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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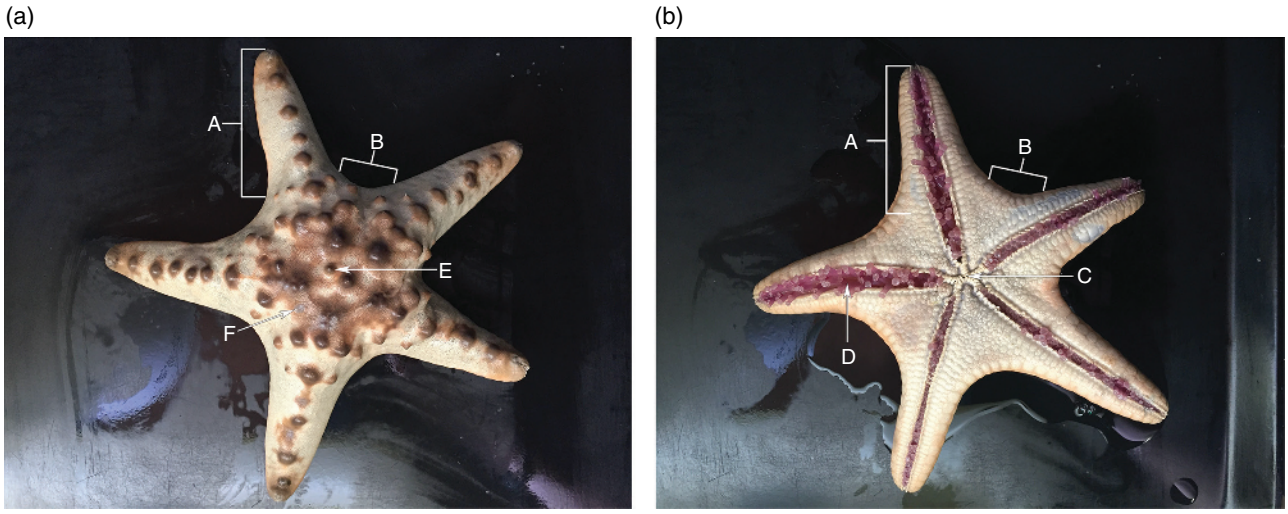


Figure 1.1 Representative image of the aboral (a) and oral (b) surface of a chocolate chip sea star (*Protoreaster nodosus*) demonstrating pentamerous symmetry. Labels include (A) radius, (B) interradius, (C) mouth, (D) ambulacral groove, (E) anus, and (F) madreporite.

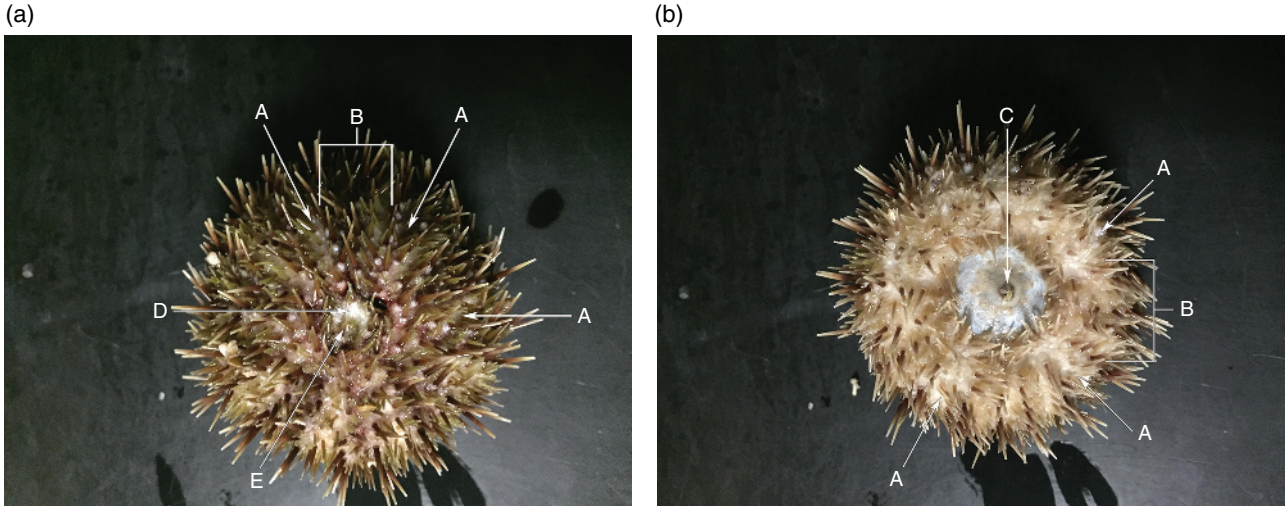


Figure 1.2 Representative image of the aboral (a) and oral (b) surface of a purple urchin (*Arabacia punctulata*) demonstrating pentamerous symmetry. Labels include (A) ambulacral plates, (B) interambulacral plates, (C) mouth, (D) anus, and (E) madreporite.

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.

