Chapter 3

The Shell and Ligament

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INTRODUCTION

The literature on the shell of the Ostreidae is voluminous (Baughman 1947; Stenzel 1971; Joyce 1972; Breisch and Kennedy 1980; Kennedy and Breisch 1981). It includes studies of the valves of many species undertaken during the last three centuries. Within the last four decades, the macrostructure, microstructure, biocrystallography, biochemistry, and formation and mineralization of shell have been examined in increasing detail. In part, this emphasis has reflected the advent of new procedures and instrumentation.

Here I present a synthesis, based on Galtsoff's (1964) monograph, of the literature appearing primarily during the last three decades on the ontogeny of form, structure, microstructure, biomineralogy, biochemistry, and physiology of shell and shell formation in larval, juvenile, and adult eastern oysters, *Crassostrea virginica* (Gmelin). (For a description of general anatomy, see Eble, Chapter 2.) The analysis examines mineral and organic components of the valves, periostracum, hinge and ligament, adductor muscle scar, shell-secreting epithelia, extrapallial space, shell deposition, biomechanics, weathering and dissolution, environmental effects, regeneration, anomalies, economics, and important problems on the biology of the shell awaiting study. Where little or no information exists on the shell of *C. virginica*, I draw upon available knowledge from other species of Ostreidae.

LARVAL SHELL

Several investigators have described the shell of early stages of *C. virginica* examined by light microscopy. This research was reviewed by Rees (1950); Carriker (1951); Stenzel (1971); Dinamani (1976); and Waller (1981). With the exception of one micrograph by Dinamani (1976) of the hinge of a larva 250 μm long, no published reports existed on the microstructure of the valves of larvae and newly hatched spat of *C. virginica* before the publication by Carriker and Palmer (1979a). Microstructural investigations of valves of larval stages of other species of oysters were similarly limited (Dinamani 1976) until the appearance of Waller's (1981) superbly illustrated paper on the larval valves of the European flat oyster *Ostrea edulis*.

Several developmental features in larval shells, not seen by early malacologists with light microscopy, have been revealed by electron microscopy (Carriker and Palmer 1979a; Waller 1981). Knowledge of these microstructures will be useful in micromorphological, physiological, genetic, and ecological studies in which the valves serve as indicators of larval condition.

Terminology

Various terms are in common use in description of the bivalve larval shell (Galtsoff 1964; Carriker and Palmer 1979a; Waller 1981). These terms are defined here as background for the sections that follow (Fig. 1).

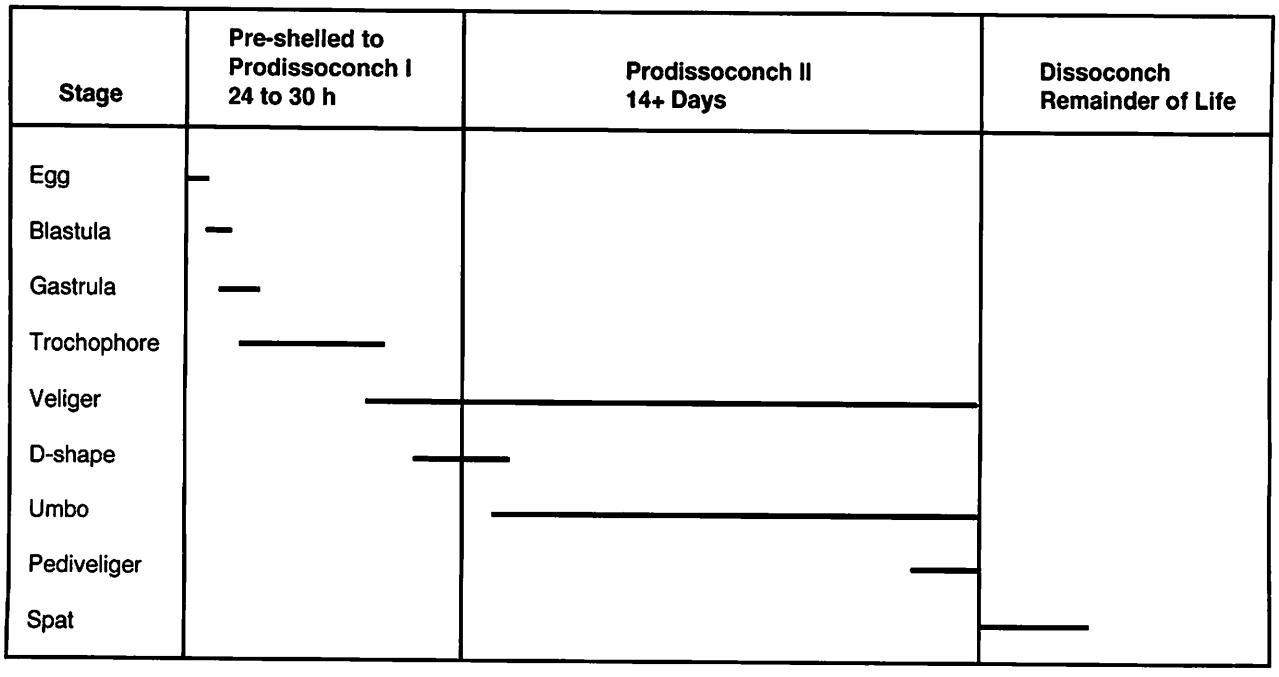


Figure 1. Association of terms used to describe early stages of *Crassostrea virginica*. Based on Waller (1981). Duration of stages, especially prodissoconch II, can be highly variable, depending on temperature, food supply, and other factors.

Anterior and ***posterior***, directions parallel to the hinge and toward the sites of anterior and posterior larval adductor muscles.

Antimarginal, sculptural and structural features of the shell that are nearly perpendicular to the shell margin at all points (Waller 1986).

Beak, the earliest part of each dissoconch valve.

Commarginal, sculptural or structural features of the shell that are parallel to the shell margins or to previous traces of the shell margin.

Commissure, the line along which edges of the valves are in contact.

Dissoconch, begins at the metamorphic line after metamorphosis, ends the prodissoconch II stage, and continues for the duration of adult sessile existence. The end of the planktonic stage and attachment of the pediveliger to a hard substratum has been termed "setting," "settling," "settlement" or "spatfall"; these different expressions are used interchangeably and are synonymous (Galtsoff 1964). Because the word *setting* has been employed commonly by American biologists and oyster growers, this term will be used here.

Dorsad, toward the larval hinge.

Height, maximal dorsoventral dimension perpendicular to the hinge.

Length, maximal anterior-posterior dimension of the shell parallel to the hinge line.

Pediveliger, the swimming-crawling larval stage (Carriker 1961) that develops toward the end of prodissoconch II stage and serves as the transitional form between planktonic and benthic existence.

Prodissoconch I, extends from the first appearance of the larval shell material to the first meeting of valve edges, forming a straight-hinged, D-shaped shell. Stenzel (1971) referred to this stage as "phylembryo" or "protostracum veliger."

Prodissoconch I/II boundary, a narrow, faintly commarginally striated transitional band sandwiched between prodissoconch I and II regions of the valves.

Prodissoconch II, extends ontogenetically from the prodissoconch I/II boundary to metamorphosis; the latter is identified by the metamorphic line in the valves. An abrupt change occurs beyond this line in shell microstructure and mineralogy,

marking the appearance of the spat or early dissoconch stage. Prodissoconch II valves exclude prodissoconch I shell but include new shell deposited on the inside of prodissoconch I valves as well as that added beyond the margins of prodissoconch I valves. The exterior shell surface of prodissoconch II bears conspicuous commarginal growth striae that clearly distinguish it from the faint microsculpture of the shell surface of prodissoconch I. Generically, the prodissoconch (I plus II) is the entire larval shell formed before metamorphosis and is synonymous with the term "larval shell." The term "veliger" refers to any shelled molluscan larva that possesses a velum, the ciliated swimming organ. The term "veliconcha," referring to the shelled veliger (including both prodissoconch I and II), was employed in the earlier literature, but is not much used now (see also Jablonski and Lutz 1980). Stenzel (1971) referred to the prodissoconch as the "prodissoconch veliger," omitting the I and II stages.

Radial, elements of each valve that radiate from the umbo and are approximately perpendicular to the shell margin midventrally.

Shell field, an area of ectodermal cells in the presumptive dorsal region of the developing embryo secreting the embryonic shell: the central part of the shell field temporarily invaginates to form the shell field invagination (earlier known as the "shell gland"), then evaginates during the shell-forming process.

Umbo (plural, umbos or umbones), the rounded elevated oldest part of each valve located to each side of the hinge and atop the beak. Anterior and posterior ends of the umboned larval oyster shell are identified as follows: the umbo of the left valve is larger and projects dorsally farther than the right one; thus, when the hinge line is viewed from above, the larger of the two umbones is to the left, the smaller to the right, and anterior is forward and posterior is to the back.

Ventrad, away from the hinge.

Width, maximal dimension between exterior surfaces of right and left valves. The terms "depth" and "convexity" are sometimes used synonymously with "width" (Chanley and Andrews 1971; Waller 1981) defined by Waller (1981) as the maximal transverse width perpendicular to the plane of commissure.

Dimensions

Sibling larvae of *C. virginica* maintained under identical conditions of culture grow at widely different rates and metamorphose at different times (Loosanoff and Davis 1963; Newkirk et al. 1977; Carriker and Palmer 1979a). For this reason larval stages have been identified primarily by form and size rather than by age. At normal summer temperatures in the Mid-Atlantic area, oyster larvae generally start setting about 2 weeks after fertilization, although specific rate of development varies with temperature; the same applies in laboratory culture of the larvae. Considerable variation has also been reported on the maximal size of larvae along the Atlantic seaboard, there being a tendency for them to set at a larger size in northern than in southern latitudes. A range of maximal length of 248 to 400 µm has been recorded by various investigators (Carriker 1951). Comparison of larvae collected in estuaries (Carriker 1951, 1959) with those cultured in the laboratory (Carriker 1959; Epifanio 1982) indicates that their external morphology is similar, and that laboratory culture does not seem to alter maximal sizes or result in deformed valves.

Dimensions of larval valves of *C. virginica* during development from prodissoconch I to the setting stage were recorded by Chanley and Andrews (1971) for larvae from Virginian estuaries as follows (see also Loosanoff and Davis 1963; Galtsoff 1964; Forbes 1967):

Length: 60 to 350 µm.

Height: initially 10 µm less than length, increasing to equal length at 90 to 100 µm, and eventually exceeding length by as much as 15 µm.

Width: 35 to 40 µm less than length, increasing to 100 µm less than length in late stages.

Hinge line: 45 to 50 µm.

Metamorphosis: at lengths ranging from 310 to 350 μm.

Shape: periphery of valves rounded at a valve length of 80 to 100 μm, and asymmetric umbones begin to form at a length of about 85 to 125 μm.

Identification

As development proceeds, the left valve of *C. virginica* larvae becomes increasingly convex and the left umbo projects and overhangs the umbo of the right valve, resulting in a conspicuously asymmetric shell (Figs. 2 to 8)[1]. Thus, with low-magnification light microscopy it is relatively easy to distinguish late-stage eastern oyster larvae from other molluscan veliger larvae in estuaries where no other species of oysters are present (Chanley and Andrews 1971). However, identification with light microscopy of early stages of most species of bivalve larvae can be exceedingly difficult, if not impossible (Rees 1950; Carriker 1951, 1959; Jablonski and Lutz 1980). Lutz et al. (1982), using scanning electron microscopy in combination with light microscopy, are developing an identification system based on characteristics of the hinge structure (provinculum) (see also LePennec 1980) that promises to facilitate bivalve larval recognition, especially of early stages.

Stages

Embryonic Shell

Initial shell formation has not been examined in the eastern oyster. The following discussion is based on the few studies of this process in other bivalves (Raven 1966 and La Barbera 1974 for the giant clam, *Tridacna squamosa*; Kniprath 1979, 1980, for the Mediterranean mussel, *Mytilus galloprovincialis*; Eyster and Morse 1984 and Eyster 1986 for the surf clam, *Spisula solidissima*; also L.S. Eyster, Tufts University, pers. comm.) These studies may be indicative of how shell formation occurs in preprodissoconch I stages of *C. virginica* (Fig. 1).

