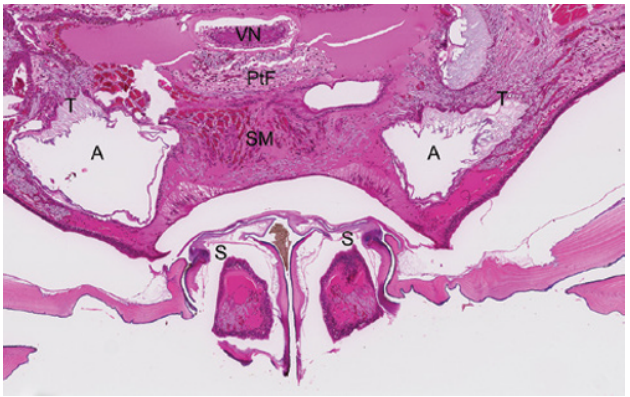


Figure 10.18 Spiracles of a giant African millipede (*Archispirostreptus gigas*). Spiracles (S) are openings in the body wall that lead to dilated, air-filled chambers, called atria (A). Numerous tracheae (T) branch from the atria. PtF, parietal fat body; SM, skeletal muscle; VN, ventral nerve cord. 50×. HE.

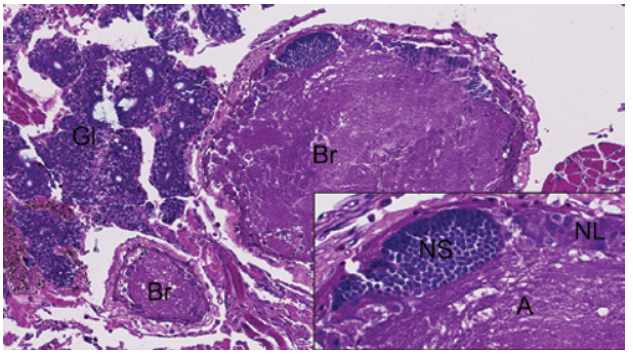


Figure 10.19 Longitudinal section through the cerebral ganglion (brain) of a giant African millipede (*Archispirostreptus gigas*) demonstrating two lobes (Br); the thin isthmus connecting the lobes is not apparent in section. On higher magnification, each lobe has an outer rim of neurons, both large (NL) and small (NS), and an inner mass of axons (A). Gl, glands. 17×; inset 400×. HE.

10.3.9.1 Spiracles

The spiracles are respiratory openings in the cuticle that are surrounded by a sclerotized ring, the peritrema. They have variable shape (round or oval) and the diameter can vary depending on the species and the body segment (approximately 20–300 µm in diameter) (Figure 10.18).

10.3.9.2 Tracheae

Tracheae originate from the atria of the spiracles. They are hollow, air-filled tubes lined by cuticle that is continuous with the body wall. The cuticular lining is shed with the rest of the cuticle during molting. The branching (or lack thereof) of the tracheae is dependent on the species. The tracheae generally range from 6 to 8 µm in diameter. The innermost extensions of the tracheae are called tracheoles. These are blind-ending and ramify into the organs to which they supply oxygen. The tracheoles are associated with cells (i.e., tracheolar cells) that may be multinucleated (Minelli 1990). Tracheoles do not shed during molt.

10.3.10 Nervous System

Unlike other species, the nervous system is not strongly cephalized. The CNS in diplopods and chilopods consists of cerebral ganglia (also called brain), a subesophageal ganglion, and ganglionated ventral nerve cord. The cerebral ganglia have two main lobes with a slightly narrowed isthmus between them. They are composed of a peripheral layer of neurons of variable sizes and a central mass of axons (Figure 10.19). The cerebral ganglia are connected to the subesophageal ganglion by a circumesophageal connective (Miley 1930). The subesophageal ganglion is composed of neuromeres of the mandibles and gnathochilarium only. The ventral nerve cord extends the length of the body

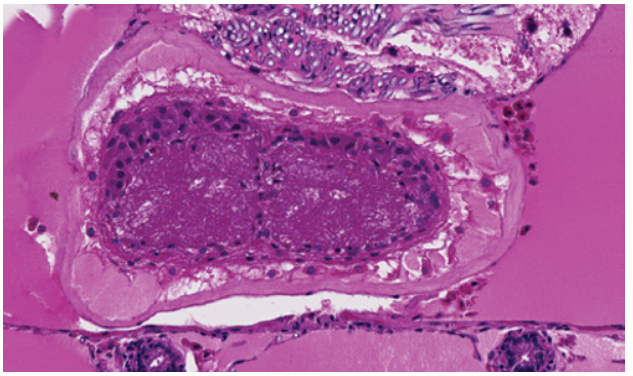


Figure 10.20 The ventral nerve cord of a giant African millipede (*Archispirostreptus gigas*). There is an outer neuronal layer and an inner axonal layer, similar to the brain. 200×. HE.

in the ventral midline of the coelom and has prominent paired ganglia at each segment (Figure 10.20). In dipolopods, the ventral nerve cord has a pair of ganglia for each of the first four trunk segments and two pairs for each diplosegment.

10.3.11 Reproductive System

Myriapods are gonochoric. Sperm transfer is indirect with spermatophores (i.e., packets of sperm). The gonads are tubular and extend the length of the trunk posterior to the third (genital) segment.

In diplopods, the paired fused ovaries lie ventral to the midgut and dorsal to the nerve cord in females. Two oviducts (uteri) extend anteriorly to the genital segment and open into a pouch-like vulva behind the coxa of the second pair of legs. Inside the vulva, there is a short groove that leads to a seminal receptacle. The testicles are in a similar

position to the ovaries but have transverse connections between the sides. Anteriorly the testis tapers into a sperm duct near the genital segment. This opens through a penis on the genital segment. Diplopod eggs are fertilized as they are laid in groups of anywhere from 10 to 300 at a time. Many millipedes build nests. Eggs hatch in several weeks.

Symphylans and paruopods have a similar gonadal arrangement to diplopods. In female symphylans, the ovary extends from the fourth to the 12th segment. The testicles extend from the fourth segment for nearly the entire length of the animal. The male genital opening is covered by curved plates which serve as a feature of sexual dimorphism (Minelli 2011). The copulatory organs are the gonopods on the seventh trunk ring. Males deposit stalked sperm drops in the absence of females. Females bite off these sperm drops and store them in gnathal pockets. At the time of oviposition, females remove eggs from the genital chamber using mouthparts and smear them with stored sperm just after laying to achieve fertilization. Females tend the nests until larvae emerge (Minelli 2011). Parthenogenesis is common in symphylans (Ruppert et al. 2004).

In pauropods, gonopore openings are present between the second pair of legs. There is a single opening in females and paired openings in males. Females have a single ovary that extends from the fifth to the 10th segment depending on follicular development. Typically, 4–6 eggs develop simultaneously with numerous undeveloped follicles evident. Testicles consist of four paired sacs in the fourth to sixth segments. Four vasa deferentia leave the testicle and continue toward the sixth segment where they fuse to form paired ejaculatory ducts that extend to the genital papillae. Eggs are spherical and laid singly or in aggregates. Sperm are flagellate. Sperm transfer is indirect, with sperm deposited on webs between soil particles. Females take up the sperm drops and fertilize the eggs after which they are laid between soil particles (Minelli 2011).

In chilopods, few species are known to have parthenogenesis. Sexually dimorphic features include increased numbers of trunk and leg segments in females, and gonopods (appendages used for oviposition) in some species. Except in scutigeromorphs, males make a web into which spermatophores are deposited, which is a few millimeters in diameter. The female uses gonopods to pick up the spermatophore and insert it into her gonopore. After fertilization, the female burrows a small cavity in soil or decayed wood, deposits her eggs, then wraps herself around the egg mass until hatching and dispersal of young (Ruppert et al. 2004).

10.3.11.1 Male Reproductive Tract

In diplopods, testes form two long tubes, which often have multiple connections between the tubes.

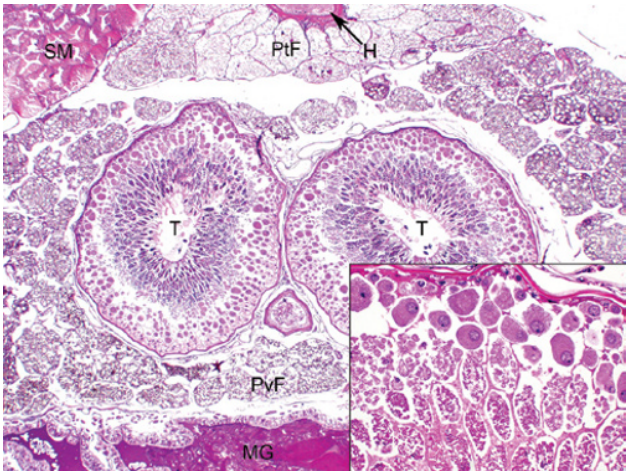


Figure 10.21 Testes of a giant desert centipede (*Scolopendra heros*). The testes (T) are paired and located dorsal to the midgut (MG) and ventral to the heart (H). PtF, parietal fat body; PvF, perivisceral fat body; SM, skeletal muscle.100×; inset 400×. HE.

In chilopods, there is variation in testis morphology; males have one to many testes that are dorsal to the midgut (Figure 10.21). In all centipedes, the testes empty into vasa efferentia, which fuse to form vasa deferentia, which later divide prior to emptying in the ejaculatory duct. All centipedes have some form of glandular tissue that contributes secretory material to the sperm by emptying into the vas deferens. These glands are accessory sex glands, seminal vesicles, or a combination of the two. Geophilomorpha have paired, dorsal, fusiform testes that terminate in vasa efferentia that fuse to form the vas deferens which is lined by simple epithelium on a thick basement membrane. In Scolopendromorpha, the testes are numerous (4–26 in number) and each is continuous with a vas efferens and vas deferens, which becomes a highly coiled epididymis. Lithobiomorpha have long, coiled tubular testes with narrow vas efferens and short vas deferens. Scutigeromorpha have paired testes, but one is a macrotestis that produces large sperm cells and the other is a microtestis that produces small sperm cells. The testes are continuous with coiled vasa efferentia that fuse to form an even more coiled vas deferens. Mature sperm are the longest of any animal and up to 2 mm in length (Minelli 1990).

10.3.11.2 Female Reproductive Tract

In contrast to male gonads, the female gonads are fairly uniform across species. Chilopods typically have a single ovary dorsal to the gut, the caudal pole of which ends in the oviduct (Figure 10.22), which splits caudally to allow passage of the gut and unites again prior to terminating in the

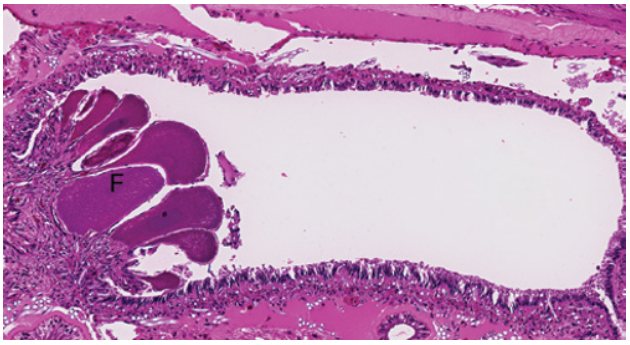


Figure 10.22 Ovary and oviduct of a giant African millipede (*Archispirostreptus gigas*). The ovarian follicles (F) bulge into the lumen, which is surrounded by a simple layer of columnar cells. 120×. HE.

genital atrium. The ovary is composed of an outer epithelium covered by connective tissue (Figure 10.23). Ovarian follicles are adhered to the ventral wall of the ovary and surrounded by a layer of epithelial cells and a follicular membrane. During vitellogenesis, yolk begins to accumulate in the oocyte. Nearly mature oocytes have a thick 10–15 µm chorion replacing the primary membrane. All chilopods have a pair of seminal receptacles, and either one or two pairs of accessory glands. The seminal receptacle is a coiled, cuticle-lined duct that invaginates from the epidermis (Minelli 1990).

In diplopods, ovaries are typically paired and lie in the dorsal coelom, lateral to the midline. In symphylans, ovaries are sac-shaped and contain synchronously developing oocytes characterized by a spherical to oval nucleus, homogenous eosinophilic cytoplasm and outer coating of granular basophilic material. Vitellogenic follicles demonstrate increased nuclear size, with yolk spheres in the peripheral cytoplasm due to micropinocytosis. Microvilli form on the oocyte surface and ultimately yolk spheres fuse, forming larger aggregates. Eggs when developed are spherical with an outer shell (chorion).

10.3.12 Special Senses

10.3.12.1 Eyes

Many species lack eyes but are negatively phototaxic due to integumental photoreceptors that respond to light. In diplopods, eyes may be lacking or there may be 2–90 lateral eyes (ocelli) derived from ommatidia loosely clustered beside the base of each antenna. Eyes may be a single ommantidium or the result of a few ommatidia fusing. In chilopods, some animals have simple eyes and a few species (of the order Scutigeromorpha) have "pseudofaceted eyes," which are similar to insects who have true compound (faceted) eyes. Pseudofaceted eyes have a corneal lens (Figure 10.24), which is a region of cuticle that is modified to a lenticular shape, subtended by a single ommatidium. The ommatidium is a cone-shaped structure composed of photoreceptor cells, supporting cells, and pigment cells (Figure 10.25). The superficial portion (below the corneal lens) is the "vitreous body," which is a hyaline region representing extensions of the peripheral pigment cells. Deep to the vitreous body is the bilayered rhabdom that acts as the photoreceptor apparatus. The rhabdom is

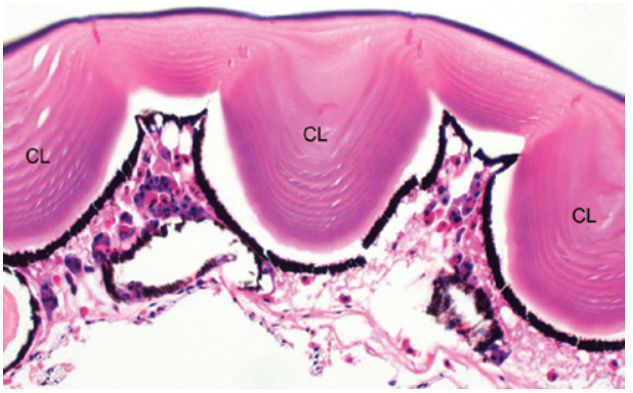


Figure 10.24 Corneal lenses of a giant African millipede (*Archispirostreptus gigas*). The corneal lens (CL) is a modified cuticle organized into multiple layers. 200×. HE.

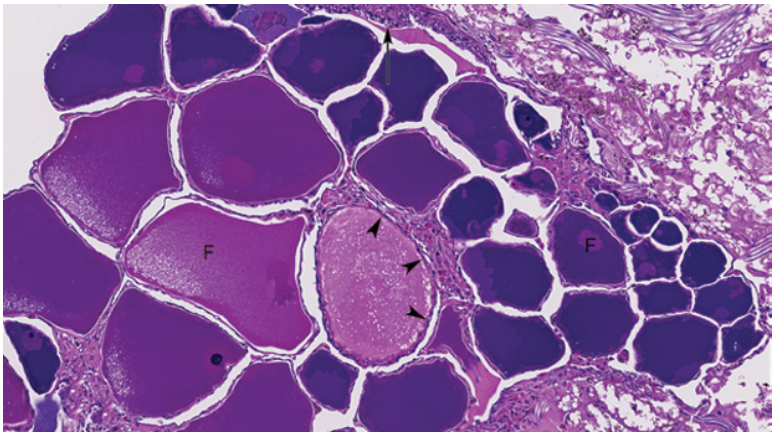


Figure 10.23 Ovary of a giant African millipede (*Archispirostreptus gigas*). Ovarian follicles (F) are in various stages of folliculogenesis. Follicles are surrounded by a thin membrane (*arrowhead*). The entire ovary is surrounded by a thin layer of epithelium (*arrow*) and connective tissue. 170×. HE.

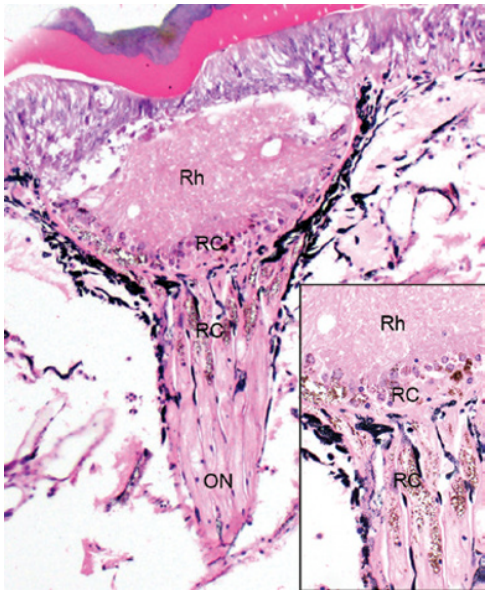


Figure 10.25 Eye of a giant desert centipede (*Scolopendra heros*). The corneal lens is out of the plane of section. The cuticle is subtended by cell processes of the rhabdom (Rh), which is subtended by two layers of pigmented retinular cells (RC). Retinular cells are continuous with the optic nerve (ON).

surrounded by supporting cells. Symphylans and pauropods lack eyes (Ruppert et al. 2004).

10.3.12.2 Tömösváry Organ

This is a sensory structure in anamorph centipedes. It may sense humidity and odors (pseudoculus in pauropoda). It is located on the ventral aspect of the cephalic shield. This organ is composed of a cluster of glandular and sensory cells that traverse the epidermis. Sensory cell processes extend through the cuticle and are covered only by the thin epicuticle.

10.3.12.3 Antennae

Centipedes and millipedes have highly sensitive antennae covered with numerous tactile and chemosensory setae (Brusca and Brusca 2003). Mechano- and chemoreceptors present have a similar histologic structure to those reported in insects. In millipedes each antenna has eight sections (articles). The apical article is small and contains four cone-shaped sensilla (Hopkin and Read 1992).

10.3.12.4 Coxal Organs

Most centipedes except Scutigeromorpha have coxal organs (Kenning et al. 2019). The function of these organs is unknown, but may be related to excretion or defense. Grossly, the typical appearance of these glands is swollen hind leg coxae with numerous pores that correspond to subcuticular coxal glands. Coxal organs of chilopods are modifications of the epidermis (unlike the "coxal glands" of arachnids and diplopods). Coxal organs are composed mainly of tall columnar epithelial cells with basilar nuclei admixed with glandular cells and thin junctional cells. The coxal organs are subtended by a subepithelial sinus (Minelli 1990).

Disclaimer

The views expressed in this chapter are those of the authors and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or US Government.

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11

Arthropoda

Decapoda

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11.1 Overview

Decopod crustaceans (Decopoda) are classified as an order of crustaceans in the phylum Arthropoda, class Malacostraca. There are more than 10 000 species in the order that includes two important suborders: Dendrobranchiata (prawns) (which do not incubate their eggs on the ventral abdomen) and Pleocyetmata that includes the infraorders Stenopodidea and Caridea (shrimps), Astacidea (lobsters, crayfish, and burrowing shrimp), Thalassinidea (mud shrimps), Palinura (spiny lobsters), and Anomura (crabs) (Barnes and Harrison 1992). While most decapods are marine, several species are also found in fresh water (crayfish and some shrimp) and some species of crabs can primarily live on land. Decapod crustaceans are a source of food for humans and other animals. Some species, such as shrimp and prawns, can be effectively cultured. Other species, such as American and European lobsters, are carnivorous and cannot be economically cultured. Several species of decapods are captured and/or bred for the aquarium trade.

The external surface of a decapod crustacean consists of a nonliving cuticle or shell, which is shed periodically to allow for growth of the internal tissues. Decapoda all have five pairs of legs (pereiopods) although modification of the first pair is commonly noted. Because decapod crustaceans are similar grossly, this work will use the lobster (*Homarus americanus*, the American lobster) as the model in most discussions (Table 11.1), then describe important anatomic differences that occur in other decapods.

11.2 Gross Anatomy of Adults

11.2.1 External Gross Anatomy

Anatomy of the adult form has been described (Bell and Lightner 1988; Felgenhauer 1992a; Johnson 1980; Phillips et al. 1980). The body is covered by a nonliving cuticle also termed a carapace (Figure 11.1). The cuticle is formed from two types of tissues. First is the hard carapace that makes up the majority of the cuticle. The second is the soft rubber-like carapace, termed arthrodial membrane, which connects the hard carapace pieces and, together with muscle, etc., provides for flexing of the hard carapace, thus forming joints. Externally the lobster's body is elongated and divided into a cephalothorax and an abdomen. The hard carapace that makes up most of the cephalothorax is further divided into head and thoracic regions by various indentations on the carapace. The dorsum of the head forms a hard arrow-like protuberance termed a rostrum that covers the eyes and other parts of the head. Crabs and some other decapods lack a rostrum. Both articulated large and small spines and nonmovable spines, as well as other protective and sensory projections of the carapace, are noted on various species of decapods. Embryologically, appendages of decopod crustaceans originate as a protopodite that attaches to the body. Projecting from the protopodite are both a endopodite and an exopodite. If the appendage contains both of these branches it is termed biramus. If either the endopodite or exopodite regresses during development, the appendage is termed uniramus. The protopodite itself is further divided into two segments, the lateral coxopodite (coxa) and the medial basiopodite

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Table 11.1 Organs for histologic evaluation in decapods.

Organ system		Organs
Body wall/musculoskeletal		Carapace, arthrodial membrane, ecdysis, molt stages, tegmental gland, setae
Digestive	Alimentary canal	Gastric and pyloric stomach, ceca, midgut, hindgut, gastric mill, pyloric filter press
	Digestive organs	Hepatopancreas
Excretory		Kidney (green gland)
Circulatory		Heart, artery, sinusoids
Immune		Hemocytes
Respiratory		Gills
Nervous		Ventral nerve cord, ganglia of the eye and anterior body
Reproductive	Male	Testes, seminiferous tubules, spermatophore
	Female	Ovaries, seminal receptacle
Special senses/organs		Eyes

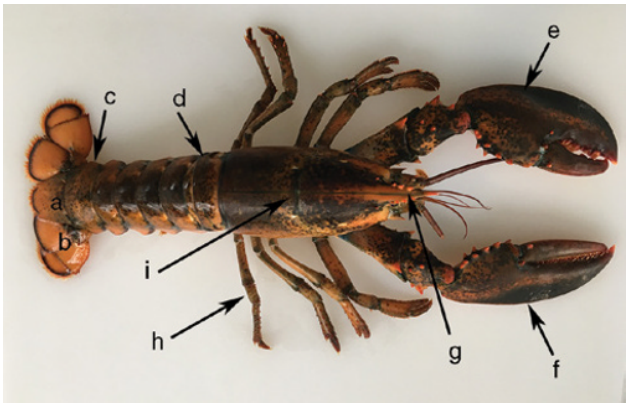


Figure 11.1 Dorsal view of *H. americanus*. (a) Telson; (b) uropods; (c) somite no. 7; (d) somite no. 1; (e) left pereiopod no. 1 (crushing claw); (f) right pereiopod no. 1 (cutting claw); (g) rostrum; (h) pereiopod no. 5; (i) cephalothorax.

(basis). As the decapod crustacean larva develops, each of these portions of the appendage further differentiates into one or more specific structures (i.e., antennae, gill, gill bailer, etc.) (Pearse et al. 1987).

Decapod crustaceans have two pairs of antennae (Figure 11.2). Each pair is composed of both a small and large antenna. The smaller of the two is located just below the eyes and is characterized by two flagella of about equal length extending from the protopod. The larger antennae are lateral to the smaller and the protopod of each contains the prominent nephridiopore.

The bilateral mandibular appendage forms the ventrum of the mouth and has surfaces that both grind and cut food. A palp extends anteriorly from the mandibles in American lobsters (Figure 11.2). The bilateral first and second maxilla form the ventral parts of the mouth. The scaphognathite



Figure 11.2 Ventral view of the mouth parts of *H. americanus*. Maxillipeds were removed from the left side of the mouth. (a) Right third maxilliped; (b) right second maxilliped; (c) right first maxilliped; (d) left second maxilla; (e) gill bailer; (f) left first maxilla; (g) left mandible and palp; (h) left nephridiopore; (i) right second antenna; (j) right first antenna.

(gill bailer) originates from the endopod of the second maxillae and is used to ventilate the gills.

The thorax is formed by the fusion of eight embryological segments (somites). The first three segments are termed maxillipeds (Figure 11.2) and are biramous. From the coxa,

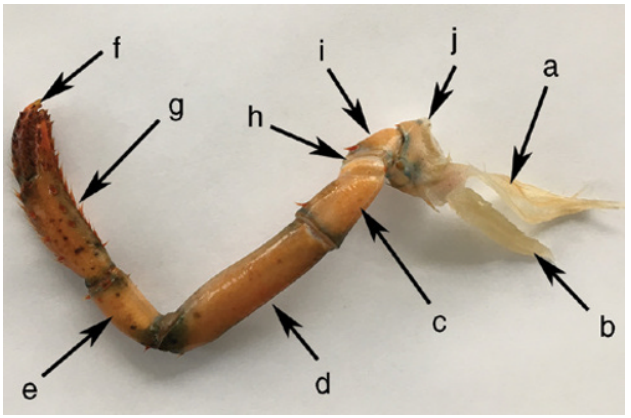


Figure 11.3 Second right pereiopod of *H. americanus*. (a) Epipodite; (b) gill; (c) ischiopodite; (d) meropodite; (e) carpopodite; (f) dactylopodite; (g) propopodite; (h) plane of autotomy; (i) basiopodite; (j) coxopodite.