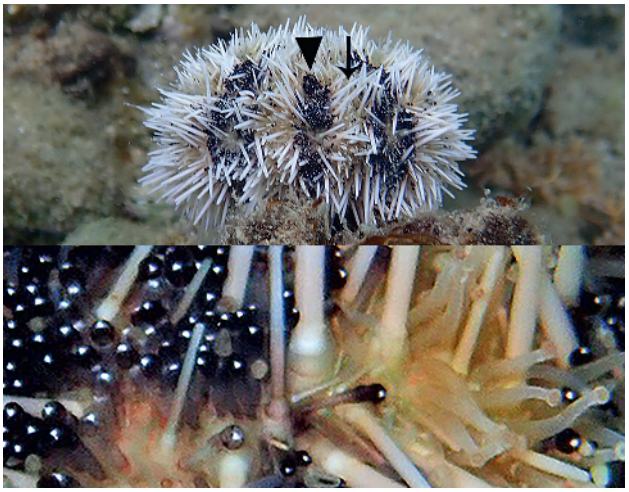


Figure 1.3 Image of a white sea urchin (*Tripneustes ventricosus*) demonstrating the distinction between ambulacral (arrow, inset right) and interambulacral (arrowhead, inset left) plates; tube feet are lacking in the latter where black-pigmented pedicellariae predominate.

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

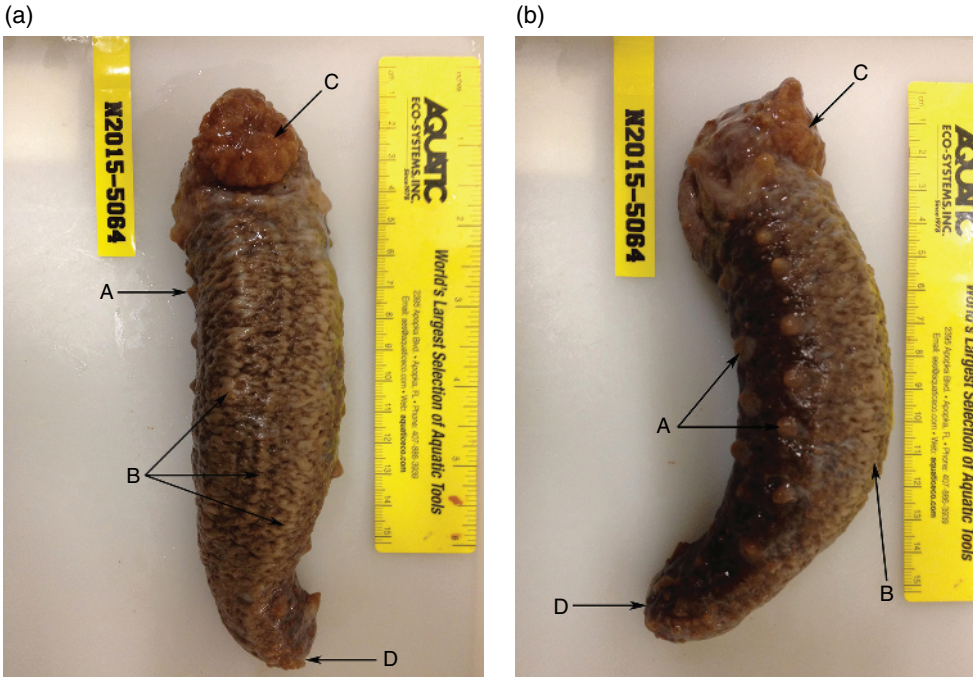


Figure 1.4 Representative image of the ventral (a) and lateral (b) aspects of a California giant sea cucumber (*Parastichopus californicus*). Labels include (A) dorsal ambulacra, (B) ventral ambulacra, (C) buccal podia, and (D) anus.

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).

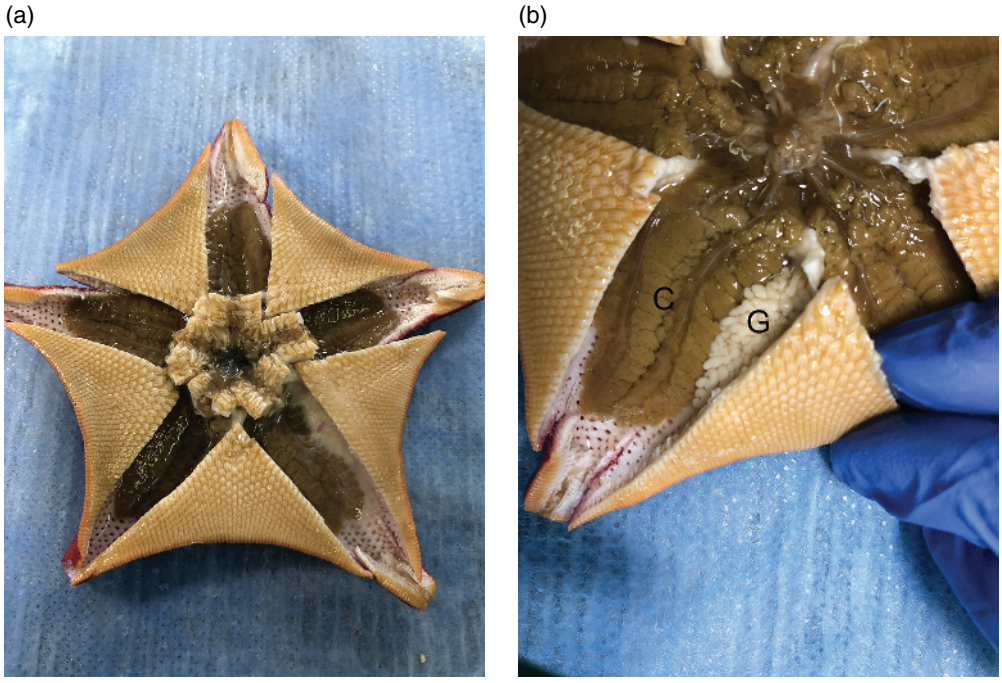


Figure 1.5 Gross necropsy image (a) of a bat star (*Patiria miniata*) open at necropsy. Higher magnification of one of the arms is provided (b) and shows the pyloric cecae (C) and the gonads (G). *Source:* Image courtesy of L. Abbo, Marine Biological Laboratory.