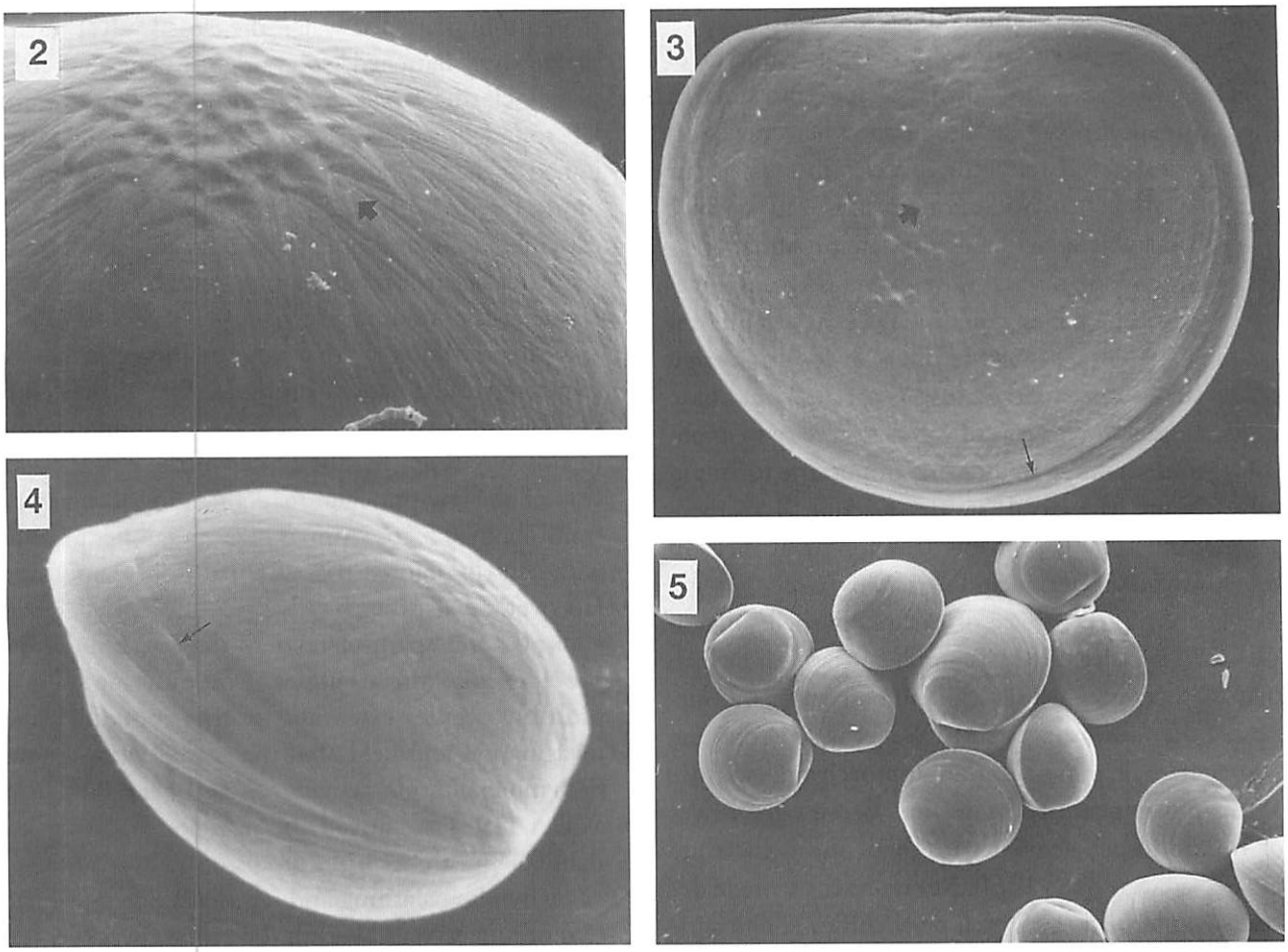
The first appearance of the shell field occurs in early embryogenesis when gastrulation is not yet complete (Moor 1983). This tissue field consists of a thickened cap of ectoderm, one cell thick, that forms over the presumptive dorsal side (post-trochal) of the embryo. As embryogenesis proceeds, the center of the shell field invaginates to form a deep, transitory "shell field invagination" (term suggested by Eyster [1983], earlier called the "shell gland") with an elongated pore to the exterior. Initial organic shell material is then secreted by cells of the shell field near the outside of the pore, forming a transparent pellicle (precursor of prodissoconch I periostracum) over the shell field and across the pore of the invagination. Next, the invagination completely evaginates and shell field epithelial cells flatten and multiply by cell division, spreading over the embryonic surface and forming the beginning of what will become the right and left mantle lobes. Probably during evagination, shell-field cells begin to secrete inorganic shell materials (calcium and other materials) into the extrapallial space sandwiched between the pellicle and the shell field epithelium, and mineralization of the embryonic shell commences. Whether calcium is secreted by cells lining the invagination or by cells that are part of the recently evaginated region of the shell field has not been documented for any mollusc (Eyster, pers. comm.). With further development of the embryo, mineral-secreting cells migrate over the right and left sides of the embryo in the wake of pellicle-secreting cells, continuously sheltered beneath the pellicle. The extrapallial space remains closed against the exterior environment by adherence of the pellicle edge to marginal cells of the shell field, forerunners of the mantle. There is probably only a single shell field and invagination in bivalves, the two embryonic shell valves being secreted by cells that migrate from the single shell field invagination to produce an initial center of mineralization beneath each forming valve. Bandel (1988) believes that the mantle in bivalves differentiates without in- or evagination. Organic shell material along the dorsal line where right

[1] All figures are of *Crassostrea virginica*, and photographs are scanning electron micrographs, unless otherwise noted. Sizes in micrometers are shell heights. HFW = horizontal field width of figure.

and left valves meet remains uncalcified; this line forms the ligament of the larval shell.

In the early trochophore of *Spisula solidissima* at 15°C, first organic shell material appears at an age of 27 h, and first minerals at 33 h. The new mineralized shell is homogeneous, with no layers or compartments. Electron microprobe analyses indicate that embryonic shells are composed almost entirely of calcium, with only small, variable quantities of Na, Mg, Al, P, S, Cl, and K (Eyster 1986). Bidwell et al. (1986) and A.M. Kuzirian (Marine Biological Laboratory, pers. comm.) demonstrated that strontium appears to be essential for the normal development of the larval shell and of larval behavior in widely separated molluscan taxa. Embryos grown in artificial seawater without strontium chloride became larvae with normally appearing soft tissues but with deformed shells and statoliths.

Shell and statolith formation was sensitive to strontium concentration differences as low as 1 ppm; a concentration of 5 to 8 ppm (near its oceanic concentration) was required for complete mineralization. In the California sea hare, *Aplysia californica,* the critical period of exposure for the required strontium be-



Figures 2-5. (2) Prodissoconch I (65 µm), punctate-stellate pattern [arrow] on external surface of one valve soaked for 75 min in a 2% v:v solution of domestic 5.25% sodium hypochlorite (bleach). (3) Prodissoconch I (65 µm), side view. Punctate-stellate pattern. Narrow rim of prodissoconch II shell forming along edge of shell [arrow]. 2% bleach 10 min. (4) Prodissoconch I (75 µm), oblique end view. Rim of prodissoconch II shell forming [arrow]. 2% bleach 6 min. (5) Prodissoconch II. Valve heights range from 95 to 130 µm. 5% bleach 10 min.

gan 72 h after oviposition and lasted no longer than 24 h; in the hard clam, *Mercenaria mercenaria,* it occurred between hours 15 to 20 of embryogenesis. Bidwell et al. (1986) suggested that strontium could be a cofactor of an enzyme involved in initiating mineralization during formation of the prodisssconch I shell. Kunigelis and Wilbur (1987) determined that inorganic phosphates at concentrations of 15 and 150 ppm inhibited embryonic shell growth in *C. virginia.* Shell calcium carbonate in molluscan embryos that have been analyzed is aragonitic (LaBarbera 1974; Eyster and Morse 1984; Eyster 1986). The mineralization of the early embryonic shell of *C. virginia* has not been determined.

According to LaBarbera (1974), in embryos of *Tridacna squamosa,* areas of most rapid and heaviest shell formation occur at the hinge (where valves articulate and larval retractor muscles insert), at the shoulder above the adductor muscle, and at valve margins. Muscle attachment sites are presumably areas of greatest stress when valves are closed; reinforcement of rims of valves would be necessary to prevent warping and ensure integrity of valve closure. Design of the shell form observed in this species could have evolved to reinforce areas of valves necessary to establish the mechanical framework important for rapid valve closure (the larval "startle" reaction [LaBarbera 1974]); the same is probably true for larval shells of other bivalve species, including *C. virginica.*

The dimpled surface of each early shell valve of *O. edulis* (Waller 1981) is similar in appearance to that in *C. virginica* (Fig. 2). The pattern of the surface of the valves suggested to Waller (1981) that the two centers of mineralization approach one another at the non-mineralized hinge line. Because the halves of the dumbbell-shaped pattern of the pellicle are connected, it is likely the halves represent a single developmental field (L. Eyster, pers. comm.). The curiously sculptured and textured surface of the first larval valves undoubtedly reflects changes taking place in the shell field epithelium during cellular differentiation in the transition from invagination to evagination and subsequent spread from the center of the field (Waller 1981).

Prodissoconch I

This stage begins with the onset of mineralization, ends with valve closure and complete covering of the larval organs by translucent valves (Waller 1981), and is completed in 24 to 30 h at summer temperatures (Andrews 1979). The straight-hinged (D-shaped) outline of prodissoconch I valves of *C. virginica,* when seen in side view using a scanning electron microscope (Fig. 3), resembles the microscope-based illustrations of this stage prepared by earlier investigators (Loosanoff and Davis 1963). Not so evident in these illustrations, however, is the considerable width of the larva at this early stage; rather than a thin, flattened wafer, the closed valves resemble an oval, flattened globe (Fig. 4). The first completely closing larval shell is formed into right and left rounded, vaulted valves of equal size and shape, joined at the anteriorly placed hinge. Valves are aragonitic (Stenzel 1964; Carriker and Palmer 1979a), extremely thin, and under low optical magnification the exterior surface appears relatively uniform and translucent to transparent.

Most striking in this stage is the conspicuous punctate-stellate pattern on the surface of the center of each valve when viewed with scanning electron microscopy (Fig. 2). The center of the pattern consists of shallow punctate marks ranging in diameter from about 0.5 to 3 μm. These merge peripherally with acutely pointed triangles that radiate and overlap over most of each valve. Commarginal striae, about 0.3 μm apart, blend with the radial lines from the apexes of the triangles. The punctuated area probably overlies extensions of the embryonic shell invagination and field, and the triangles possibly reflect mineralization activity of cells of the shell field. Waller (1981) removed the pellicle (probably less than 0.1 μm thick) from prodissoconch valves of *O. edulis* by plasma ashing and revealed a polygonal pattern of tiny crystals beneath the pellicle over the shell field, and marginal, incremental mineralization beneath the stellate-radial external sculpture of the valves. Waller (1981) concluded that change from shell-field cells to mantle epithelium is probably gradual and occurs well before the prodissoconch I/II boundary. Formation of the faintly commarginally

striated band between prodissoconch I and II, about 7 to 8 μm wide in *C. virginica*, probably represents adjustment of mantle edges to valve secretion of the shell past the embryonic stage (Figs. 3, 5, 6).