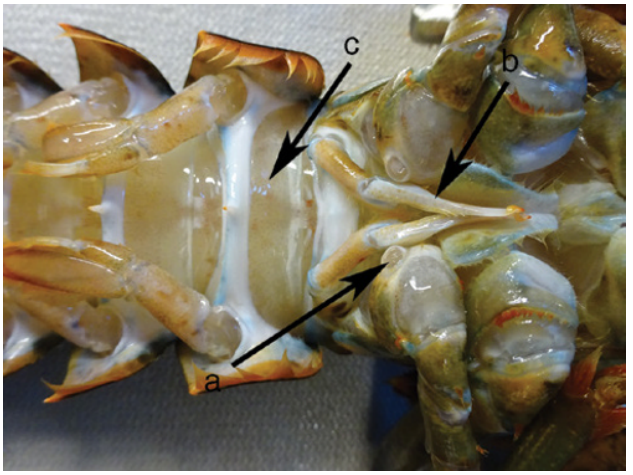


Figure 11.4 Ventral surface of a male *H. americanus*. (a) Gonadopore; (b) modified pleopod; (c) arthrodial membrane.

in maxillipeds two and three, extends a gill and an epipodite. Laterally from the basis of maxilliped three extends a prominent leg-like segmented structure that is used in breaking down food and in grooming (Figure 11.2) (Felgenhauer 1992a; Phillips et al. 1980; Ruppert et al. 2004).

Five pairs of legs, termed pereiopods, extend ventrally from the posterior five cephalothorastic segments (somites). They are considered uniramous since they do not have an exopod originating on the protopodite. Each leg can be divided (proximally to distally) into the following segments: coxa (coxopodite), basis (basipodite), ischium (ischiopodite), merus (merepodite), carpus (carpopodite), propodus (propopodite), and dactylus (dactylopodite) (Figure 11.3). In some swimming crabs (such as the blue crab, *Callinectes sapidus*), the propodus and dactylus are flat and broad and provide oar-like structures.

A cheliped (claw) is formed by a rigid extension (finger) of the propodus that interacts with the dactylus (movable finger). In many lobsters and crabs, the first three pair of legs are modified and form chelipeds. In the American lobster, the first pair of chelipeds are greatly enlarged and are differentiated into a crusher and a cutter claw. Some species show a subchelate claw in which the dactyl folds against the propodus to form a claw-like structure.

In males, bilaterally, at the base of the fifth leg on the medial side of the coxa are the gonadopores (Figure 11.4). The female gonadopores are located on the medial side of the coxa of the third walking legs (Figure 11.5). A seminal receptacle, composed of two lateral chitinized walls with a deep anterior to posterior linear pocket that extends dorsally into the body of the animal, is located on the midline between the fourth and fifth pereiopod in female lobsters and other similar decapods (Figures 11.5 and 11.6). It is used for the storage of spermatophores. In some species, such as

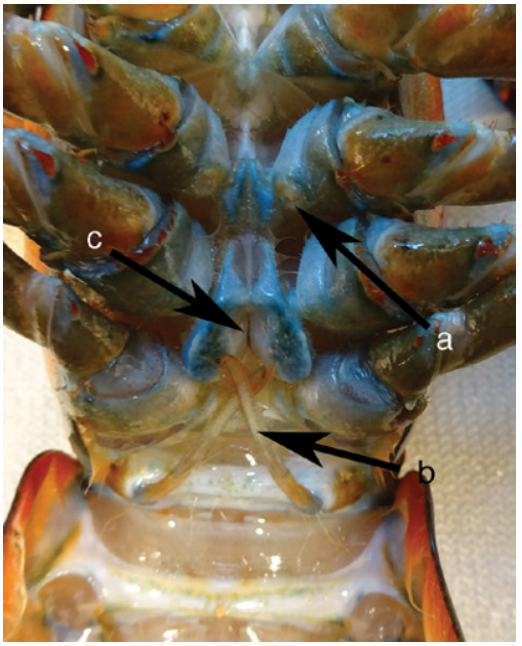


Figure 11.5 Ventral surface of a female *H. americanus*. (a) Gonadopore; (b) modified pleopods; (c) seminal receptacle.

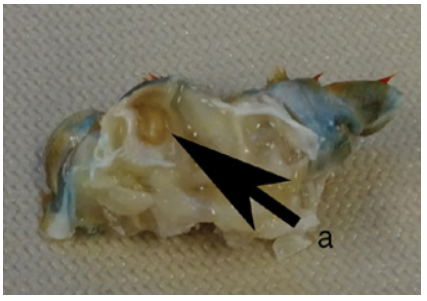


Figure 11.6 Sagittal cut through the seminal receptacle. (a) Receptacle pouch extending internally from the receptacle orifice.

the ghost crab (*Inachus phalangium*), spermatophores are placed into a seminal receptacle located within the end of the gonadopore. Seminal receptacles are storage chambers that retain sperm till spawning occurs (Krol et al. 1992).

Dorsally and medially, the carapace covers internal organs. The dorsal surface of the carapace shows indentations which demarcate underlying organ locations. These indentations are especially prominent on crabs. Laterally, the thoracic carapace extends from the body wall to cover the gills. This extension is termed the branchiostegite, and forms the outer wall of the large bilateral branchial chambers. The inner wall of the chamber (the lateral wall of the cephalothorax) is formed by a portion of the carapace termed the epimeron. Branchia (gills) extend from the base of each of the bilateral thoracic appendages and adjacent ventrolateral carapace (arthrodial membrane of the epimeron) and extend dorsally within the branchial chamber. In some crabs, the branchiostegite is expanded laterally and the thinly chitinized membrane lining the inner surface of the expanded branchiostegite forms respiratory lobules that project into the seawater-bathed space around the gills, thus forming "lungs" lateral to the gills.

Epipodites originate from each coxa adjacent to the branchia of the second and third maxilliped and all five thorasic segments. Epipodites in each segment partially separate and maintain the branchia (gills) (Figure 11.7). The branchia (1–4 gills per segment/leg) are named according to their origin as a podobranch which originates from the coxa of each segment, arthrobranchia which originate from the arthrodial membrane between the coxa and the body wall (up to two may be present), and a pleurobranch which originates from the body wall (epimeron). Rarely are all four possible gills present in each segment.

Three different basic types of gills are noted in decopod crustaceans (Fox 2001; Ruppert et al. 2004; Taylor and Taylor 1992). Trichobranchiate gills are noted in lobsters, crayfish, and several crab species (Figure 11.8a,b). In these species, the gills taper to a point as they reach the dorsum of the branchial chamber. Phyllobranchiates, which include many types of crabs and shrimp, have gills composed of leaf-like lamellae that extend from the central core (axis) of the gill (Figure 11.8c,d). Dendrobranchiate gills found in penaeid shrimp are composed of a central gill axis from which bilateral secondary lamellae and tertiary filaments extend (Figure 11.8e,f). The secondary lamellae tend to curve toward each other distally from the gill axis.

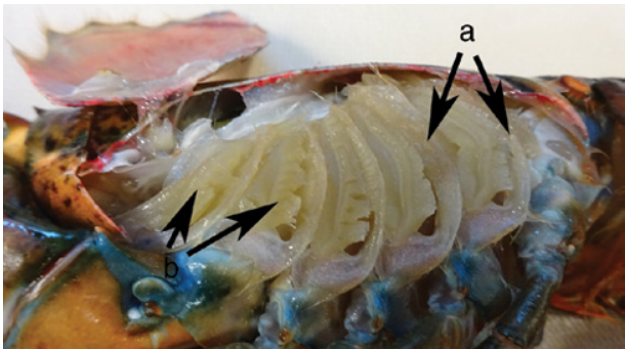


Figure 11.7 Lateral view of trichobranchiate gills of a *H. americanus* with branchiostegite removed. (a) Epipodites; (b) branchia.

Oxygen-carrying water enters the branchial chamber ventrally at the base of the cheplid (modified first legs) and between the remaining legs, and flows over the gills and out via the branchial chamber through exhalant pathways. Exhalant spaces are located dorsally to an area lateral to the mouth. This arrangement can produce one-way water flow that may increase oxygenation. The scaphognathite of the secondary maxilla helps to create a current that pumps the water in and out of the branchial chamber.

The abdomen is divided into six segments (somites). Dorsally, in lobsters, each segment is covered by a hard carapace. The segments are connected to each other by arthrodial membranes that allow for overlapping of the segments and flexion of the abdomen. Ventrally, the abdominal carapace is composed of bands of hard carapace separated by wide bands of the arthrodial membrane (Figures 11.4 and 11.5). Bilaterally and ventrally are the pleopods (swimmerets). The second to fifth pair are biramous with flat rami (endopod and exopod) edged with a setal fringe (setae are described later). The pleopods help with swimming in the water column. In the Pleocyetmata, fertilized eggs are attached to the pleopods until they hatch. The first pair of pleopods are modified for reproduction. In male lobsters and most other decapods, the first pair of pleopods are highly modified to form gonopods (Figure 11.4), which are chitinized thin structures with a central depressed canal used for transferring spermatophores from the male gonadopore to the female. The canal size and shape vary by species from being a central depression in the lobster to an almost totally enclosed canal in some decapods. The first pleopods in females are smaller than pleopods 2–5, and similar in shape to the male gonopods, but are not highly chitinized as in the male (Figure 11.5).

The tail fan is composed of a central telson and bilateral uropods. The anus empties ventrally on the telson. The bilateral uropods (highly modified pleopods) extend from the base of the telson. Each uropod is composed of a protopod that attaches to the telson base and two large flat leaflets (highly modified endopod and exopod) (see Figure 11.1).

Unlike most other decapods, the cephalothorax of crabs is dorsoventrally flattened and laterally widened (Johnson 1980). In crabs, the abdomen is greatly reduced

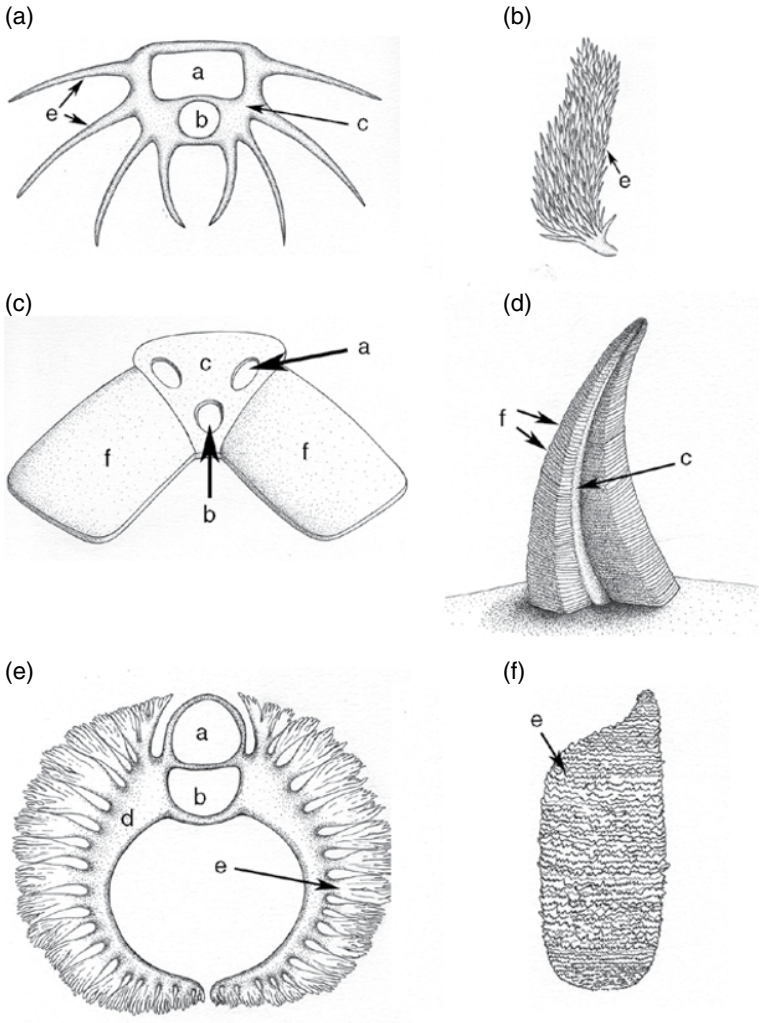


Figure 11.8 Line drawings of three types of decopod crustacean gills. *Source:* After Calman from Ruppert et al. (2004). (a) and (b) Filamentous or trichobranchiate gill of lobsters and crayfish; (a) is a transverse section, and (b) is a lateral view of the entire gill. (c) and (d) Lamellar or phyllobranchiate gill of brachyuran crabs and shrimps; (c) is a transverse section, and (d) is a lateral view of the entire gill. (e) and (f) Dendrobranchiate gill of panaeid shrimp; (e) is a transverse section, and (f) is a lateral view of the entire gill. a, afferent channel; b, efferent channel; c, primary (central) axis; d, secondary lamellae; e, filaments; f, lamellae. *Source:* Original drawing by Alicia Schickle.

in size and is retroflexed back against the ventrum of the cephalothorax. The first and second abdominal segment are modified for reproduction. The remaining abdominal segments together form a broad flap (in females) or wedge-shaped abdominal flap ending in a pointed telson (in males) (Figure 11.9). A thin cuticle covers the retroflexed ventral surface of the abdomen. Through this cuticular surface, muscle can be noted running parallel and on either side of the intestine, which empties at the anus on the telson. Pleopods are not present on segments 3–6 of the male, but are present in the female and are used for egg attachment.



Figure 11.9 Ventral surface of *Hemigrapsus sanguineus* (Japanese shore crab). The reflexed abdomen of the male (a) is pyramidal in shape while that of the female (b) is broad. The chelipeds of the male *H. sanguineus* are enlarged compared to those of the female.

11.2.2 Internal Gross Anatomy

The body wall (the thin membrane lining the carapace) can be noted upon dissection and can be easily removed from the inner surface of the carapace. Chromatophores are often noted in the membranes underlying cuticles (Figure 11.10). Internally within the cephalothorax, the heart is noted dorsally and posteriorly. Underlying it are the gonadal tissues (Figures 11.11 and 11.12).

The ovaries of female decapods are bilateral and may be composed of both anterior and posterior lobes (Figure 11.12). In the lobster, the green anterior and posterior lobes

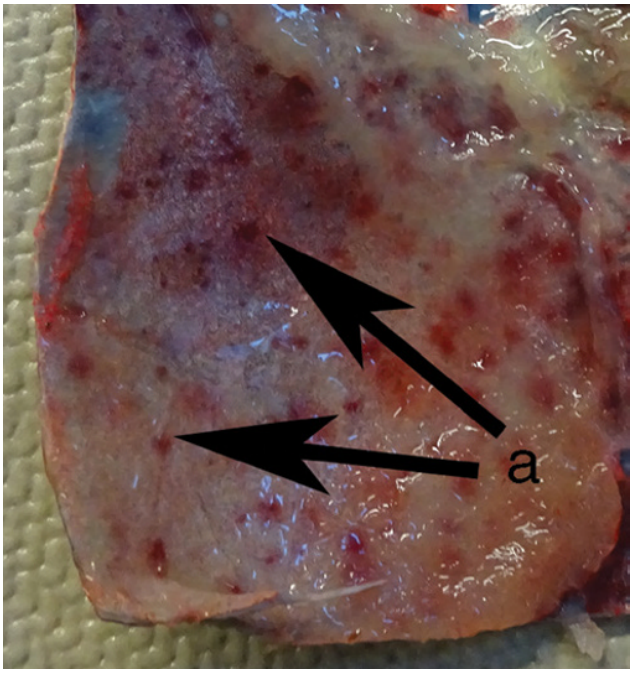


Figure 11.10 Ventral surface of hard carapace showing chromatophores (*arrows*) located in sinusoidal connect tissues.

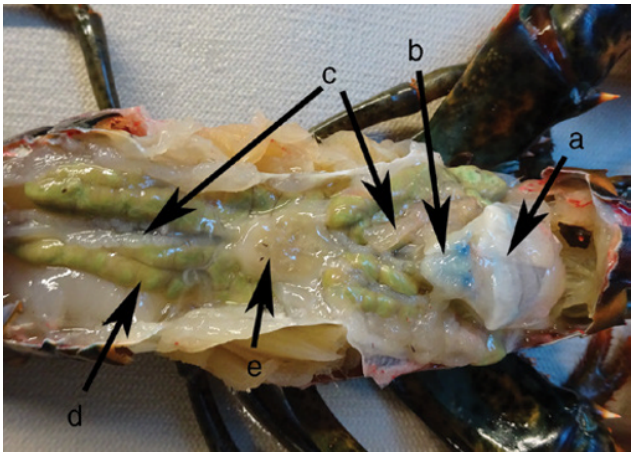


Figure 11.11 Dissection of male *H. americanus*. (a) Cardiac stomach; (b) pyloric stomach; (c) male gonad; (d) hepatopancreas; (e) heart.

are connected by a bridge underlying the heart forming an H-shaped organ. When the ovary is filled with ripe eggs, the lobes of the gonad extend into the dorsal abdomen. Oviducts move eggs ventrally to the gonadopores. In males (Figure 11.11) the white, knobby string-like lobes of the testes, as in the female, extend anteriorly and posteriorly and connect to form an "H" ventral to the heart. Convoluted, long, white, bilateral vasa deferentia move sperm to the gonadopores. The number of lobes of ovary and testes vary

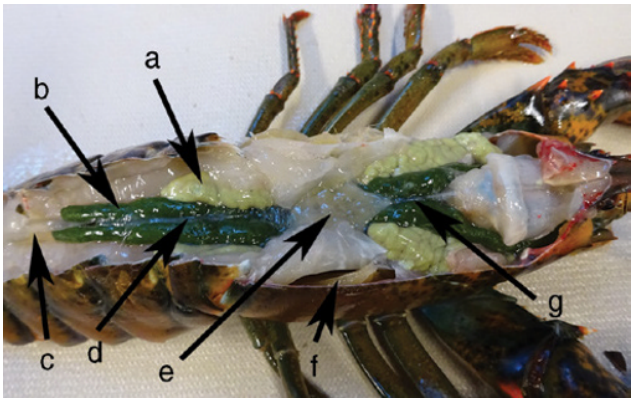


Figure 11.12 Dissection of a female *H. americanus*. (a) Hepatopancreas; (b) ovary; (c) midgut; (d) posterior aorta; (e) heart; (f) branchial chamber; (g) anterior aorta.

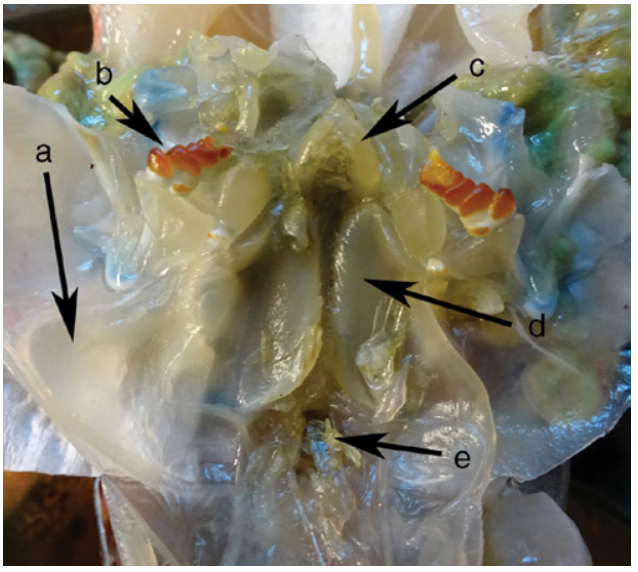


Figure 11.13 Dorsally opened stomach of *H. americanus*. (a) Uncalcified gastrolith; (b) gastric mill; (c) pyloric (ampulatory) filter press covering the underlying pyloric (ampulatory) filter; (d) cardiac ridges with setae along the edges; (e) ventral esophageal opening into the cardiac stomach.

between types of decopod crustacean. Shrimp, crabs, and some lobsters have multiple lobes.

Ventrally, the esophagus opens into the cardiac stomach. The cardiac stomach is lined externally by a membrane (similar to a serosa) and internally by a thin layer of chitin. Laterally in the gastric stomach wall, oval white gastroliths, which can store calcium before molting, can be identified (soft if postmolt and mineralized if premolt) (Figure 11.13). At the dorsolateral side of the junction between the cardiac stomach and the pyloric stomach are the two parts of the gastric mill (described later) (Figures 11.13 and 11.14). Ventrally within the pyloric stomach is the winged portion

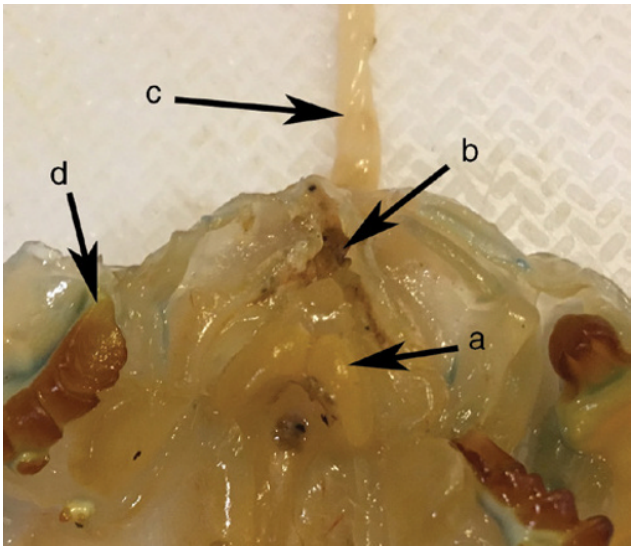


Figure 11.14 Dissection of a female *H. americanus*. Dorsally opened stomach. (a) Filter press; (b) dorsal chamber of the pyloric stomach with pyloric fingerlets (large setae) in the compaction zone in the pyloric stomach; (c) midgut; (d) gastric mill.

(pyloric filter press) of the ampullary filter complex, which conducts fine particles of food to the hepatopancreas. Ventral and lateral to the gonadal tissues is the large lobulated light green hepatopancreas. The dorsal chamber of the pyloric stomach leads to the midgut which continues dorsally through the abdomen to the anus on the ventral surface of the telson. In the head portion of the cephalothorax, just underlying the antennae and applied to the carapace membrane is the flattened, discoid dark green (in the American lobster) antennae gland (kidney). Ventromedial to the green gland and antennae is the supraesophageal ganglion and the circumesophageal connectives that lead around the esophagus to the subesophageal ganglion. The neural cord extends from the subesophageal ganglion and continues along the ventral body wall parallel and adjacent to the ventral thoracic and abdominal arteries (Figure 11.15).

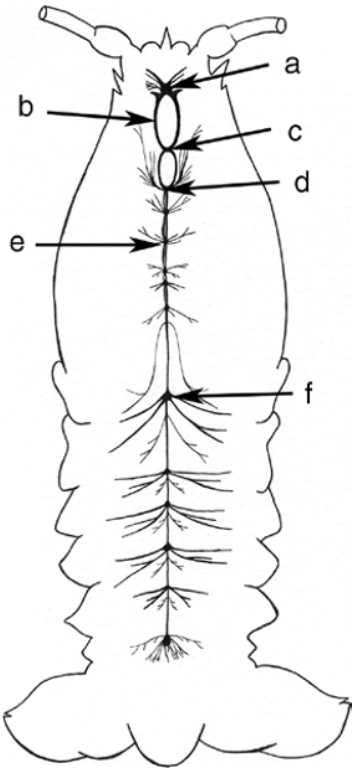


Figure 11.15 Line drawing of the dissected neural cord of *H. americanus*. *Source:* After Bullock and Horridge from Phillips et al. (1980). (a) Cerebral ganglia; (b) circumesophageal connectives; (c) postesophageal commissure; (d) subesophageal ganglion; (e) 2nd thoracic ganglion; (f) 1st abdominal ganglion. *Source:* Original drawing by Alicia Schickle.

11.3 Histology

11.3.1 Cuticle

The cuticle (carapace, shell) forms the outer surface of the animal as discussed above. The carapace is a complicated structure that undergoes molting (ecdysis) periodically in order for the internal tissues to grow. It acts both as an external skeleton (hard carapace) to which muscles and other connective tissues attach on the inner surface and a softer "rubber" like skeleton (arthrodial membrane) that provides for joint function (Figure 11.16). Both types of

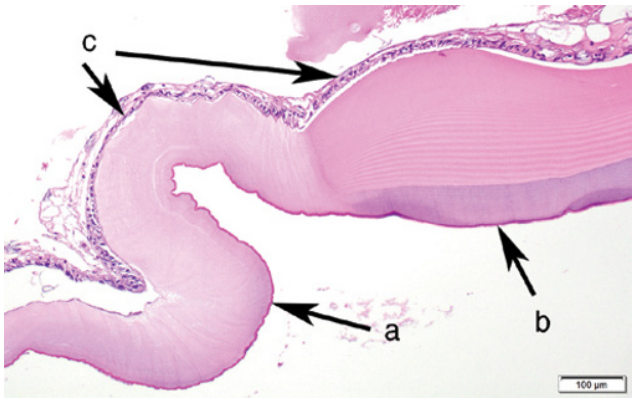


Figure 11.16 Junction of the hard and soft carapace of *H. americanus*. (a) Arthrodial (soft) carapace; (b) hard carapace; (c) carapace epithelium. Decalcified HE.

cuticle are formed by a simple columnar epithelium. The epithelium is stimulated to produce layers of carapace by endocrine hormones. Underlying the carapace epithelium is the "spongy" hypodermis composed of large vacuolated cells (the glycogen contents are removed during histologic processing). Also found in the hypodermis are sinusoids,

arterioles, nerves, and other connective tissues (fibrocytes, muscle cells, etc.) (Johnson 1980; Roer and Dillaman 1993).

Cuticle formation has been studied extensively in *H. americanus*. The production of the hard carapace occurs in layers (see below) and production of each layer is associated with parts of the molt cycle. The entire cuticle is produced by the underlying epithelial cells, which can be cuboidal to tall columnar, depending on the stage of molt. The epithelial cells are affected by endocrine hormones that instruct the cells as to the type of cuticular layer to produce. The layers of the cuticle are composed of tightly adhered cuticular cylinders produced by the epithelial cell. The cuticle is composed of chitin crystals with associated proteins (Aiken 1980; Neville 1975). The crystals orient in a helical fashion as the chitin is produced by each epithelial cell, resulting in a histologic appearance of successive lamallae of chitin in each layer of the cuticle. Tiny extensions of the epithelial cells (pore canals) originate from the epithelial cell edges and form cellular canals that traverse the cuticle above each cell in newly molted animals (Halcrow 1993). It is through these canals that mineralization of the chitin/protein deposits occurs post molt. The pore canals collapse as the chitin is mineralized.