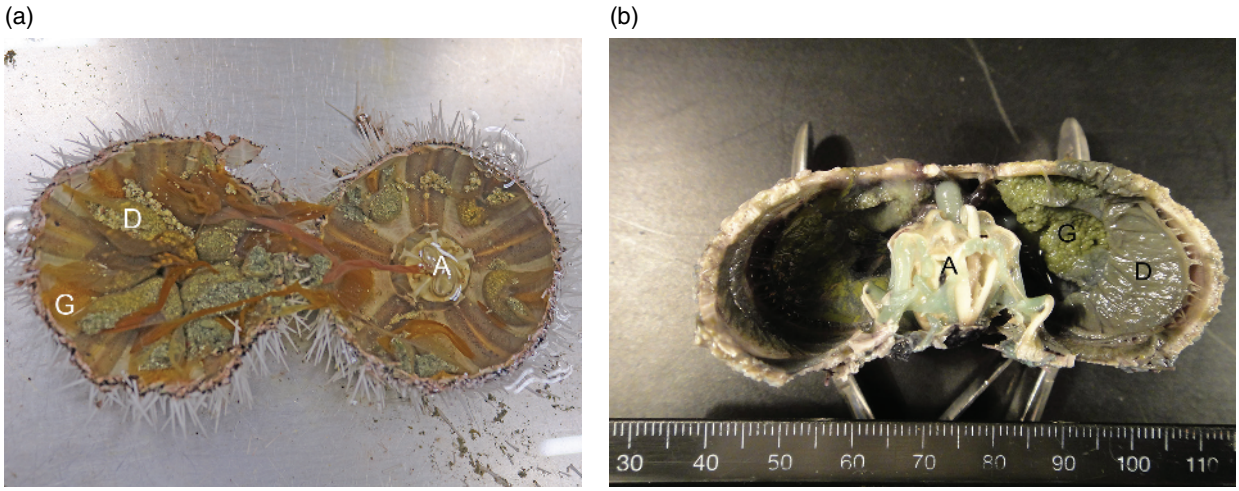


Figure 1.6 Gross necropsy images of urchin open at necropsy. Images include a white sea urchin opened at the equator and submerged in sea water (a) and a purple urchin (*Arabacia punctulata*) opened dorsoventrally (b), showing the gonads (G), digestive tract (D), Aristotle's latern (A), and ampullae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

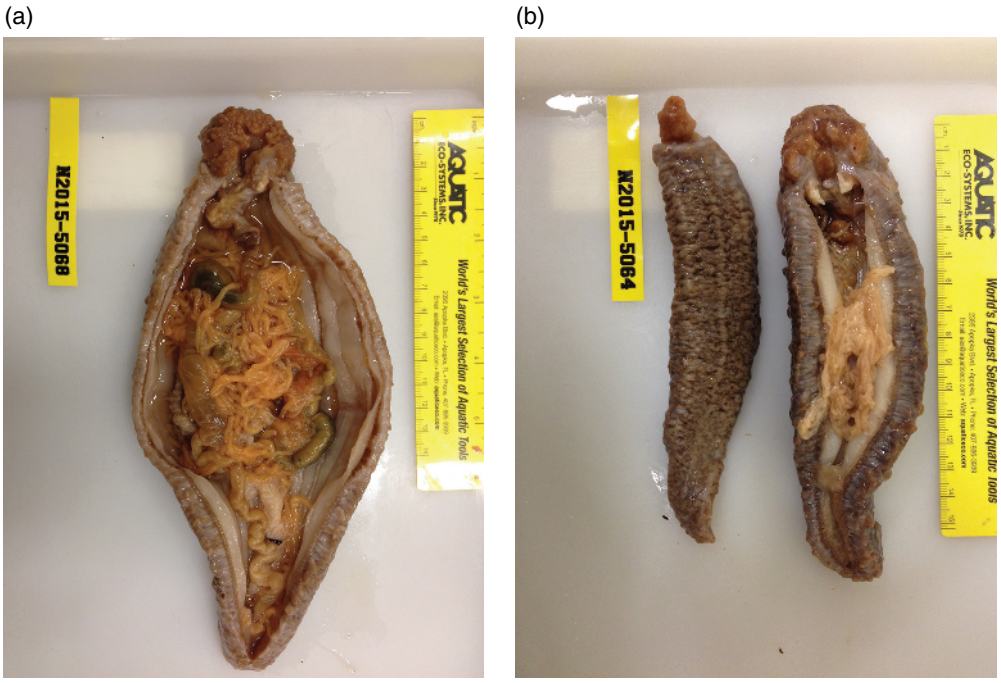


Figure 1.7 Gross necropsy images of a California giant sea cucumber opened along the dorsum removing the dorsal ambulacrum. Images include an animal that has not spontaneously eviscerated prior to death (a) and one that has spontaneously eviscerated (b).

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.