Prodissoconch II

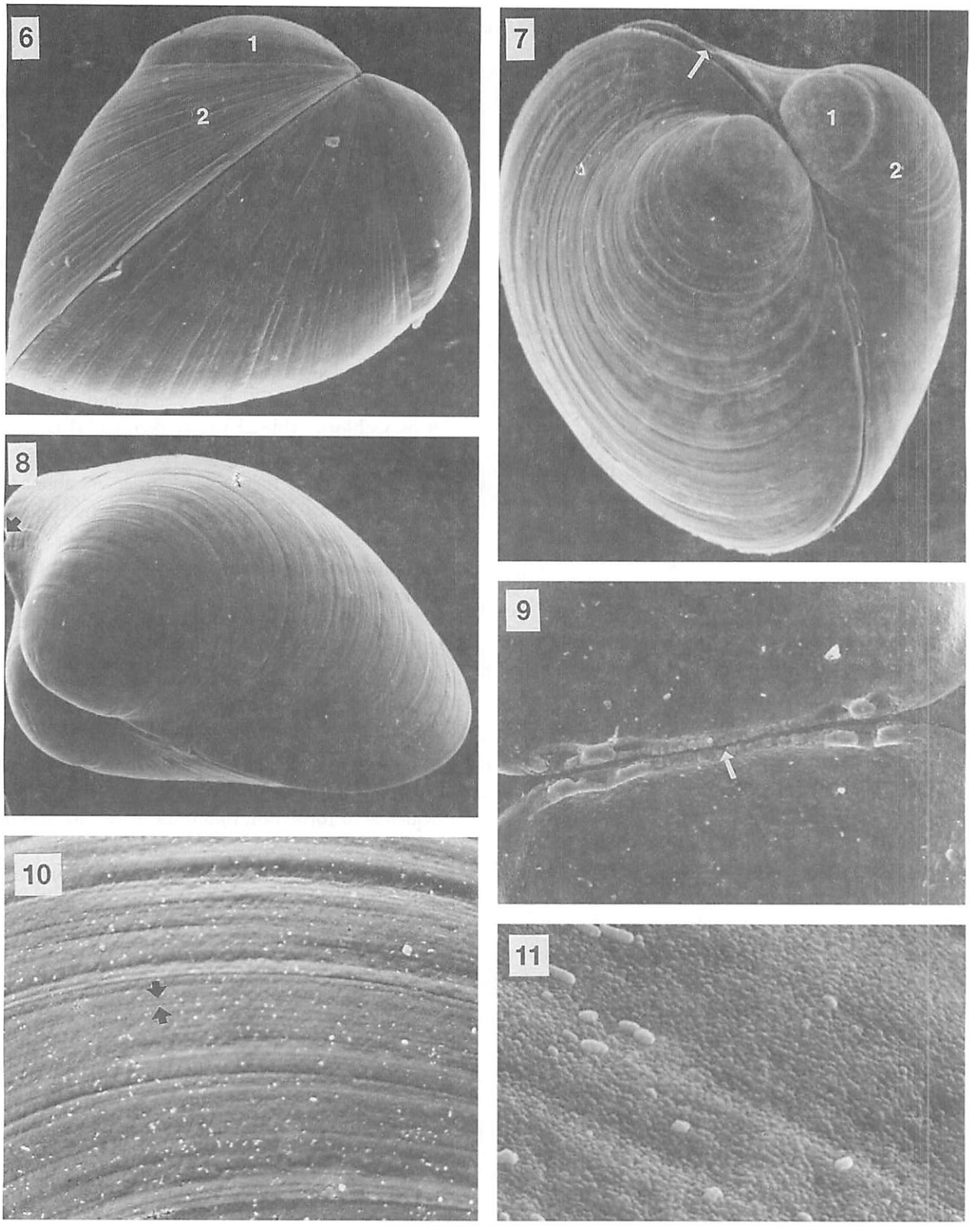
As veligers increase in size, their valves develop unequally, the left valve (the future attached valve of the spat) becoming considerably wider (i.e., in the right-left dimension) than the right one (Fig. 6). Simultaneously on either side of the hinge area there appears an increasingly prominent umbo, the left being larger and projecting farther above the hinge than the right one (Fig. 7). In the process of marginal incremental growth of the valves (maximal shell growth occurring midventrally), previously formed commarginal shell annuli are gradually displaced dorsolaterally on either side of the hinge, resulting in the dorsally projecting umbones and pointed dorsal ends of the valves (Fig. 8). As growth progresses, the umbones "bend" slightly toward the posterior (Fig. 7). This is the result of greater increments of shell deposition along the anterior-anteroventral margins of the valves than along the posterior margins and the concomitant shift in position of the axis of rotation through the interumbonal length and differential migration of the ligament (T.R. Waller, Smithsonian Institution, pers. comm.). Valves become increasingly heavier than those of prodissoconch I, and as seen under optical magnification, although still translucent, increasingly obscure the organs within. With further increase in size of the shell, umbones come close together medially (Fig. 7), preventing valves from opening widely, and anterior and posterior sides of the valves flare broadly (Fig. 8). The hinge develops into a complex larval provinculum (Fig. 9). In larvae of the Pacific oyster, *Crassostrea gigas*, comparative rates of shell deposition are higher than those reported for the adult (Maeda-Martinez 1987).

The anterior adductor muscle develops first in the early prodissoconch I. By the end of this stage, at a shell height of about 100 μm, both adductor muscles are present and are of nearly equal size. At the surface of the adductor muscle scar, adductor muscle fibers split into several components and terminate on an intermediate layer of modified mantle tissue; within this layer of myo-epithelial tissue, dense bundles of fibrils pass from the shell surface to the junctional aspects of the muscle fibers (Elston 1980; see also Bonar 1978). After setting of the pediveliger, the anterior muscle is resorbed, and the posterior one moves anteroventrally to occupy its definitive position and become the single adductor muscle of the adult oyster (Galtsoff 1964; Stenzel 1971; see Eble, Chapter 2).

A prominent feature of exterior surfaces of prodissococh II valves of *C. virginica* treated with sodium hypochlorite (bleach) (Carriker 1979) is closeset, conspicuous, commarginal annulations (Figs. 6, 7, 8). The finest of these growth striae are about 0.8 μm apart (Fig. 10). Removal of the periostracum from the valves exposes microstructural shell units, which are granular in nature and vary in size from 0.1 to 0.2 μm (Fig. 11).

In *O. edulis*, typical shell striae are formed in prodissoconchs cultured at constant temperature and illumination without mechanical disturbance, and thus would appear to result from an endogenous rhythm (Millar 1968). In tropical bivalve mytilid larvae, growth striae are produced twice daily regardless of temperature or salinity differences (Siddall 1980). Frequency of formation of annuli in *C. virginica* has not yet been related to environmental factors.

The polymorph (a specific crystalline form of a compound that can crystallize in different forms) of calcium carbonate in the valves of prodissoconch II, like that of prodissoconch I larvae, is also aragonite (Stenzel 1964; Carriker and Palmer 1979a). Why larval Bivalvia possess aragonitic shells is unclear (Carriker 1988). Stenzel (1964) conjectured that aragonitic valves could be more advantageous than calcitic ones to motile veligers because aragonite is harder, has greater strength as a structural material, and is less prone to breakage by cleavage. Calcitic valves, on the other hand, could be more advantageous than aragonitic valves to bivalves permanently immobilized on the bottom, like adult *C. virginica*, because calcite is less soluble in seawater (Simkiss and Wilbur 1989) and is secreted more economically than aragonite (i.e., calcite fills a larger volume per mole than does aragonite). However, it has been reported that



Figures 6-11. (6) Prodissoconch II (120 µm), anterior view. Prodissoconch I valve [1], prodissoconch II valve [2]. 5% bleach 23 min, 3 min low power sonication. (7) Prodissoconch II (300 µm), umbones viewed obliquely from right side. Arrow points to posterodorsal notch of left valve. Prodissoconch I valve [1], prodissoconch II valve [2]. 5% bleach 45 min. (8) Prodissoconch II (250 µm). Left anterodorsal view. Arrow points to posterodorsal notch and fasciole of left valve. 5%

bivalve larvae of at least one species possess partly calcitic valves (Medakovic et al. 1989).

The prodissoconch I valves of *O. edulis* incubating in the mantle cavity of the parent contain up to 4% calcite and 7% aragonite, whereas prodissoconch II valves just before release of the larvae from the parent are almost entirely aragonitic with only a trace of calcite (Medakovic et al. 1989). These authors suggest that calcitic crystallites play a role in the crystallization of aragonite. Distribution of calcite and aragonite in the larval valves also points to a possible linkage between free-living bivalve larvae and an aragonitic shell. If this is true, bivalve larvae, like those of the Chilean oyster *Ostrea puelchana*[2] (Walne 1963; Chanley and Dinamani 1980), which remain the full larval period within the parent (Chaparro et al. 1993), should possess partly, or perhaps totally, calcitic larval valves. This interesting possibility has not been investigated.

Mantle and Valve Margins. Most of the bilobed mantle of veligers of *C. virginica* is a single cell thick. At margins, the mantle thickens to two to four cells and splits into two folds from between which the newly elaborated layer of periostracum emerges and on which the shell is deposited. The mantle also thickens at the dorsal region where right and left lobes merge and secrete the periostracal material that forms the ligament-like continuous external layer between the hinged sides of the valves. Cells of the mantle are typically flattened and contain dense granular cytoplasm with abundant profiles of rough endoplasmic reticulum (Elston 1980). In all stages of larvae of *O. edulis*, the margin of the mantle also consists of two folds (Waller 1981).

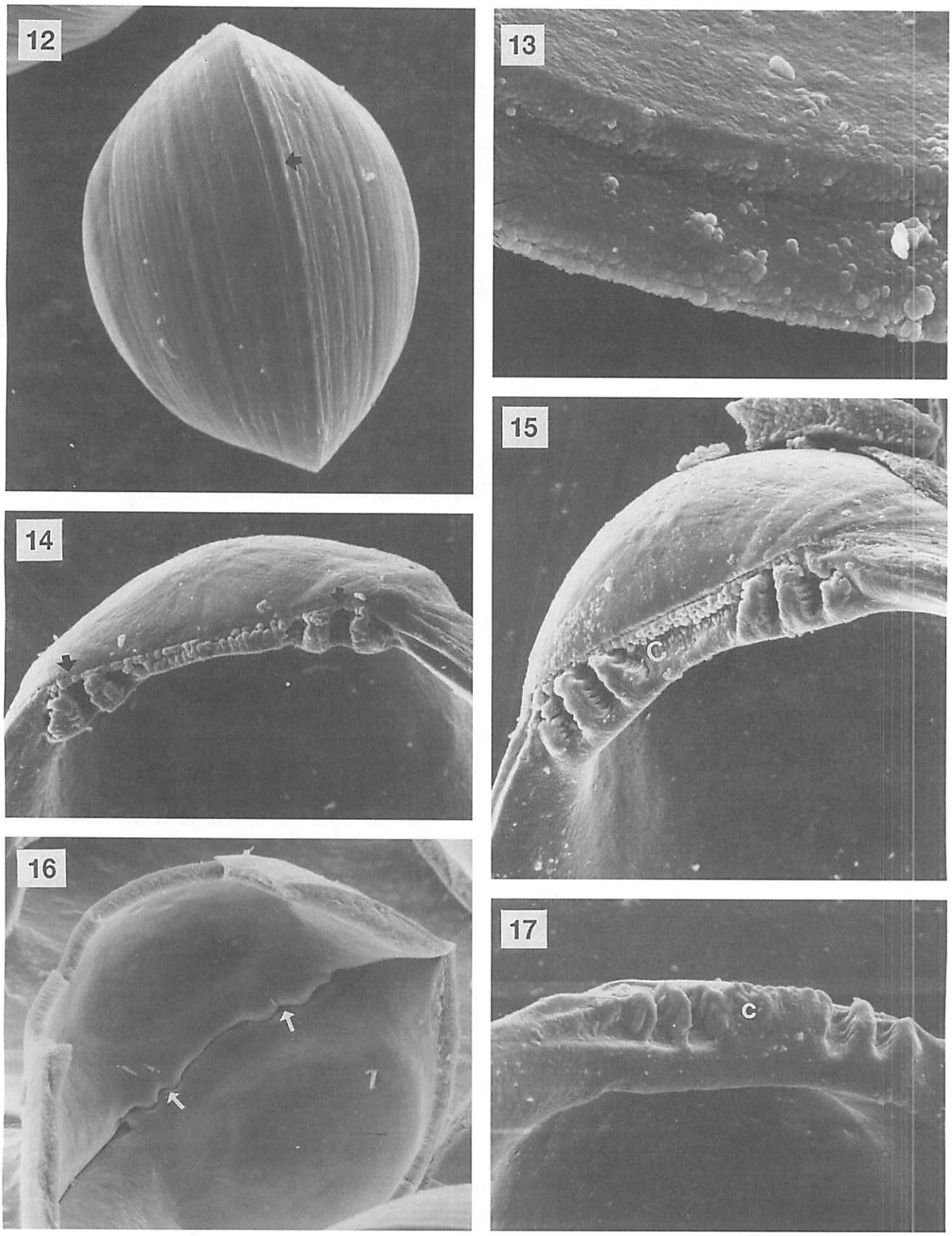
The contact of valve edges of *C. virginica* is tight (Figs. 6, 8, 12). The rims are terraced internally and produce a step that runs around each valve to the provinculum (Fig. 13). Terracing is revealed by dissolution of the periostracum with bleach. Shell units on valve edges are granular and range in diameter from about 0.1 to 0.5 μm. In all prodissoconch II stages of *O. edulis*, valves likewise fit together tightly at the edges in a tongue-and-groove fashion, the right valve rim possessing the groove and the left valve rim the tongue (Waller 1981).

