Chapter 2

General Anatomy

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INTRODUCTION

The eastern oyster *Crassostrea virginica* is a monomyarian lamellibranch with a pronounced bilateral asymmetry and a restricted coelom typical of the class (Seed 1983). "With the loss of the head, the most convenient anteroposterior axis (Fig.1) in a bivalve is represented by a line running from the mouth through the middle of the posterior adductor (near the anus)" (Morton and Yonge 1964). The primitive position of the isomyarian bivalve dorso-ventral axis was a line running from the hinge (mid-dorsal) through the foot (mid-ventral). Those bivalves that retained the postlarval byssus complex, however, showed a gradual reduction (anisomyaria) and eventual loss (monomyaria) of the anterior adductor (Seed 1983). As a consequence, the hinge began to migrate towards the anterior end of the animal. This resulted in a more advantageous mechanical advantage with respect to the enlarging posterior adductor. The anteroposterior axis of the eastern oyster is located by a line running through the mouth and adductor muscle to the posterior margin of the shell (Fig. 1). The dorsoventral axis can best be represented by a line perpendicular to the anteroposterior axis running from the dorsal surface to the ventral surface (ancestral position of the foot).

There are several works that describe eastern oyster anatomy and histology in varying degrees of detail: Moore (1898), Brooks (1905), Churchill (1920), with the most comprehensive being Galtsoff (1964). Techniques to demonstrate oyster anatomy, including the circulatory system (Eble 1960; Shuster and Eble 1961), are available. Throughout the text, I refer to my own unpublished observations to supplement literature reports. Unless otherwise indicated, I used Alcian blue, pH 2.6 - periodic acid Schiff stain during my histological studies.

THE SHELL

Details of shell formation, composition and growth are discussed by Carriker in Chapter 3; only the general appearance and axes of symmetry will be mentioned here. The shell of the oyster consists of two calcareous valves joined by a resilient hinge ligament. Valves are asymmetrical, the left being larger and more deeply cupped than the right. Because the oyster invariably settles on its left valve, the right valve is always uppermost (Fig. 2)

THE MANTLE

Anatomy

Internal organs are covered with a fleshy fold of tissue called the mantle or pallium. Left and right lobes of the mantle are joined at their posterior margins in the region of the cloacal chamber and at the so-called "oral hood" (the anterior end of the mantle forms a cap and covers the mouth and labial palps); elsewhere the lobes are unattached and follow the curvature of the valves. The mantle is always in contact with the valves but is not attached to them. The large central cavity bounded by the mantle lobes is the pallial cavity and contains the palps and gills on the ventral side and the rectum on the dorsal side; specifically, the rectum opens dorsally to a special portion of the dorsal pallial cavity known as the cloacal chamber (Figs.1, 2). The right lobe of the mantle is separated from the visceral mass to form the promyal chamber (Fig. 2); the left lobe is fused to the visceral mass.

The pallial cavity is subdivided into two cavities. The cavity formed by the fusion of the mantle dorsally with the visceral mass and ventrally with the bases of the gills is known as the epibranchial chamber and continues posteriorly as the cloacal chamber. The large cavity containing the gills and bounded by the two mantle lobes is the hypobranchial chamber.

Radial muscles originate in the visceral mass, course through the mantle, and insert by fan-like enlargements in the margin at the base of the sensory tentacles that form the distal margin of the mantle (Fig. 3). Most of the radial muscles are accompanied along their length by blood vessels and nerves. Slen-

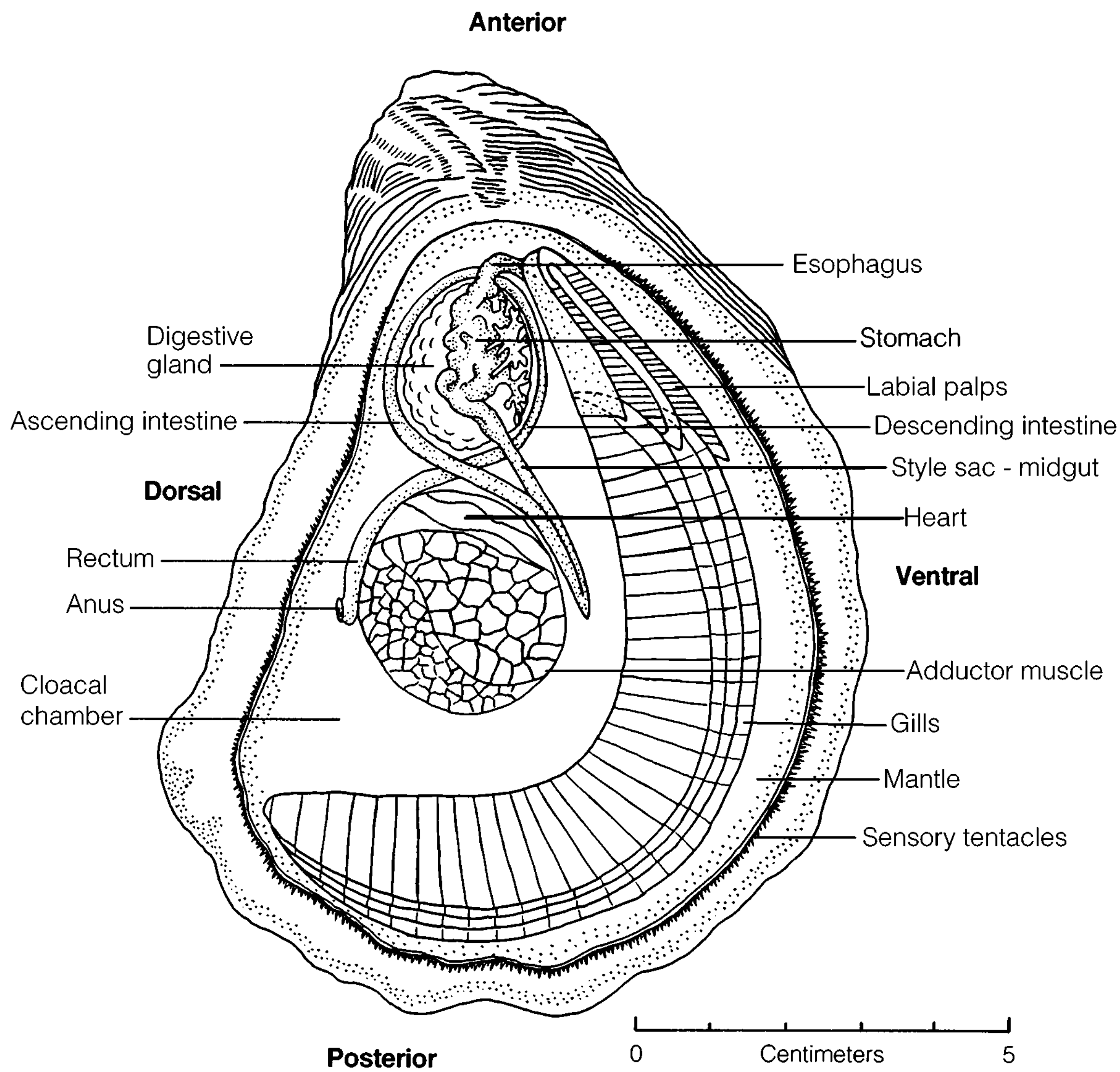

Figure 1. Oyster in left valve showing cardinal axes and digestive system including labial palps and gills. Redrawn from Galtsoff (1964).