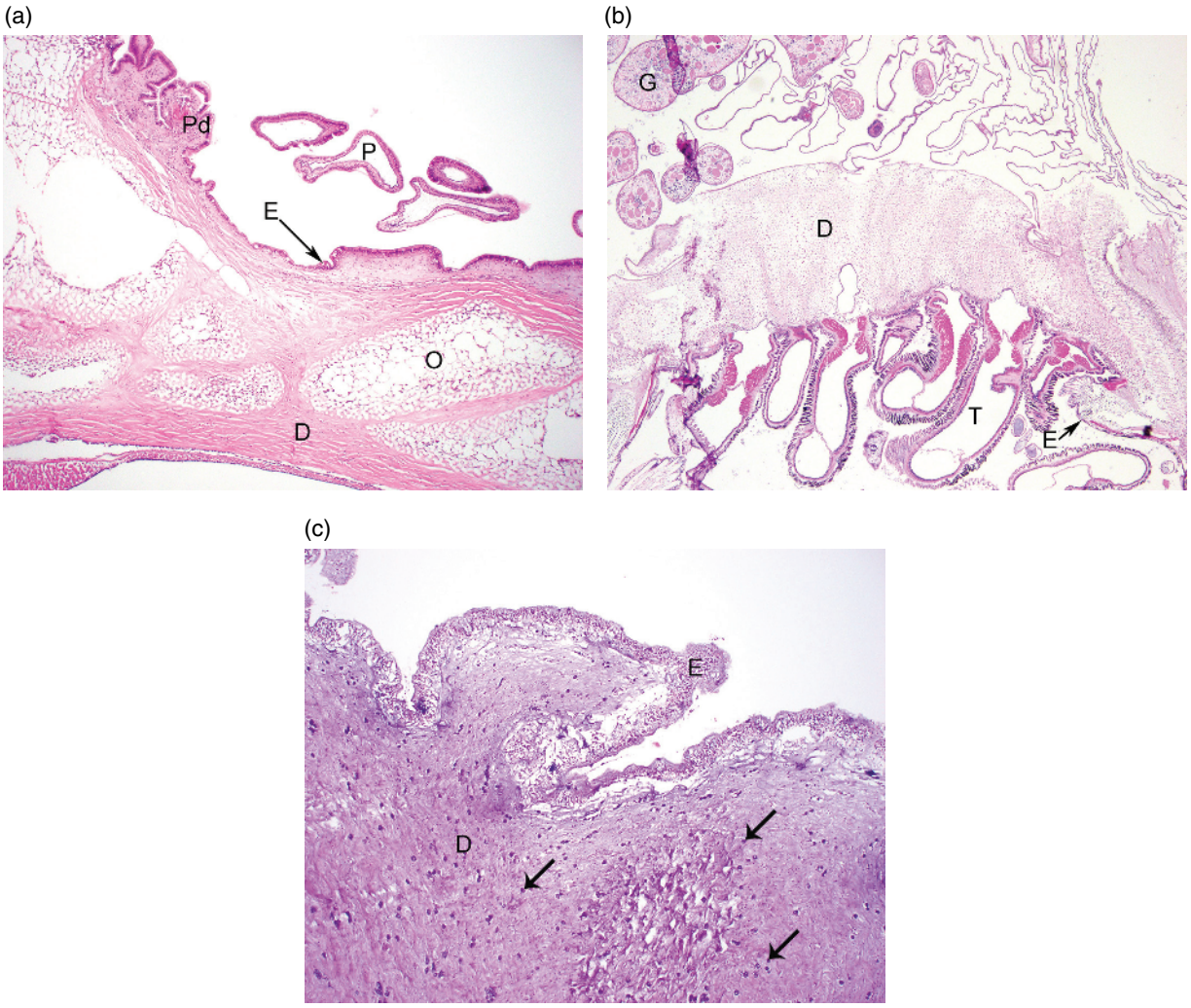


Figure 1.8 Low-magnification image of the histology of the body wall of an (a) ochre sea star (*Pisaster ochraceus*), (b) white sea urchin, and (c) California giant sea cucumber. Hematoxylin & eosin (HE), 100×, 40×, 100×, respectively. D, dermis; E, epidermis; G, gonads; O or arrows, ossicles; P, papulae;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

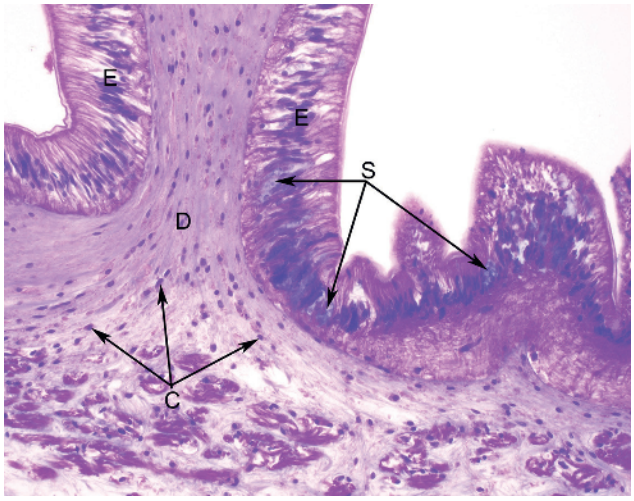


Figure 1.9 Histology of the epidermis of a sunflower sea star (*Pycnopodia helianthoides*). Individual cell types are difficult to discern with light microscopy. The columnar epidermis (E) has occasional secretory cells (S). The subjacent dermis (D) contains many coelomocytes (C). 400×, Lee's methylene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