Provinculum. As valves of prodissoconch II develop, the provinculum thickens and rudimentary swellings on anterior and posterior ends form into well-defined interlocking teeth about 4 to 5 μm long (Fig. 9), and shallow corrugations between them soon develop into minute denticles. By the early prodissoconch II stage the provinculum is thus well formed, the large terminal rectangular taxodont teeth about equally developed on each end of the provinculum (Fig. 14). Terminal teeth are slanted toward the median at an acute angle (Fig. 15). Length of the provinculum is about 45 μm, and remains about the same dimension throughout prodissoconch II stage; valve margins gradually encroach upon and outflank hinge ends (Stenzel 1971). There are no flanges, or lateral or special teeth beyond the ends of the provinculum, the strong closely articulating terminal teeth serving to guide the hinged valves (Fig. 16).

By mid-prodissoconch II, terminal teeth are fully formed (Fig. 15); two at each end of the provinculum of the right valve (Fig. 17), and three at each end of the provinculum of the left valve (Fig. 18). Number and placement of teeth in larval *C. virginica* cor-

[2] Note that the taxonomic status of austral ostreid species is controversial. These authors originally identified this species as *Tiostrea chilensis*, but the editors have renamed it following the convention of Carriker and Gaffney (Chapter 1).

bleach 25 min, 6 min sonication. (9) Provinculum of prodissoconch II, slightly more developed than that in Figs. 7 and 8, viewed from interior ventral side. Provinculum 48 μm long, denticles [arrow] starting to form between larger interlocking terminal teeth. 2% bleach 10 min. (10) Prodissoconch II, pediveliger. Exterior surface of valve. Note the commarginal annulations, the finest of which are 0.8 μm apart [see arrows]. 5% bleach 45 min. (11) High magnification of Figure 10 to show granular structure of exterior shell surface with peristracum removed. Granules about 0.1 μm in diameter. 5% bleach 45 min.



Figures 12-17. (12) Prodissoconch II (125 µm). Ventral view of valves showing tightly apposed margins (arrow). 5% bleach 23 min, 3 min sonication. (13) Prodissoconch II, pediveliger. Interior of edge of right valve to show terracing after treatment with 5% bleach for 40 min. HFW = 20 µm. (14) Prodissoconch II. Provinculum of left valve, 50 µm long. Socket

respond to that in other species of larval oysters that have been examined (Imai 1977).

Terminal teeth bear conspicuous transverse grooves on their sides (Fig. 18). These grooves and ridges minimize sheer on the hinge of shells as valves gape (Stanley 1978; Waller 1981). By the end of prodissoconch II and the early pediveliger, substantial quantities of shell material have been added beyond the ends of the provinculum (Figs. 15, 17) and a cardinal plateau about 20 μm wide, has been deposited between the terminal teeth on the right valve (Fig. 17); this plateau interlocks with a socket between terminal teeth on the left valve (Fig. 15). Denticles are still present, but now confined to the outer rim of the cardinal socket (Fig. 19) and plateau (Fig. 17), left behind by deposition of new shell on the interior of the valves. With further growth of the larval shell, the provinculum of the late pediveliger fills with newly deposited shell material, gradually obliterating the terminal teeth and denticles (Fig. 19). By setting time, or early afterwards, the process of obliteration has been completed (Fig. 20). Deposition of shell begins at the posterior end of the provinculum and gradually advances anteriorly. Thus, provincular teeth are short lived and function only as larval structures; when their role has ended, they are entombed in new shell material.

Larval Ligament. The earliest hinge ligament in prodissoconch I is the thin, exterior, nonmineralized periostracal layer secreted by the dorsal mantle epithelium. At first, right and left valves articulate only on this layer. The interior of the hinge of early prodissoconch valves shows no evidence of an inner larval ligament; however, at a later stage (Fig. 21) of larval development, a small, button-shaped, inner larval ligament forms about 50 μm anterior to the anterior terminal teeth on the inside of the hinge. By the pediveliger stage, the inner larval ligament, now about 20 μm in diameter, is a prominent feature of the inside of the hinge (Fig. 21), and appears to be continuous with the external ligament now forming anteriorly over the presumptive dorsal hinged area of the pivotal axis of the dissoconch hinge (Fig. 22).

Posterodorsal Notch. A distinctive external shell feature, the fasciole, first described in bivalves by Tanaka (1960), is conspicuous on the left valve (Figs. 23, 24) of prodissoconch II of *C. virginica.* This mineralized structure is a flattened, solid, somewhat cornucopially shaped elevation of the shell surface. It arcs smoothly ventromedially from the outer limits of the prodissoconch I/II boundary to end abruptly at the metamorphic line between prodissoconch II and the spat (dissoconch) valve in a curved posterodorsal notch (Fig. 25). The fasciole is the track left by the notch as the valve grows. A slight depression is reflected on the interior of the left valve opposite the more fully developed right notch. Dissolution of the periostracum with dilute bleach reveals the typically granular form of the shell units (0.2 to 0.4 μm in diameter) in the rim of the notch and valve (Fig. 24) (Carriker and Palmer 1979a; Waller 1979).

The function of the posterodorsal notch can only be hypothesized at this time. In veligers of *O. edulis* the notch, described and named by Waller (1979, 1981), forms exactly adjacent to the postanal ciliary tuft. This suggested to Waller (1981) that beating of these cilia deflects the thin periostracal envelope of the margin of the valves at this point, the deflection made permanent by subsequent mineralization. The postanal tuft is a continuation of the telotroch, a posterior crown of cilia on the velum (Raven 1966), present during the trochophore stage and thus is there throughout the prodissoconch I

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between two terminal teeth on left and right [arrows], 2.5 μm wide. 5% bleach 40 min. (15) Prodissoconch II, early pediveliger. Provinculum of left valve, showing cardinal socket [c] (23 μm wide at top) ventral to denticles and between terminal teeth. 5% bleach 25 min, 6 min sonication. (16) Prodissoconch II, middle stage. Interior of provinculum (47 μm long) showing closeness of articulation [arrows] of terminal teeth. Left valve at top. 5% bleach 23 min, 3 min sonication. (17) Prodissoconch II, early pediveliger. Provinculum of right valve showing cardinal plateau [c] between terminal teeth. Umbo is hidden behind hinge line. 5% bleach 40 min. HFW = 60 μm.

phase of larval shell development; the quite sudden appearance of the notch near the prodissoconch I/II boundary signals the onset of complete valve closure and first contact of valve margins with the beating postanal cilia. The notch disappears at the prodissoconch-dissoconch boundary, perhaps because the postanal tuft also disappears at metamorphosis. The notch is likely a prominent feature throughout the Ostreidae, and absent or only poorly developed in planktotrophic larval valves of other bivalve taxa. Waller's (1981) interpretation of the presence of the notch probably also holds for the notch in larval *C. virginica* (Carriker and Palmer 1979a), which also possesses a postanal ciliar tuft (Elson 1980). The possible function of postanal cilia in aiding setting activities of *C. virginica* has not been studied.

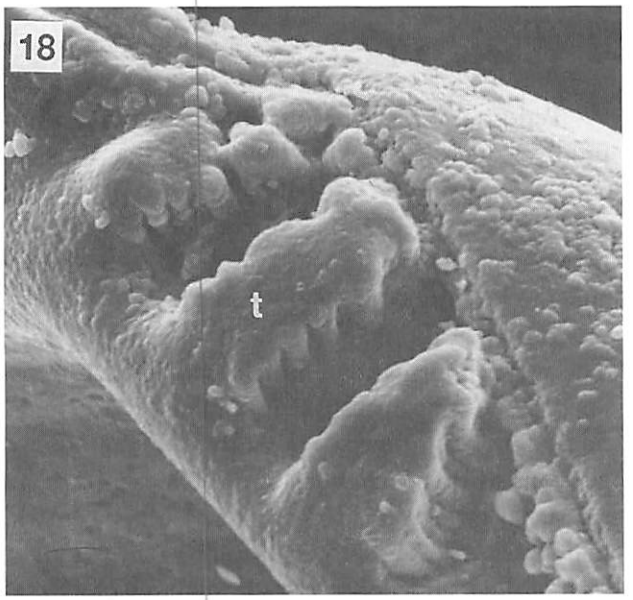
Internal Valve Microstructure. The interior margin of a bleach-treated prodissoconch I valve of *C. virginica* contains a distinct outer stratum of shell about 0.5 μm thick (Fig. 26). Inside this, the shell consists of granular shell units ranging in diameter from 0.1 to 0.5 μm in surface view. A fractured section of an early prodissoconch II valve (Fig. 27) is about 1 μm thick and composed of an exterior layer of granular shell units about 0.7 μm thick (probably prodissoconch I) and an inner layer about 0.3 μm thick (possibly prodissoconch II shell). Granular units in the fractured section range in diameter from about 0.05 to 0.1 μm.

Valves of prodissoconch II larvae, though stouter than those of prodissoconch I, are still surprisingly thin, ranging from 4 μm in the mid-prodissoconch stage (Fig. 28) to 6 μm thick in a late pediveliger (Fig. 29). The structural characteristics of aragonite (i.e., harder, stronger, and less prone to breakage than calcite, Stenzel 1964) could have contributed to the evolution of extreme thinness of bivalve larval valves. Small mass and weight of valves facilitate suspension in the water column for the duration of planktonic larval life, and articulating, closing valves may provide some protection from extreme abiotic environmental conditions. The structure of the shell is homogeneous, composed of small granules ranging in diameter from 0.1 to 0.5 μm (Figs. 28, 29). Granules tend to be arranged in discrete columns (Fig. 28), particularly in the inside layer. Those in the central part of the fracture section are of different sizes and appear randomly mixed (Fig. 30).

As prodissoconch II valves thicken during development, shell granules in fractured sections near the center of the valves appear to become arranged in three indistinct layers; the inner and outer ones (Figs. 28, 29) resemble the prismatic-like strata described by Waller (1981) in the prodissoconch valves of *O. edulis*. Whether these layers in C. *virginica* are prismatic is difficult to determine from the micrographs available; this needs to be resolved by further research.

The hypothetical relationship between distinctive microstructural layers in the larval shell of *O. edulis* and cellular differentiation during transition from the shell field to the mantle suggested by Waller (1981) probably applies equally well to that in developing larval valves of *C. virginica*. The hypothesized sequence is as follows: (a) initial embryonic shell formation by shell field cells; (b) transition from shell field to mantle epithelium during the early to middle prodissoconch I stage; (c) early phase of shell formation by the mantle epithelium during the middle to