A fully formed carapace is designated as stage C_4 and includes the occurrence of the membranous layer. It is also termed the "resting stage," the intermolt stage, or anecdysis (Figure 11.17). This stage can be very short in young larvae and juveniles (just a few weeks to months) and years long in large, older decapods. It is during this stage that the animal goes about its daily functions and grows/expands its internal tissues. The first part of stage D, termed D_0 (passive premolt), is often included with stage C_4 (usually written as C_4/D_0). During D_0, autotomized limbs begin to regenerate and gastrolith mineralization begins. As the internal tissues grow and become constrained by the carapace, molting hormones are produced. Molting starts with stage D_1, active premolt. During stage D_1, a new epicuticlar layer is produced by the epithelial cells. The new epicuticle is deposited between the membranous layer and the now hypertrophic (tall columnar) cuticular epithelium. During D_2, a new exocuticle (not yet mineralized) is formed by the epithelium on the inner surface of the new epicuticle. In stage D_3, absorption of mineral from the old cuticle's inner surface and breakdown of the membranous layer occur, creating a space throughout the body between the old carapace and the newly formed epicuticle (Skinner 1985).

The act of molting (ecdysis) occurs in two phases. In the first (passive) phase, the lobster absorbs water and becomes diffusely edematous. In the second phase, the arthrodial membrane between the dorsal portion of the cephalothorax and the dorsal portion of the first somite of the abdomen ruptures. The lobster then begins to withdraw from the old carapace through this hole, first by withdrawing the cephalothorax, then the abdomen. The carapace of the newly molted animal consists of the newly formed epicuticle and the exocuticle. Immediately post ecdysis (4–8 hours), molting stage A_1, the animal continues to absorb water into its tissues. Because the animal's tissues are edematous, the soft carapace stretches and takes on a larger shape. These layers have not yet been mineralized so the animal is "soft" and easy prey for other carnivores. Newly molted crustaceans usually hide in a burrow of some type to avoid predation. In stage A_2, the animal begins to mineralize the exocuticle. As the animal mineralizes the new exocuticle, this larger "house" is maintained and the animal's tissues begin to eliminate the widespread edema. Once the exocuticle is mineralized, the animal will begin foraging again.

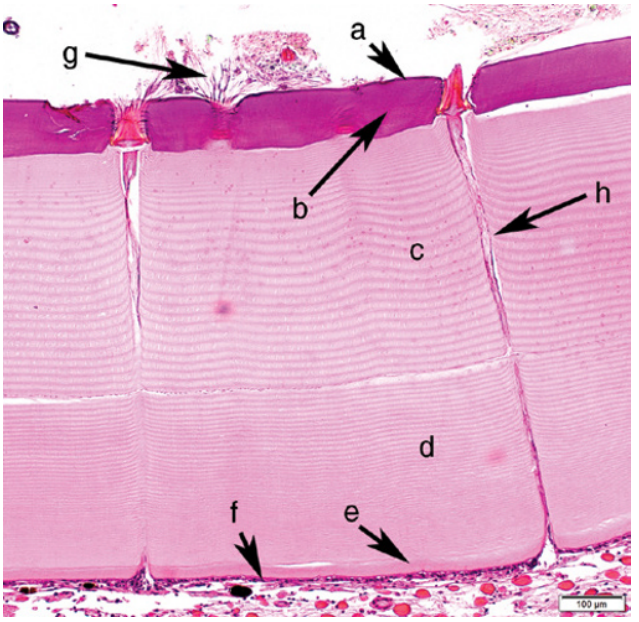


Figure 11.17 Histology of the carapace of a lobster in C_4/D_0 phase of development. (a) Epicuticle; (b) exocuticle; (c) calcified endocuticle; (d) uncalcified endocuticle; (e) membranous layer; (f) cuticular epithelium; (g) plumose setae (debris is associated with the plumes and plumes are missing from two of the setae); (h) setal canal containing a setal nerve. Decalcified HE.

In stage B, secretion of the new endocuticle begins and the outer portions of the endocuticle are mineralized (calcified endocuticle). In stages C_1 to C_3, further production of the endocuticle occurs and calcification of the outer layers of the endocuticle continues. The inner layers of the endocuticle (uncalcified endocuticle) are never strongly mineralized.

The stages leading up to ecdysis (D_1 to D_3), ecdysis, and stages immediately post ecdysis (A_1 to B) occur relatively quickly (1–2 days). Ecdysis itself should not take more than an hour even in very large animals.

Coursing in channels through the cuticle are two basic types of structures: setae and tegmental gland ducts. Setae (Figure 11.17) are composed of two parts. One part is the projection of the carapace that forms a fine spine-like to feather-like extension that may or may not have a movable socket at its base (providing articulation). Each of these projections is innervated by extensions of nerves that run through the channel in the carapace from the hypodermis. There are three types based on function: mechanoreceptor, chemoreceptor, and grooming setae, but each type of seta may participate in more than one function. There are numerous setal forms on any one species of decapod crustacean (Felgenhauer 1992b; Roer and Dillaman 1993; Stevenson 1985). Setae that participate in either or both mechanoreception or chemoreception are innervated by bipolar neurons and a dendrite extends to the tip of these setae. The dendrite is surrounded along its course through the channel by enveloping cells (forming a dendritic sheath) (Felgenhauer 1992b). Some setae are open at the tip (especially in chemoreceptive setae) to allow for direct contact with the environment. Large setae involved with grooming are predominantly found in the mouthparts and stomach.

Tegmental glands (Figure 11.18) are present in the hypodermis of all decapod crustaceans. They are described as rosettes of cuboidal to pyramidal cells that produce a waxy secretory product. A small, approximately 3 µm duct is formed by a central cell in the rosette. This long duct (the second type of channel through the carapace), formed by this cell, extends from the glandular lumen to the cuticular surface. Product moves up the duct to the surface of the epicuticle where it is distributed over the surface. The ducts are lined by an epicuticular-like material, producted by the ductal cell (Felgenhauer 1992b). Tegmental glands are more commonly found in areas around the mouth, pereiopods, pleopods, gills, eyestalks, esophagus, and hindgut. The products, components, and function may vary based on the molt cycle and the location of the glands. Atrophy of tegmental glands have been noted in late C_4/D_0 phases of molting (Arsenault et al. 1979; Smolowitz et al. 1992, 2005). Around the mouth, tegmental glands secrete acid mucopolysaccharides that may lubricate the mouthparts. Glands within the pleopods secrete a cement-like substance that firmly attaches spawned eggs to the pleopods in species that incubate eggs (Felgenhauer 1992b).

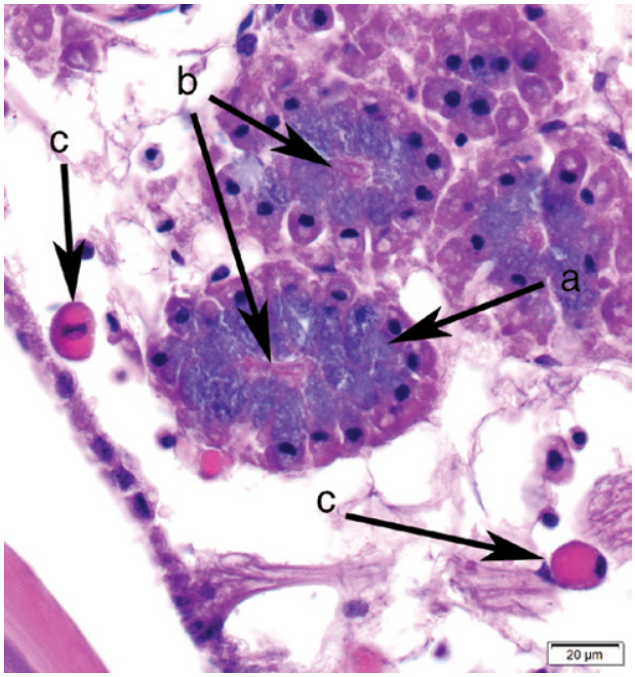


Figure 11.18 Histology of a tegmental gland of *H. americanus*. (a) Pyramidal glandular cells; (b) chitinized tube formed by the central collecting ductal cell; (c) RI cells. HE.

RI cells (reserve inclusion cells, reserve cells, cyanoctyes) (Figure 11.18) are stationary cells found on the edges of sinusoids and are noted in abundance in the hypodermis of the cuticular epithelium of decapods in intermolt. In hematoxylin and eosin-stained tissues they demonstrate a large uni- or multiloculated inclusion in the cytoplasm. They are also found in hypodermis and connective tissues surrounding the hepatopancreatic and gonadal tissues. The cells decrease in size during premolt and are not detectable histologically when ecdysis occurs. The cell inclusions contain glycoproteins, including hemocyanins, and are important in carapace formation and molting (Johnson 1980; Roer and Dillaman 1993).

Muscles attach to the cuticular epithelium and the base of the overlying cuticle in agonistic bundles. In several locations, apodemes occur. Apodemes are projections of the carapace into the body that provide a surface attachment for larger muscles. Muscle fibers in decapod crustaceans are striated and multinucleated (Phillips et al. 1980; Ruppert et al. 2004)

11.3.2 Gastrointestinal Tract

The gastrointestinal tract of decapods is divided into the esophagus, foregut (stomach), midgut, hindgut, anterior and posterior ceca, and digestive gland (pyloric ceca). The fore- and hindguts are lined by chitin. At molt, these protective internal layers are shed and replaced (Phillips et al. 1980). The short esophagus often is thrown into ridges (Felgenhauer 1992b) and empties into the anterior/ventral foregut ventrally via a valve. The foregut is divided into a cardiac and a pyloric stomach (Figures 11.13, 11.14, 11.19, and 11.20). The cardiac stomach contains a large lumen and several hard, twig-like ossicles (spicules) that are areas

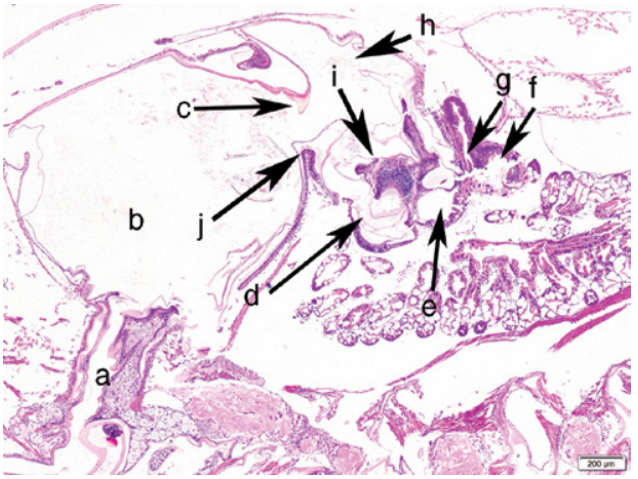


Figure 11.19 Histology of the stomach of a lobster. (a) Esophagus; (b) cardiac stomach; (c) gastric mill; (d) pyloric filter; (e) primary hepatopancreatic duct; (f) midgut (intestine); (g) opening to the anterior midgut ceca; (h) dorsal pyloric stomach; (i) pyloric filter press; (j) cardiac ridge. HE.

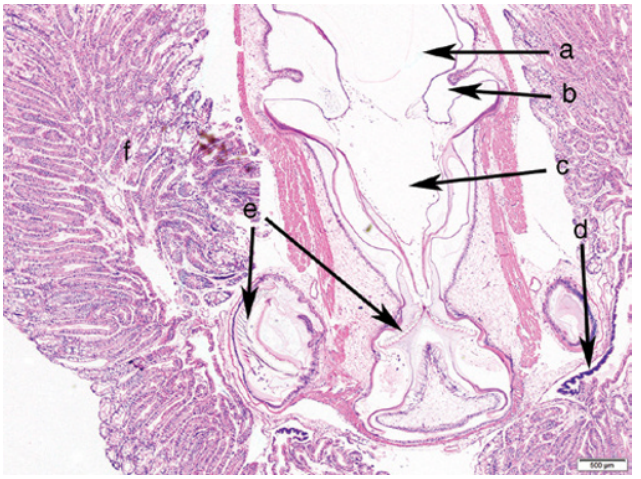


Figure 11.20 Histology of the plyloric stomach of a crayfish. (a) Dorsal chamber; (b) pyloric filter press; (c) intermediate chamber; (d) primary duct to hepatopancreas; (e) filter channels covered by filter setae (note: the epithelium is separated from the overlying structures in several locations due to processing artifact). HE.

of mineralization in the chitin lining. Ossicles provide structure and help muscles of the cardiac stomach to macerate food. At the posterior-dorsum of the cardiac stomach, a localized area of the epithelium produces a gastric mill. The two parts of the mill (one on either side of the cardiac stomach) have interlocking washboard-like projections that help to finely grind food.

Ventrolateral channels formed by ridges (cardiac ridges) occur along the sides of the cardiac stomach and are covered by rows of fine setae (Figures 11.13 and 11.19); the ridges help to direct small particles of ingesta ventrally and retain the large particles dorsally in the cardiac stomach.

The pyloric stomach is highly compartmentalized (Figures 11.19 and 11.20) and is divided into ventral, medial and dorsal compartments by lateral folds in the walls of the stomach. The dorsal compartment of the pyloric stomach compacts large particles of ingesta retained by the cardiac setal screen in the cardiac stomach (Figures 11.14, 11.19, and 11.20). These materials are dehydrated and compacted with help by the pyloric fingerlets (larger seta-like structures that project into the lumen) then enter the midgut through a pylorointestinal valve made of cuticular ridges (Icely and Nott 1992). Ingesta entering the midgut is bolus-like in formation.

Small particles of ingesta move from the ventrum of the cardiac stomach (after falling through the setae of the cardiac ridge) to the preampulatory then the ampulatory chamber where the pylorohepatic valve is located. Ingesta that did not fall through the cardiac ridges, and that is not very large, is further macerated and moved posteriorly to the winged pyloric filter press and its underlying filter.



Figure 11.21 Histology of the hepatopancreas of a crayfish. Blind-ended digestive tubules extend from a secondary duct. (a) Secondary duct; (b) arteriole; (c) primary duct. HE.

With the action of the stomach and the pyloric filter press, ingesta is pressed in the medial compartment, resulting in the movement of small particles (approximately 100 nm) through the filter. These particles join with the material that traveled from the ventrum of the cardiac stomach (material that previously fell through the setae between the cardiac ridges). The filter bifurcates and each bifurcation leads to a primary duct of one of the hepatopancreas lobes (Figures 11.20 and 11.21). Basically, the filter is a sieve that allows fine particles of ingesta into the antechamber (primary ducts) of the hepatopancreas. Macerated ingesta that does not enter through the filter to the hepatopancreas

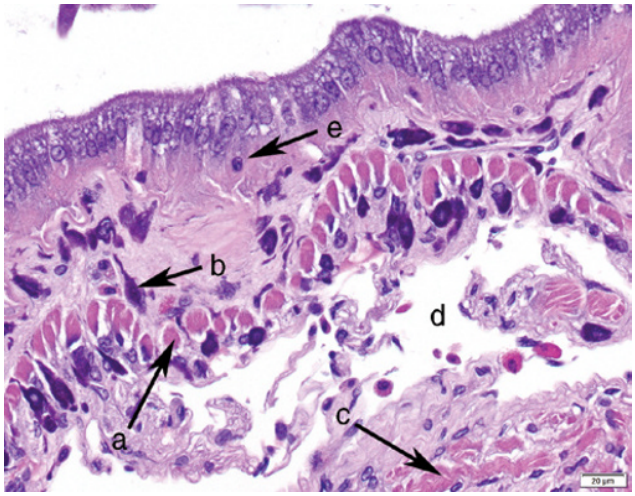


Figure 11.22 Histology of the midgut of *H. americanus*. (a) Circular muscle; (b) glandular cell; (c) longitudinal muscle; (d) sinusoidal lumen containing hemocytes and fixed RI cells; (e) basal cell. HE.



Figure 11.23 Histology of the hindgut of *H. americanus*. (a) Chitin lining the epithelial cells. (b) longitudinal muscle layer. HE.

is redirected from the medial compartment to the dorsal compartment of the pyloric stomach and becomes a component of the boli formed as described above.

Most recently evolved Pleocymata and all Dendrobranchiata have both ossicles and gastric mills, but more archaic Pleocymata may lack ossicles and/or gastric mills. The diet of each species also influences the occurrence and size of these structures (Icely and Nott 1992).

Epithelium lining the midgut (Figure 11.22) is simple columnar, often granulated, contains lipid droplets, and sits on a thick basement membrane. Granules in the epithelial midgut cells are the source of the thin layer of chitin that coats the boluses (dehydrated fecal balls) traveling through the midgut. Smaller, basal cells are found interspersed between the columnar cells and are thought to be either endocrine cells or regenerative cells that replace the columnar cells. Digestive and absorptive actions are not as important in the midgut as in the hepatopancreas.

The epithelium of the junction between the hindgut and midgut is thrown into folds forming a valve and is developed to varying degrees in each species of decapod. The hindgut is composed of simple columnar epithelium with basal folding that allows for dilation of the lumen. Chitin lines the surface of the epithelium and is shed during molting (Figure 11.23). Circular muscle fibers are present in the hypodermis along with tegmental glands. The hindgut dilates and can draw in fluid in an antiperistaltic action, thus providing fluid lubrication to the hindgut and midgut.

The occurrence of ceca is common in decapods. Anterior and posterior ceca are long blind-ended pouches with narrow lumens that extend dorsally from the midgut at either its posterior and/or anterior end. In American lobsters, large paired posterior midgut ceca extend anteriorly from their connection at the hindgut (McLaughlin 1983), but anterior ceca are also present (Figure 11.19). Brachyurans (crabs) often have large anterior midgut ceca that extend anteriorly into the cephalothorax from their origins on the anterior midgut. Ceca are similar histologically to the midgut. Their function is not known, but they may contribute digestive or pH-modifying enzymes to the midgut (Icely and Nott 1992).

The hepatopancreas of decapods is very similar between all species. The hepatopancreas (also termed the midgut gland, pyloric ceca, liver) originates at the antechamber of the pyloric stomach and forms bilateral blind-ended, arborizing, tubular glands (Figure 11.21). In lobsters, the hepatopancreas is divided into two bilateral lobes. The lobes of the hepatopancreas lie in a defined sinusoid (i.e., the hepatopancreas is surrounded by a sinusoidal space defined by a surrounding membrane). The ducts and tubules themselves are surrounded by both longitudinal and circular muscle fibers and connective tissues (Ceccaldi 1989; Vogt 2019). Between the tubules, well-defined arterioles empty hemolymph into the sinusoid (Figure 11.21).

The walls of the antechamber (atrium) of the hepatopancreas are formed by the primary ducts of the hepatopancreas (composed of columnar epithelium) and extend into the hepatopancreas (Icely and Nott 1992; Vogt 2019) (Figures 11.19 and 11.20). Three secondary ducts originate from each primary duct. Each secondary duct enters one of

three sublobes where it divides into numerous smaller ducts. Extending from each small duct are numerous blind-ended tubules where digestion and absorption occur. The number of lobes of the hepatopancreas may vary between decapod species.

Five types of epithelial cells have been described lining the tubules: E cell, F cell, B cell, R cell, and M cell. The number of each type of cell varies with the time after feeding and the molt stage. E cells are found only at the distal end of the tubule (Figure 11.24). These low cuboidal to squamous cells undergo mitosis and are the source of new cells in the tubules. Columnar, ciliated F cells (Figure 11.24) are found in the mid to distal end of the tubules. F cells mature into large ciliated columnar B cells (Figure 11.25) which are noted in the proximal portions of the tubules. F cells contain small enzyme vacuoles in their cytoplasm. B cells contain large vacuoles with basally compressed nuclei. Periodically after eating, B cells secrete digestive enzymes from the vacuoles into the tubular lumen and into the stomach. Both F and B cells absorb some nutrients by pinocytosis and transfer these nutrients to the hemolymph and hemocytes in the surrounding sinusoids (Icely and Nott 1992). Columnar, ciliated R cells are the predominant cell type in the tubules and are found in the proximal portions of the tubules (Figure 11.25). They ingest and digest larger particles from the tubular lumens and store lipid, glycogen, and metals in vacuoles in their cytoplasm. M cells are sparse, small basal cells located between the surrounding tall columnar cells lining the tubules and are thought to be endocrine cells (Vogt 2019).



Figure 11.24 Histology of the blind end of a hepatopancreatic tubule. E–E cells; F–F cells; (a) undifferentiated cell. HE.

11.3.3 Cardiovascular System

The outer wall of the heart is composed of an spongy adventitia. The inner "walls" of the heart are composed of loosely organized striated muscle trabeculae (Figure 11.26). The surfaces of the muscle fibers are lined by an intima (noncellular). Hemolymph (blood) enters the heart through valves

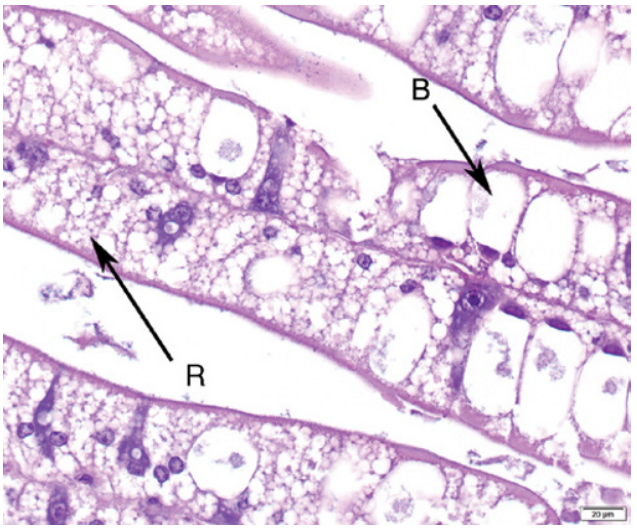


Figure 11.25 Histology of a proximal part of a hepatopancreatic tubule of a crayfish. B–B cells; R–R cells. HE.

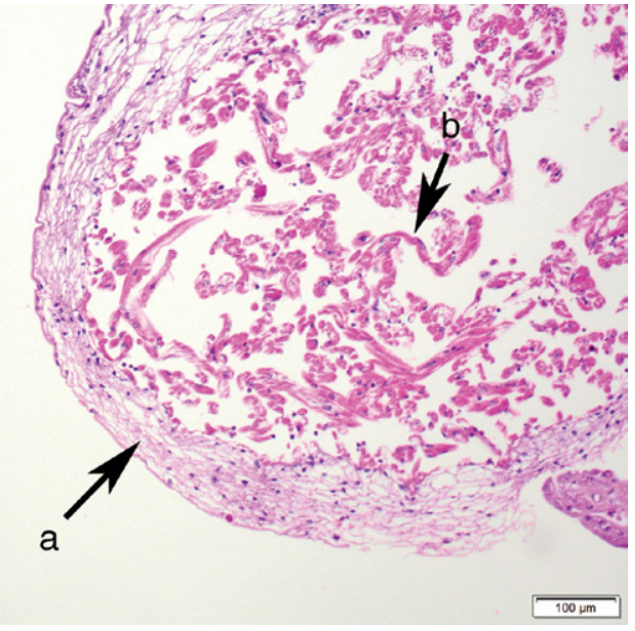


Figure 11.26 Histology of the heart of a crayfish. (a) Adventitia; (b) striated trabecular muscle. HE.

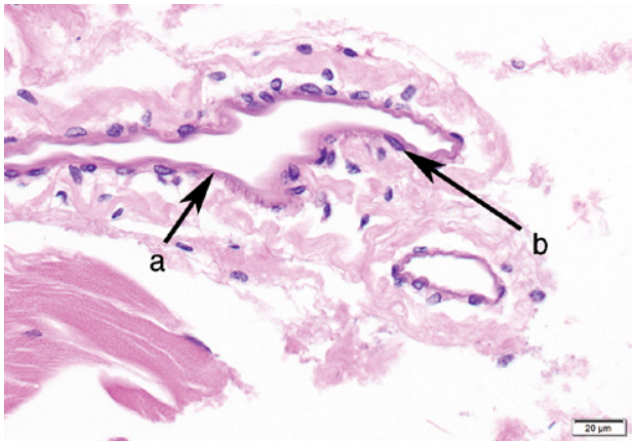


Figure 11.27 Histology of a medium-sized artery. (a) Internal intima (basal lamina); (b) endothelial cell. HE.

(ostia) in the heart wall from the pericardial space surrounding the heart (there are no atria). Three pairs of valves enter the heart of decopod crustaceans. In lobsters, two of the pairs are located laterally and one ventrally. The location varies between species of decopod. Hemolymph is pumped into anterior, posterior, and sternal arteries from the heart. The sternal artery supplies the ventral abdominal artery. The heart is surrounded by a pericardial sac that receives hemolymph from the sinusoids throughout the body. The pericardial sac acts as an atrium.

Walls of medium to large arteries (Figure 11.27) are composed (from the lumen outward) of an acellular inner intima, endothelial cells, loose connective tissues, and an outer intimal layer. In some other species of decapods, striated muscle cells may be noted in large arteries adjacent to the inner intima (Johnson 1980; Martin and Hose 1992). In lobsters and some other decapods (some shrimp and crab species), a cor frontale is present in the anterior aorta (McLaughlin 1983; Martin and Hose 1992; Johnson 1980). The cor frontale consists of stomatogastric ganglion and supporting connective tissue structures that project into the lumen of the aorta. The nerves travel from the cor frontale to innervate the valve between the heart and the aorta. The purpose is to increase the amount of hemolymph entering the aorta when needed by the cerebral nervous system (Johnson 1980). Similar structures may be present in the ophthalmic arteries.

Small arterioles, such as those noted between hepatopancreatic tubules, consist of a lumen surrounded by a thin intima and a few squamous endothelial cells circumferentially surrounded by fixed phagocytes. The sinusoids are lined by a thin intima (membrane) that covers all the organs. Defined venules and veins are not identified and hemolymph is pumped back to the pericardium by the action of the muscles of the body.