der, concentrically arranged muscle bands parallel the free edge of the mantle and intersect the radial muscles at right angles (Galtsoff 1964; Morrison 1993). The prominent circumpallial artery (Fig. 3) runs in the margin of the mantle a few millimeters central to the fringing tentacles (Figs. 3, 4); this artery gives off many fine branches to the tentacles as well as larger branches to the broad face of the mantle. The circumpallial nerve (Figs. 3, 4) lies just peripheral to the circumpallial artery; it receives many fine branches from the tentacles and relays them either to the cerebral or visceral ganglia (it is not known which) via the radial nerves (Galtsoff 1964).

The border of the mantle is divided into three projecting lobes (Galtsoff 1964). The outer or shell lobe (Figs. 3, 4) is narrow and lies in contact with the margin of the shell; it is visualized best in rapidly growing oysters where it can be seen protruding beyond the edge of the valves. The middle lobe bears short and long sensory tentacles. The former are slender and numerous, the latter are thick and occur in a ratio of about 5 short to 1 long. The middle lobe is separated from the shell lobe by a deep cleft, the periostracal groove (Fig. 4). The inner lobe or pallial curtain (Nelson 1938) bears long, thick tentacles. When muscles of the inner lobes contract, the pallial curtain projects inward approximately at right angles to the mantle surface; the mantle lobes of both sides are brought into juxtaposition and the long tentacles of both sides of the pallial curtain interlock, effectively sealing the entrance to the mantle cavity. The valves can be open but no exchange of water can take place where the pallial curtain is sealed; by selectively opening only certain areas of the pallial curtain and then contracting the adductor muscle, the oyster can direct strong jets of water from the mantle cavity for such activities as spawning eggs or removing rejecta (pseudofeces).

Histology

The microscopic anatomy of the mantle consists of two epithelia, shell-side and pallial cavity-side, with connective tissues in between. The shell-side epithelium is composed of non-ciliated cells with microvilli that vary from tall columnar to cuboidal; because cell height is so variable, the basement membrane presents a wavy appearance because all cells are in contact with the valves. Unicellular glands are frequently located in zones of tall columnar cells where the body of the gland is situated near the basement membrane and a short neck connects to the free surface. I found that the surface in contact with the shell is coated with acid glycosaminoglycans (mucopolysaccharides). Unicellular gland cells are present, with one type containing neutral glycoproteins and another type containing a mixture of acid glycoproteins

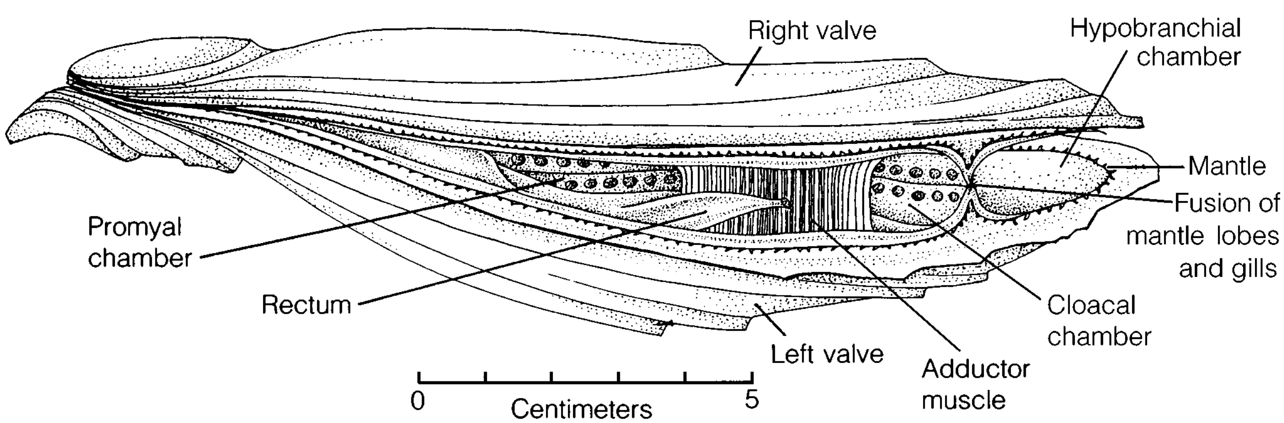

Figure 2. Diagram of dorsoposterior fusion of mantle lobes; the posterior extremity of the gills is found at this point. Openings to the two exhalant regions, the promyal chamber and the cloacal chamber, are clearly seen. From Galtsoff (1964).

rich in carboxyl groups and neutral polysaccharides; the latter outnumber the former by about a 3:1 ratio.

My histological sections show that the pallial cavity-side epithelium is simple, ciliated cuboidal in the dorsal area of the pallial cavity. In the ventral region, however, cilia are restricted to "ciliary tracts": groups of 8 to 10 cuboidal or columnar cells that have long cilia (Fig. 5) and unicellular glands (Morrison 1993). One type of gland cell contains neutral glycoproteins and another has acid glycosaminoglycans rich in carboxyl groups; the latter outnumber the former by about a 4:1 ratio. The cuboidal epithelium on either side of ciliary tracts lacks both cilia and unicellular glands. A subepithelial blood sinus is associated with both epithelia. Connective tissue consists chiefly of vesiculated cells (Leydig cells) that function as storage parenchyma as well as connective tissues; Leydig cells are omnipresent.