Figures 18-22 (opposite page). (18) Prodissoconch II, early pediveliger. Terminal teeth [t] on posterior side of provinculum of left valve showing deep sockets between teeth and grooves on sides of teeth. 5% bleach 25 min, 6 min sonication. HFW = 25 μm. (19) Prodissoconch II, late pediveliger. Provinculum of left valve partly obliterated by deposition of shell [arrow]. 5% bleach 25 min, 6 min sonication. HFW = 65 μm. (20) Early dissoconch. Opened matching pair of valves of spat showing resilium [arrow], one half in each resilifer in hinge apparaus. Left valve in upper right. HFW = 245 μm. (21) Prodissoconch II, late pediveliger. Interior of provinculum in normally gaping valves to show inner larval ligament [l] and cardinal socket [c]. Right valve at top. Cleaned microbiologically. Valves 295 μm long. (22) Prodissoconch II, late pediveliger. Provinculum of left (to left) and right (to right) valves spread open showing anterior terminal teeth [t], outer ligament [l], cardinal socket [c], and cardinal plateau [p]. Inner larval ligament obscured by break in shell surface. HFW = 220 μm.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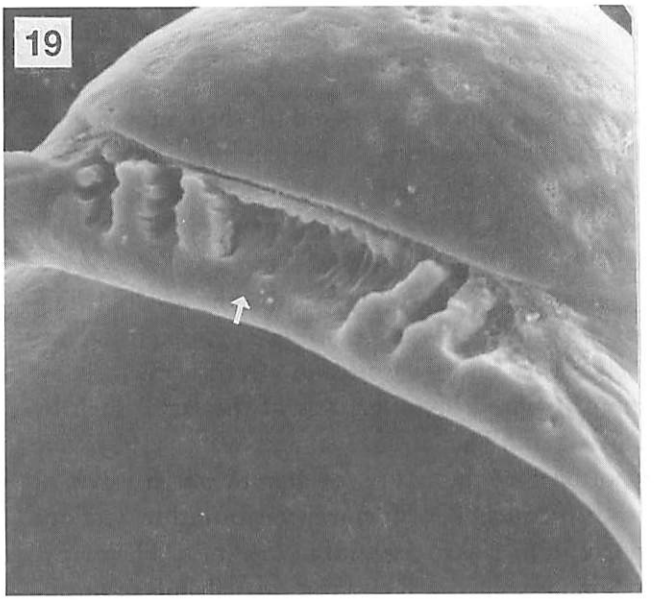
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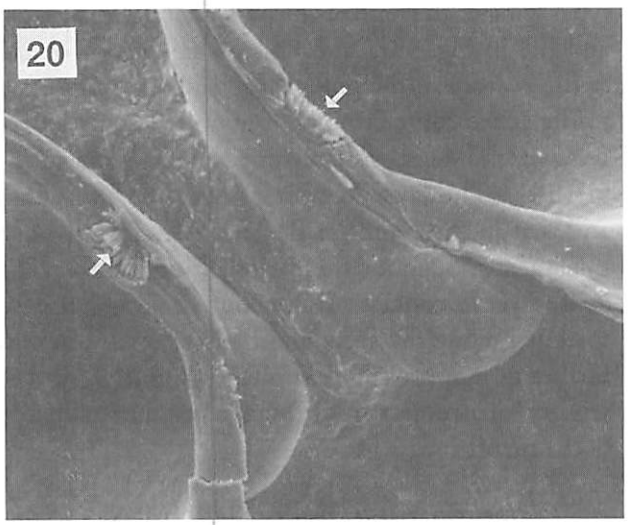
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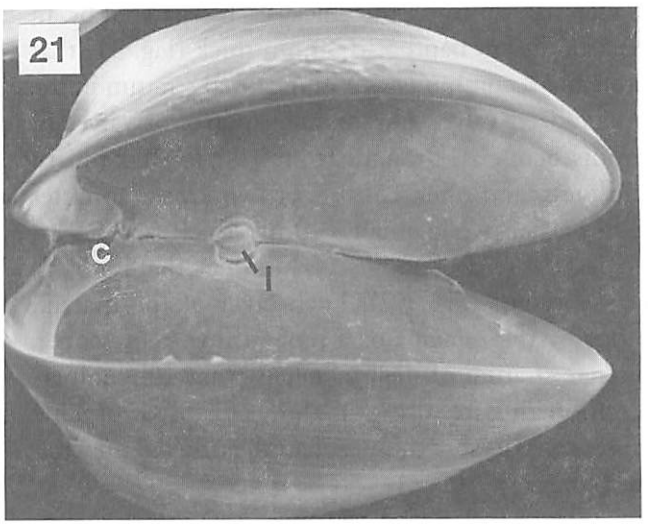
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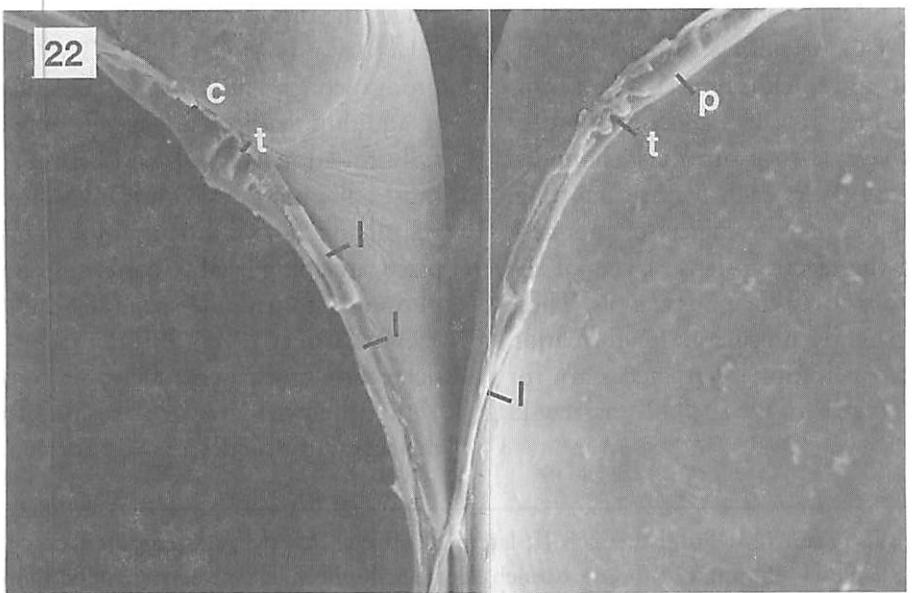
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late prodissoconch I stage; and (d) shell formation by the mantle epithelium during the prodissoconch II stage. Cells at the margin of the shell field secrete the pellicle, followed by cells at the margin of the mantle secreting the thin periostracal layer.

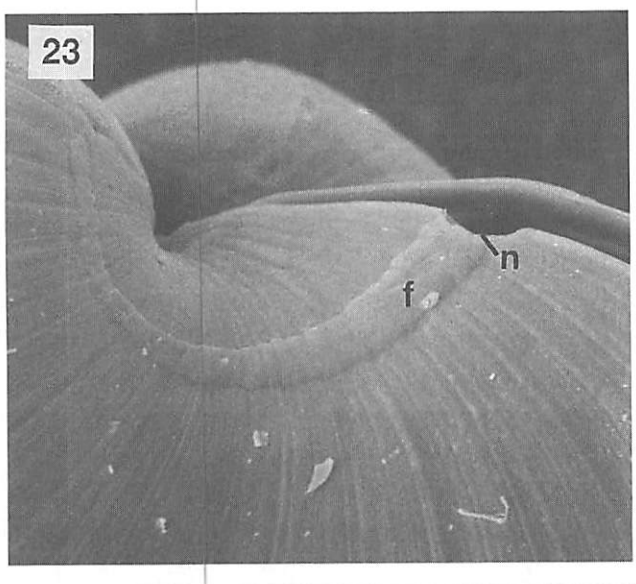
Tomaszewski's (1981) study of the insoluble matrix of the valves of veligers of *C. virginica*, using histochemistry and transmission electron microscopy, complemented investigations of the larval shell by Carriker and Palmer (1979a) and Waller (1981). He reported that the matrix of demineralized valves of prodissoconch I in dorsoventral ultrathin sections ranges from 100 to 500 nm in thickness. The matrix is composed of 7 or 8 electron-dense bands of organic material, 24 to 45 nm thick, each separated by an electron lucent band 5 to 30 nm thick (Fig. 31) that probably accommodates a mineralized stratum in the mineralized valve. The organic matrix of valves of prodissoconch II ranges from 170 to 290 nm in thickness, and often consists of two layers, an outer electron-dense one about 140 nm thick, and an inner less dense layer about 100 nm thick that is not always present (Fig. 32). Each layer of the matrix, probably the remains of a growth line, represents a discrete stratum of shell material removed by mineralization thus resulting in thin, collapsed, organic layers considerably thinner than the original mineralized strata.

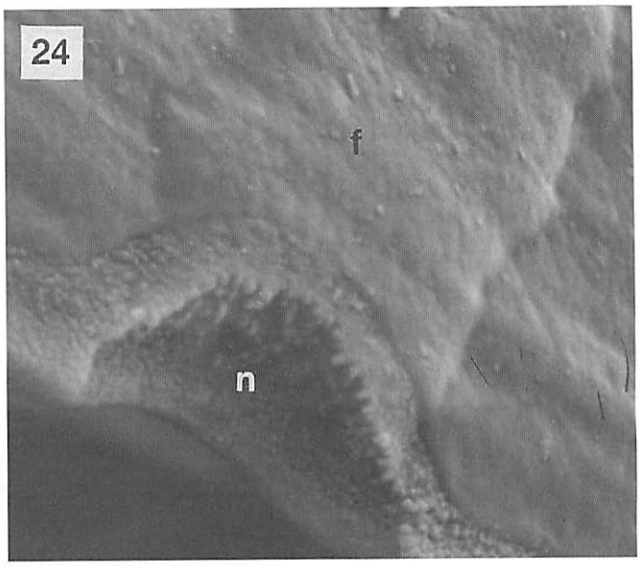
Periostracum. The thin pellicle that covers valves of prodissoconch I of *C. virginica* is a single electron-dense layer about 10 nm thick (Fig. 31). The periostracum of prodissoconch II valves consists of a central electron-lucent layer 8 to 9 nm thick, bounded by two electron-dense layers each 5 nm thick (Fig. 32). Layers of both the organic matrix of prodissoconch I valves and the electron-dense layers of the periostracum stain positively for polyphenols. These compounds are apparently precursors for formation of quinones, the cross-linking agent necessary for tanning the organic matrix of the shell (Tomaszewski 1981).

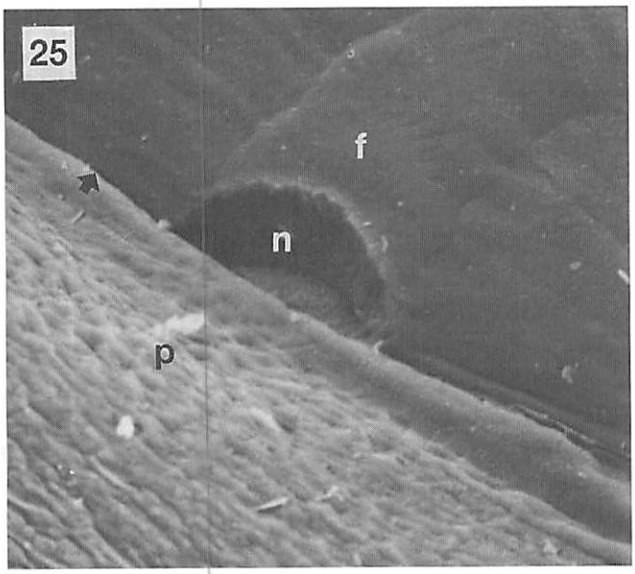
Periostracal formation has not been examined in prodissoconch II stages of *C. virginica*, but was studied by Cranfield (1974) in the pediveliger of *O. edulis*. The extrapallial space is confined to the region between pallial attachment (near the shell margin) and the periostracum (Fig. 33); only epithelium in this region of the mantle appears to form the shell. The remaining mantle epithelium is poorly developed. The extrapallial space is bordered on the mantle side by a columnar epithelium. The mantle margin consists of an outer and inner fold, with the periostracal groove located at the base between the folds. The periostracal sheet forming at the margin of each of the mantle lobes arises from a row of two cell types lying at the base of the periostracal groove ([of] and [if] in Fig. 33), and remains highly convoluted at the bottom of the groove, probably an adaptation to the active contractibility of mantle margins. In *C. virginica* the sheet possesses three layers (Tomaszewski 1981). The layer in contact with the inner fold and that in the center of the sheet remain the same thickness throughout the periostracum, whereas the dense layer in contact with the outer fold increases in thickness along the sheet and then abruptly thickens further as it approaches the shell margin. Groups of sensory cilia project from the apex of the inner fold of the mantle.