11.3.4 Hemocytes and Inflammation

It is generally agreed that there are two basic types of hemocytes (blood cells) in the hemolymph of a decapod crustacean: hyaline cells (hyalinocytes/agranulocytes) and granulocytes (Figure 11.28). Granulocytes are divided into semigranulocytes (small granule cells) and large granulocytes (refractile or large granular cells). While function studies contradict each other to some extent, Martin and Hose (1992) determined that hyaline cells are primarily involved in aggregation (a function similar to that of platelets in vertebrates) and coagulation. Hyaline cells appear to react to inciting events by undergoing cytolysis and releasing coagulation factors that transform the hemolymph in the area into a gel-like substance. Granulocytes are larger than hyalinocytes. Small granulocytes are both smaller and contain markedly smaller granules than the large granulocytes. However, small granulocytes are more numerous. Granulocytes do not lyze but do expulse their granules containing lysosomal enzymes upon stimulation, after which they are primarily responsible for phagocytosis of the inciting cause. When inciting causes are too large for phagocytosis or the area of necrosis/infection is large, a typical inflammatory reaction termed encapsulation occurs. An encapsulation (equivalent to a granuloma in mammalian terms) is characterized by a central melanized zone surrounded by layers of reactive hemocytes and degranulated, flattened (transformed) hemocytes. Intact hemoctyes are noted at the periphery of the lesion (Figure 11.29). The encapsulations primarily result from granulocytes which have degranulated and then formed the closely adhered layers of flattened cells (Martin and Hose 1992).

The melanization-like response of tissues is a common inflammatory response. Molecules of prophenoloxidase are present in the hemolymph and granulocytes (primarily

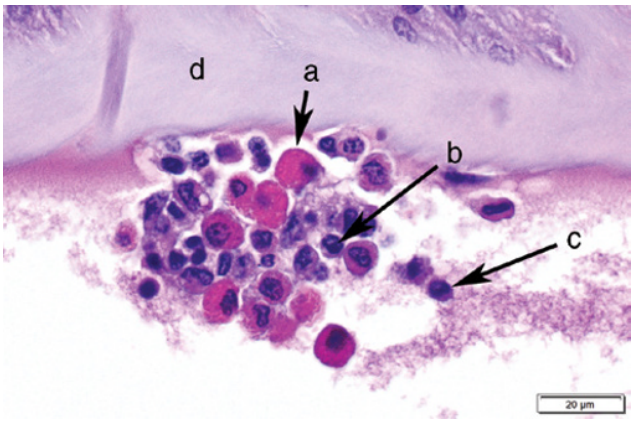


Figure 11.28 Hemocyte types adjacent to the internal intima of the dorsal posterior aorta. (a) Granulocyte; (b) hyalinocyte (agranulocyte); (c) semigranulocyte; (d) intima of the artery. HE.

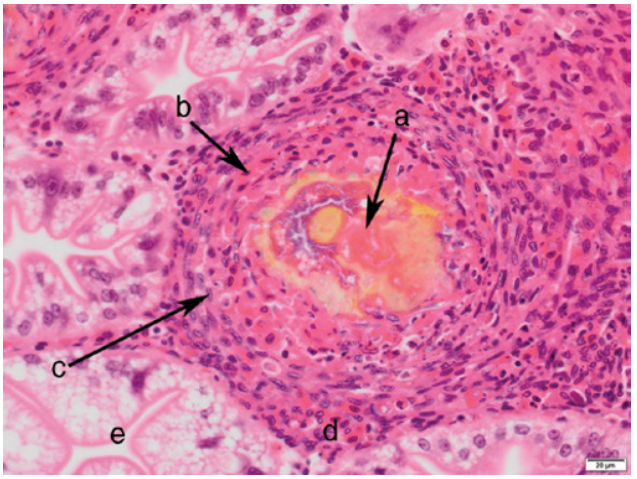


Figure 11.29 Encapsulation (decapod granuloma). (a) Central melanization (upon demineralization the color becomes pink/yellow instead of brown/black); (b) melanizing reactive granulocytes; (c) transformed reactive granulocytes; (d) migrating granulocytes; (e) hepatopancreas tubule. HE.

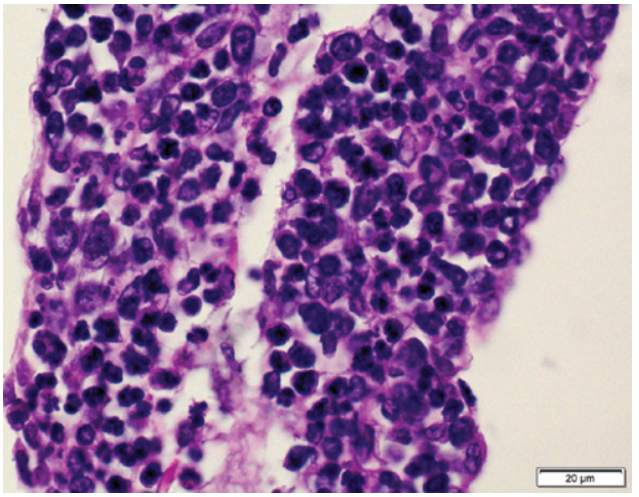


Figure 11.30 Histology of a focus of hematopoietic tissue surrounding a small arteriole in a crayfish. HE.

large granulocytes). When infection occurs, prophenoloxidase is released from granulocytes and activated by lipopolysaccharides (components of bacterial cell walls) or 1,3-glucans (fungal cell walls) forming phenoloxidase in the hemolymph. Phenoloxidases catalyze the hydroxylation of monophenols to diphenols (dopamine and dopa-like molecules) then oxidize the diphenols to an orthoquinone. Orthoquinones are highly reactive molecules that destroy and cross-link protein (and form the melanized foci in the inflammatory response) (Figure 11.29).

The melanization response is also used to harden portions of the carapace that may experience the most wear, such as the tips of claws, and is termed sclerotization or tanning (Roer and Dillaman 1993). In the process of sclerotization, prophenoloxidase and other proteins and enzymes are deposited in the upper layers of the cuticle as it formed. Just after ecdysis, a tightly controlled system of prophenoloxidase-activating factors is stimulated resulting in the production of a serine protease that converts prophenoloxidase previously deposited in the forming exocuticle into phenoloxidase (Budda and Shafer 2005). Sclerotization then follows similar chemical changes as described for inflammation. This action results in cross-linking of histidine and lysine amino acids in proteins deposited in the new cuticle producing the melanization (blackening) of the outer cuticular layers.

Hemocytes are produced in hematopoietic tissues which vary in location in different subclasses. Lobsters and most decapods have areas of hematopoiesis associated with the dorsal and lateral sides of the foregut. In all decapods, hematopoietic tissue contains early (stem cell) and mid to late stages of differentiating hemocytes and mitotic cells. Hematopoietic lobules (lobsters, shrimp) or nodules (crab) occur in specialized hematopoietic arteries that primarily appear to originate from larger arteries. The hematopoietic tissues are surrounded by loose layers of collagen and spongy connective tissues through which the newly formed hemocytes migrate into the surrounding hemosinuses (Figure 11.30). The activity of the hematopoietic tissues varies with the molt cycle, with an increase in hematopoiesis just before molt (Johnson 1980; Martin and Hose 1992).

Hemoglobins are not present in decapod crustaceans. Instead, high molecular weight molecules of hemocyanin are used to transport oxygen throughout the tissues. There is controversy concerning where hemocyanins are produced. Johnson (1980) proposed that hemocyanins are produced in reserve inclusion cells (RI cells), but Durstewitz and Terwilliger (1997) and Lehnert and Johnson (2002) showed the F cells of the hepatopancreas appear to be the only source of hemocyanin in decapod crustaceans.

11.3.5 Excretory System

The excretory system of a decapod crustacean is the primary means of eliminating nitrogenous wastes and is composed of several parts: a coelomosac, a labyrinth, a bladder and bladder duct, a nephridiopore, and the surrounding hemosinus. Urine produced by marine decapods is isosmotic with its hemolymph, unlike the urine of freshwater decapods. Hemolymph is delivered directly to the coelomosac by an antennal artery that originates from the heart. In the coelomosac, it divides into small arterioles that empty into the sinusoids within the coelomosac and labyrinth. The coelomosac develops embryologically from

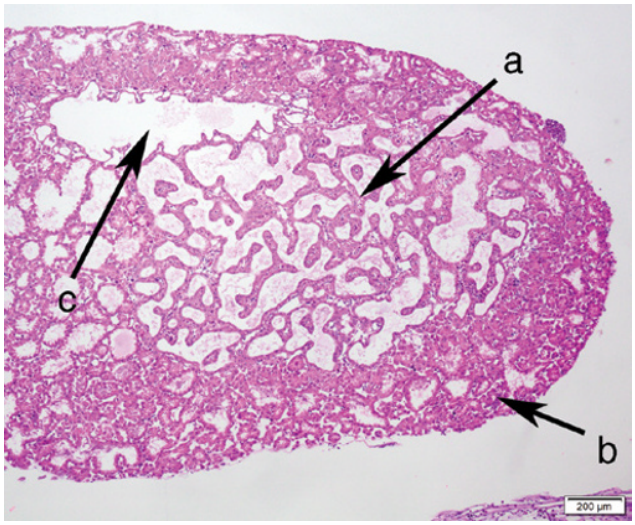


Figure 11.31 Histology of the kidney (green gland) of a crayfish. (a) Coelomosac cells; (b) labyrinth cells; (c) urinary space. HE.

the coelom and is a series of branching tubules lined by cells considered analogous to podocytes in vertebrates (Figure 11.31). The usually highly vacuolated cuboidal epithelium is active in protein secretion and absorption. Large inclusions can sometime be noted in apical vacuoles in coelomocytes. The epithelium lies on a thin basal lamina.

The labyrinth, as the name indicates, is a convoluted series of branching tubules. Labyrinth cells are cuboidal to columnar and function in the ionic modification of urine. Johnson (1980) noted both an active (columnar, dense epithelial cells) and inactive (cuboidal to low columnar) state of labyrinth cells in the blue crab but was not able to correlate it with molting or other processes. Peterson and Loizzi (1974) and others have divided the labyrinth epithelium into proximal and distal portions (labyrinth I and labyrinth II) based on ultramicroscopic morphology. In many areas, the coelomic epithelium is separated from the labyrinth epithelium only by a sinusoid. In histologic sections, the sinusoids throughout the gland appear very small and/or are collapsed. Occasionally, urate crystals are noted in the urine lumens in the labyrinth.

From the labyrinth, urine is deposited in the bladder. The bladder is extensive (contains three lobes in crabs) and is lined by cuboidal to columnar epithelium similar to that noted in the labyrinth. It may be pigmented in some crab species. The bladder is destroyed in dissections. In freshwater decapods, a nephridial tubule connects the labyrinth and the bladder. Proximally the nephridial tubule is lined by cuboidal cells and distally by columnar cells (indicating slightly different functions). The overall function of the nephridial tubule is reabsorption of solute (against the osmotic gradient), thus increasing the ions present in the hemolymph of freshwater decapods (Peterson and Loizzi 1974). Urine is eliminated from the bladder through the nephridiopore.

11.3.6 Respiratory System

The primary function of the gills of decapod crustaceans is respiration, but they are also used in osmotic and excretory functions (Taylor and Taylor 1992). All three types of gills, trichobranchiate, phyllobranchiate, and dendrobranchiate, move hemolymph through a central core of each gill into filaments (and lamellae) of the gill by afferent and efferent vessels (efferent channels). The vessels are separated by a septum (axial septum, central filament, longitudinal septum) composed of connective tissue cells. The microscopic structure and cells making up the filament are similar between species. The filaments are lined by simple cuboidal to almost squamous epithelium that is covered by a thin cuticle. Pillar structures (cells) help to maintain the open lumen of the channels in the filaments. Tegmental glands and nephrocytes are noted in the axial tissues. Nephrocytes (branchial glands, branchial kidneys, nephrophagocytes) are podocyte-like, large (20–50 µm), mostly singularly occurring cells in the connective tissue of the gills. They progressively develop vacuoles filled with yellow material until molt and are empty post molt. Their function is unknown, but they are thought to be similar to fixed phagocytes.

Trichobranchiate gills (primarily found in lobsters and crayfish) (Figure 11.8a,b) are morphologically unusual compared to other decapods. The filaments on each gill are divided into inner filaments (inner being closest to the body wall [epimeron] and outer filaments being closest to the branchiostegite) (Taylor and Taylor 1992). In each filament (Figures 11.32 and 11.33), hemolymph moves through an afferent branchial vessel (channel) of the gill core to the afferent canal of the outer filament. Hemolymph then flows into an extensive lateral lacuna of the filament. From the lateral lacuna, hemolymph is directed to the efferent canal in the filament which joins with other efferent canals to form a mantle canal in the gill (not connected to the afferent or efferent vessels). From the mantle canal, hemolymph is directed into afferent canals of inner filaments, then moves into lateral canals of the inner filament and into the efferent canal of the inner filament and then finally to the efferent vessel (channel) of the gill core. It is important to note that there is an incomplete separation between each canal in the filaments. After circulation through the gills, oxygenated hemolymph is directed back to the pericardial sac.

Middle filaments are present in some species including *H. americanus*. *Jasus novaehollandiae* (rock lobster) is the best studied (Figures 11.34 and 11.35). Middle filaments

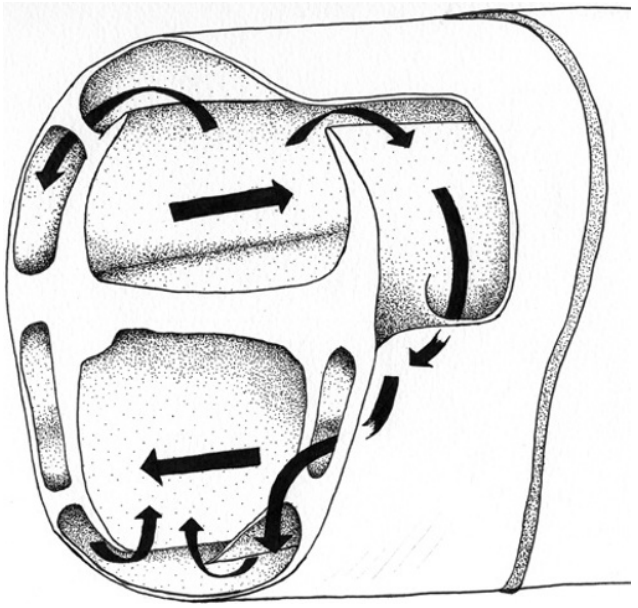


Figure 11.32 Line drawing of the hemolymph circulation in an outer filament of the rock lobster (*Jasus* sp.) *Source:* After Rogers from Taylor and Taylor (1992). Arrows indicate the direction of hemolymph flow entering through the afferent canal then moving into the lateral canal and from there into the efferent canal. *Source:* Original drawing by Alicia Schickle.

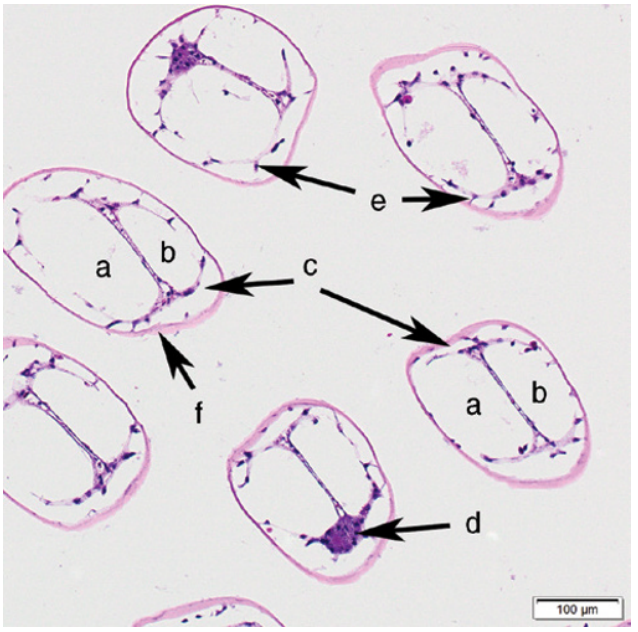


Figure 11.33 Histology of a cross-section of an outer gill filament of a crayfish. (a) Afferent canal; (b) efferent canal; (c) lateral canal; (d) tegmental gland; (e) pillar cell; (f) cuticle. HE.

have afferent, middle, and efferent canals. A modification occurs from the circulation described above in that hemolymph leaves the efferent canals of the outer filaments and flows to an expanded lateral canal within the gill core.

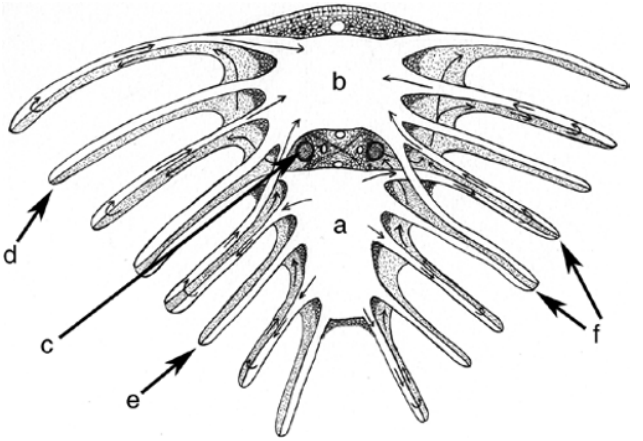


Figure 11.34 Line drawing of the filaments of *Janus novaehollandiae* (rock lobster) showing hemolymph flow through the inner, outer, and middle filaments of a gill. *Source:* After Rogers from Taylor and Taylor (1992). (a) Afferent channel; (b) efferent channel; (c) lateral canal; (d) inner filament; (e) outer filament; (f) middle filaments. *Source:* Original drawing by Alicia Schickle.

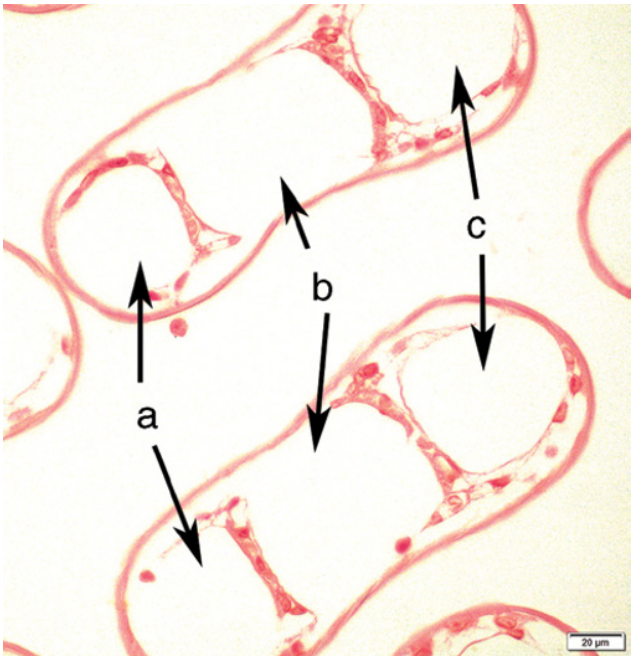


Figure 11.35 Histology of a cross-section of a middle gill filament of *H. americanus*. (a) Afferent canal; (b) middle canal; (c) efferent canal. HE (stain is substantially faded).

From there the hemolymph flows to both the middle canal of the middle filament and the afferent canal of the inner filaments. Hemolymph exits via the efferent canal of the inner filament back to the efferent vessels of the filament core. Meanwhile, the afferent canal of the middle filament also receives hemolymph from the gill core's afferent vessel (channel). This hemolymph is circulated through the

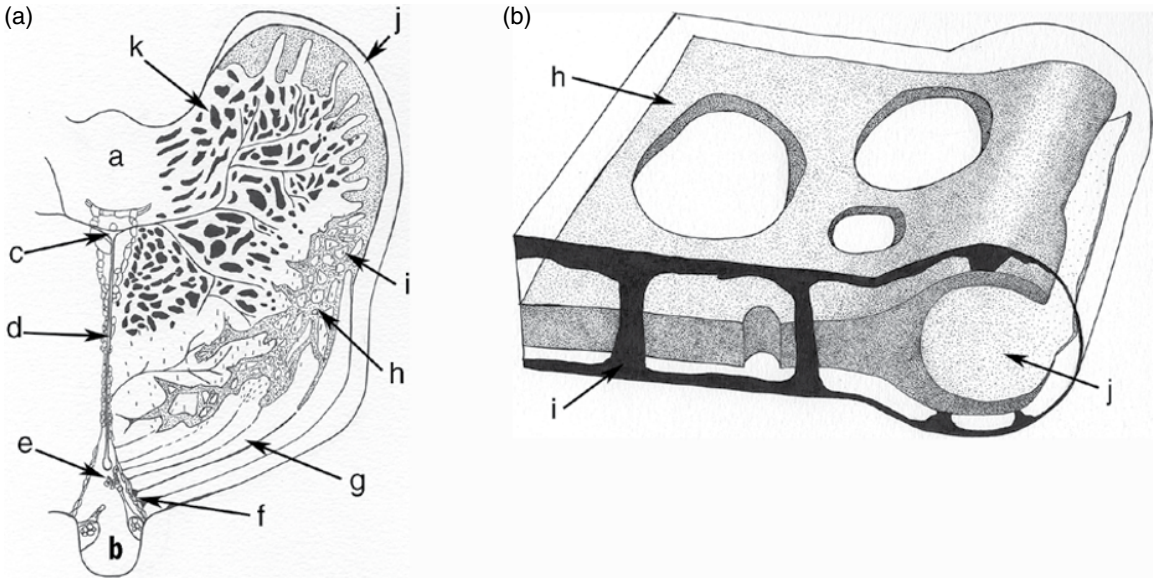


Figure 11.36 Line drawing of a phyllobranchiate gill lamellae of a crab (*Carcinus maenas*). *Source:* after Taylor and Greenway from Taylor and Taylor (1992). (a) Transverse section of a lamellae. (b) Dorsal-ventral section through the marginal canal. a, afferent branchial vessel (channel); b, efferent branchial vessel (channel); c, artery; d, afferent shaft; e, efferent shaft; f, efferent valves; g, efferent canals; h, fenestrated lamellar septum; i, pillar cell; j, marginal canal; k, ionocytes (function in maintaining the osmotic balance of the hemolymph). *Source:* Original drawing by Alicia Schickle.

efferent canal of the filament into the efferent vessel of the gill core. As mentioned above, the partitions between these channels are fenestrated and very leaky (Taylor and Taylor 1992). This unusual flow pattern may increase the oxygenation of the hemolymph and/or ion exchange and excretion.

Phyllobranchiate gills are found in brachyuran crabs, most anomurans and shrimps (Figure 11.8c,d). Each phyllobranchiate gill is composed of an elongated flattened axial tube from which flat lamellae extend producing ventral to dorsal piles of "butterfly wings" (Taylor and Taylor 1992) (Figure 11.36a). The afferent branchial vessel (channel) is located at one side of the axis and is separated by connective tissue and mesodermal cells (including nephrocytes, ionocytes, and branchial arterioles) from the efferent branchial vessel at the other side. Each of the wings extends laterally from the flattened shaft (branchial axis). Each wing is composed of two parallel sheets of cuboidal epithelium and associated cuticle, separated from each other by pillar cells. Along the outer edges of the wings, a fenestrated lamellar septum partially partitions the lumen inside each wing. Along the distal edges of each lamella (wing) is a marginal canal (a semicircular expansion of the lumen) (Figure 11.36b). Hemolymph enters through the afferent vessel (channel) and flows into the lumen of the lamellae in a semicircular manner, ending at the efferent canals which empty into the efferent vessel (channel).

Dendrobranchiate gills of penaeid shrimp (Figure 11.8e,f) consist of a primary axis which divides into secondary lamellae. From the secondary lamellae, several flattened tertiary filaments arise. Each tertiary filament has a similar structure to a "wing" of the phyllobranchiate gill described above but is much smaller. Afferent and efferent hemolymph vessels (channels) carry hemolymph from the axes to the canals present in each filament. Afferent and efferent canals are separated from each other by a thin septum in each filament (Bell and Lightner 1988).

11.3.7 Neuroanatomy

The central nervous system of lobsters extends anteriorly to posteriorly and lies ventrally in the body tissues (Figure 11.14). It is composed of a supraesophageal ganglion (often termed the brain) that is just anterior to the esophagus. Circumesophageal connectives leave the supraesophageal ganglions and extend around the esophagus. They fuse to form a postesophageal commissure from which two large parallel nerves proceed posteriorly to join the subesophageal ganglia. Extending posteriorly from the subesophageal ganglion is a neural cord interspersed with segmental paired, but fused, thoracic and abdominal ganglia (McLaughlin 1983; Phillips et al. 1980). The number of abdominal ganglia and the amount of fusion of the ganglia vary by species. The fusion of segmental ganglia is most developed in crabs in which thoracic ganglia are fused into

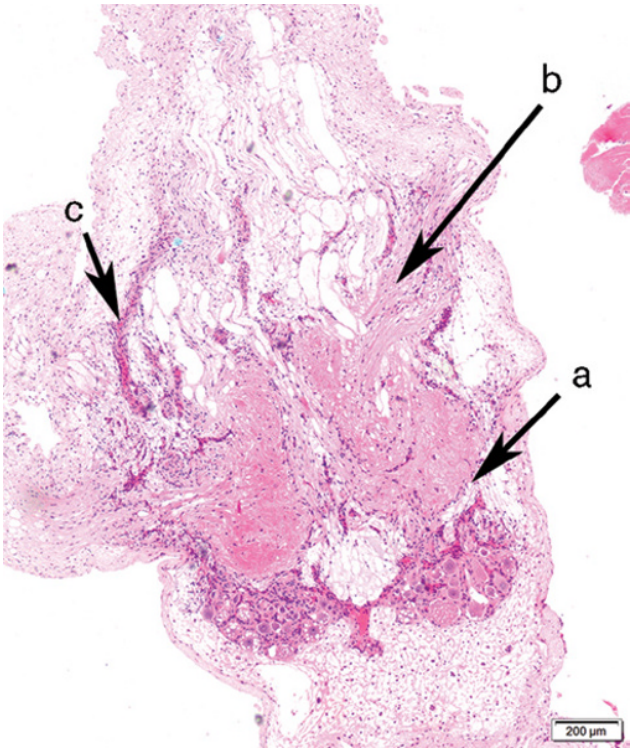


Figure 11.37 Photomicrograph of a thoracic ganglia. (a) Ganglia; (b) nerve; (c) arteriole. HE.