Mantle margin epithelia in my sections vary among the three lobes: (1) *pallial curtain (inner lobe)* — the inner surface is lined with an epithelium identical to pallial cavity-side mantle epithelium with which it is contiguous. The outer surface is covered with a simple, cuboidal epithelium; (2) *sensory lobe (middle lobe)* — the inner surface is highly folded simple cuboidal epithelium with many unicellular glands,

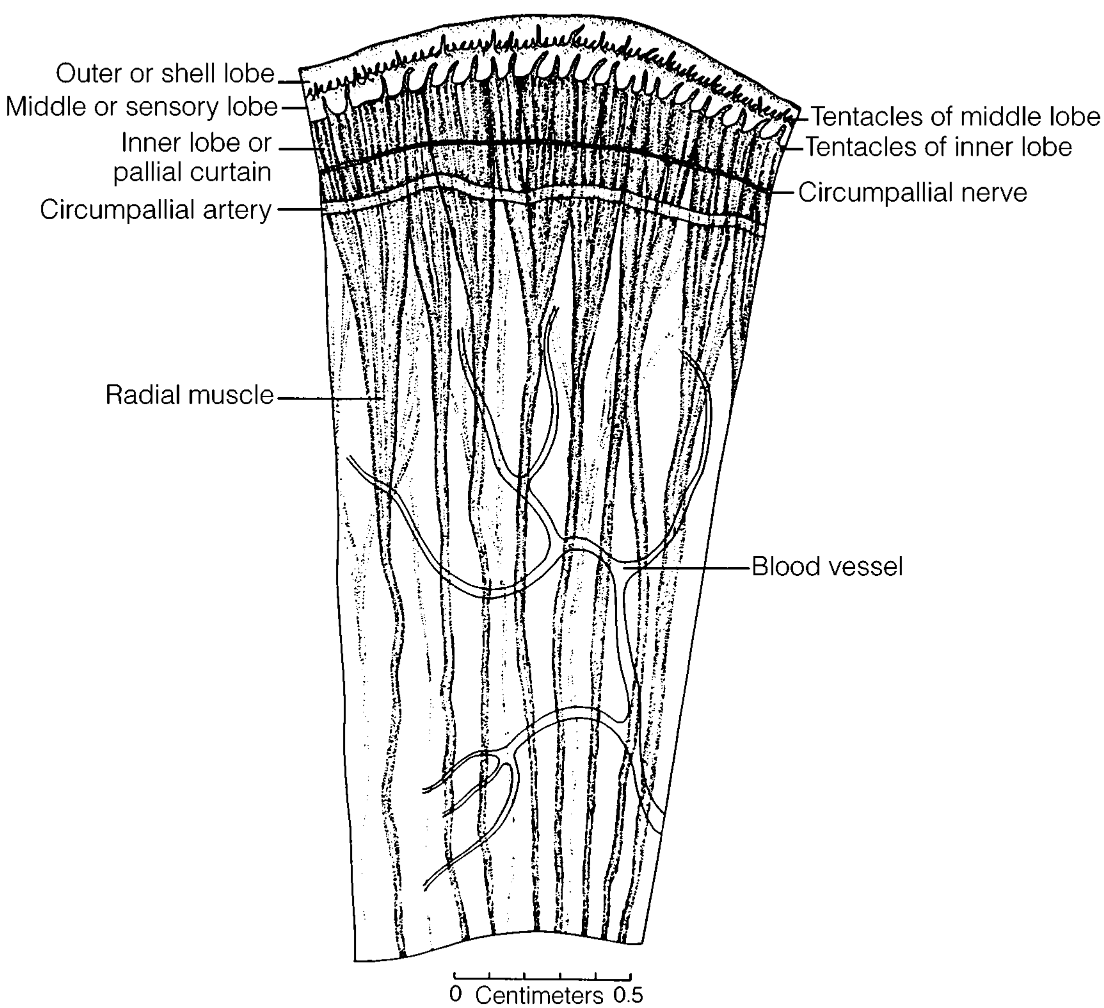

Figure 3. Whole mount of a portion of the mantle from about the middle to the margin. Most connective tissue was removed by maceration. From Galtsoff (1964).

some containing neutral glycoproteins and others acid glycoproteins rich in carboxyl groups; epithelial cells usually contain a dark-brown pigment. The outer surface has a simple, ciliated cuboidal epithelium lacking unicellular glands; (3) shell lobe (outer lobe) — the inner surface has a simple, ciliated cuboidal epithelium; many unicellular glands are present near the tip of the lobe and contain glycosaminoglycans rich in carboxyl groups. The outer surface has an epithelium continuous and identical to the shellside epithelium of the mantle; unicellular glands rich in carboxyl glycosaminoglycans are present deep in folds of the basement membrane. An in-depth discussion of the histology, scanning, and transmission electron microscopy of the mantle, including mantle lobes, is given by Morrison (1993).

ADDUCTOR MUSCLE

Anatomy

The adductor muscle, a prominent organ situated in the posterior region of the body, consists of an anterior translucent larger part and a smaller, white, crescent-shaped region. The adductor muscle func-