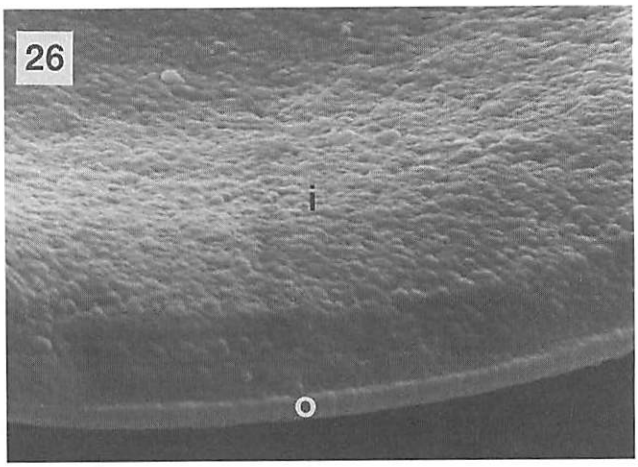
Pediveliger Attachment. The pediveliger concludes the prodissoconch stage in most species of bivalve larvae (Carriker 1990). The pediveliger retains prodissoconch I and II valves and ciliated velum, and in addition develops an actively crawling foot with a fully functional byssal gland. In *C. virginica*, as in most other molluscs with planktonic larvae, the pediveliger functions primarily in searching for a setting place, attaching itself to hard substrata and metamorphosing into juvenile spat (Nelson 1924; Prytherch 1934; Carriker 1986). Initial attachment is by a slender, nearly transparent, sticky byssus thread (Carriker 1990), followed later by adhesion of the left valve to the substratum with a tanned protein material simi-

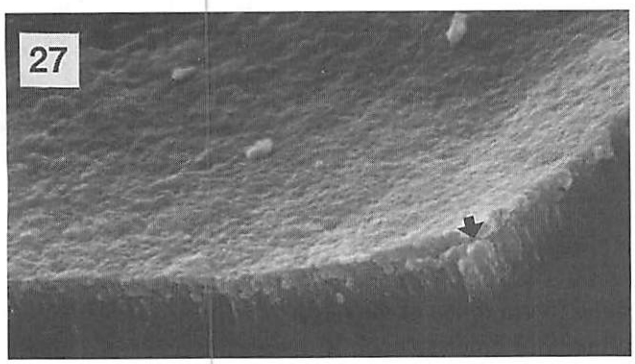
Figures 23-28 (opposite page). (23) Prodissoconch II, late pediveliger, left valve, posterodorsal notch [n], fasciole [f]. Notch 15 μm wide. 5% bleach 45 min. (24) Prodissoconch II, late pediveliger, left valve, posterodorsal notch [n] and fasciole [f]. High magnification of posterodorsal notch (10 μm wide), end view. 5% bleach 45 min. (25) Dissoconch, early

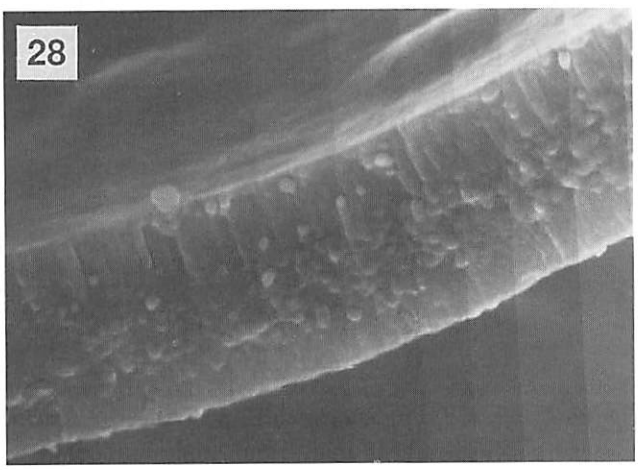








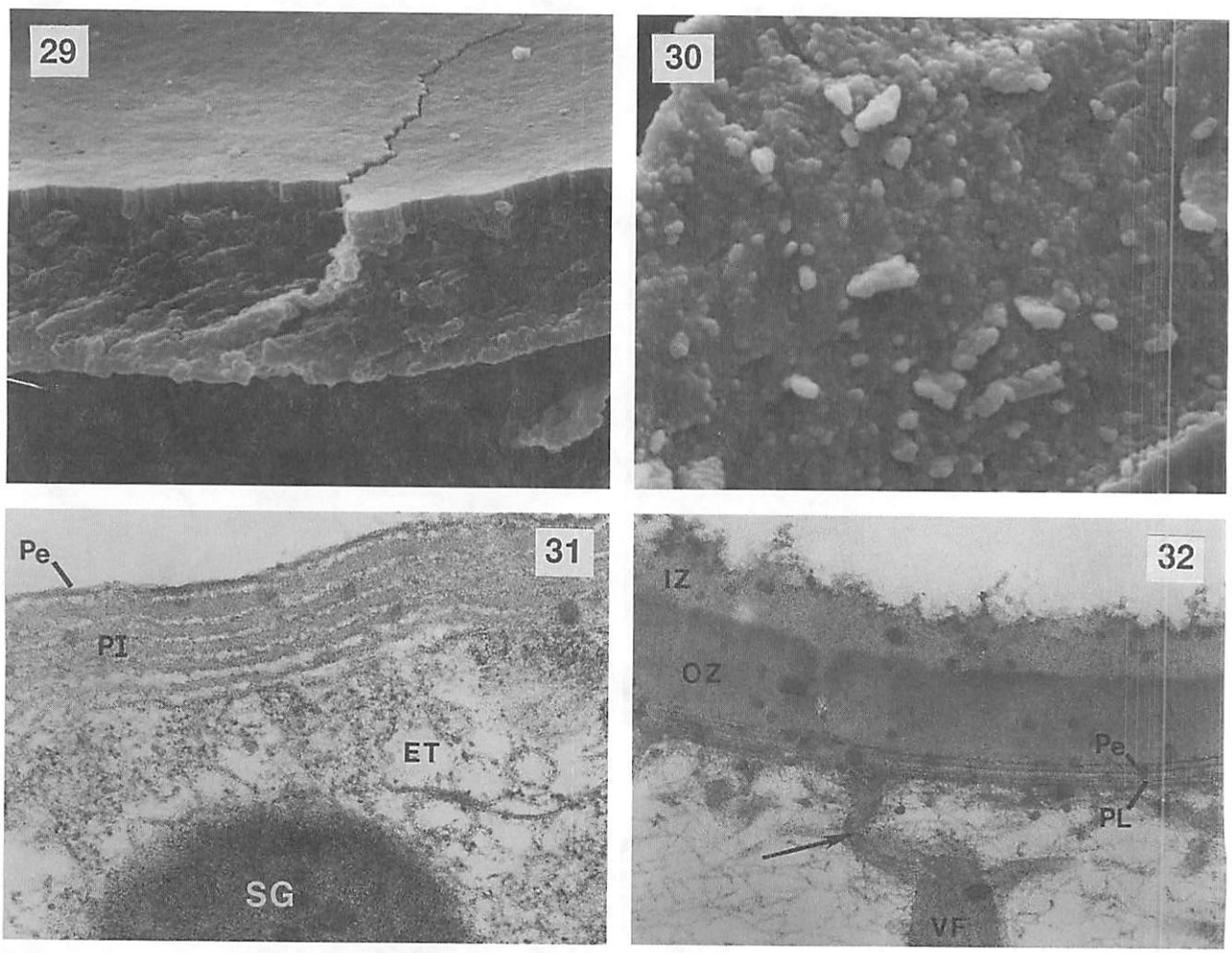




stage, left valve. Fasciole [f] and posterodorsal notch [n] terminating at metamorphic juncture [arrow]. Beginning of prismatic shell [p]. Notch 14 µm wide. 5% bleach 30 min. (26) Prodissoconch I-II. Interior view of edge of valve showing thin outer layer [o] (0.5 µm thick) of prodissoconch I valve; inner surface of granular shell units of prodissoconch II valve [i]. Valves 60 µm high. 2% bleach 10 min. HFW = 12 µm. (27) Prodissoconch I-II. Fractured section [arrow] (1 µm thick) of valve and view of interior surface of valve. Valves 65 µm high. (28) Prodissoconch II, mid stage. Fractured section (4 µm thick) of middle of valve, anteroposterior plane. Cleaned in 5% bleach 23 min, 3 min sonication before fracturing.

lar to that of the periostracum (Cranfield 1975; Yonge 1979; Tomaszewski 1980, 1981). Both the thread and the adhesive are produced in the byssal gland in the heel of the foot.

In *C. virginica* the basal part of the foot contains the secretory cells of the byssal gland. These cells form a central mass of byssal secretion that appears well before the foot is fully formed, and empties into paired left and right ducts. These ducts are lined with secretory cells histologically similar to those of the byssal gland itself. The ducts open separately on the posteroventral side of the foot, ventral to a ciliated furrow. Byssal secretion is granular, refractile, with a proteinaceous component, and eosinophilic (Elston



Figures 29-32. (29) Prodissoconch II, pediveliger. Fractured section (6 µm thick) of middle of valve in dorsoventral plane. Cleaned in 5% bleach 25 min, 6 min sonication before fracturing. (30) Prodissoconch II, pediveliger. Fractured oblique section in dorsoventral plane, outer part of valve. Granules 0.2 to 0.5 µm in diameter. Cleaned in 5% bleach for 45 min before fracturing. (31) Transmission electron micrograph of radial section through prodissoconch I demineralized shell matrix [PI] composed of several electron dense bands. Periostracum [Pe], epithelial tissue [ET], secretory granule [SG] (0.5 µm in diameter). From Tomaszewski (1981). (32) Transmission electron micrograph of radial section through demineralized prodissoconch II shell matrix, outer [OZ] and inner [IZ] zones. Tri-layered periostracum [Pe]. Attached to the shell matrix is the larval cement, which includes the proximal layers [PL] and a branching [arrow] of a vertical fiber [VF], 0.15 µm wide, from the inner zone of adhesive pad. From Tomaszewski (1981).

1980). In *O. edulis* the byssus gland consists of four types of cells that secrete into a byssus duct from which the byssus thread is extruded. The thread is composed of two cores surrounded by a continuous sheath and contains sulphated acid mucopolysaccharide, nonsulphated acid mucopolysaccharide, protein, glycoprotein, neutral sugars, and sulphated proteoglycan (Cranfield 1973 a, b, c).

According to Prytherch (1934), at the start of setting the pediveliger of *C. virginica*, crawling on its highly clinging ciliated foot, begins to release the byssus that tenaciously adheres to the surface over which the larva is crawling, preventing its being washed away by tidal currents. At first the byssus is relatively heavy and cylindrical in shape with a diameter of 4 μm; it then gradually thins and flattens to a width of 10 μm as setting is approached. Frequent contractions of the foot accompany the forcing of byssal secretion from the byssal duct. The total length of the byssus, or maximal distance covered by a crawling pediveliger, varies from a few centimeters to about 38 to 50 cm, according to the duration of the crawling phase, nature of the substratum, temperature, and salinity.

Finally, as its movements become slower, the pediveliger comes to a standstill, holding the shell at right angles to the surface preparatory to attachment (Prytherch 1934; Tomaszewski 1981). Next the foot is quickly protruded to its full length from the right side of the shell at about the center and flattened down against the surface. As the shell is pulled a short distance sideways by short jerky movements, there follow one or two vigorous contractions of the valves against the base of the foot that force out the adhesive fluid, or the last of the byssal substance. The shell is then quickly and forcibly pressed against the surfaces so that the left valve is held tightly in contact with the adhesive which hardens almost immediately. The foot holds the valves firmly in place for a minute or two and then slowly withdraws into the mantle cavity of the shell, completing the process of setting. The adhesive hardens in less than a minute to such a degree that the spat cannot be detached by vigorous flow of water.

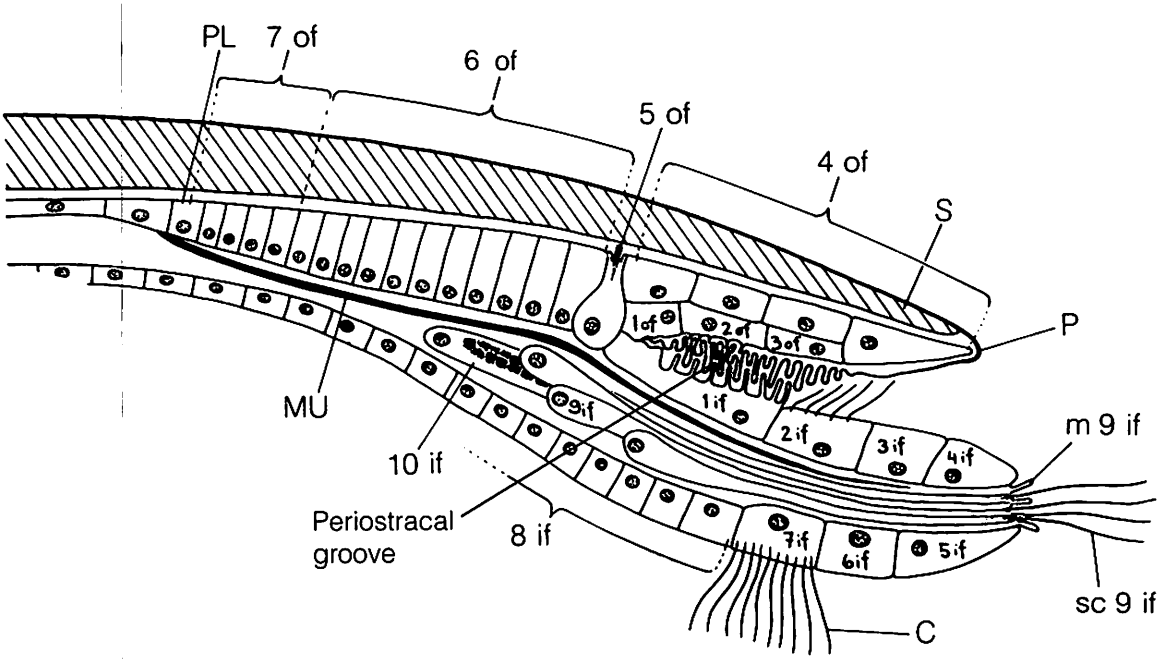


Figure 33. Generalized diagram of radial section through mantle edge of pediveliger of *Ostrea edulis*. Numerals indicate the different cell types that are histochemically and morphologically discernable. Inner fold [if], outer fold [of], cilium [C], microvillus [m], musculature [MU], periostracum [P], pallial line [PL], shell [S], sensory cilium [sc]. From Moor (1983), who modified a figure in Cranfield (1974).