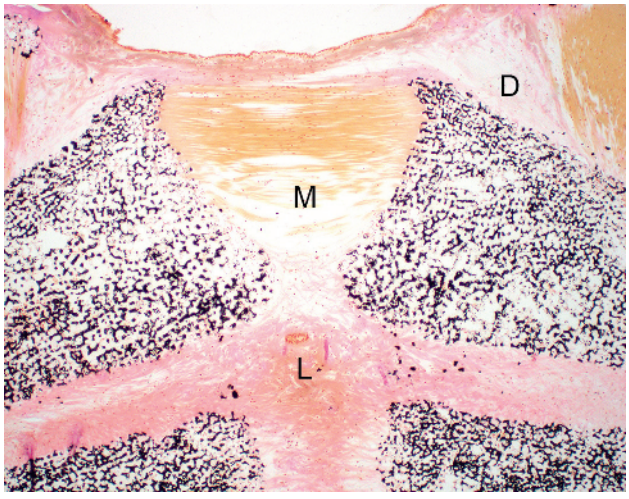


Figure 1.10 Low-magnification image of the histology of a sunflower sea star ossicle demonstrating dermal, ligamentous, and muscular attachments. 200×, von Kossa.

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

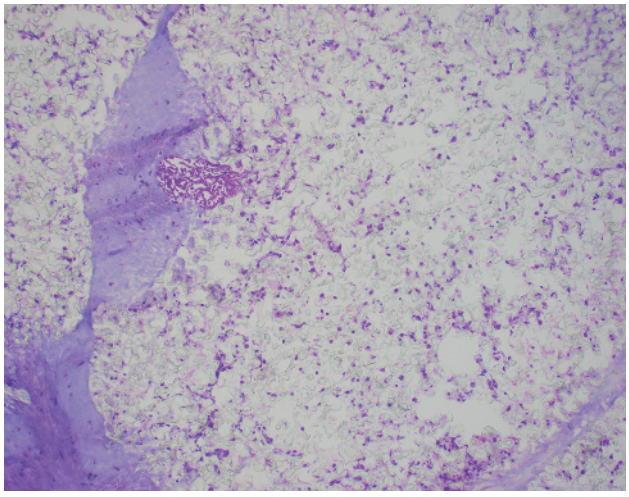


Figure 1.11 Higher magnification image of the histology of an ochre sea star ossicle demonstrating the sclerocyte lattice (plastinated section). 400×, LMB.

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

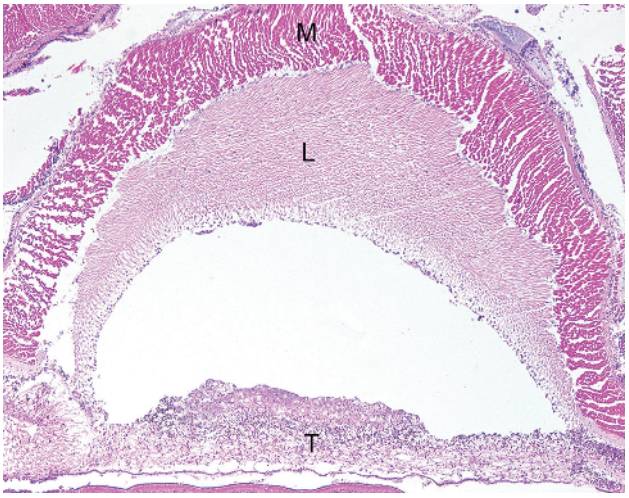


Figure 1.12 Histology of the base of a white sea urchin spine at the ball and socket joint. 400×, HE. M, muscle; L, ligament; T, test.

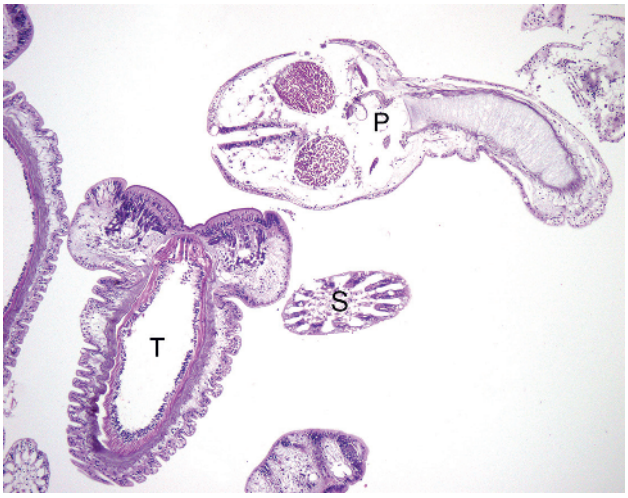


Figure 1.13 Histology of white sea urchin appendages including pedicellaria (P), spine (S), and tube foot (T). 100x. HE.