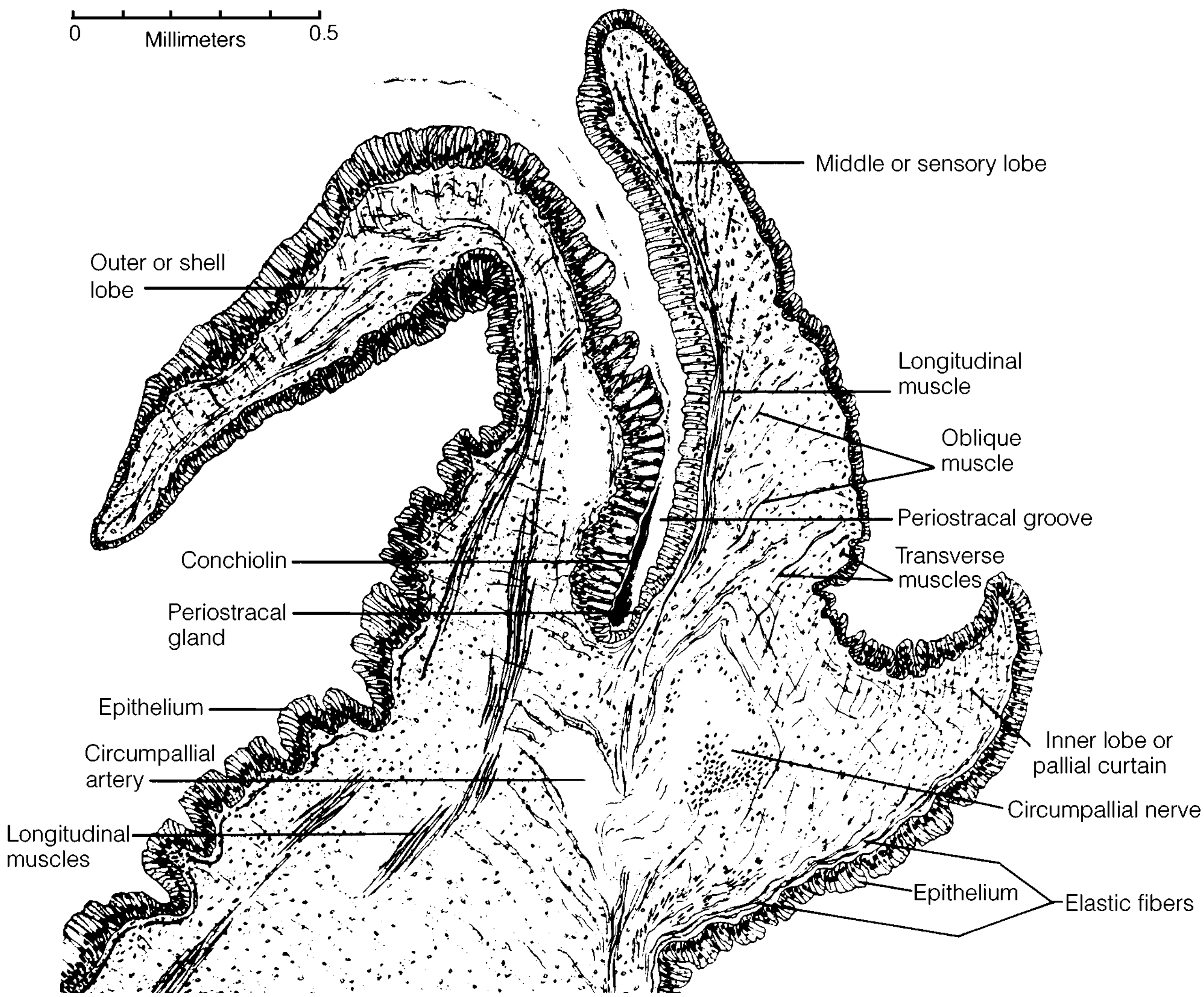

Figure 4. Transverse section of mantle margin to illustrate the three lobes of the mantle margin. The outer or shell lobe is bent as a result of fixation. From Galtsoff (1964).

tions to close the shell. Relaxation of the adductor muscle allows the valves to gape because of the resiliency of the hinge ligament. The rectum passes over its dorsal surface and the kidney is located near the anterior ventral portion. The posterior aorta runs along the anteriomedial face of the adductor muscle before entering the tissues approximately at midpoint to supply muscle fibers.

Histology

Muscle fibers of the translucent part of the adductor muscle have a unique oblique or double-oblique striation (Galtsoff 1964) with nuclei elongated and oriented in the long axis of the fibers. Widest diameter of the fibers is 3 µm (Morrison and Odense 1974). Filaments of the translucent muscle are not obviously aligned. Dense bodies are present but there is no Z membrane (the structure to which actin filaments are anchored); occasionally, dense bodies are obliquely aligned (Morrison and Odense 1974). Hemolymph sinuses are extensive and can be seen easily in well relaxed muscle (Fig. 6); delicate strands of connective tissue envelop each muscle fiber and extend across hemolymph sinuses imparting to the latter a cobweb-like appearance (Fig. 6). Arteries are composed of highly organized connective tissue and feed directly into muscle sinuses.

Muscle fibers of the white, opaque portion of the adductor muscle are smooth and much wider (8 µm) than those of the translucent part. Opaque muscle has wide, thick filaments (110 nm) that vary in diameter in transverse section. Arrangement of thin filaments (actin) around the thick filaments (myosin) is irregular (Morrison and Odense 1974). Construction of arteries and sinuses is similar to that described

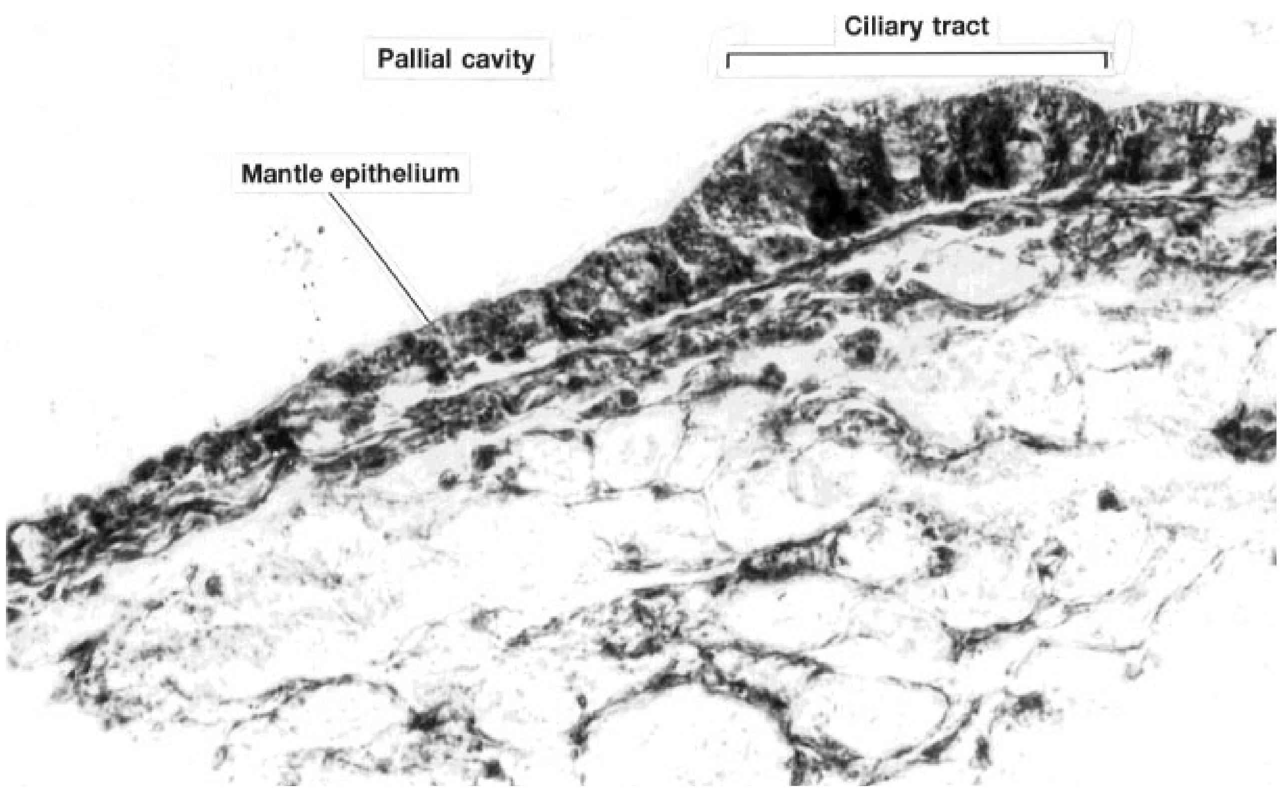

Figure 5. Section of ciliary tract in pallial cavity side of ventral mantle epithelium. Note clusters of 5 to 8 eosinophilic granule cells with cilia that constitute a ciliary tract. Adjacent low cuboidal cells lack cilia. Horizontal Field Width (HFW) = 87.5 µm.