Attachment of pediveligers to substrata is a critical phase in the life cycle of oysters; large numbers of larvae fail to find suitable substrata in nature. Even in artificial tanks and hatcheries losses are high (Bonar 1976). Setting occurs at a shell length of 260 to 350 μm, the usual size being about 300 μm, but this could be increased by growth in cool waters or delay in setting (Andrews 1979).

Pediveliger Bioadhesive. The small pad of adhesive from the byssal gland of *C. virginica* that attaches the left prodissoconch II valve to the substratum is 4.5 to 9.0 μm high (i.e., dimension at right angles to the tangent of the shell surface). Based on the appearance of the types of fibers, the following structural zones are evident in the fully formed adhesive pad (Fig. 34): a central core divisible into an inner zone close to the shell and an outer zone adjacent to the substratum, and an external thin peripheral zone (Tomaszewski 1981).

The inner zone, which ranges in height from 2.8 to 4.5 μm, occupies a little more than half the volume of the adhesive pad. The pad is characterized by vertical microfibers 100 to 160 nm wide. These fibers are polyphenol rich and test positive for tyrosine, disulfide linkages, and lysine. The fibers are enmeshed in a matrix of fine polyphenol negative fibrils. Inner zone fibers are separated from the prodissoconch valve by proximal adhesive layers, the first of which, 6 to 8 nm wide, is polyphenol positive. The outer zone consists of microfibers, 10 to 30 nm wide, enmeshed in a matrix of smaller microfibrils, that tend to orient horizontally and are polyphenol negative and test positive for tyrosine (a general protein indicator) and Alcian blue (indicates mucopolysaccharides) at pH 2.5. The peripheral zone consists of intertwining fibers, 50 to 220 nm in diameter, that extend from the larval valve to the substratum; these are rich in polyphenols, tyrosine, and lysine (Tomaszewski 1981).

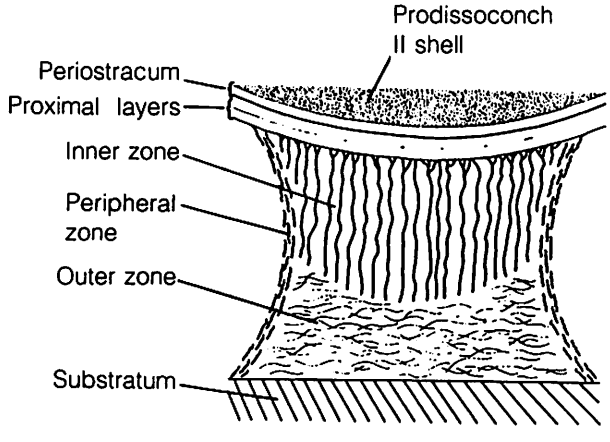


Figure 34. Diagram of radial section through larval adhesive pad, about 7 μm high. Vertical dimension exaggerated three times over width. From Tomaszewski (1981), modified from Cranfield (1975).

All the different fibers arise after the fluid adhesive has been released from the byssal gland. Presence of polyphenols and lysine suggests that the adhesive is so tanned that no free amino groups and polyphenols remain (H. Waite, College of Marine Studies, University of Delaware, pers. comm.). The outer zone is probably not tanned as it does not contain amino groups, tyrosine, or polyphenols detectable by histochemistry (Tomaszewski 1981).

Environmental Effects. The extent to which extremes of temperature, salinity, and other environmental factors normally encountered in estuaries could affect prodissoconch valves of larvae of *C. virginica* has not been reported. However, Siddall (1980) conducted such a study on tropical mytilid larvae. Shells formed at a salinity of 14 ppt (in a temperature range of 18 to 30°C) were significantly thinner than those at 28 or 42 ppt. At warm temperatures (resulting in high growth rates), growth striae were more widely spaced than those at lower temperatures. Magnesium content was highest in valves formed at temperatures above 24°C (across a range of salinity of 14 to 42 ppt), and lowest in those valves formed at 14 ppt (in a temperature range of 18 to 30°C). Strontium content of the valves did not vary over the temperature or salinity range tested.

Bivalve larval shells provide physical support for the soft internal organs, and probably some protection from mechanical impacts by suspended particles (Carriker 1986) and osmotic stress from extremes of salinity. Although the shell is probably of little, if any, protection from the numerous predatory benthic and

pelagic animals that naturally consume zooplankton (Nelson 1921; Korringa 1952; Carriker 1961; but see Purcell et al. 1991), it does under some uncommon circumstances make possible the passing alive of bivalve larvae (oysters included) through the mantle cavity and digestive tract of various invertebrates. Nelson (1921) published a striking photomicrograph of some of the 62 ready-to-set eastern oyster larvae taken from the stomach of one live adult eastern oyster; whether the larvae were alive was not stated. Transport of larvae through the alimentary canal of invertebrates, though interesting, is probably not ecologically important (Mileikovsky 1974).

EARLY DISSOCONCH SHELL

The planktonic larval life of *C. virginica* ends when the pediveliger sets. Within 12 to 24 h after setting, spat can be dislodged from smooth hard surfaces without injury, making possible the production of so-called "cultch-free" (that is, unattached) spat (Dupuy and Rivkin 1972). Reattachment can occur only by deposition of new dissoconch shell material around the shell margin; subsequent dislodgment without breakage depends upon the use of flexible cultch material with some rugosity such as fine mesh screens and plastic sheets (Andrews 1979). Recently, Coon et al. (1986) demonstrated that solutions of epinephrine or norepinephrine (10^{-4} M) induce larvae of *C. gigas* and *C. virginica* to metamorphose without settlement behavior or attachment, thus becoming cultchless. There were no apparent effects from induced metamorphosis during one year of culture.

Metamorphosis begins immediately after attachment of the pediveliger to the substratum. During this relatively rapid, complex transformation to the juvenile dissoconch (marked on the shell surface by the metamorphic line), pronounced cellular differentiation of the mantle occurs accompanied by notable physiological changes. Not the least of these is initiation of secretion of the highly microstructured, mineralized valves characteristic of the dissoconch.

Mantle

Inner organs of dissoconch bivalves are clothed by a soft, fleshy sheet of tissue, the mantle or pallium. The principal role of the mantle is secretion of the shell, ligament, and adductor muscle attachment sites. Mantle epithelium adjacent to the inner shell surface secretes the shell materials, and consists of a layer one cell thick. Right and left mantle lobes are joined together at the anteroventral margin and form a cap or hood that covers the mouth and labial palps. In a live open oyster, the margin of the mantle can be extended some distance beyond the edge of the valves; in a closed oyster, the mantle is held partly withdrawn, coming to rest about midway between the distal rim of the gills and the edge of the shell (see Eble, Chapter 2; see also Morrison [1993] for a detailed description of the mantle.)

Descriptions in the following sections of the mantle, mantle lobes, periostracum, and adhesion in the early dissoconch stage of *C. virginica* are based, in part, on Tomaszewski (1981) who studied spat ranging in height from 0.3 mm (1 d after setting) to 0.4 to 1.3 mm (8 d after setting).

The early dissoconch spat under normal conditions spends most of its time with the left mantle lobe extended beyond the ventral margin of the valve up to 20% of the total height of the shell. When extended, the mantle margin remains flush with the substratum. A disturbance will cause it to retract into the shell, but such retractions usually last for a minute at most. The right mantle also extends, but never more than 1% of the total shell height; it also undergoes retraction-extension movements, but these are rapid, as much as 20 per min. As in the late prodissoconch, the mantle margin of the early dissoconch is divided longitudinally into an outer fold adjacent to the inner shell surface, and an inner fold toward the mantle cavity. The junction of the two folds defines the groove from which the periostracum arises. Within about a month after metamorphosis, a third fold appears on the mantle lobes. This new fold probably originates from the inner face of the second, or early inner fold.

Outer Mantle Fold

As seen in histological and microstructural sections of this fold perpendicular to the mantle edge, the outer epithelium facing the extrapallial space consists of a row of cuboidal cells (Type A, Fig. 35) having short microvilli and extensive rough endoplasmic reticulum in basal and nuclear regions. Each cell possesses a Golgi body, numerous mitochondria, multivesicular bodies, and two types of secretions. The first are large, heterogeneous, spherical inclusions filled with polyphenol-positive material, and the second, small, electron dense, slightly polyphenol positive, or large and electron lucent, secretory granules.

Type B cells of the inner epithelium of the outer mantle fold line the periostracal groove and are cuboidal in shape, possess short microvilli, well developed rough endoplasmic reticulum, and two types of secretory granules, one of which contains polyphenol-positive granules.

One of the two types of gland cells (Type C) within the outer mantle fold is subepithelial, occasionally adjoins the periostracal groove, and contains globular secretion that stains positively for proteins, tyrosine, arginine, disulfide coupling, and the periodic acid-Schiff reaction (PAS). The other type of gland cell (Type D) is a granulocyte and is located along the inner face of the outer mantle fold; it opens into the distal half of the periostracal groove. Cell Type E lacks microvilli and lies deep in the base of the periostracal groove. It contains several mitochondria, large granules, and a region devoid of organelles or inclusions and is characterized by a homogeneous fibrous matrix that usually occupies one fourth of the cell volume. The newly formed periostracal material is closely appressed to the cell membrane in the vicinity of the fibrous matrix.

Inner Mantle Fold

The outer epithelium of the inner mantle fold lines the periostracal groove. The first two cells (Type

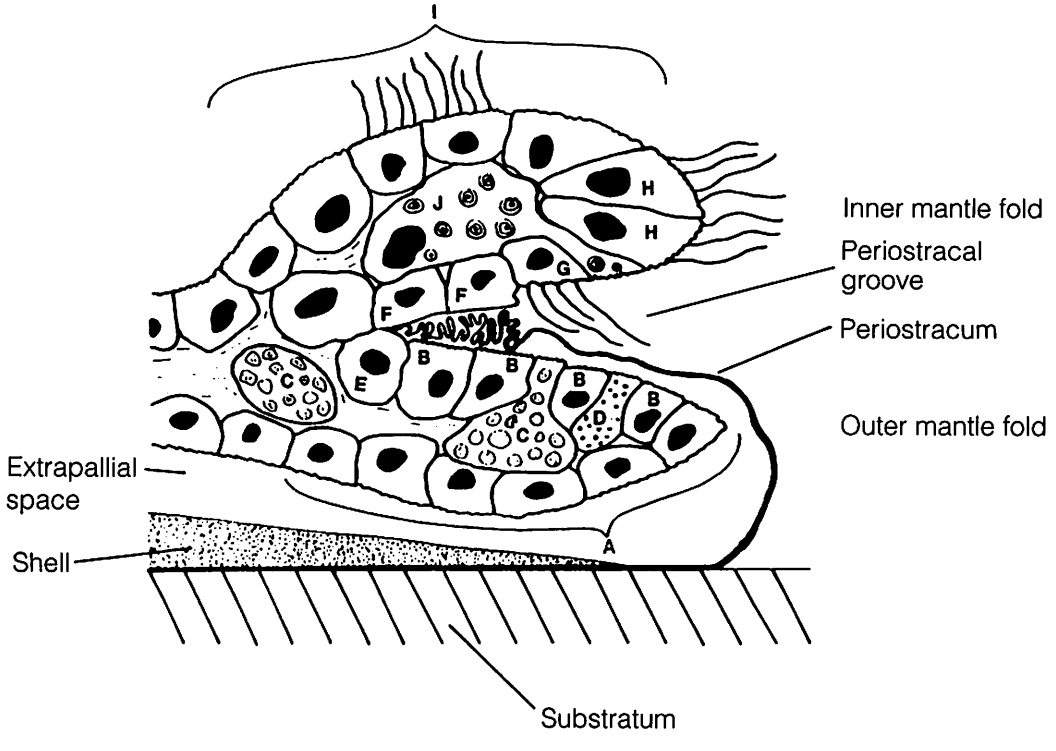


Figure 35. Diagram of radial section through ventral margin of left mantle in early dissoconch stage. Letters identify location of cell types described in text. Length of mantle folds about 20 μm. From Tomaszewski (1981).