Figure 11.38 Histology of a large nerve. (a) Small arteriole; (b) neural sheath. HE.

one large mass. Histologically, ganglia are composed of nerve cell bodies forming the periphery of each ganglion with centrally located neuropile (Figure 11.37). Axon tracts are noted in the ganglia. Ganglia and nerves are surrounded by a neural sheath (Figure 11.38) composed of fibrous connective tissue. The perineurium underlying the sheath and surrounding the ganglia and nerves contains abundant arterioles/capillaries (Govind 1992).

The supraesophageal ganglion is composed of three main parts: the protocerebrum, deutocerebrum, and tritocerebrum. The protocerebrum contains large optic ganglia which innervate the eyes as well as other ganglia that innervate other sensory organs and tissues of the head. The deutrocerebrum innervates the antennae and the tritocerebrum innervates the gastric tissues. The circumesophageal connective originates from the tritocerebrum. The subesophageal ganglion innervates the mandible and maxillipeds.

11.3.8 Reproductive System

Decapod crustaceans are dioecious. All Malacostraca contain an androgenic gland, a ductless gland associated with the posterior vas deferens. It produces androgenic hormones which result in the differentiation of developing larvae into males. If this substance is not present, the animal becomes female.



Figure 11.39 Histology of the ovary of a crayfish. (a) Connective tissue capsule; (b) sinusoid; (c) arteriole; (d) follicular cells; (e) ovarian duct epithelium. HE.

In lobsters, the ovarian wall (Figure 11.39) is composed of a thin layer of fibrous connective tissue that includes some muscle fibers. The ovary sits within a hemosinus delineated by the periovarian wall. Location of the germinal epithelium varies by species. In *H. americanus* it is located near the lumen but in other species, it may be found along the periphery of the gland. Connective tissue, containing small arterioles/capillaries, divides the ovary

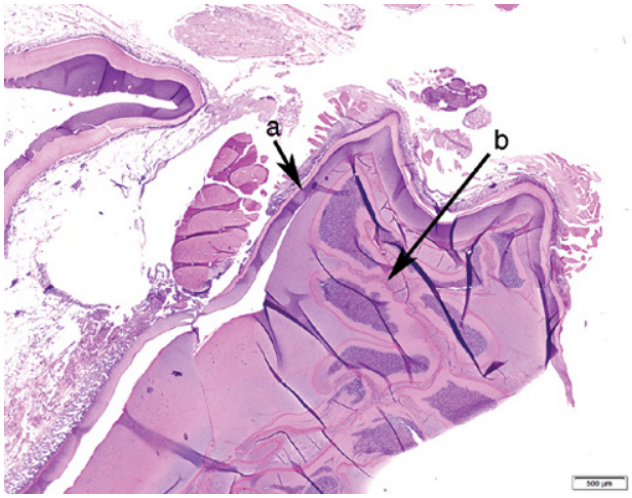


Figure 11.40 Histology of the seminal receptacle of *H. americanus*. (a) Chitin lining of the receptacle; (b) stored spermatopores. There are numerous fold artifacts (*arrowheads*) from sectioning. HE.

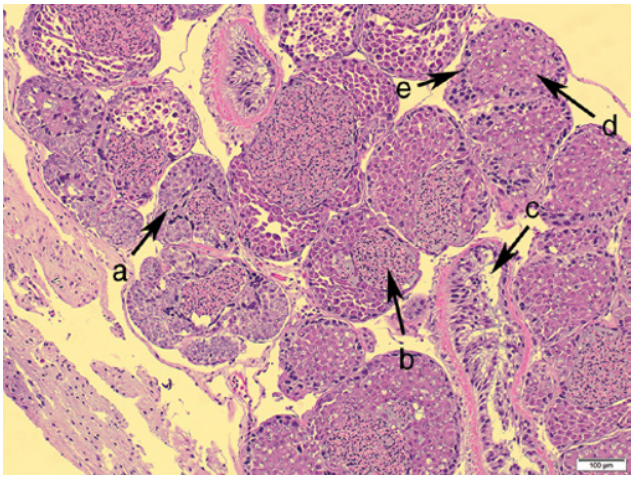


Figure 11.41 Testis of *H. americanus*. (a) Spermatogonia; (b) spermatids; (c) collecting tubule; (d) primary spermatocytes; (e) nurse cell. HE.

into "cysts" of developing oocytes. Oocyte maturation proceeds as follows: previtellogenesis, vitellogenesis, oocyte growth and maturation (Krol et al. 1992). As the oocytes grow in size, they tend to move to the periphery of the cyst and the cysts move to the periphery of the gonad. The ovarian cavity/lumen is often barely visible histologically (or grossly) but does connect to each cyst. Follicle cells (nurse cells) also originate from the germinal epithelium. As the oocyte develops, follicle cells move from the germinal epithelium and surround each oocyte. They function in the accumulation of yolk (vitellogenesis) by the oocyte and change from columnar to squamous as the egg develops. Follicular cells remain in the gonad post spawning. Oviducts connect the ovaries to gonadopores. The oviduct is lined by columnar cells with fibromuscular walls. The lumen is closed until spawning, giving the appearance of cords of cells, rather than a duct (Johnson 1980). In some species, the distal end of the oviduct is modified to form a spermatotheca (seminal receptacle) that can store spermatophores. In other decapods, such as the American lobster, the spermatotheca is an invagination of the exoskeleton and is not directly associated with the gonadopore (Figure 11.40). The spermatotheca may have glandular epithelium and/or be lined by chitin (Johnson 1980; Krol et al. 1992). These modifications, when present, are specific for each species. In some species, females only mate once and use the retained spermatophores for subsequent fertilization.

Histologically, the lobes of the testis are composed of highly convoluted tubules resulting in the appearance of multiple tubules in each lobe (Figure 11.41). The thin testicular wall is similar to that of the ovary, but no muscles are present. Spermatogonia are located in the lobe walls and sperm maturation in most species is synchronous within each tubule of the lobe. The sequence of sperm production is spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, mature spermatozoa. In decapods, sperm is often not flagellated, but sperm do often develop unusual structures. For example, spikes are noted extending from spermatozoa in deep sea crabs (*Geryon* sp.). Nurse cells (nutritive cells, follicle cells) are prominent in the seminiferous tubules and may surround the developing sperm. In several decapod species, collection tubules (often also convoluted) connect the seminiferous tubules to the proximal vas deferens. The collecting tubules in some species are lined by mucus-producing cells.

The vas deferens is divided into three or more regions depending on the species and is highly convoluted. In penaeid shrimp, it is divided into three parts: proximal, medial, and distal. Additionally, in penaeid shrimp the proximal and medial vasa deferentia are divided into ventral and dorsal lumens, both lined by columnar epithelial cells with microvillus borders. The proposed functions are to prevent the spermatids with unusual structures from becoming tangled and/or to provide nutrients for the spermatids. The lumens join together in the distal portion of the vas deferens. The distal vas deferens in decapods is the site of spermatophore development (Figure 11.42). There the spermatids are closely packed and enclosed by secretions forming an acellular wall. Spermatophores can exhibit distinctive shapes especially

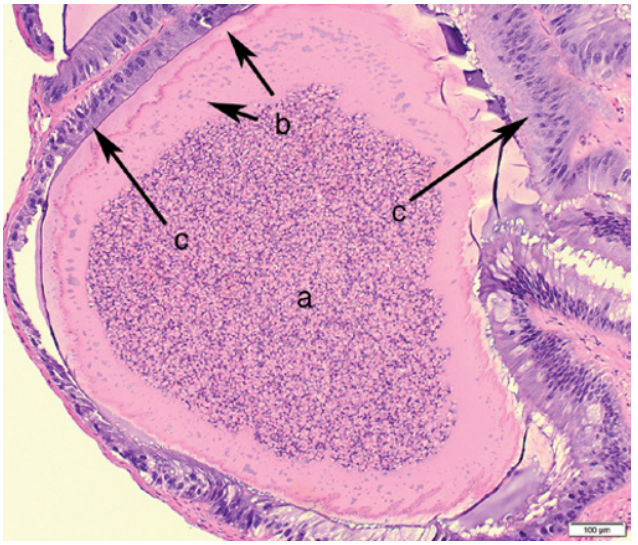


Figure 11.42 Vas deferens containing a developing spermatophore. (a) Spermatids; (b) layers of the developing spermatophore; (c) variation in epithelial types of the vas deferens. HE.

in decapods with external fertilization. In some species, spermatophores may be stored in the male until fertilization occurs.

11.3.9 Special Senses

Chemoreceptors and mechanoreceptors are common in decapods and are primarily associated with setae (discussed previously). Decapods also have well-developed eyes (Bell and Lightner 1988; Johnson 1980; Meyer-Rochow 1975; Ruppert et al. 2004; Waterman 1961). In lobsters and many other decapods, eyes are attached by a stalk to the head region just under the rostrum. In some decapods that stalk is long and movable and in others, very short and nonmotile (most crabs). Each eye is composed of many hexagonal, cone-shaped subunits; each is termed an ommatidium (Figures 11.43 and 11.44). The cornea (lens) of each ommatidium is produced by underlying corneagenous cells. The cornea is shed at molting. Light travels through the cornea (which has no ability to focus) and is directed down the crystalline cone to the retinular cells. The inverse pyramidal-shaped cones which are actually made up of four parts are produced and surrounded by the four crystalline cone cells. At the distal ends of the cones are pigment cells whose black granules move along the distal length of the cones and help the animal to adapt to variation in light in the environment.

The number of retinular cells in each ommatidium varies depending on species. These cells contain membrane-associated light-sensitive rhodopsin and black pigment

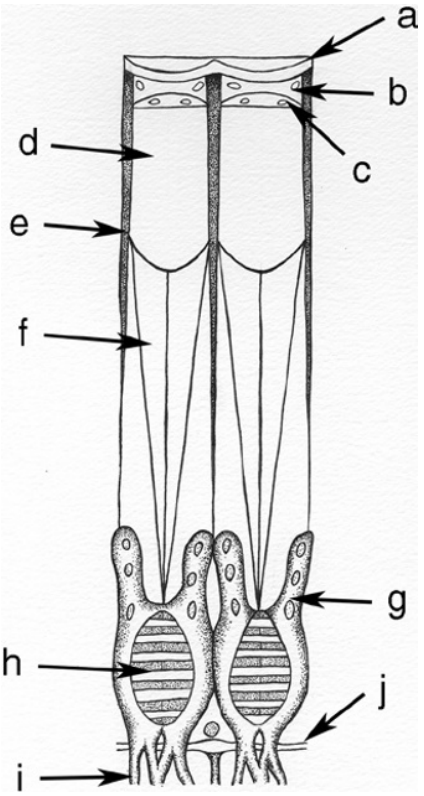


Figure 11.43 Line drawing of decapod ommatidia. *Source:* After Bernhards and Wasserman from Ruppert et al. (2004). (a) Cornea; (b) corneagenous cells; (c) crystalline cone cells; (d) crystalline cone; (e) distal pigment cells; (f) crystalline cone stalk; (g) retinular cells with pigment; (h) rhabdom; (i) axons of the optic nerve; (j) basal lamina. *Source:* Original drawing by Alicia Schickle.

granules that move from distally to proximally underneath the outer surface of the cell, depending on the amount of incoming light (Meyer-Rochow 1975). When the cell membranes are stimulated by light, a chemical change occurs in the rhodopsin. This in turn produces an electrical pulse in the exiting axons of the retinular cells. The intensity of the change is increased by a rhabdom that is formed by the retinular cells. The retinular cells in each ommatidium form a tube-like structure. On the inner surface of the retinular cell (the side that forms the tube) each retinular cell has long microvilli. The microvilli project into the tubular lumen, obliterating it. Importantly, the microvilli from adjacent cells are oriented perpendicular to each other forming the distinct area, termed a rhabdom, in the center of the tube (Figures 11.43–11.45). Rhodopsin is concentrated in the microvillar membranes, greatly amplifying the light-induced electrical impulse. The primary optic nerve fibers originate from the retinular cells and travel through a basement membrane into the lamina ganglionaris. There the axons are surrounded by hemolymph. In the lamina ganglionaris, axon fibers form bundles which

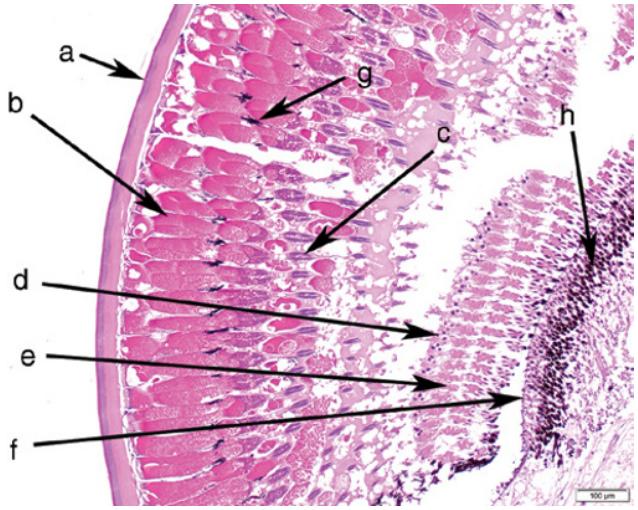


Figure 11.44 Histology of a sagittal section through the eye of a crayfish in a dark environment as determined by the lack of dispersion of pigment granules along the sides of the cones in the distal pigment cells and the lack of dispersion of pigment along the outer membranes of the renticular cells above the basal lamina. (a) Cornea; (b) crystalline cone; (c) crystalline cone stalk; (d) retinular cell nuclei; (e) rhabdom; (f) basal lamina; (g) distal pigment in accessory pigment cells; (h) distal retinular cell axons of optic nerve containing pigment. (Note: spaces between layers are artifacts of processing.) HE.

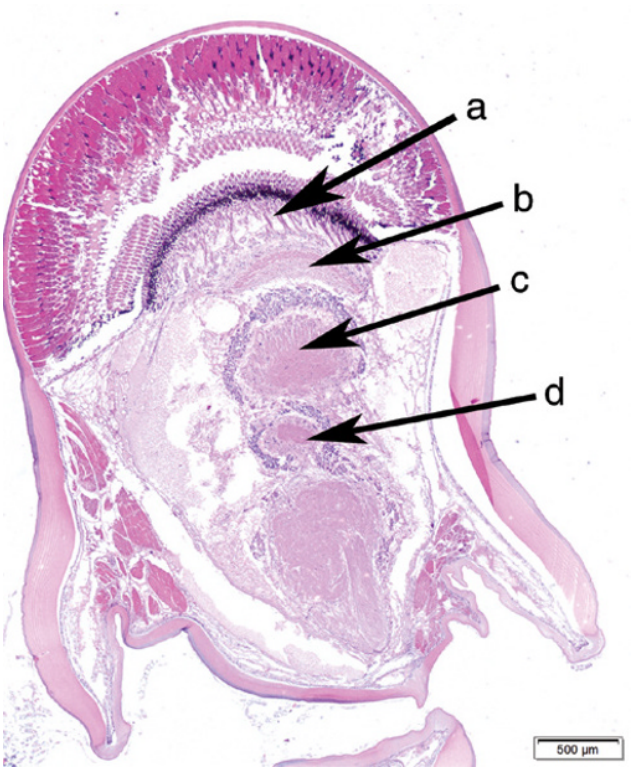


Figure 11.46 Histology of a sagittal section through the eye and eyestalk of a crayfish. (a) Lamina ganglionaris; (b) media externa; (c) media interna; (d) media terminalis. (Note: spaces between layers are artifacts of processing.) HE.

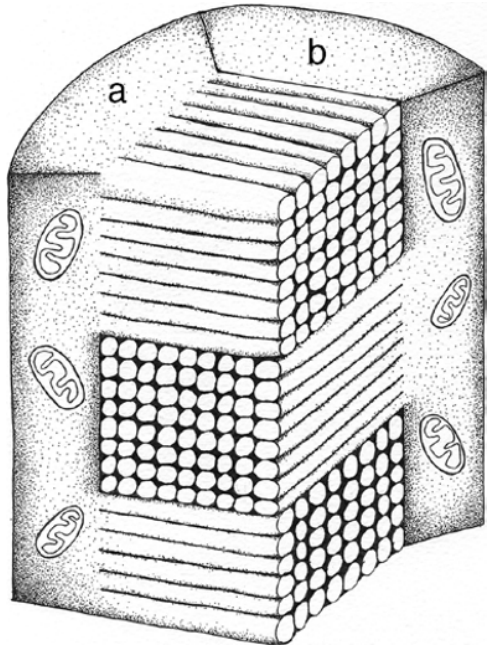


Figure 11.45 Line drawing of the rhabdom of the compound eye of crayfish (*Procambarus clarkia*) showing perpendicular orientation of the microvilli projecting from two retinular cells (a) and (b). *Source:* After Eguchi and Burr from Ruppert et al. (2004). Original drawing by Alicia Schickle.

travel to the distal optic ganglia. The optic ganglia are composed of a (distal) medulla externa, a medulla interna, and a (proximal) medulla terminalis (Figure 11.46).

11.3.10 Endocrine System

The effects of endocrine hormones on molting and reproduction have been well studied in decapods. One set of neuroendocrine glands prevents molting. Between the medulla externa and the medulla interna is the sinus gland, a neurohemal organ. It is located at the junction of the smaller inner and larger outer hemosinus of the eye. The gland is composed of small, round cells which surround tubules that branch from the inner sinus. The gland projects into the outer sinus of the eye stalk. The sinus gland contains several endocrine compounds but some important hormones are the molt-inhibiting hormone (MIH), mandibular organ-inhibiting hormone (MOIH) and gonadal-inhibiting hormone. MIH is produced in the X-organ which is composed of neurosecretory cells in the proximoventral portion of the medulla terminalis. MIH is transferred from there to the sinus gland by long axons (Fingerman 1992; Hartenstein 2006). Eyestalk ablation is sometimes used to

decrease the abundance of MIH in the hemolymph in intermolt time periods and thus promote molting.

The Y-organ is usually a flattened oval and is found bilaterally in the head region associated with the epithelium of the maxillary segment of the head (ventral and lateral to the eyestalks) (Johnson 1980). It is composed of small cells that undergo changes during the molt cycle by developing larger nuclei and increased amounts of cytoplasm. The nuclei regress in size and the cell has minimal cytoplasm post molt. The Y-organ produces ecdysone which stimulates molt. Ecdysone gradually increases in abundance in the hemolymph in the premolt time period, becomes most abundant at molt then decreases quickly immediately after molt. The production of ecdysone is inhibited by MIH (Johnson 1980; Fingerman 1992).

The mandibular organ (MO) produces methylfarnasoate (MF) (Figure 11.47). It is located bilaterally ventral and anterior to the Y-organ, and is adjacent to the ventral epidermis and the muscles of the first maxilla and mandible. The gland is ductless, large, highly vascularized and primarily composed centrally of larger (20×35 µm) cells, often polygonal or spherical in shape with acidophilic granules. In lobsters, smaller cells with a high mitotic index are noted at the periphery of the gland. It is lobulated in some species such as lobsters. MF is similar to juvenile hormone identified in insects and is proposed to have the same functions of regulating growth/molting and reproduction. MF production is inhibited by the hormone named MOIH which is released by the sinus gland (Fingerman 1992; Hartenstein 2006; Kwok et al. 2005).

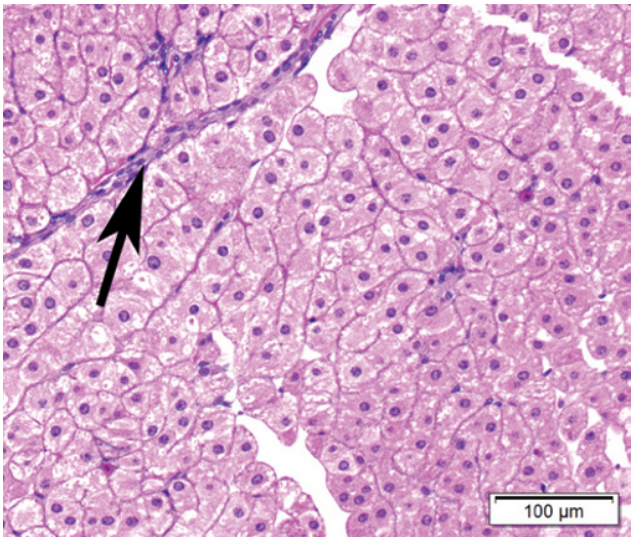


Figure 11.47 Histology of the polygonal cells of the mandibular organ (arrow points to a small arteriole). HE.

Acknowledgments

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12

Arthropoda

Insecta

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12.1 Introduction

Insects are the most diverse group of animals in the world. They are members of the phylum Arthropoda and are identified by three pairs of jointed legs, three body segments, an exoskeleton, one pair of antennae, and usually two pairs of wings. Several families have particular importance for human and environmental health, particularly Apidae (e.g., bees), Drosophilidae (e.g., *Drosophila* sp.), and Culicoidea (e.g., mosquitoes). Two-thirds of all flowering plants depend on insects for pollination. Many insects are parasites and also serve as viral and bacterial disease vectors for humans. Additionally, insects may be the source of massive agricultural devastation, requiring intensive management for food production.

The diversity of this taxon results from their adaptation to virtually every ecologic niche, including land, air, and fresh water. Qualities that are considered the most important to their success include resistance to dessication, flight, and holometabolous development. Holometabolous insects, which include beetles, butterflies, moths, bees, and ants, have immature stages that are markedly different from adult stages (e.g., caterpillar and butterfly). This allows for resource partitioning and decreased competition as the larvae occupy separate ecologic niches from the adults.

The six largest orders of insects are Coleoptera, Lepidoptera, Hymenoptera, Diptera, Hemiptera, and Orthoptera. Coleoptera includes beetles, which have hardened wings. Lepidoptera includes butterflies and moths, which have two pairs of large wings covered with small scales. Hymenoptera includes bees, wasps, and ants, which have two pairs of thin, clear wings (some animals in this class only have wings during one life stage). Diptera includes flies and mosquitoes, which have two pairs of wings with the second pair being markedly reduced in size (and difficult to visualize). Hemiptera includes aphids and cicadas, which have short wings that form a triangle on the back. Orthoptera includes grasshoppers and crickets, which have four wings and jumping hindlimbs (Ruppert et al. 2004). Due to the marked diversity of insects, this chapter primarily uses honeybees for descriptions of anatomy and histology; comparisons to specific groups are provided when necessary.

12.2 Gross Anatomy

The external surface of insects is similar to other arthropods. It is overlain by a chitinous exoskeleton that is made up of hard plates connected by soft cuticles (or sutures). The exoskeleton functions as a protective barrier, prevents loss of water, and provides mechanical support, as skeletal muscle inserts directly to the underside of the exoskeleton. The body is divided into the three sections (or tagmata): head, thorax, and abdomen.

The head bears at least two antennae in all species. Antennal morphology is varied, and is particularly complex in moths. Paired compound eyes and up to three unpaired ocelli are on the head. A number of mouthparts form a preoral cavity, which naturally leads to the oral cavity. The anterior mouthpart is a sclerotinized plate called a labrum, which is sometimes referred to as the "upper lip," and can be raised or lowered by muscular attachments and a soft joint connecting it to the head. The posterior mouthpart is the hypopharynx, which is a soft, appendage-like extension of the head that may function as a tongue. Posterior to the hypopharynx are paired maxillae, which are toothed and have sensory palps. The lateral mouthparts

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include the mandibles. There is some variation in oral anatomy, but the general plan described above is appropriate for most orders.

The thorax consists of three segments, each bearing a pair of legs (forelegs, middle legs, and hindlegs). These thoracic segments are the prothorax (anterior), mesothorax (middle), and metathorax (posterior). The subphylum name "Hexopoda" means six legs. Each leg is divided into six, articulating articles. From proximal to distal, these are the coxa, trochanter, femur, tibia, tarsus, and pretarsus. The thorax in many species bears two pairs of wings, one pair on the mesothorax (forewings) and one on the metathorax (hindwings).

The abdomen is typically composed of 9–11 segments. These can be divided into the pregenital (1–7), genital (8 and 9), and postgenital (10 and 11) segments. The gonopore is usually on segment 8 and 9 in females and males, respectively. Some species have appendages, such as copulatory appendages and stingers, extending from the abdomen (Ruppert et al. 2004).

12.2.1 Dissection

Most insects that are processed for histology can be treated with organs *in situ*. Lateral sections of smaller insects typically provide orientation of organs and are excellent for morphology studies (Figure 12.1). Transverse sections are frequently superior for diagnostic pathology as more tissue can be examined per animal with multiple transverse sections of each body segment (Figures 12.2–12.4). Resin-embedded sections may be superior to paraffin embedded as they maintain the architecture of the chitinous exoskeleton without the need for softening solutions. If dissection of an insect is desired, excellent references are available (Dade 2009; Löw et al. 2016). Dissection typically requires a dissecting microscope, as well as a board or plate for pinning the insect.