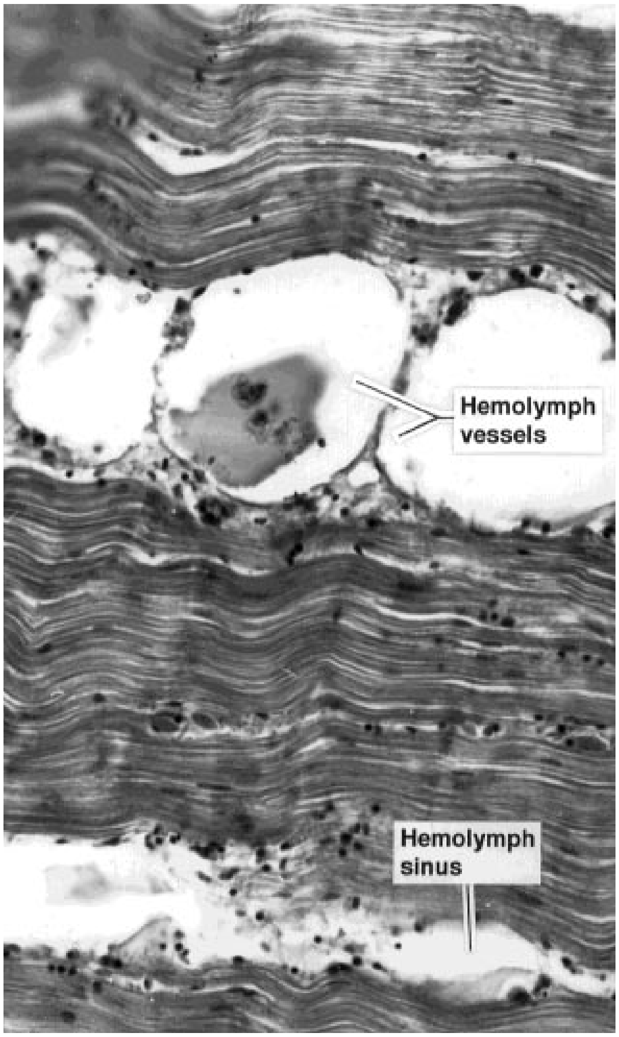

Figure 6. Transverse section of relaxed adductor muscle showing extensive vascular system consisting of delicate hemolymph vessels and broad sinuses. HFW = 190 µm.

for the translucent part. Hemolymph sinuses in the ventral part of the adductor muscle open directly into renal sinuses containing kidney tubules. The morphology and fine structure of the adductor muscle is discussed by Morrison in Chapter 4.

HEART AND CIRCULATION

Details of the heart and circulatory system are discussed by Eble in Chapter 7.

GILLS

Gills of the eastern oyster consist of four folds (demibranchs) of tissue suspended from the visceral mass and occupy much of the ventral and ventroposterior portions of the mantle cavity (Fig. 1). Together with the mantle, they are the chief organs of respiration. They create water currents, collect food particles, and move food particles to the labial palps for further sorting (see Newell and Langdon, Chapter 5, for details). Gills also serve to separate masses of eggs released from the ovary during spawning into individual ova for efficient fertilization.

Anatomy

Gills in each eastern oyster in cross section take the shape of four V's, a double V on the right, and another on the left side of the oyster (Fig. 7); each V is known as a demibranch and each arm of the V is called a lamella, with an inner descending lamella and an outer ascending lamella. Each lamella is composed of vertical filaments that, in turn, are clustered in vertical folds or plicae. Two marginally joined lamellae constitute a demibranch and two joined demibranchs are a gill. Each gill is organically attached to the body of the oyster at the open end of the VV known as the gill base; the pointed end of each V is called the gill margin and projects into the mantle cavity (Galtsoff 1964).

The gill is illustrated from its dorsolateral aspect in Fig. 7 and shows the broad face of a lamella which, as mentioned above, is composed of parallel arrays of filaments. In a eulamellibranch such as the eastern oyster, filaments are joined to each other at regular intervals by tissue connections called interfilamentar junctions; these junctions contain hemolymph vessels. The space between each of these junctions contains an ostium which is the approximately 60 µm long by 20 µm wide opening (Figs. 7, 8) through which water passes into the plical water chamber when the animal is filtering. Both lamellae of a demibranch are connected to one another by interlamellar junctions composed of tissue that contains hemolymph vessels, occurring at regular intervals from base to margin of the gill (Figs. 7, 8).