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

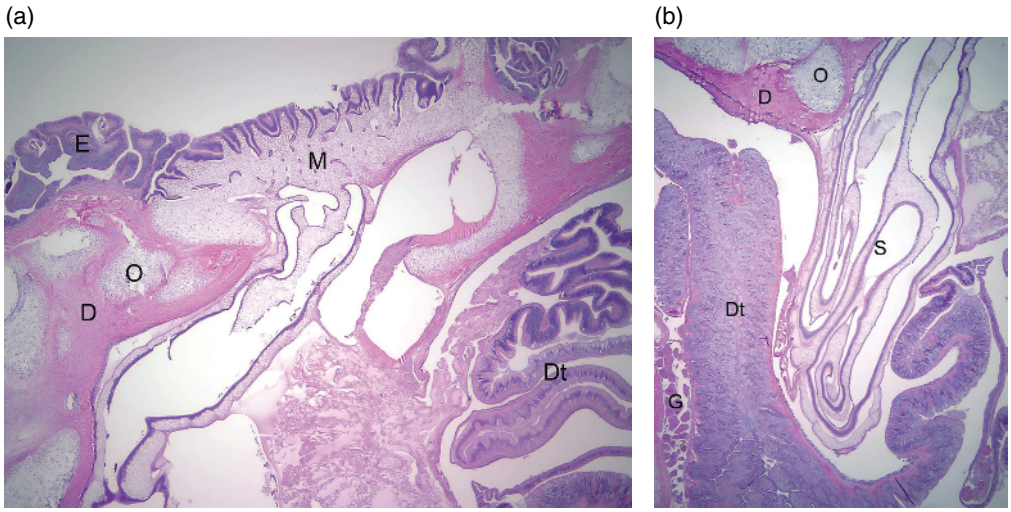


Figure 1.14 Histology of the madreporite (a) and stone canal (b) in a mottled star (*Evasterias troschelii*). 25×, 50×, HE. D, dermis; Dt, digestive tract; E, epidermis; G, gonad; M, madreporite; O, ossicles; S, stone canal.

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

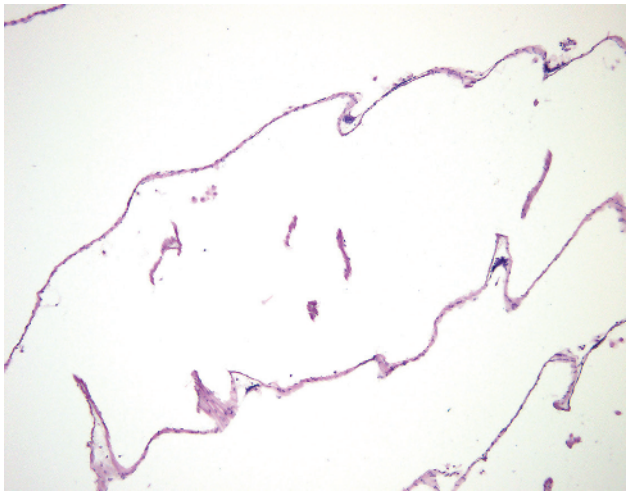


Figure 1.15 Histology of the water vascular (radial) canal in a white urchin. 200×, HE.

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

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

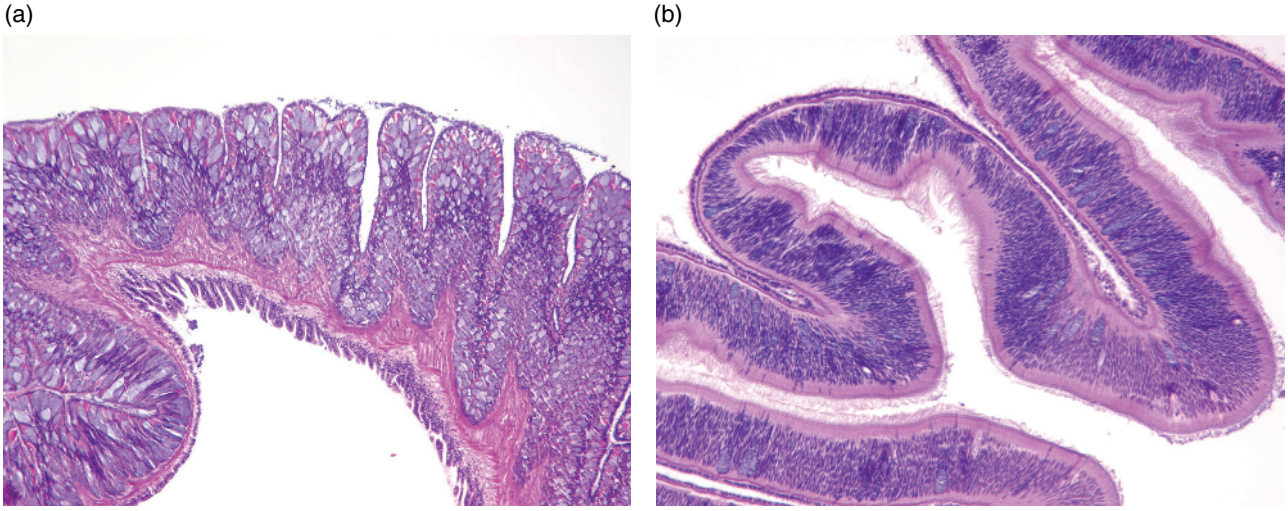


Figure 1.17 Histology of the cardiac (a) stomach in a mottled star (100×, HE) and pyloric stomach (b) of a mottled star (200×, HE).

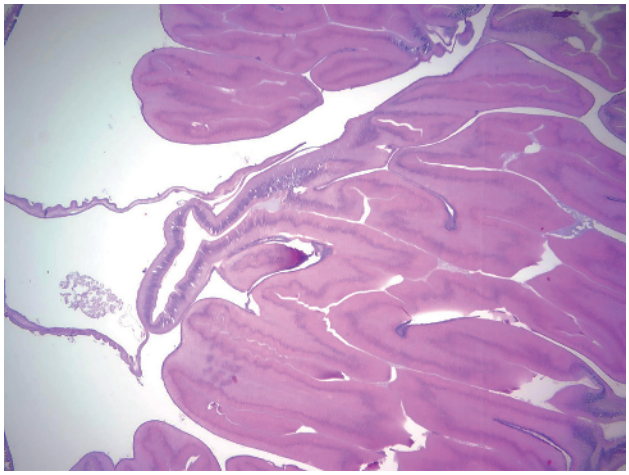


Figure 1.18 Histology of the pyloric cecae of a mottled star. 25×, HE.