12.3 Histology

12.3.1 Body Wall and Coelom

See Table 12.1 for a list of organs that may be identified histologically in insects. As in all arthropods, the exoskeleton encloses the body cavity, called a hemocoel, which contains hemolymph that bathes the viscera and muscle (Figure 12.5). The hemocoel is lined by the basal lamina of the epidermis and gut. It is divided by a perforated horizontal partition (dorsal diaphragm) into a dorsal region (pericardial sinus, which surrounds the heart) and a ventral region (perivisceral sinus, which surrounds the viscera, including the gut, gonads, Malpighian tubules, etc.). Additionally, a ventral diaphragm separates the larger perivisceral sinus from a perineural sinus, which surrounds the ventral nerve cord on the ventral-most aspect of the hemocoel. A ventral diaphragm is absent in Diptera and Coleoptera. The ventral diaphragm generally consists

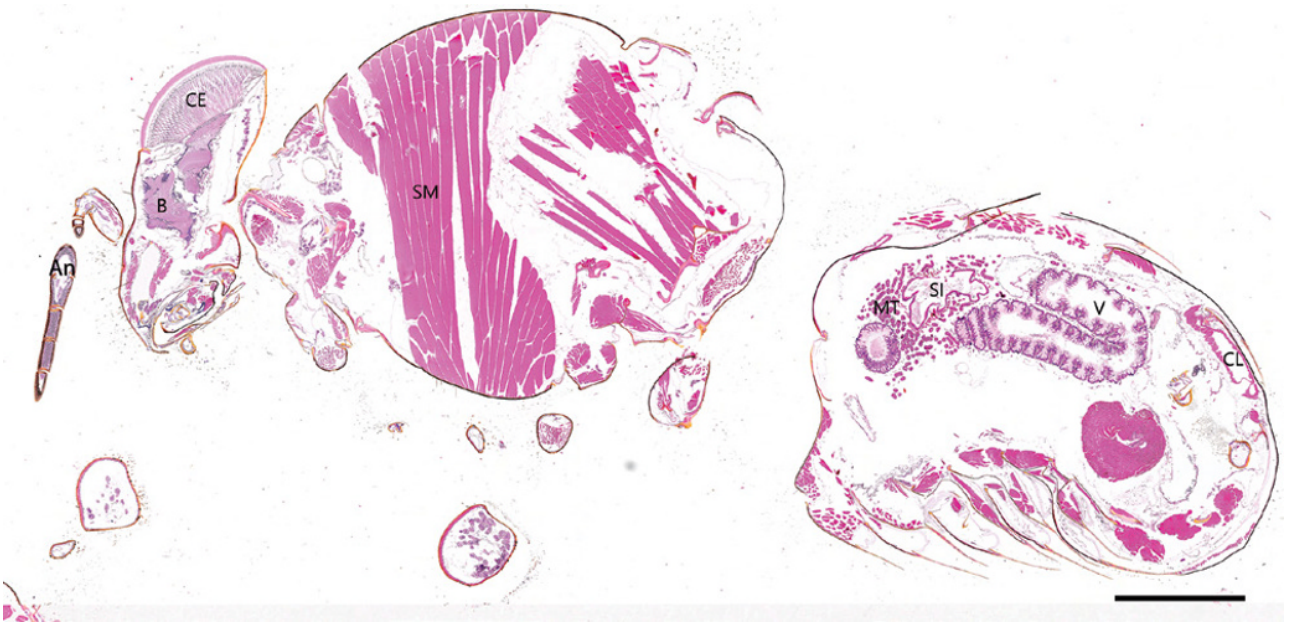
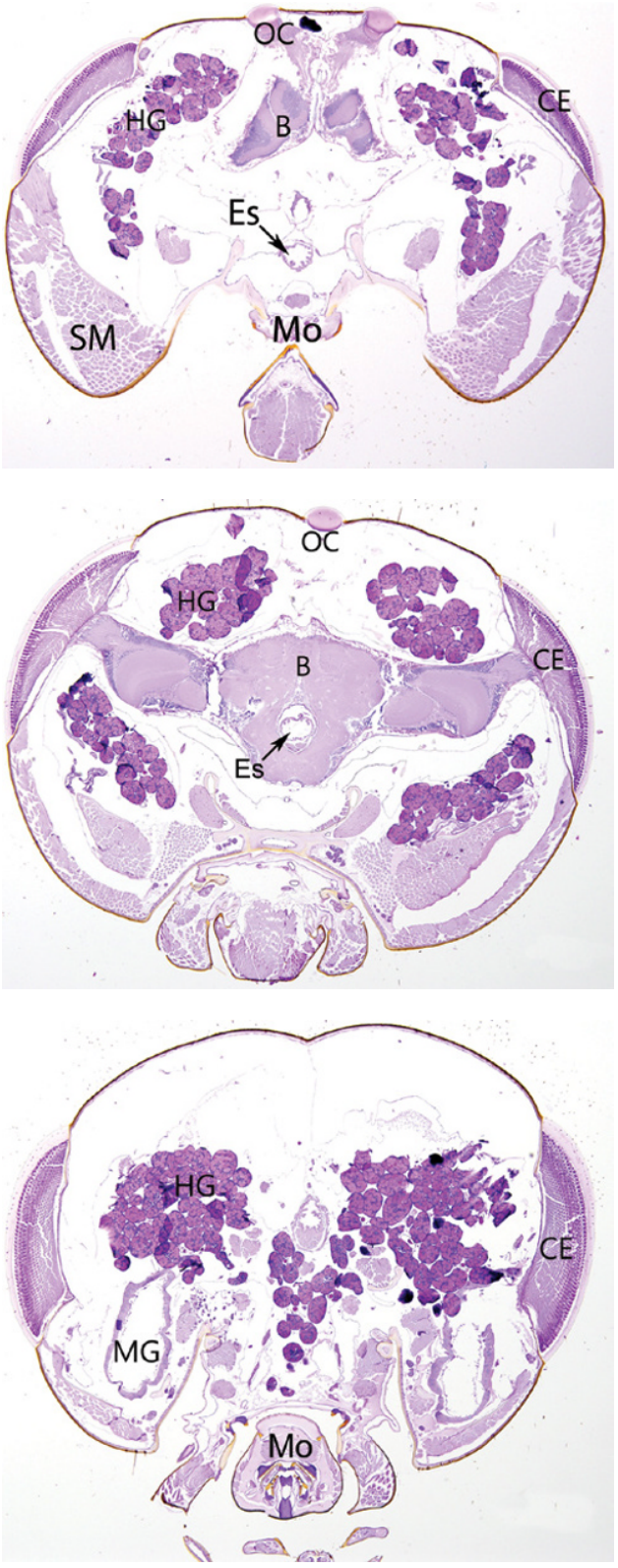


Figure 12.1 Sagittal section of a honeybee (*Apis mellifera*). Scale bar, 1.0 mm. HE. An, antenna; B, brain; CE, compound eye; CL, cardiac lumen; MT, Malpighian tubules; SM, skeletal muscle; SI, small intestine; V, ventriculus.



Figures 12.2–12.4 Transverse sections of the head of a honeybee (*Apis mellifera*). 20×. PAS. B, brain; CE, compound eye; Es, esophagus; HG, hypopharyngeal glands; MG, mandibular gland; Mo, mouth; OC, ocellus; SM, skeletal muscle.

Table 12.1 Organs for histologic evaluation in Insecta.[a]

Organ system		Organs
Body wall/musculoskeletal		Exoskeleton (*cuticle*), skeletal muscle, epidermis (*hypodermis*)
Digestive	Alimentary canal	Foregut (*stomodeum*), midgut (*mesodeum* or *ventriculus*), hindgut (*proctodeum*)
	Fat body	
Excretory		Malpighian tubules
Circulatory		Heart, aorta, sinuses
Immune		Hemocytes
Respiratory		Tracheae, tracheoles
Nervous		Brain (*supraesophageal ganglion*), circumesophageal connectives, subesophageal ganglion, ventral nerve cord
Reproductive	Male	Testes, vas deferens, accessory sex glands
	Female	Ovaries, oviducts, accessory sex glands
Special senses/ organs		Compound eyes, ocelli (may be absent in adults)

[a] Alternative names for organs are provided parenthetically, in italics.

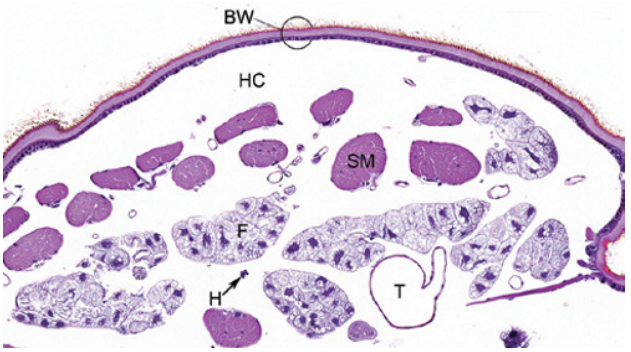


Figure 12.5 Body wall of a gypsy moth caterpillar (*Lymantria dispar*). The body wall is composed of a cuticle subtended by the epidermis. The body wall encloses the hemocoelom, which contains hemolymph. 200×. HE. BW, body wall; F, fat body; H, hemocytes; HC, hemocoelom; SM, skeletal muscle; T, tracheae.

of connective tissue attached to a neuronal cord and muscles (Harrison and Locke 1990). Perforation of both diaphragms allows hemolymph to flow between sinuses (Ruppert et al. 2004).

12.3.1.1 Exoskeleton

The exoskeleton, or cuticle, is composed of alpha chitin and protein. It is secreted by the subjacent epidermis and undergoes molting (or ecdysis) for growth, addition of limbs, etc.

An extension of the exoskeleton is continuous with a cuticular lining of multiple structures that extend deep into the body, including the foregut, hindgut, fat body, and tracheal system (Figure 12.6). The external cuticle is divided into plates called sclerites that are connected by articular membranes.

There are multiple layers of the exoskeleton, specifically a thin, outer epicuticle, a slightly thicker middle exocuticle, and a thick, inner endocuticle (Figure 12.7). The epicuticle is a water-resistant or water-proof layer that either prevents dessication (in terrestrial insects) or restricts osmostic influx (in aquatic insects). In terrestrial insects, the epicuticle includes wax produced by glandular cells in the epidermis that secrete wax through ducts in the endocuticle and exocuticle.

The cuticle is thickest during intermolt periods. Insects deposit cuticle after feeding, resulting in a thickened cuticle. At molting, the new cuticle that is deposited is substantially thinner than that during intermolt (Harrison and Locke 1990).

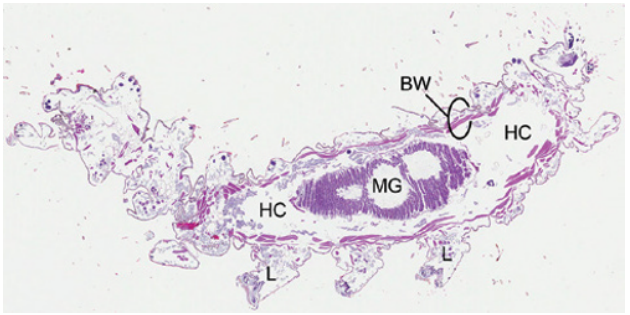


Figure 12.6 Sagittal section of a gypsy moth caterpillar (*Lymantria dispar*). The body wall encloses the hemocoelom, which contains hemolymph and internal viscera. 20×. HE. BW, body wall; HC, hemocoelom; L, legs; MG, midgut.



Figure 12.7 Body wall of a gypsy moth caterpillar (*Lymantria dispar*). Higher magnification of the previous figure revealing three layers of cuticle: outer epicuticle (Ep), middle exocuticle (Ex), and inner endocuticle (En). The epicuticle has numerous small setae. The epidermis (*arrow*) is cuboidal with basal nuclei. Beneath the basement membrane of the epidermis is the hemocoelom (HC). 400×. HE.

12.3.1.2 Epidermis

The epidermis is a simple layer of polygonal cells on a basal lamina. In most regions, the epidermis is columnar, but it can be flattened. The epidermis is referred to in some texts as "hypodermis" as it is subjacent to the cuticle, which is secreted by the epidermis. The epidermis is separated from the subjacent hemolymph by only a basement membrane, which allows the epidermis to phagocytose materials directly from the hemolymph.

The epidermis contains microtubules that are attached to pigment granules. Pigment can move rapidly in the microtubules to produce color changes. During intermolt, the epidermis is active, laying down cuticle and replicating. Dividing epidermal cells may fail to completely separate after replication, resulting in multinucleated and polyploid epidermal cells (Harrison and Locke 1990).

12.3.1.3 Epidermal Glands

Acinar glands are multifocally embedded in the epidermis. Cells comprising these glands are referred to as Class 3 cells. These cells have abundant variability in their organization. In all cases, however, the inner aspect of the cells connects with the outer cuticle via a duct that extends between epidermal cells and through the cuticle. The duct is surrounded by canal cells (which are modified epidermal cells) as it passes through the epidermis (Figure 12.8). The duct is lined by inner endocuticle throughout its length. It can be uniform in diameter throughout its length, or have multiple bulbous distensions.

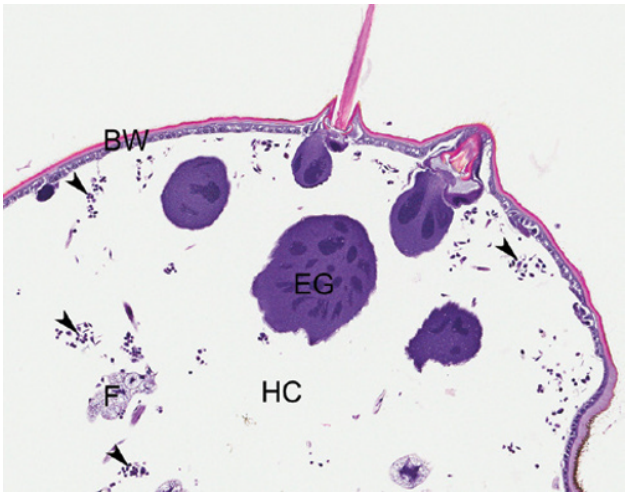


Figure 12.8 Body wall of a gypsy moth caterpillar (*Lymantria dispar*) with epidermal glands. Epidermal glands are present beneath the body wall and empty through ducts in the body wall. Aggregates of cells in the hemocoelom (HC) are hemocytes (*arrowheads*). 100×. HE. BW, body wall; EG, epidermal glands; F, fat body; HC, hemocoelom.

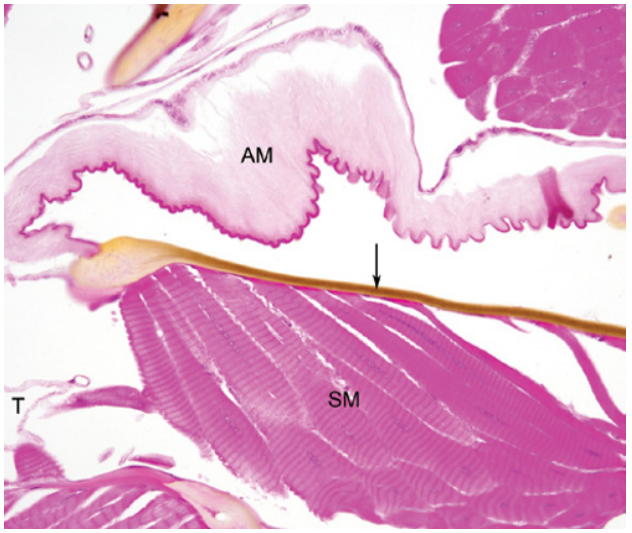


Figure 12.9 Skeletal muscle of a honeybee (*Apis mellifera*). The skeletal muscle (SM) attaches directly to the underside of the sclerite (*arrow*). An arthrodial membrane (soft portion of the cuticle; AM) is adjacent to the site of attachment. T, tracheae. 400×. HE.

In addition to distinct acinar glands, some epidermal cells are modified for glandular secretions. These cells secrete directly into the cuticle and do not have an associated duct. Glandular secretions are typically under hormonal control, and less commonly under nervous control (Harrison and Locke 1990).

12.3.1.4 Connective Tissues

The interior surface of the head portion of the exoskeleton has an elaborate number of apodemes (which are invaginations of the exoskeleton to form inner projections), collectively called the tentorium, which serve as attachment sites for muscles. Skeletal muscles attach directly to the underside of sclerites. Skeletal muscles frequently attach to adjacent sclerites, passing over articular membranes, to allow for contraction and movement. Skeletal muscles are striated (Figure 12.9).

12.3.1.5 Wings

Wings are very thin folds of the body wall. They are composed of a double sheet of epidermis and cuticle (Figure 12.10). There are multiple, arborized veins throughout the wings that are hollow structural elements containing tracheae, nerves, and hemolymph. Large flight muscles in the thorax facilitate wing movement. Flight muscles either insert directly on the wings (i.e., direct muscles) or do not have direct connection (i.e., indirect muscles). Flight muscles may be so large that they occupy the majority of the thorax in some species (Ruppert et al. 2004).

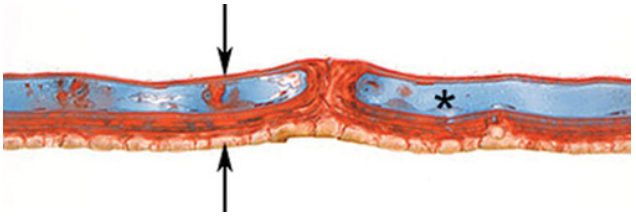


Figure 12.10 Forewing of a beetle (unspecified species). The wing is composed of double sheets of epidermis and cuticle (*arrows*) enclosing hemolymph in the hemocoel (*asterisk*). 200×. Modified trichrome.

After metamorphosis, the wings are initially filled with abundant hemolymph, similar to the remainder of the coelom. Once the wings are opened/spread for the first time, the heart beats in the reverse direction, resulting in hemolymph being sucked back into the abdomen (this process may take several hours). Concurrently, there is postecdysial diuresis, resulting in increased viscosity of the hemolymph, which causes negative hemolymph pressure and expansion of the respiratory tree, specifically the air sacs. Together, these actions cause almost complete loss of hemolymph from the wings, leading to direct apposition of the upper and lower wing laminae. The only hemolymph that remains in the wings is in the wing veins (Harrison and Locke 1990).

12.3.1.6 Air Sacs

There are air sacs occupying a large volume of the coelomic cavity, usually around the waist, in some insects. Smaller air sacs are present in the head, wing veins, and legs. In Lepidoptera, large air sacs occupy the metathorax and first abdominal segment. In some Diptera, large air sacs occupy the first abdominal segment. Air sacs function as an "aerocoel," which partitions the hemocoel.

12.3.2 Digestive System

As in all arthropods, the alimentary tract can be divided into the foregut, midgut, and hindgut. The foregut is generally responsible for storage and some mechanical digestion prior to enzymatic digestion and absorption in the midgut. Salivary glands surround the foregut and are composed of acini supported by abundant ducts (Figure 12.11). In worker bees, there are large glands in the head called hypopharyngeal glands that produce food (royal jelly) for bee larvae (Figures 12.12 and 12.2–12.4). The hindgut functions in fecal formation and resorption of water (Ruppert et al. 2004). The fat body is analogous to the vertebrate liver, but has a greater functional diversity (Harrison and Locke 1990).

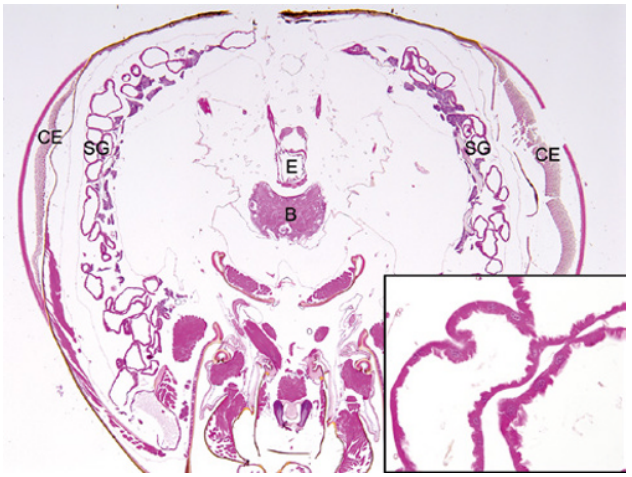


Figure 12.11 Transverse section of the head of a honeybee (*Apis mellifera*) at the level of the compound eyes (CE). The esophagus (E) and brain (B) are in the center of the head, and are surrounded laterally by the salivary glands (SG; inset). 20×. HE.

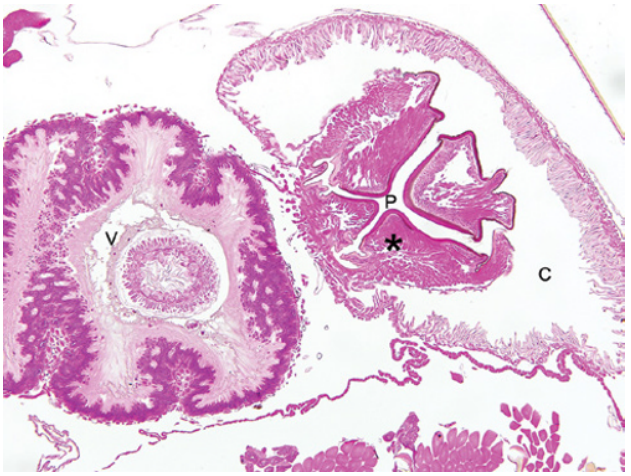


Figure 12.13 Junction of the crop (C), proventriculus (P), and ventriculus (V) in a honeybee (*Apis mellifera*). The gizzard is lined by cushions (*asterisk*) that narrow the lumen and act as a sieve to prevent large particles from entering the ventriculus. 100×. HE.

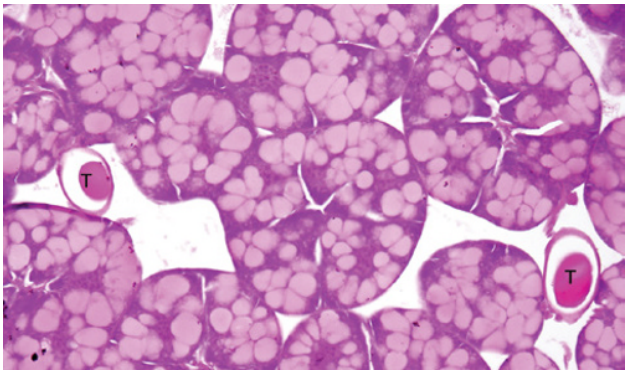


Figure 12.12 Hypopharyngeal glands in a worker honeybee (*Apis mellifera*). 400×. HE. T, tracheoles.

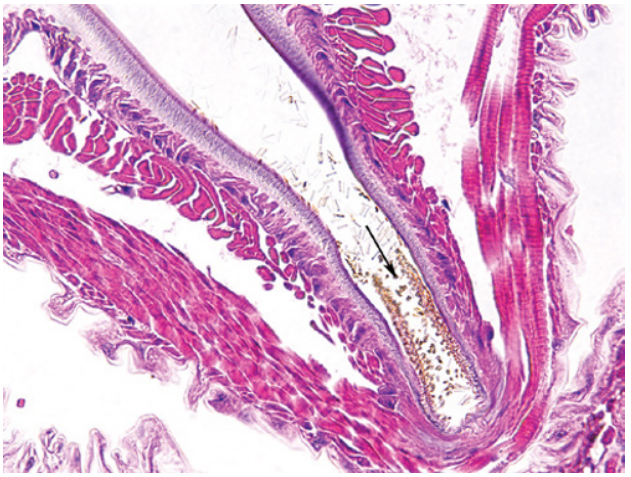


Figure 12.14 Proventriculus of a honeybee (*Apis mellifera*). The cuticular lining of cushions has numerous setae (*arrow*) that act as a sieve to prevent large particles from entering the ventriculus. 600×. HE.

12.3.2.1 Foregut

The foregut (also called stomodeum) can be divided into four parts: pharynx, esophagus, crop, and gizzard (proventriculus). The pharynx has muscles embedded on the frontal dorsal part of the head. The esophagus is thin and short. The crop is large and occupies much of the abdominal hemocoelom. In some Diptera, the crop forms a lateral diverticulum for storage (Harrison and Locke 1990). The gizzard (proventriculus) is a bulge at the termination of the crop and is bordered posteriorly by digestive ceca in some species (Löw et al. 2016; Ruppert et al. 2004). The termination of the proventriculus has soft cushions (also called pulvilli) that have short setae that act as a sieve to prevent larger particles from entering the midgut (Figures 12.13 and 12.14) (Löw et al. 2016).

The foregut is derived from the ectoderm and has an inner cuticular lining subtended by a simple epithelium on a basement membrane (Figure 12.15). These are further surrounded by an inner longitudinal and outer circle muscle layer. In most of the foregut, the cuticle is nonsclerotized and flexible. In insects with biting mouthparts, the foregut cuticle is sclerotized in the proventriculus. The proventriculus may bear sclerotized (toothed) plates (or teeth) or spines that facilitate continued mechanical breakdown of food.

Labial glands produce saliva and are present in the thorax with their ducts emptying adjacent to the hypopharynx in most terrestrial insects, except those of Coleoptera. In Lepidoptera, labial glands make silk and the saliva is produced by mandibular glands. Labial and mandibular glands are ectodermal, and the duct is lined internally by a cuticle. These glands may be tubular or racemose. They are

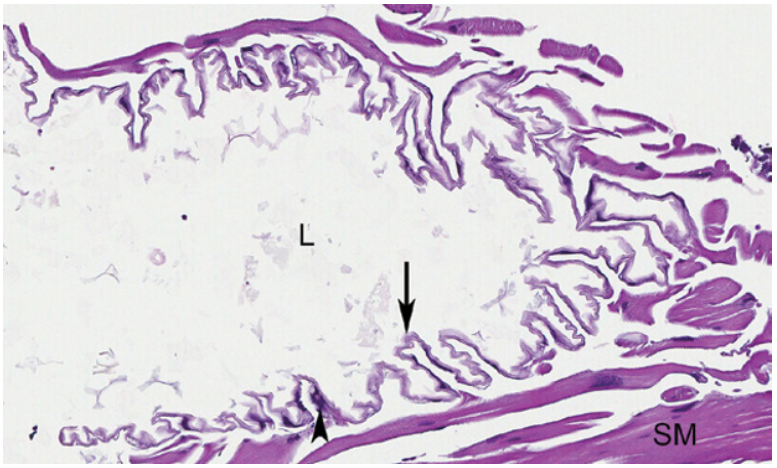


Figure 12.15 Foregut of a gypsy moth caterpillar (*Lymantria dispar*). The lumen (L) is surrounded by thin, simple epithelium (nucleus of an epithelial cell is highlighted with an arrowhead) with an inner cuticle (*arrow*). The epithelium is further surrounded by layers of striated muscle (SM). 200×. HE.