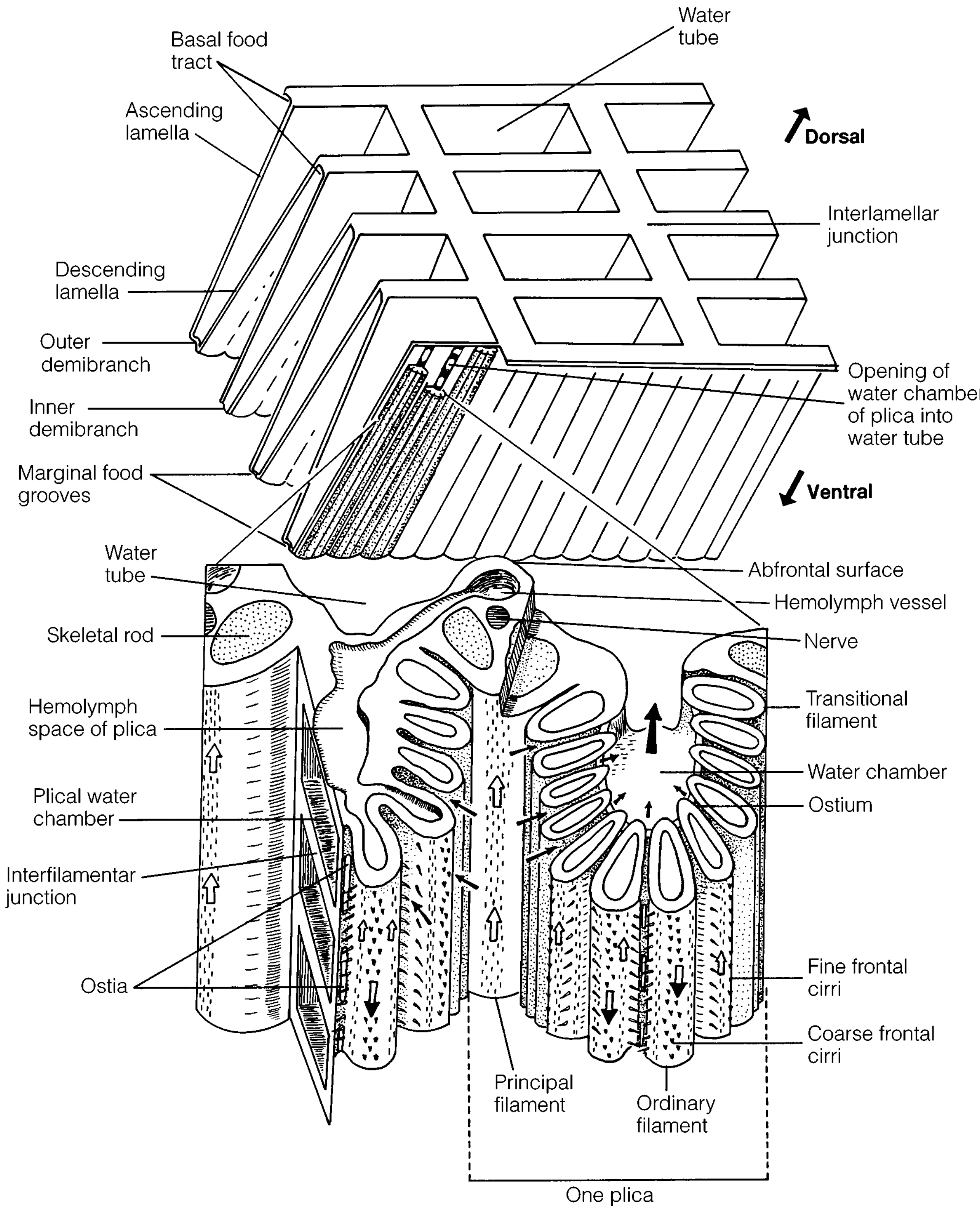

Figure 7. Stereodrawing of part of two gills of *C. virginia* (upper) with expanded detail of a plical fold (lower). The direction of particle movement on the coarse and fine frontal cilia is indicated by the black headed and open arrows, respectively. The direction of water movement between the filaments, through the ostia and into the water tubes is indicated by the solid black arrows. Based on drawings by M.R. Carriker in Nelson (1960).

Filaments of each lamella are arranged in a regular series of folds called plicae. Each plica consists of a large principal filament located at the base of the fold and a series of ordinary filaments; that filament next to the principal filament is termed the transitional filament (Figs. 7,8).

Histology and Fine Structure

Histology

Each gill filament consists of a simple epithelium whose cells on the frontal and lateral surfaces bear cilia of various sizes: contrary to the diagrams in Nelson (1960), my own histological observations indicate that cells on the abfrontal surface lack cilia (Figs. 7, 8, 9).

The marginal food groove is a ciliated trough formed at the apex or margin of each demibranch, the free edge where the ascending and descending lamellae join (Fig. 7). The epithelium varies from a stratified ciliated columnar type, from three to four cells deep near the edges of the groove to only one or two cells thick at the center of the groove. Two types of goblet cells are present usually near the edges of the groove: a more dominant form that secretes acidic glycoproteins and one that secretes neutral glycoproteins (Eble, pers. obs.). Cilia are long in the terminal groove and average 10 to 12 µm, especially

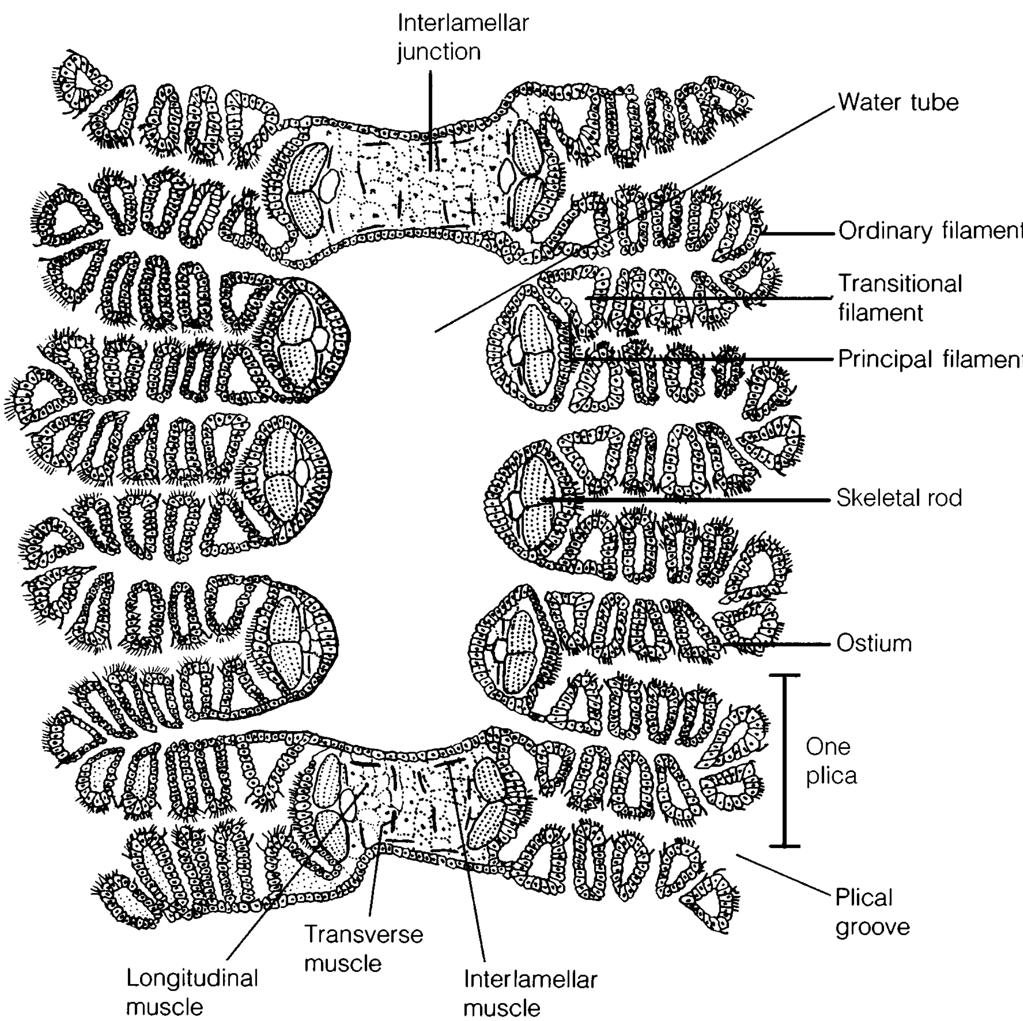

Figure 8. Frontal section through a gill demibranch showing plicae and two interlamellar junctions. × 500. Redrawn from Galtsoff (1964).

near the edges. The direction of beat is towards the palps.