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)

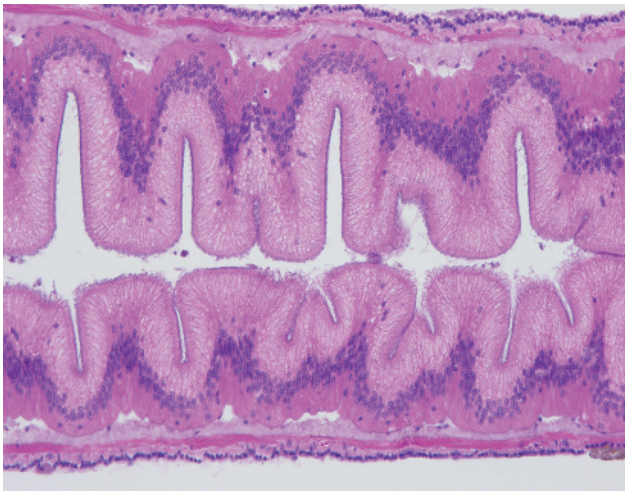


Figure 1.19 Histology of the large intestine of a white urchin. 200×, HE.

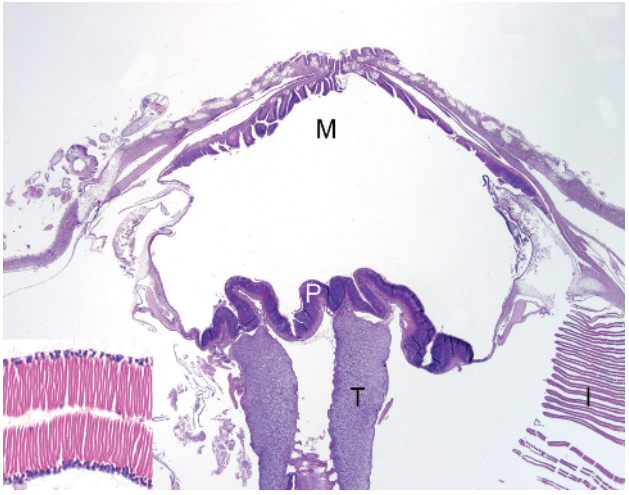


Figure 1.20 Low-magnification histology of anatomy of Aristotle's lantern in a white urchin. Inset shows closer view of interpyramidal muscle. 20×, HE. I, interpyramidal muscle; M, mouth; P, pharynx; T, teeth.

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

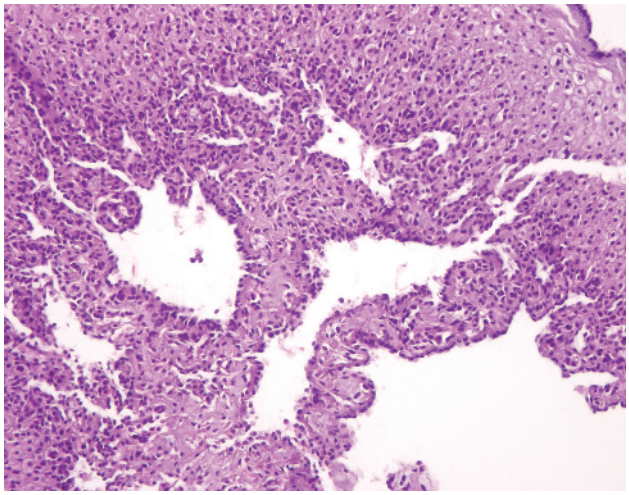


Figure 1.21 Axial gland in a white urchin. 400×, HE.

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

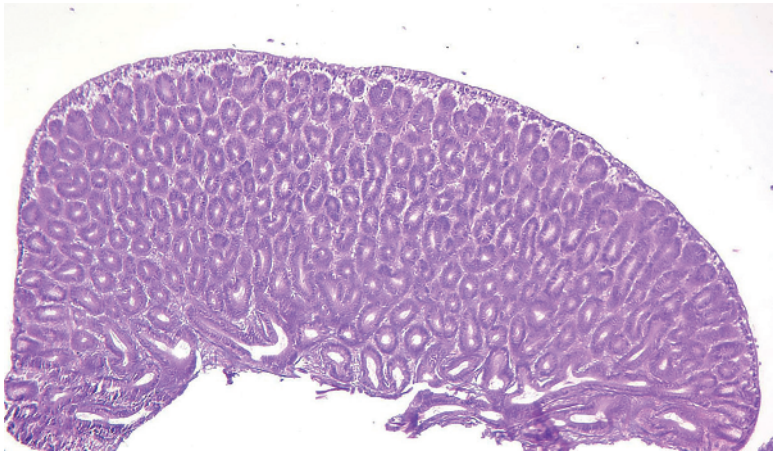


Figure 1.22 Tiedmann's body in a mottled star. 100×, HE.