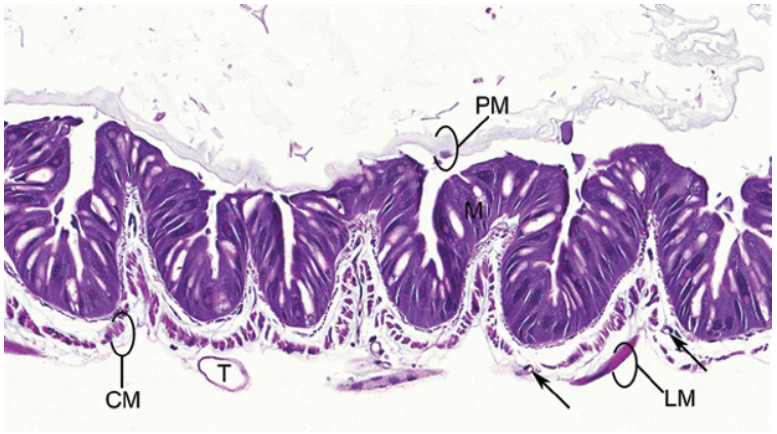


Figure 12.16 Midgut (ventriculus) of a gypsy moth caterpillar (*Lymantria dispar*). The mucosa (M) is overlain by a peritrophic membrane (PM). Circular (CM) and longitudinal (LM) muscle layers surround the midgut. Tracheae (T) are larger diameter respiratory channels. Tracheoles (*arrows*) are smaller diameter respiratory channels that ramify into the tissues. 400×. HE.

composed of tubules and acini of secretory and parietal cells. Secretory cells have granules and produce saliva. Mandibular glands also produce pheromones in bees. Parietal cells are smaller with basal nuclei; these may be involved in replacing damaged secretory cells or in ion exchange. Salivary glands of mosquitoes are particularly well studied due to their involvement of human disease transmission. In mosquitoes, salivary glands enlarge and fill with secretory product in adults. Age-dependent degeneration of salivary glands follows with degeneration, cytoplasmic vacuolation, and a decrease in size. Saliva of mosquitoes contains anticoagulant to prevent the blood meal from clotting (Harrison and Locke 1990).

12.3.2.2 Midgut

The midgut is also called the mesodeum or ventriculus (Stell 2012). A peritrophic membrane overlies the mucosa of the midgut and surrounds food boluses in most insect species (Figures 12.16 and 12.17). It is a thin, mesh-like acellular layer composed of chitin and proteins, through which midgut epithelial microvilli sometimes protrude. The peritrophic membrane protects the midgut mucosa as this region of the intestinal tract is not covered in cuticle

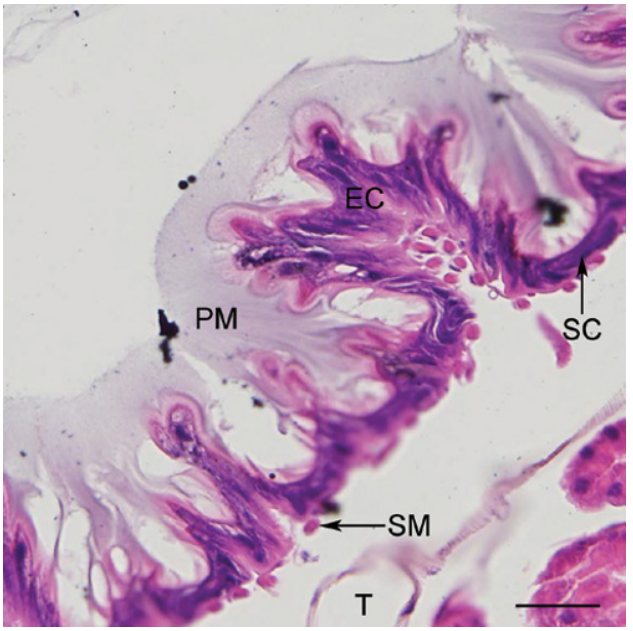


Figure 12.17 Ventricular mucosa of a honeybee (*Apis mellifera*). EC, epithelial cells of the mucosa; PM, peritrophic membrane; SC, stem cells; SM, striated muscle; T, tracheae. Scale bar, 50 µm. HE. Source: Contributed by Dr Colby Klein.

(unlike the rest of the enteric tract). The lumen contains symbiotic organisms, some of which provide vitamins to the host that the host is otherwise incapable of producing. The peritrophic membrane permits the passage of electrolytes, water, and solutes across the midgut mucosa, which is where the majority of absorption occurs (Harrison and Locke 1990).

The midgut is generally composed of simple cuboidal to columnar cells with occasional interspersed goblet cells, neuroendocrine cells, and regenerative cells (Harrison and Locke 1990; Löw et al. 2016). Regenerative cells can occur individually or in clusters, and are often associated with endocrine cells. Regenerative cells are basally located stem cells surrounded apically by tall columnar secretory cells. Endocrine cells are variably shaped (e.g., pyramidal, oval, fusiform) and lie on the basement membrane with only a thin neck of cytoplasm extending to the lumen. The basement membrane is surrounded by very thin circular and longitudinal muscle layers (Figures 12.16 and 12.17). In some species, the midgut has multiple digestive ceca branching off the anterior portion; these allow for increased surface area for water and other absorption. Digestive ceca have similar histology to the rest of the midgut except they do not contain food boluses and lack a peritrophic membrane (Löw et al. 2016). Midgut cells sometimes contain multilayered mineral concretions with various metals. The number and size of these increase with age, and they may represent storage.

12.3.2.3 Hindgut

The hindgut serves in both food absorption and osmoregulation as the contents of the Malpighian tubules empty into this segment. It is composed of an anterior intestine (comprising ileum and colon) and a posterior rectum, both of which are covered by a cuticle (Figure 12.18). The intestine may be separated from the midgut by a muscular sphincter and the Malpighian tubules. The length of the hindgut reflects the animal's diet; omnivorous animals typically have long hindguts, while herbivorous and blood-sucking insects typically have short hindguts. Termites have symbiotic flagellates in the hindgut that aid in digestion of cellulose. The hindgut terminates at the anus, which opens at the posterior abdomen and is controlled by a muscular sphincter (Harrison and Locke 1990; Ruppert et al. 2004). The rectum is a primary location for water resorption and also functions in salt balance (along with the Malpighian tubules). Rectal pads (also called rectal papillae) in some species are specialized epithelium in the rectal mucosa to facilitate water resorption (Löw et al. 2016). In the majority of the hindgut, the epithelium forms a single layer but in the rectal pads, it can be complex and multilayered. Variations of the rectal pads are many, including simple pads that are an outpouching lined by simple epithelium, to extensive, multilayered epithelia that interdigitate with Malpighian tubules.

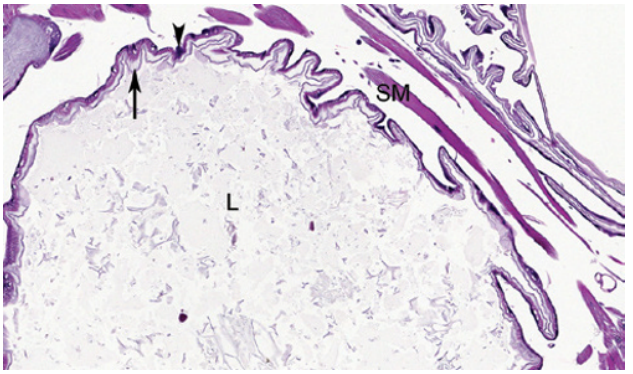


Figure 12.18 Hindgut of a gypsy moth caterpillar (*Lymantria dispar*). The lumen (L) contains plant material and other digesta. The mucosa is simple (nucleus of a mucosal epithelial cell is highlighted with an arrowhead) with an internal cuticle (*arrow*). The epithelial basement membrane is surrounded by layers of striated muscle (SM). 150×. HE.

The internal surface of the hindgut is lined by a semipermeable cuticle that is continuous with the body wall. The epithelium of the intestine is low cuboidal and that of the rectum is columnar. Inner circular and outer longitudinal muscles surround the intestines, and are perforated by the tracheal system supplying oxygen to all layers of the hindgut. Muscular layers are relatively thin in the anterior hindgut. Muscular layers are surrounded by a connective tissue sheath.

12.3.2.4 Fat Body

The fat body is analogous to the vertebrate liver in that it synthesizes and stores lipid, carbohydrates, proteins, and excretory products. Glycogen stores in the fat body are used as a source of glucose. The fat body fills most of the body cavity. It can be divided into the visceral fat body, which surrounds the gut, and the parietal fat body, which is internal to the body wall (Ruppert et al. 2004). The primary cell is the adipocyte (also called trophocyte), which is a large polygonal cell with a large amount of finely vacuolated cytoplasm filled with glycogen and lipid droplets (Harrison and Locke 1990; Löw et al. 2016). There are four sequential arrangements of adipocytes that correspond to development: single-layered ribbons, double-layered ribbons, separate cells, and lobes attached to tracheae. In larvae, fat bodies proliferate in a ribbon plane, forming long, single-layered ribbons the length of the animal. After this, in the premetamorphic animal, adipocytes proliferate at right angles until the entire fat body is two cell layers thick.

During pupation, the adipocytes separate. In adult insects, adipocytes are reorganized into lobules that are

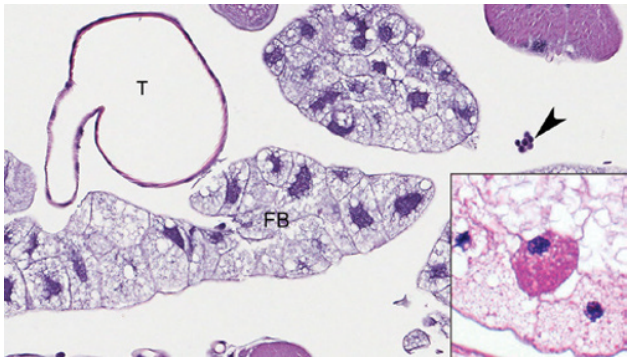


Figure 12.19 Fat body of a gypsy moth caterpillar (*Lymantria dispar*). The fat body (FB) adipocytes (also called trophocytes) are polygonal with numerous cytoplasmic vacuoles representing lipid, glycogen, and other storage products. A trachea (T) and small cluster of hemocytes (*arrowhead*) in the hemocoelom are also present. Inset: An oenocyte (large polygonal cell with eosinophilic granules) is present admixed with adipocytes, which contain numerous calcium spherules, in the fat body of a Madagascar hissing cockroach (*Gromphadorhina portentosa*) with bacterial sepsis. 400×. HE.

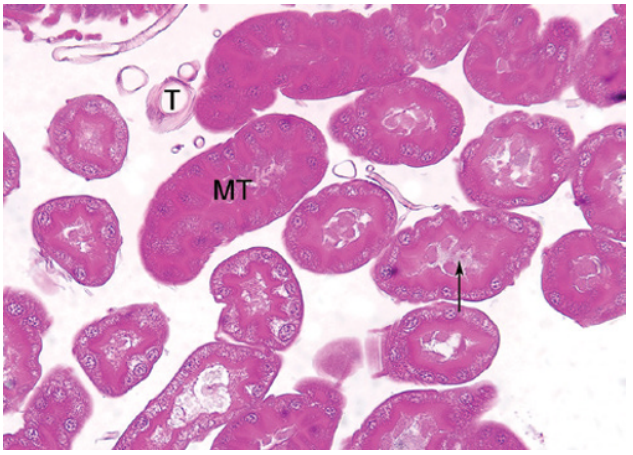


Figure 12.20 Malpighian tubules of a honeybee (*Apis mellifera*). The tubules (MT) are lined by a simple layer of cuboidal cells on a basement membrane; no muscle layers are present. Some lumina contain excretory product (*arrow*). 400×. HE. T, tracheae.

variably distinguishable depending on the species. The entire fat body is invested in a basal lamina. There are numerous granules in adipocytes (Figure 12.19). Half of the volume of the fat body is due to glycogen and lipid (Harrison and Locke 1990). Some adipocytes store urates (urocytes) or symbiotic organisms such as bacteria (mycetocytes). In honeybees and some other insects, the fat body is a mixture of adipocytes and oenocytes. Fat bodies in all insects have numerous tracheae and tracheoles, but no obvious innervation (Harrison and Locke 1990). The fat body also functions in the synthesis of vitellogenin for ova production, and the synthesis of some components of the humoral immune system (e.g., antimicrobial peptides) (Hillyer 2016).

12.3.3 Excretory System

The primary excretory system is the Malpighian tubules, which are blind-ended, tubular diverticula that branch from the terminal portion of the midgut (Figure 12.20). These tubules are bathed in hemolymph (as are all viscera), from which they absorb ions, water, toxins, and nitrogenous wastes such as uric acid. They create hyperosmotic urine that is then excreted into the tubular lumen. From there, the wastes are transported to the hindgut (or in some species midgut), where the acidic pH leads to precipitation of the wastes, which are refractile under polarized light (Ruppert et al. 2004). The rectal epithelium resorbs sugar, ions, and water from the urine and returns it to the hemolymph. Malpighian tubules are small tubules lined by a simple cuboidal epithelium on a thin basement membrane. In cross-section, Malpighian tubules may have approximately 2–12 epithelial cells (Löw et al. 2016). Malpighian tubules are densely tracheated, with tracheoles spread widely and evenly over the surface of the tubules (Harrison and Locke 1990).

Malpighian tubules are present in most insects except for aphids. Insects lacking Malpighian tubules have urate cells in the fat body that concentrate uric acid, and/or saccate nephridia in the head for excretion. Aquatic insects excrete ammonia rather than uric acid (Ruppert et al. 2004). There is enormous diversity in the organization of Malpighian tubules. Broadly and in most species, Malpighian tubules contain more than one cell type; segmented is the most common organization pattern (see below) and most segments have a large primary cell and fewer, smaller, secondary (or stellate) cells. Primary cells commonly contain crystalline inclusions that are concentric, often spherical, mineralized lamellations.

Four general categories of organization have been reported: (i) segmented with uniform cells, (ii) nonsegmented with diverse cells, (iii) segmented with diverse cells, (iv) bilateral asymmetry of cell types. Segmentation of the Malpighian tubules means that cells are differentiated for various functions depending on the location of the segment. Most insects have segmented Malpighian tubules with diverse cells. In animals with uniform cells per segment, the most distal, secretory segment (which produces urine) may have a single, binucleate cell type with cytoplasmic crystals, while the proximal segments are composed of uninucleate cells without cytoplasmic crystals. Nonsegmented tubules have homogenous composition along their entire length. These typically have a consistent ratio of intermixed stellate and primary cells. Primary cells

produce urine and have intracellular crystals. Stellate cells are small and thin without crystals; their function is unclear, but may be related to ion exchange. In some insects, the tubules on opposite sides of the animal are not identical; this is called bilateral asymmetry and occurs in many flies, including *Drosophila* spp. (Harrison and Locke 1990).

In addition to Malpighian tubules, some apterygotes (wingless insects) also have labial kidneys, which function in ultrafiltration and reabsorption, similar to vertebrate kidneys. Labial kidneys are absent in pterygotes (winged insects). Labial kidneys are paired and have three parts: sacculus, coiled tubule, and excretory duct. The sacculus is lined by a simple epithelium composed of podocytes that bulge into the lumen. These podocytes ultrastructurally have foot processes creating filtration slits with the basement membrane, analogous to the vertebrate glomerulus. The coiled tubule is lined by a simple epithelium ranging from low to tall columnar with an apical nucleus. The excretory duct is lined by a simple epithelium with an apical nucleus and a thin inner cuticular lining.

12.3.4 Circulatory System

As with all other arthropods, the circulatory system includes the heart, aorta, sinuses, and hemolymph. The heart is a tubular organ composed of circular muscles and lies in the pericardial sinus (Figure 12.21). Paired ostia are perforations in the heart that allow for transport of hemolymph into or out of the heart. Alary muscles in each segment suspend the heart in the pericardial sinus and contract, leading to diastole, or expansion of the cardiac lumen to fill the heart with hemolymph. Contraction of the cardiac muscles results in systole and pushing of the hemolymph into the aorta (Harrison and Locke 1990; Löw et al. 2016).

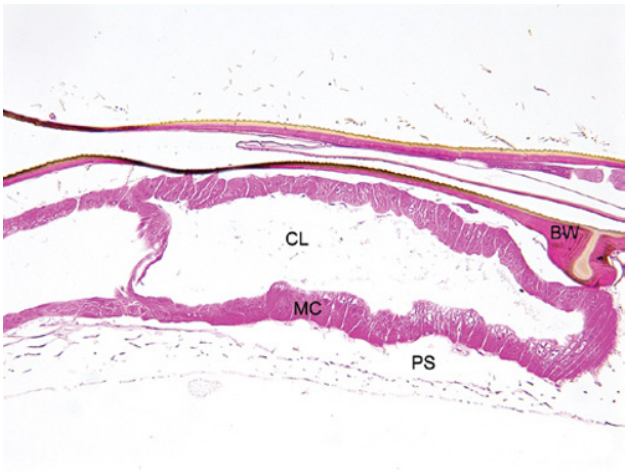


Figure 12.21 Heart of a honeybee (*Apis mellifera*). The heart is a tubular organ enclosed in the pericardial sinus (PS) in the dorsal aspect of the hemocoelom. 150×. HE. BW, body wall; CL, cardiac lumen; MC, myocardium.

In an open circulatory system, cardiac pumping alone is not sufficient to supply hemolymph to the appendages such as antennae, mouthparts, legs, and wings. Special structures allow either passive transport of hemolymph to appendages or, more often, active transport via accessory pulsatile organs that are located at the base of the appendages. Some insects also have accessory diaphragms that help partition the hemocoel of the appendages into sinuses in hemolymph distribution and flow. Vessels extending into many appendages are associated with ampullae and muscles that pump hemolymph and comprise the accessory pulsatile organs. In some insects, muscular swellings of the aorta comprise the pulsatile organs. Muscular diaphragms may also serve as accessory pulsatile organs. Vessels may branch off the aorta or be discontinuous (Harrison and Locke 1990).

12.3.4.1 Heartbeat Reversal

Most holometabolous insects have one-way ostia valves allowing for influx, but preventing efflux, of hemolymph. Some insects have two-way ostia to allow a unique pumping mechanism known as heartbeat reversal, which is retrograde pumping of the heart. The primary function is to remove hemolymph from various parts of the body. This can occur regularly in some adult insects to remove hemolymph that has accumulated in venous parts of the body, and in postmetamorphosis winged insects to remove hemolymph from the wings (Harrison and Locke 1990; Slama 2010). Periodic heartbeat reversal may also help to facilitate respiration by changing hemolymph volume and thus exerting negative pressure on the tracheal system (Harrison and Locke 1990).

12.3.4.2 Pericardial Cells or Athrocytes

Athrocytes are cells suspended in soft tissue strands attached to either the dorsal or ventral sides of the heart and the body wall or salivary glands. Suspensory tissue is a combination of muscle and connective tissue. These cells have intimate contact with hemolymph, from which they pinocytose macromolecules that can traverse the basal lamina covering them. Pinocytosed material is stored in athrocytes' cytoplasm in storage granules. Athrocytes synthesize protein and have a role in iron metabolism. They have an extensive reticular system that is expanded and relaxed with the pumping of the heart; this allows for increased contact with the hemolymph for pinocytosis. Athrocytes are arranged in clusters. They can become markedly large in size as they typically increase in size, not

number, over an animal's lifespan. They are frequently binucleate (Harrison and Locke 1990).

12.3.5 Immune System

The primary immune cells of insects are hemocytes. Hemocytes drive both cellular immune responses (e.g., phagocytosis) as well as humoral immune responses (e.g., melanization). Hemocytes differ from all other tissues in their lack of a basal lamina. This allows them to perform both pinocytosis and phagocytosis. Hemocytes are present both in circulation (i.e., circulating hemocytes) and attached to tissues (i.e., sessile hemocytes). Hemocytes tend to aggregate around the heart, where hemolymph flow is highest and they are most likely to encounter pathogens. This is also a focus of hematopoiesis in adults; in larval stages, hematopoiesis occurs in many locations. Cellular immunity can result in phagocytosis (i.e., engulfment by an individual cell) or in the formation of capsules and nodules; all can be associated with melanization.

Cellular encapsulation (i.e., formation of a capsule) occurs with pathogens that are too large for phagocytosis or nodulation. During encapsulation, hemocytes attach directly to a large pathogen (e.g., nematode, parasitoid, etc.) or foreign body via specific integrins and form a layer around it, effectively isolating the pathogen or foreign body. Nodulation (i.e., formation of a nodule) occurs when hemocytes attach to each other to surround a smaller pathogen (usually bacterial aggregate) and discharge their contents, encasing the pathogen in antimicrobial substances. Nodulation and encapsulation appear similar (to identical) morphologically. In some species, distribution of the hemocytes in capsules and nodules is organized and in others it is random. For example, in some species the first hemocytes to attach are granulated (sometimes called granulocytes) and the later hemocytes surrounding the outside are not granulated (sometimes called plasmatocytes).

Melanization is involved in the killing of pathogens (e.g., bacteria, fungi, parasites) via oxidation and/or starvation. Melanization causes a pigmented, proteinaceous capsule to surround an invading pathogen. Melanin precursors (i.e., prophenoloxidase) and enzymes involved in melanin formation are produced by hemocytes, specifically called oenocytoids (i.e., cells with cytoplasmic phenoloxidase precursors) in some species. Humoral immunity contributes to pathogen killing via lysis, which causes rupture of the pathogen's cellular membrane due to antimicrobial peptides, lysozymes, etc. (Hillyer 2016; Lavine and Strand 2002)

Generally, all immune cells in insects can be called "hemocytes" but distinct types of hemocytes have been categorized based on function for some animals. Hemocyte categorization and nomenclature is not standardized among Insecta. For example, the fruit fly (*Drosophila melanogaster*) has phagocytic plasmatocytes and encapsulating lamellocytes, while lepidopteran larvae have phagocytic granulocytes and encapsulating plasmatocytes (Hillyer 2016; Lavine and Strand 2002)! The various types of immune cells are difficult to discern using bright-field light microscopy, and most descriptions elaborating on the different hemocytes of insects rely on ultrastructural features.

12.3.6 Respiratory System

The tracheal system is essentially an invagination from the body wall, and as a result is lined internally by cuticle. The system is a series of tubes (tracheae) that conduct oxygen to the tissues by fine branches of the system (tracheoles) that ramify directly into the tissues. In aquatic larvae and small insects, the tracheal system is closed and the tracheae branch at both gills and other tissues. In terrestrial insects, the tracheal system is open as tracheae communicate directly with the outside through spiracles. Spiracles are paired ostia in the body wall that lead to air-filled tubes (tracheae) that deliver oxygen to tissues. There are usually 10 pairs of spiracles (two thoracic and eight abdominal) that are each controlled by muscles that facilitate opening and closing of the spiracles. There are usually four main pairs of longitudinal tracheal trunks in the abdomen, and a more complex system in the thorax. Tracheae branch repeatedly and become smaller as they approach their target organ, where they become tracheoles. The density of the tracheal system is directly proportional to the metabolic demands/oxygen requirements of the tissue.

There are some modifications to the spiracle-tracheae-tracheole system in certain insects. This is predominantly true in subadult stages of aquatic insects, some of which lack spiracles and tracheoles, or have modifications of either or both (Ruppert et al. 2004). Tracheal "gills" are present in many aquatic larvae and in the pupae of some trichopterans (caddisflies).

12.3.6.1 Spiracles

Each spiracle has an external opening on a plate (or peritreme) that leads to longitudinal and transverse tracheal trunks. The peritreme may have spines or hairs that act as a filter for the air. Spiracles vary widely in number for species with this system. Apterygotes lack spiracles.

12.3.6.2 Tracheae

Tracheae are a system of cuticle-lined tubes (Figure 12.22). The cuticle is continuous with that of the integument. In larger tracheae, the cuticle is shed during molting and with each molt the tracheal diameter increases. The primary components of the tracheae are the cuticle and a basal lam-



Figure 12.22 Tracheae (T) in the superficial aspect of the hemoceolom of a gypsy moth caterpillar (*Lymantria dispar*). 200×. HE. BW, body wall.

ina that is up to 300 nm wide. The basal lamina is widest in portions of the tracheae that are not embedded in tissue, and thinnest in embedded portions where the lamina can be as thin as 15 nm.

12.3.6.3 Tracheoles

Tracheoles are fine, approximately 1–4 µm tubes within the cytoplasm of tracheole cells, which are present at the terminal branching points of tracheae (Figure 12.23). Tracheole walls are <0.1 µm diameter, allowing for gas diffusion (Löw et al. 2016). Besides being associated with cells, another difference between tracheae and tracheoles is that the cuticle of tracheoles is not shed during molting.

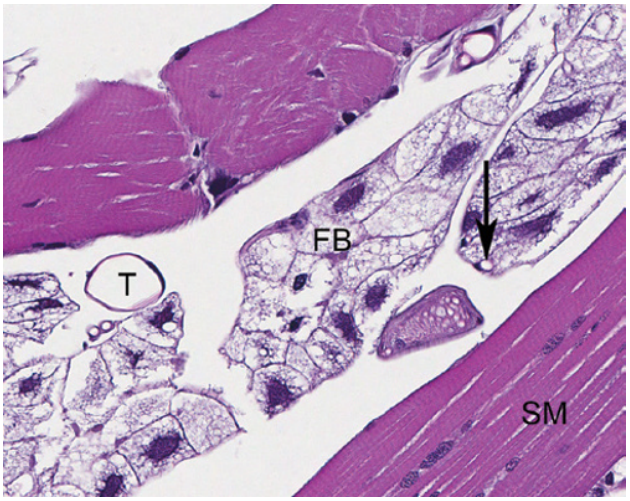


Figure 12.23 Tracheoles in a gypsy moth caterpillar (*Lymantria dispar*). Tracheoles (*arrow*) ramify directly into the fat body (FB). Tracheae (T) are larger diameter air-filled tubes. 400×. HE. SM, striated muscle.

12.3.6.4 Tracheal Gills

Tracheal gills are outgrowths, most commonly on the abdominal segment, that are thin lamellae or narrow filaments, depending on the species. These gills allow for absorption of additional oxygen from the environment. Gills are covered by a thin cuticle (approximately 0.6 µm thick) covering densely packed subcuticular tracheoles. Tracheal gills in some species have a central hemocoel surrounded by epidermal cells. Rarely, specialized cells for osmoregulation (chloride cells), as well as fat body cells, are also present in tracheal gills (Harrison and Locke 1990).