A pair of skeletal rods is present in all types of filaments; although the older literature refers to them as chitinous, they are composed of a fibrous protein rich in collagen (Brown 1952; Rudall 1955; LePennec et al. 1988). Skeletal rods are found under the epithelium on the frontal and lateral surfaces (Figs. 8, 9); connective tissue can frequently be seen between the rods. The remainder of the interior of the fila-

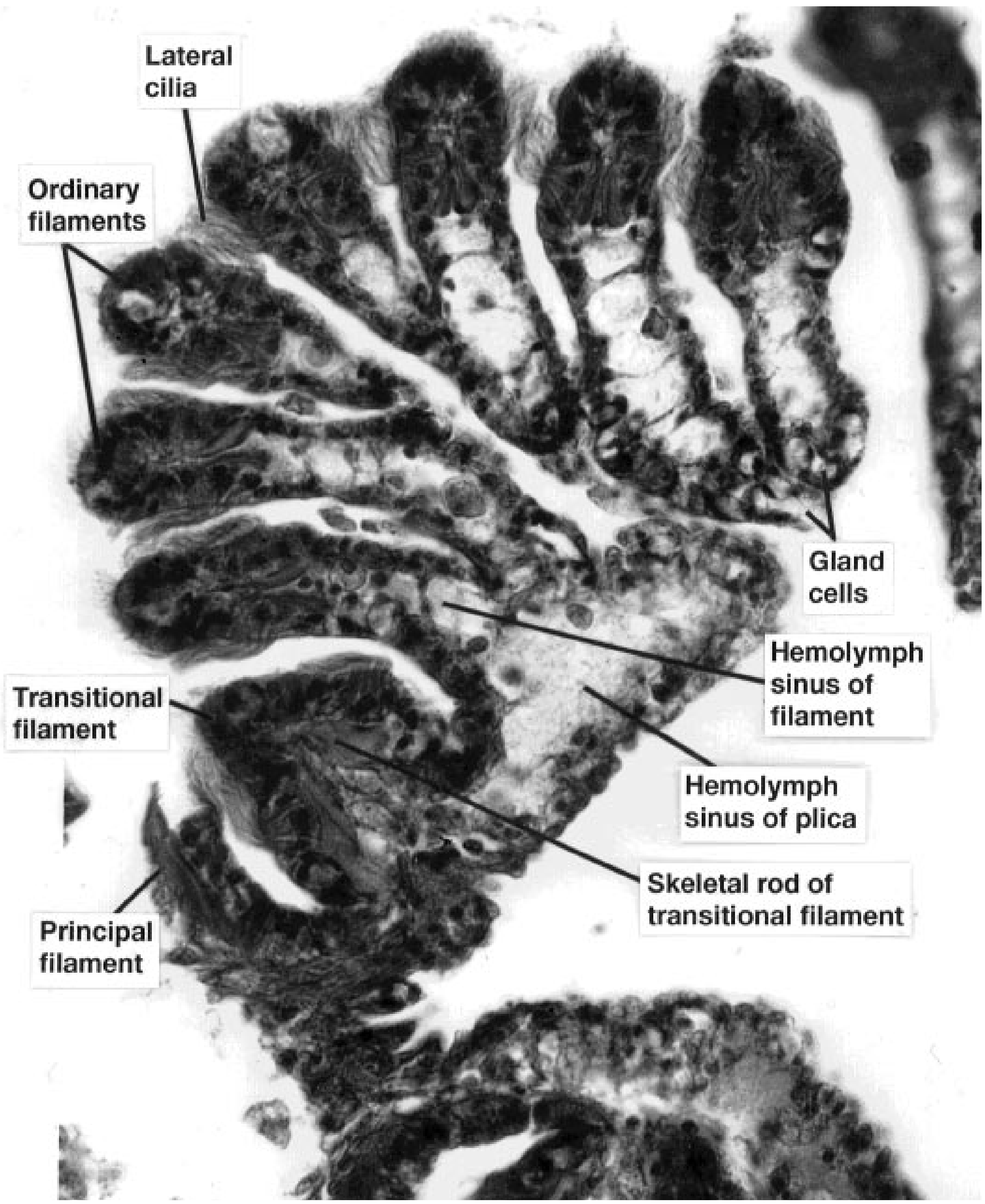

Figure 9. Frontal section through a gill plica to show general structure. Note how blood sinuses of ordinary filaments become contiguous with blood sinus of plica. HFW = 71 µm.

ment is a hemolymph sinus spanned by slender lacunar cells (Figs. 9, 10).

My sections also show two types of goblet cells on gill filaments: a large cell (26 µm) filled with coarse granules composed of acidic glycoproteins located on the frontal surface and smaller cells found near the lateral and abfrontal areas that secrete neutral glycoproteins. According to Bernard (1974), the large, granular mucous cells in *C. gigas* elaborate a zone of mucus about 12 µm thick and 20 µm wide; unicellular glands on the lateral and abfrontal surfaces respond to disturbance (either tactile, temperature, or light stimuli) and form a "rejection" mucous sheet 250 to 400 µm thick (see Newell and Langdon, Chapter 5 for discussion of current theories of particle capture). Atkins (1938) figures two types of mucous glands in European flat oyster *Ostrea edulis* but she does not discuss their histochemistry.

Fine Structure

The epithelium of gill filaments of *C. virginica* consists of two broad categories of cells, those with and those without cilia (Fig. 11). Both cell types possess microvilli and may contain mucus droplets.