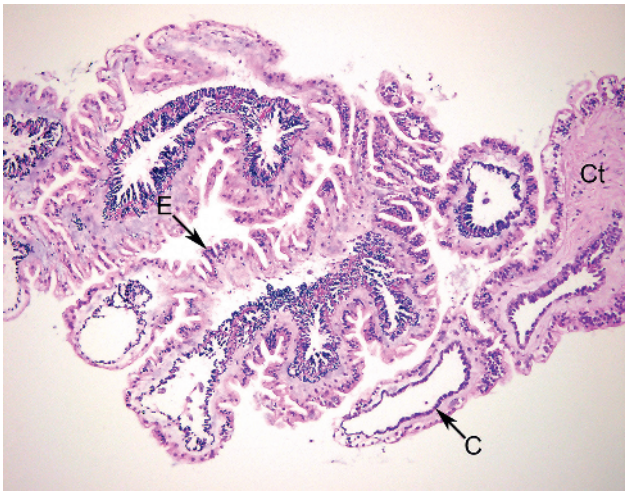


Figure 1.23 Histology of gills (papulae) in a white urchin showing epidermal surface (E), supported by connective tissue (Ct), and a central lumen lined by coelomic epithelium (C). 200×, HE.

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.

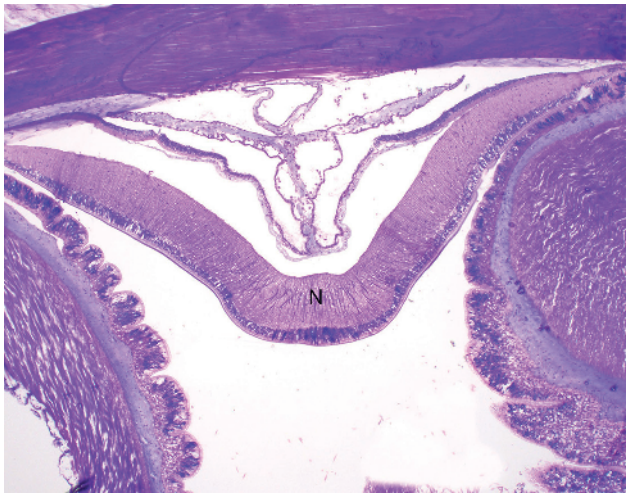


Figure 1.24 Histology of the ventral nerve cord (N) in a mottled star. 100×, LMB.

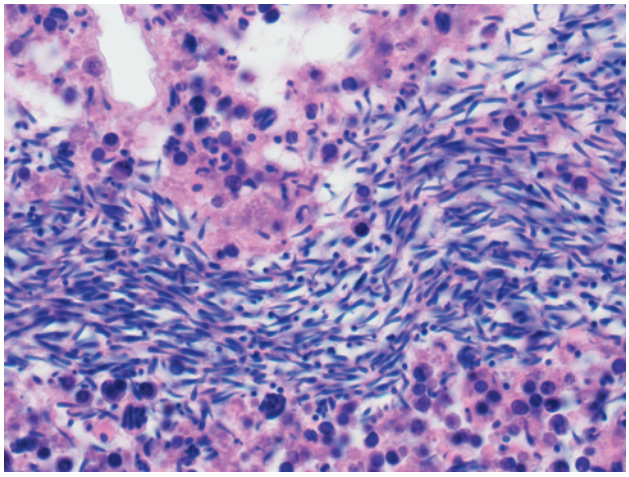
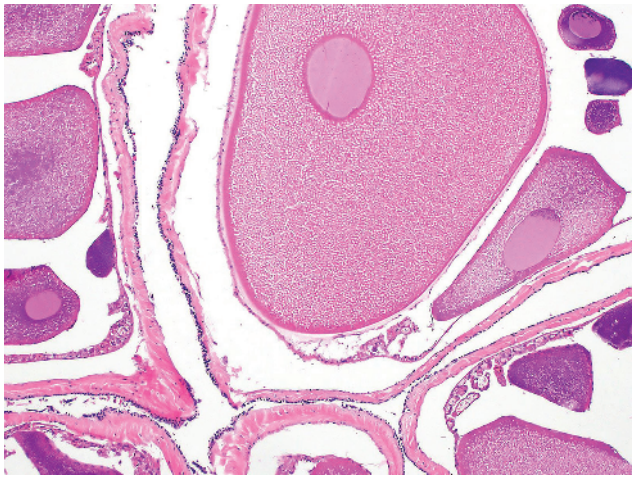


Figure 1.26 Nutritive support cells in the gonad of a sand dollar. 100×, HE.

(a)



(b)

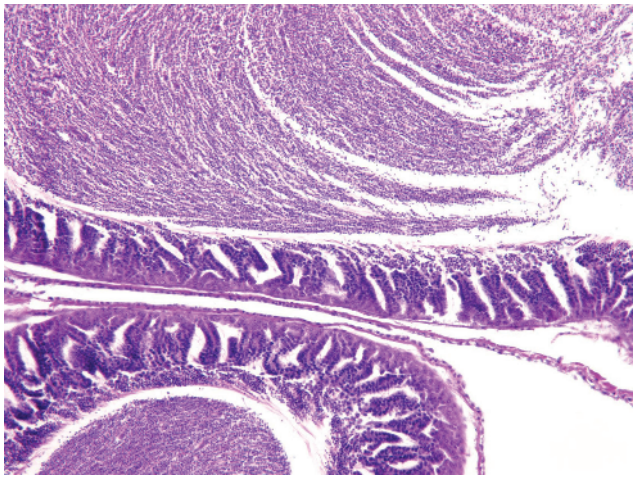


Figure 1.25 Histology of the ovary (a) in a Caribbean thorny star, and testicle (b) in a mottled star. 200×, HE. *Source:* (a) Image courtesy of Elise LaDouceur.

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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