12.3.7 Nervous System

The central nervous system consists of a brain (also called supraesophageal ganglion), circumesophageal connectives (a ring of nervous tissue encircling the foregut), subesophageal ganglion, and ventral nerve cord. The brain in most insects consists of, in order from anterior to posterior, a protocerebrum (innervates optic system), deutocerebrum (innervates antennae), and tritocerebrum (innervates labrum). The subesophageal ganglion is connected to the tritocerebrum by the circumesophageal connective, which forms a ring around the foregut (see Figure 12.3). The ventral nerve cord is the posterior extension of the subesophageal ganglion and extends the length of the body (Figures 12.24 and 12.25). At each body segment, the ventral nerve cords bulge due to ganglia, from which nerves radiate laterally (Löw et al. 2016;

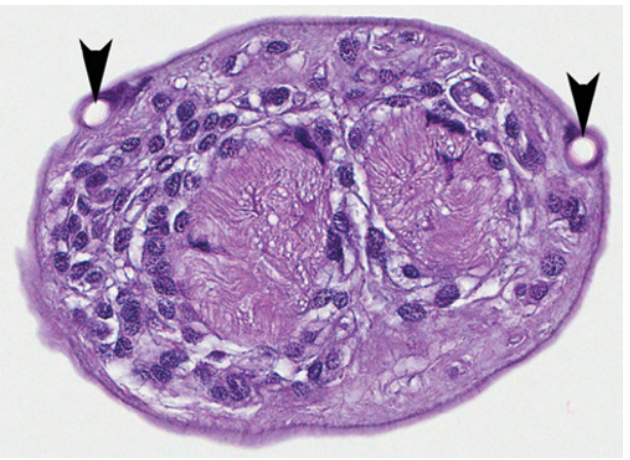


Figure 12.24 Transverse section of a ventral nerve cord of a gypsy moth caterpillar (*Lymantria dispar*). The tracheoles (*arrowheads*) ramify into the tissue. 300×. HE.

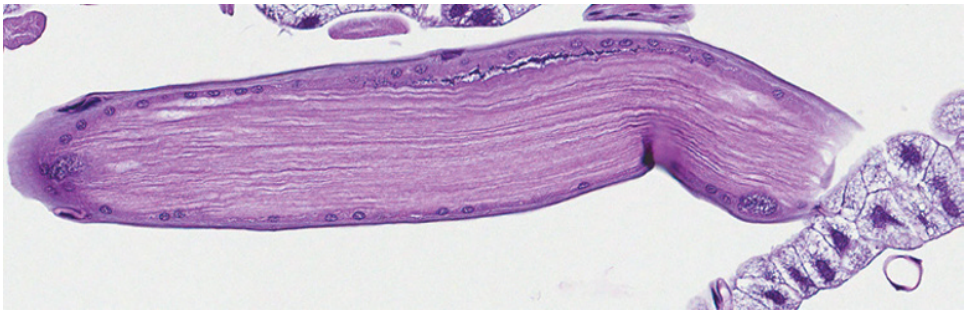


Figure 12.25 Longitudinal section of a ventral nerve cord of a gypsy moth caterpillar (*Lymantria dispar*). 300×. HE.

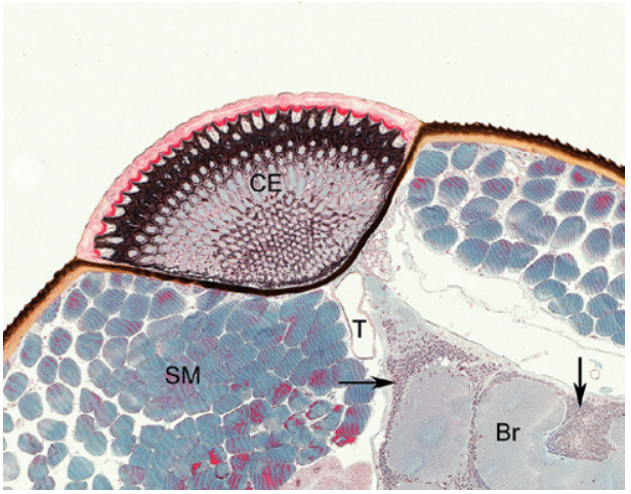


Figure 12.26 Section through the head of a carpenter ant (*Camponotus* sp.). The outer layer of neurons (*arrows*) of the brain (Br) are small and densely clustered. 100×. Modified trichrome. CE, compound eye; SM, skeletal muscle; T, tracheae.

Ruppert et al. 2004). Neuroendocrine tissue that controls molting, reproduction, heart beat, etc. is located posterior to the protocerebrum.

The brain is surrounded by a thin connective tissue capsule. Near the surface of the brain, neurons are small and densely packed with minimal cytoplasm; these neurons are called mushroom body cells or Kenyon cells (Figure 12.26).

12.3.8 Reproductive System

Insects are sexually dioecious. Sperm transfer is via direct transfer (i.e., copulation) in most species. Fertilization is internal within the abdomen (Ruppert et al. 2004).

12.3.8.1 Female Reproductive System

Females have paired ovaries and respective oviducts in the dorsal aspect of the abdominal cavity. The ovary is composed of ovarioles, which are tubular organs that are proximally thin at the germaria (center for germ cell mitosis and differentiation of oogonia into primary oocytes), and distally large at the vitellarium (where yolk is added to the maturing oocyte) (Figure 12.27). Only in *Drosophila* spp. and related species do germaria have functioning stem cells throughout the animal's reproductive life. In most insect species, the germarium is filled with oocytes and arrested stem cells. In these animals, the developing oocyte is surrounded by a cellular layer that secretes the chorion; the size of the germarium decreases throughout the animal's life due to the lack of functioning stem cells. In termite queens and coccids, stem cells may be reactivated to produce new ovarioles throughout the animals' reproductive life. In some insects, including gall midges, ovaries are reduced to small germ cell clusters that develop free in the body cavity.

The ovariole is surrounded by a layer of cells, muscle, and tracheoles, collectively called the ovariole envelope. In some species, the ovariole envelope persists and in others it is substantially reduced over a lifetime. The oviducts join to form the vagina, from which branch accessory glands and a seminal receptacle. The vagina opens via a ventral gonopore on the eighth body segment in most species. Oviposition occurs either via appendages (i.e., ovipositors) on segments 8 and 9, or via telescoping of segments 6 through 9 (Ruppert et al. 2004).

12.3.8.2 Male Reproductive System

Males have paired testes that pass sperm to vasa efferentia that terminate in seminal vesicles, which store sperm (Speigel et al. 2013). The seminal vesicle has three layers of epithelial cells that correspond to the outer epithelial layer of the seminal vesicle, the invaginated inner layer of the seminal vesicle, and the circular epithelial layer of the inserted vasa deferentia. Sperm leave the seminal vesicle via the vasa deferentia, which connect to a common ejaculatory duct that opens through the gonopore on a penis (also called aedeagus) on segment 9. Testes are composed of tubules of developing germ cells surrounded by connective tissue (Figure 12.28). Anteriorly, testes are composed of spermatogonia that progress in development posteriorly to spermatocytes, spermatids, and sperm. Spermatozoa are long and

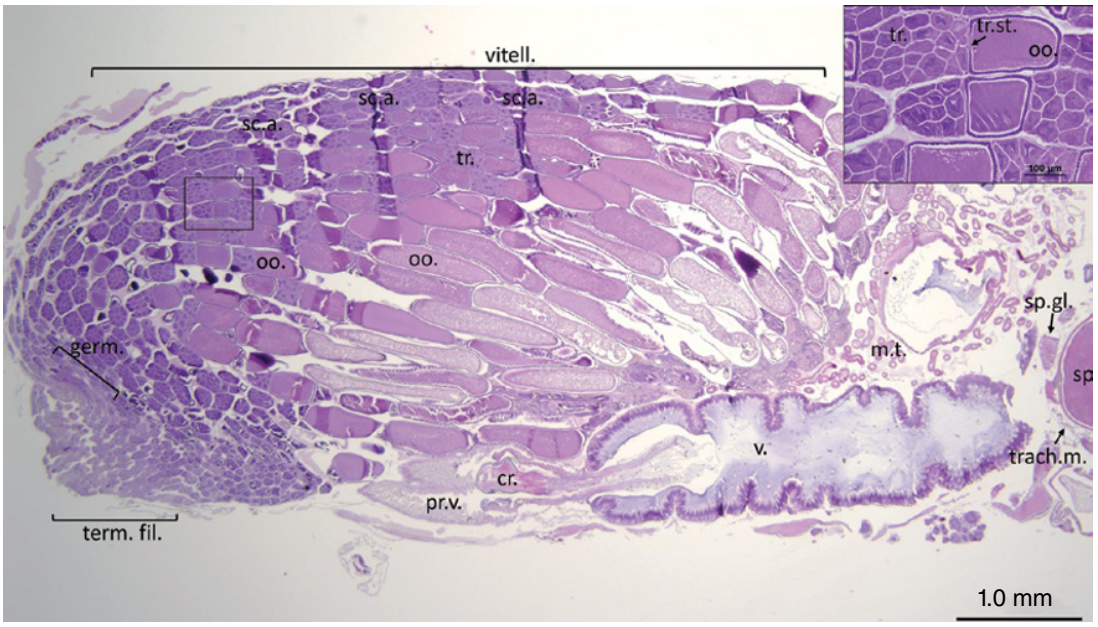


Figure 12.27 Longitudinal section of the abdomen of a mated honeybee queen. Most of the abdominal cavity is occupied by the paired ovaries, which are subdivided into three sections based on the oocyte developmental stages. The most cranial terminal filament (term. fil.) is composed of undifferentiated cystoblast cells. The germarium (germ.) is composed of disorganized proliferating cystoblasts producing germline cells (cystocytes), often forming rosettes. The largest portion of the ovary is vitellarium (vitell.) composed of a linear arrangement of developing oocytes (oo.), each followed by a cluster of trophocytes (tr.). Each oocyte is connected to one trophocyte cluster by a single trophic stalk (tr.st.) (inset). Caudal to the ovary there is a spermatotheca (sp.) which stores sperm acquired during mating with multiple drones. The spermatotheca is surrounded by spermathecal glands (sp.gl.) and tracheal meshwork (trach.m.). pr.v., proventriulus; cr., crop; v., ventriculus; m.t., Malpighian tubules; box, enlarged area in the inset (*top right*). Source: Contributed by Dr Ivanna Kozii. HE.

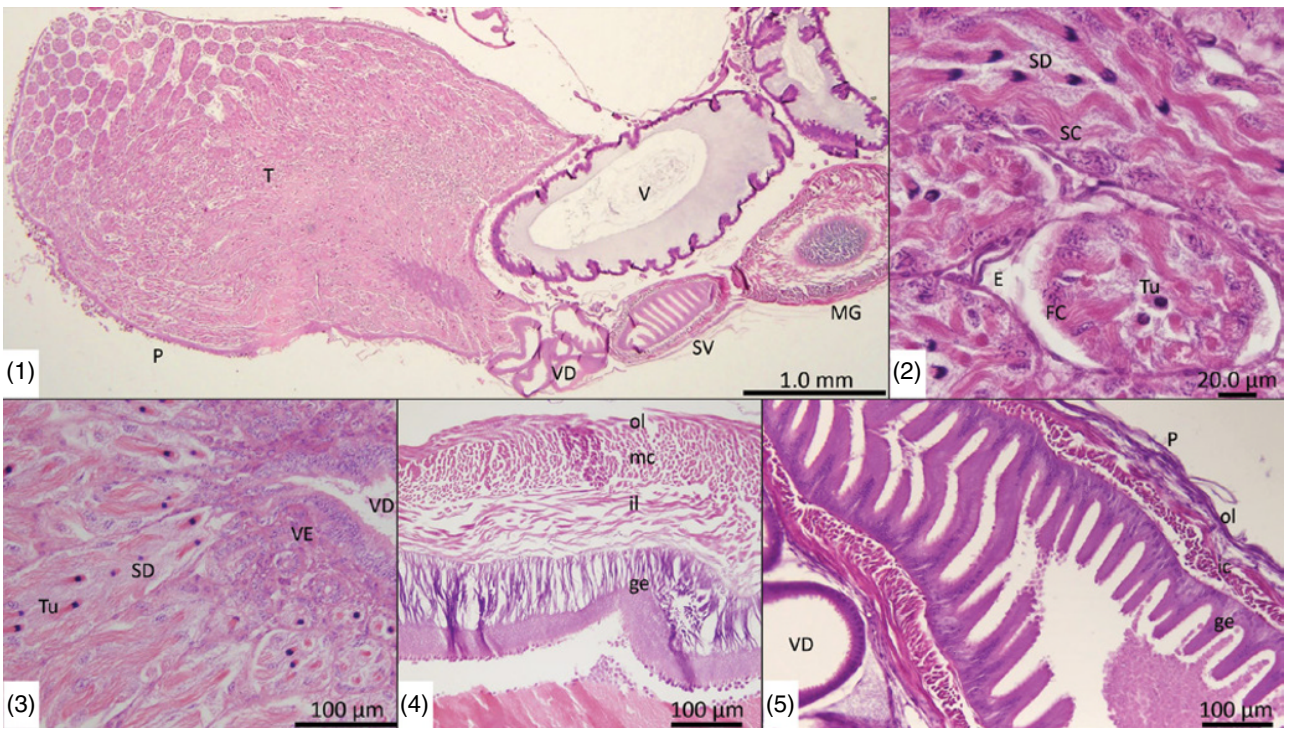


Figure 12.28 Photomicrograph of a coronal section of a newly emerged honeybee drone: abdomen (1), testes (2, 3), mucous gland (4), seminal vesicles (5). Most of the abdominal cavity (1) is occupied dorsally by the paired testes (T), and ventrally by the mucous gland (MG), and seminal vesicles (SV) connected to testes by the vas deferens (VD). The peritoneum (P) covers all reproductive organs and gastrointestinal tract (V, ventriculus). The testes (2, 3) are composed of 150–200 tubules (Tu), housed within a peritoneal sheath. The tubules are composed of an outer attenuated epithelial layer (E) and an inner cuboidal follicular cell layer (FC) which hold developing gametes within intracellular spermatocysts (SC). The lumen is filled with spermatodesms (SD) – the bundled spermatozoa that are released when the spermatocysts rupture. Spermatodesms are passed into the vas efferens (VE), then into the common vas deferens (VD) and seminal vesicle. The sperm remain within the seminal vesicle until time of mating, when they are ejected via the ejaculatory duct with the abundant secretions of the mucous gland. Both mucous glands (4) and seminal vesicles (5) are lined by glandular epithelium (ge), surrounded by muscular layers (inner circumferential muscle [ic], outer longitudinal muscle [ol], medial circumferential muscle [mc]), and covered by peritoneum (P). Source: Contributed by Dr Colby Klein.

filiform. The flagellar axoneme generally follows the standard 9+2 microtubule pattern, though some modifications exist, especially in Protura (Harrison and Locke 1990). Accessory glands (called mesadenia) may branch from the ejaculatory duct. Accessory glands are multilobed and composed of multiple tubules lined by a simple epithelium and contain abundant, brightly eosinophilic secretory product to facilitate sperm transfer (Elelimy et al. 2017).

In addition to the penis, segment 9 (and possibly 10) may have appendages that aid in either positioning during copulation or sperm deposition. These appendages are retractable in some species and may not be visible externally (Harrison and Locke 1990).

12.3.9 Special Senses

12.3.9.1 Eyes

The head has both a pair of compound eyes (Figure 12.29) and up to three, unpaired ocelli, or rudimentary eyes (Figure 12.30). Many adult insects lack ocelli. Compound eyes have many, closely packed, light-receiving units, called ommatidia (Figure 12.31). Ommatidia are rod-shaped and occur in close, parallel opposition to one another, perpendicular to the exoskeleton. Each ommatidium has a focusing system composed of a corneal lens, which is continuous with the exoskeleton. Corneageous cells of the epidermis produce the corneal lens. Subjacent to the lens is a transparent crystalline cone that transmits light to the photoreceptor cells, which are called retinular cells. These cells contain rhodopsin, a photosensitive pigment, in their microvilli, which they orient toward a central axis (called a rhabdom) to concentrate the photosensitive pigment. Retinular cells terminate on axons that synapse with optic ganglia (Ruppert et al. 2004).

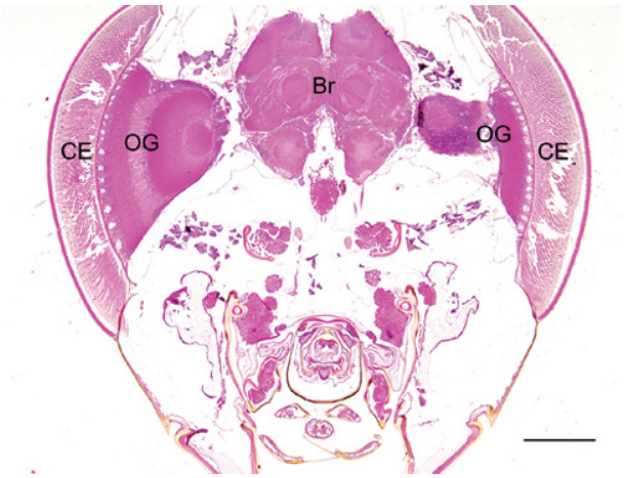


Figure 12.29 Transverse section of the head of a honeybee (*Apis mellifera*). The lateral portions of the head have compound eyes (CE), which are continuous with optic nerves (ON) that extend to the brain (Br). 2×. HE. Scale bar, 500 µm.

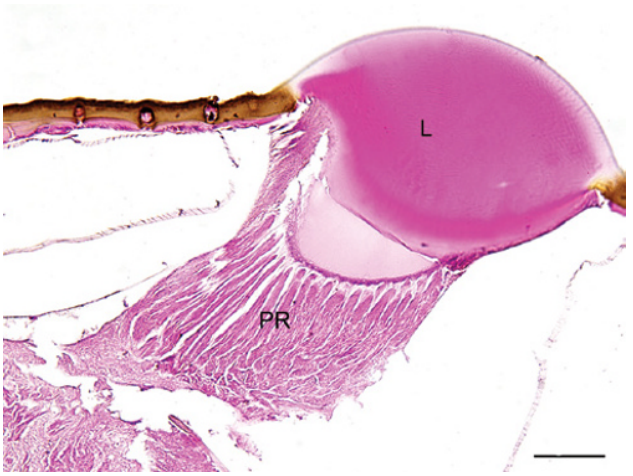


Figure 12.30 Section through an ocellus in a honeybee (*Apis mellifera*). The ocellus is a simple eye composed of a modified cuticle comprising the lens (L) subtended by photoreceptor cells (PR). 10×. HE. Scale bar, 50 µm.

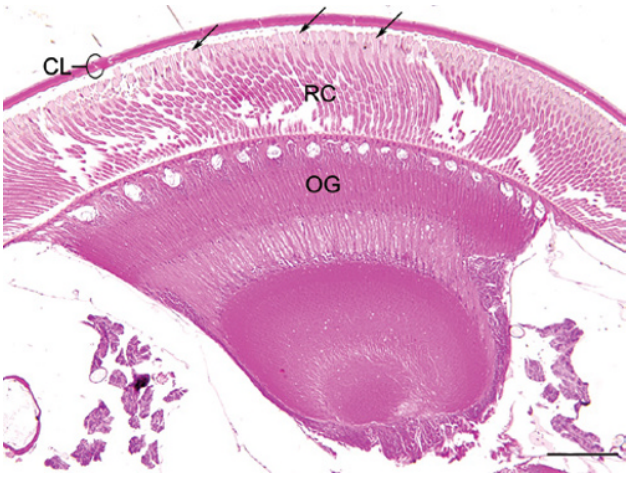


Figure 12.31 Section through a compound eye in a honeybee (*Apis mellifera*). The cuticle is modified into a corneal lens (CL). Subjacent to the lens are numerous crystalline cones (*arrows*). Below these cones are numerous retinular cells (RC). Deep to the compound eye is the optic ganglion (OG) of the brain. 4×. HE. Scale bar, 200 µm.

12.3.9.2 Cuticular Receptors

Numerous sensilla are located throughout the exoskeleton, particularly on the antennae and legs. These sensilla include mechanoreceptors, chemoreceptors, proprioreceptors, thermoreceptors, and others. Antennae and tarsi are major sensory receptors for most insects. Antennal sensilla include olfactory, gustatory, mechano-, thermo-, and hygroreceptors (Figure 12.32). The tarsi sensilla include gustatory and tactile receptors.

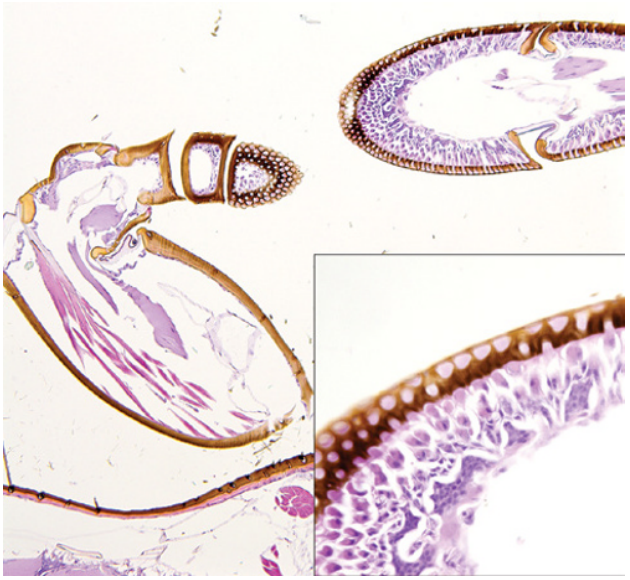


Figure 12.32 Sensilla of a honeybee (*Apis mellifera*). 2×. HE. Inset, 30×.

12.3.10 Endocrine System

12.3.10.1 Corpora Allata

These are paired glands that lie caudal to the brain. They secrete "juvenile hormones" which play a role in postembryonic development. Histologically, the organ is composed of packets of cells surrounded by a connective tissue capsule. Multiple cell types have been identified. Many cells are closely packed with a small amount of granular, basophilic cytoplasm. Additionally, Diptera have a few large, hypertrophied cells and glandular-like epithelium around a central cavity.

12.3.11 Silk Glands

The best known insects that produce silk are lepidopterans, including the silk worm and silk moths. There are also many other insects that spin silk during some point of their life cycle. Different groups of insects use different silk-producing organs, which include labial glands, dermal glands, silk glands derived from Malpighian tubules, and accessory sex glands (Harrison and Locke 1990).

12.3.11.1 Labial Gland

This is the most common silk-producing organ in insects, and is present in lepidopterans, as well as some other insect classes. Labial glands vary in shape from a simple "N" shape to a highly convoluted tubular structure of varying diameter. Labial glands are paired and usually segmented with the posterior portion secreting fibroin, the main component of silk. The middle portion secretes sericin in *Bombyx* spp. The anterior portion is a nonsecretory duct with a thick cuticular intima that forms a radial structure histologically. The ducts fuse to form a common duct that empties into the spinnerets located in the apical labium of the mouth cavity. In mature *Bombyx* spp., nuclei of the posterior portion are highly ramified. Generally, silk gland nuclei of lepidopterans become more highly branched ("arborescent") and complex as caterpillars age, and may become several hundred micrometers in diameter! The complexity and size of silk gland nuclei are due to numerous (up to 20) endomitotic cycles (i.e., increases in the number of chromosomes without nuclear division) (Harrison and Locke 1990).

12.3.11.2 Dermal Silk Gland

Dermal silk glands, also called tarsal glands, are present in Embioptera and Diaspidina. They secrete both fibroin and sericin, which are ejected through a complex cuticular projection. In Embioptera, tens of dermal glands are clustered on the tarsal segments. Dermal glands are multinucleated cells on bristles arising from the hypodermis. In Diaspidina, up to 100 dermal glands insert in the epidermis of the abdomen and project into the body cavity. Each gland is 1–2 cells (Harrison and Locke 1990).

12.3.11.3 Malpighian Tubule-Derived Silk Glands

Malpighian tubules in many species serve multiple functions. In larval Neuroptera, Raphidioptera, and certain Coleoptera, segments of the Malpighian tubule epithelium secrete silk.

12.3.11.4 Accessory Sex Gland

Accessory sex glands of multiple insect orders produce silk for eggs and their associated structures, such as egg stalks of Neuroptera, egg cocoon in *Hydrophilus acuminatus*, and ootheca (egg case) of Mantodea.

12.3.12 Venom Gland

Some species of insects can bite or sting, including apids. The venom gland of honeybees is composed of tubular organs in the caudal abdomen. The venom glands empty into the venom sac (Figure 12.33), which leads to the bulb of the sting. The bulb is a dilated portion at the base of the sting that holds venom (Stell 2012).

Disclaimer

The views expressed in this chapter are those of the authors and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or US Government.

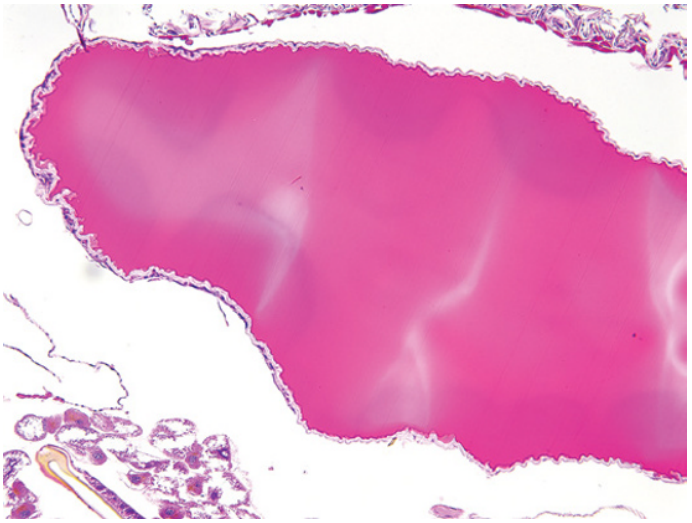


Figure 12.33 Venom sac of a honeybee (*Apis mellifera*). The venom sac is lined by a simple epithelium with a basement membrane. The venom is hypereosinophilic. 10×. HE.

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