Microvilli average 0.7 µm in length and 0.1 µm in diameter; their external surface is covered by a fine filamentous "cell coat" or glycocalyx (Fig. 12). Microvilli are also covered by three to five fine strands of mucus that run parallel to the surface of gill filaments; the thickness of these strands is, on the average, 20 nm. When several microvilli are examined in cross-section, they demonstrate a close contact with

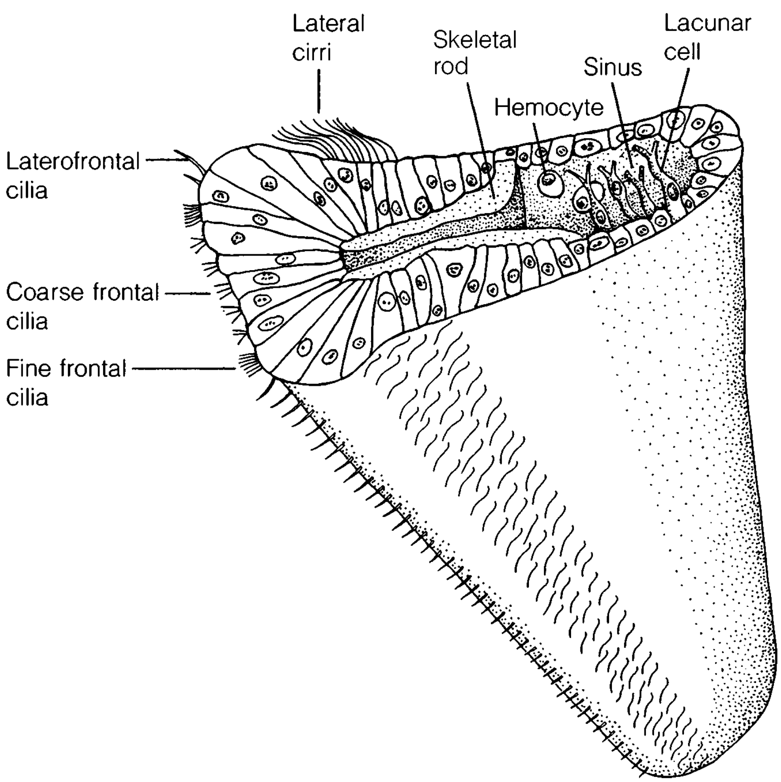

Figure 10. Stereodiagram of an ordinary filament of the gill. Note slender lacunar cells spanning the hemolymph sinus. $\times$ 20.

each other (and with adjacent cilia) via the mucus, which imparts a web-like appearance to the microstructure of the gill surface (Fig. 12; pers. obs.).

The cytoplasm of most ciliated epithelial cells contains many round or ovoid mitochondria that are located primarily in the apical portion of cells (Fig. 13); mitochondria are more numerous in ciliated cells than in non-ciliated cells.

The cytoplasm of some non-ciliated epithelial cells is filled with large, round, secretion granules of varying size whose profiles range from 0.70 to 1.30 µm; secretion granules are surrounded by a unit membrane. Based on their ultrastructural difference, these granules can be divided into two morphological types: Type-A granules contain homogeneous electron-dense material and Type-B granules are moderately homogeneous and moderately electron dense (Fig. 13). These two types of granules may represent different maturation stages of a secretion granule or two different types of secretory granules.

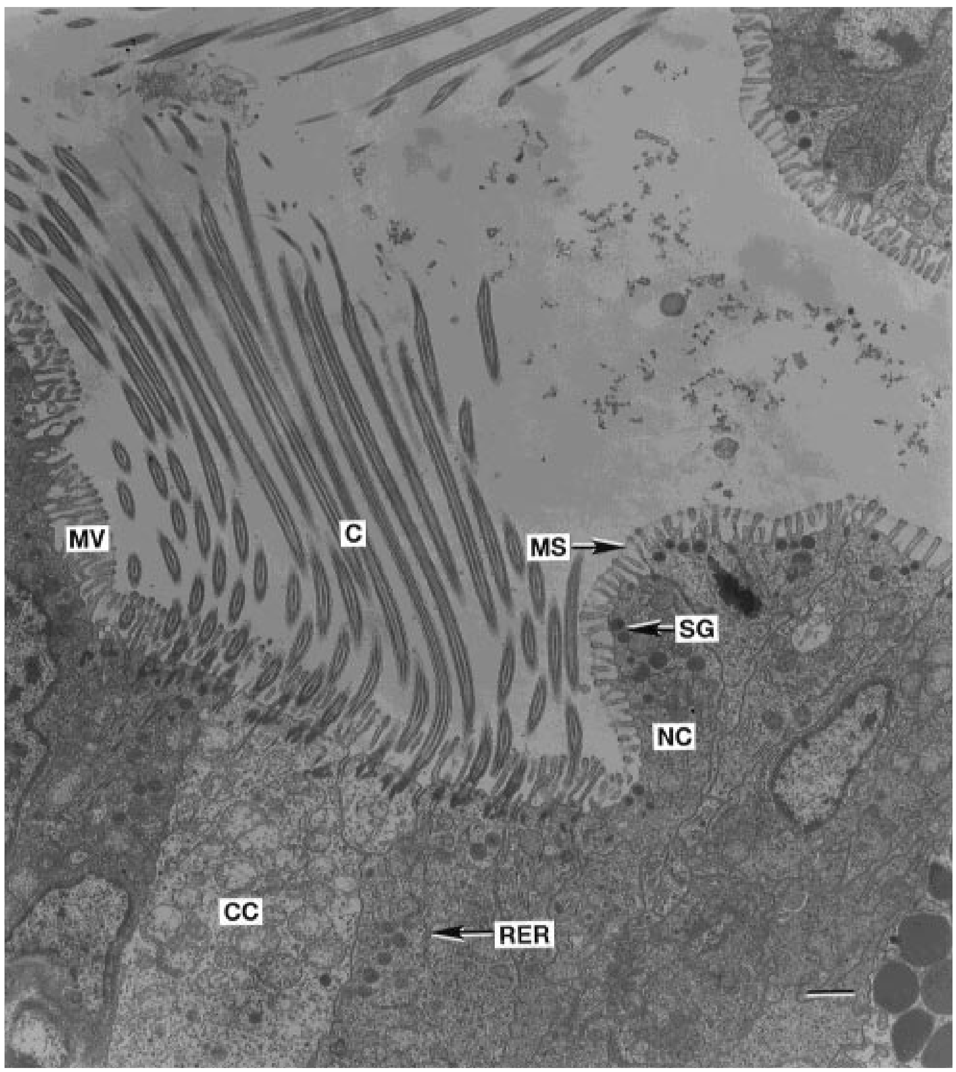

Figure 11. Abrupt transition between lateral ciliated [CC] and non-ciliated [NC] cells in a gill filament. Cilia [C], mucous strands [MS] between microvilli [MV]. Rough endoplasmic reticulum [RER], secretory granule [SG]. × 9,075. Bar = 1 µm.