F) as seen in section lie next to the basal area of the groove. They lack microvilli and contain a large nucleus. The supranuclear area contains mitochondria and some rough endoplasmic reticulum. Presence of granules and vacuoles indicates apical secretory activity.

A ciliated cell with long microvilli (Type G), located halfway along the outer epithelium of the inner mantle fold, lies at the point in the periostracal groove where the convoluted periostracal sheet begins to straighten. This type of cell contains many mitochondria, several Golgi bodies, multivesicular bodies, and polyphenol-rich secretory granules. Cells of Type H, carried on the distal tip of the inner mantle fold, possess long microvilli and cilia and are actively secretory with two types of secretory bodies. Type I cells, some ciliated and some not ciliated, line the inner face of the inner mantle fold. Gland cell Type J, deep within the inner mantle fold, possesses a long slender apical process that opens into the outer part of the periostracal groove. It is cuboidal, contains numerous mitochondria, rough endoplasmic reticulum, and an occasional Golgi body. The apical end contains vacuoles, spherical inclusion bodies, and large multivesicular bodies. Secretory granules contain L-DOPA (3,4-dihydroxyphenylalanine) oxidase-positive material. Secretions, which become increasingly L-DOPA oxidase-intense, pass through the process into the periostracal groove.

Periostracum

As it arises from the basal cells at the bottom of the periostracal groove, periostracal material takes the form of a highly convoluted sheet that then straightens as it passes out of the groove (Figs. 35, 36). Three layers are distinguishable ultrastructurally in the forming sheet: (a) a dense pellicle facing the inner mantle fold, 3 to 5 nm thick, which becomes the external layer of the periostracum on the shell surface; (b) an intermediate layer 3 to 11 nm thick; and (c) a wide opaque layer facing the outer mantle fold, which becomes the surface on which the shell is formed. Layer c thickens from 10 nm as the sheet moves distally in the periostracal groove to 20 to 30 nm as it emerges from the groove at the mantle edge.

On the basis of his study, Tomaszewski (1981) hypothesized that periostracal material, for covering the external surface of the valves as well as for adhesion to the substratum after setting, probably is formed as follows in early dissoconchs of *C. virginica*. Cells E and B synthesize and secrete the initial periostracal material, cells B forming the innermost layer of the periostracum (next to the shell). Polyphenols (probably in the form of L-DOPA-containing proteins; Waite, pers. comm.) secreted into the groove, upon oxidation to quinones by phenol oxidase, initiate, or possibly augment, tanning of the periostracal material. The periostracal sheet differentiates into its characteristic three

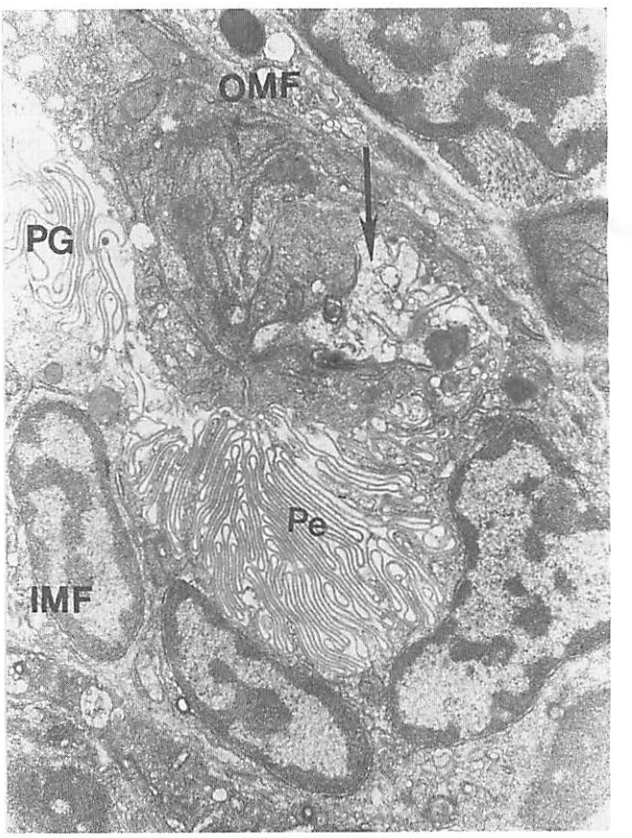


Figure 36. Transmission electron micrograph of basal cell [arrow]. Radial section through region of periostracal groove in ventral margin of left mantle of early dissoconch. Cell type E (see Fig. 35) and early convoluted periostracum [Pe], inner mantle fold [IMF], outer mantle fold [OMF], periostracal groove [PG]. HFW = 6 μm. From Tomaszewski (1981).

layers soon after periostracal material leaves the basal cells and enters the periostracal groove (Fig. 36). Secretions of cells F and G serve as lubricants on the external surface of the periostracal sheet and as wetting agents to promote close contact between the periostracal surface and the substratum. Ciliated cells G aid in moving the periostracal sheet out of the groove and straightening the convolutions as the sheet emerges from the groove (Fig. 35). The degree to which the mantle folds are inflatable and extensible by hemolymph pressure could also affect smoothing of the sheet. Ciliated cells H are sense organelles with a mechanoreceptive function, aiding the left mantle as it makes contact with the substratum during the process of adhesion at setting. Large subepithelial gland cells J synthesize phenol oxidase, which is discharged onto the external surface of the passing periostracum, possibly initiating the tanning reaction of the periostracum as it is pressed against the substratum forming a permanent adhesive bond. Subepithelial gland cells in the outer mantle fold and cells along the inner face of the outer mantle fold probably contribute materials to the periostracum as well as enhance affixation of the periostracum to the outer surface of the newly forming shell margin. Waite (1977, 1983) and Saleuddin and Petit (1983) summarized the process of periostracal formation in other molluscs.

As has been suggested by several authors (reviewed by Tomaszewski 1981), the lack of a pallial attachment of the mantle to the shell in *C. virginica* could explain the deeply convoluted nature of the early periostracal sheet. Convolutions could be an adaptation to the extreme contractibility of the mantle. The mantle in oysters is attached to the shell only at the periphery of the adductor muscle (Galtsoff 1964); the convolutions would permit the mantle to withdraw deeply into the mantle cavity without breaking continuity of the periostracum secured to both the shell edge and the periostracal groove. This could well be the case in early dissoconchs. It is unlikely to apply to older individuals because the extent of withdrawal of the mantle is so extreme (see p. 118 for further discussion). Waller (1980) notes that a convoluted periostracal sheet within the base of the periostracal groove seems to be a general feature within the Bivalvia.

Attachment

In young dissoconchs the periostracal sheet emerging from the periostracal groove of the left mantle margin serves the following three purposes: (a) it provides the organic base upon which the left valve develops; (b) it furnishes the organic covering of the external surface of the left valve; and (c) it simultaneously serves as the adhesive that affixes the early dissoconch to the substratum after initial attachment by the pediveliger (Fig. 37). This adhesive is thus periostracum, and is not mineralized as was believed by earlier investigators.

Tomaszewski (1981) suggested the following mechanism for adhesion of spat to substrata after setting. The left mantle (Fig. 38; numbers refer to processes described in figure) is extended beyond the ventral margin of the left valve, and with the aid of mechanoreceptors (1) located on the distal tip of the inner mantle fold, the mantle edge makes contact with the substratum; this closes off the periostracal groove from the external environment. The periostracal sheet is advanced out of the periostracal groove (2) aided by cilia of the inner mantle fold (3). Cells of the inner face of the outer mantle fold (4) secrete polyphenols, proteins, and phenol oxidase for tanning and elaborating the periostracum. As the sheet passes by gland cells J of the inner mantle fold (5), it receives L-DOPA oxidase which coats the external surface of the periostracum and accelerates tanning. The sticky periostracal sheet is simultaneously applied to the substratum as it leaves the groove (6). Because of its semi-fluid nature, the periostracal sheet conforms to the microtopography of the substratum, making close adhesive contact with smooth areas and filling in crevices. The L-DOPA oxidase quickly accelerates hardening of the periostracal sheet into a permanently tanned bond.

Meanwhile, formation of the prismatic layer of shell is initiated, resulting in permanent affixing of the periostracal sheet to both the substratum and the left valve (Fig. 37). Close left valve-substratal attachment continues so long as the left valve is secreted close to the substratum. Tight adhesion can persist for only a short distance, as when spat set on gravel or other small objects, or over a longer interval as

when setting is on a larger, relatively smooth, flat surface. Waller (1990 and pers. comm.), however, considers that the idea of adhesion of periostracum to both substratum and shell is not well founded, because different sides and parts of the periostracum are involved. For example, the columnar prismatic layer of the shell could be formed by nucleation within the inner layer of the periostracum, and prism walls, essentially the squeezed-out residue left by expanding nucleation points, could therefore actually be periostracal in nature. In this regard, Harper (1991) in a study of young individuals of several ostreid species (excluding *C. virginica*) demonstrated that the periostracal sheet is secreted throughout the attachment process, and proposed that the sheet is permeable, allowing "leakage" of mineral components (remarkably similar to those of the shell) in the extrapallial fluid into the space between the periostracum and the substratum, where mineralization and cementation are completed. Whether "leakage" need be postulated, in view of the development of crystallities within the periostracal sheet (Carriker et al. 1980a), is unclear (see "Prism Formation," p. 130).

Gland cell J of the inner mantle fold is also found in the right mantle of *C. virginica*. Consequently, the oyster theoretically could fasten either valve to the substratum. However, oysters attach exclusively by the left valve (Yonge 1979), never extending and applying the right mantle to a substratum even if lying on their right side (Galtsoff 1964). Hence, though the periostracal sheet of the right valve emerges sticky, it never serves the function of attachment.

Shell Microstructure

Metamorphic Line

During early adhesion to the substratum, the young dissoconch bivalve initiates development of the adult form of the shell. From homogeneous aragonitic prodissoconch II valves, the shell metamorphoses abruptly beyond the metamorphic line to prismatic calcite (externally) and foliated calcite (internally) (Figs. 39, 40; Carriker and Palmer 1979a). The reason for the sharp transition in shell mineralogy from aragonite to calcite, while islands of aragonite remain as muscle scars and resilial fibers in the ligament (Stenzel 1962, 1963; Galtsoff 1964; Taylor et al. 1969), is unclear (but see discussion on Prodissoconch II on p. 81).

Change in shell microstructure and mineralogy during metamorphosis probably could not occur without some modification of the secretion of shell materials by the mantle epithelium. Epithelial cells, which in planktonic larval stages secreted aragonitic granules, quite suddenly are reconstituted in the early spat to form a microstructure and orientation of strikingly different biocrystals at the rim of the valves. A further reconstitution occurs in the epithelium behind valve edges where foliated calcite is secreted, in some places in the form of tightly packed laths, and

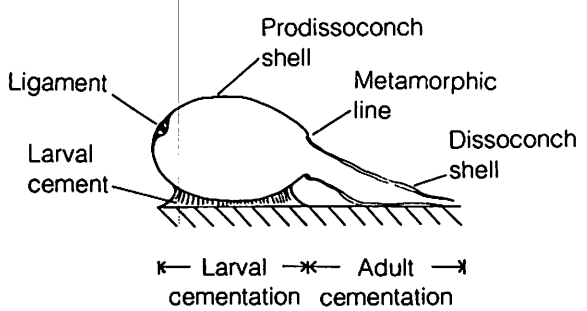


Figure 37. Diagram of a radial section through early dissoconch stage, showing prodissoconch and dissoconch shell and larval adhesive pad. Approximately 100 ×. From Tomaszewski (1981).

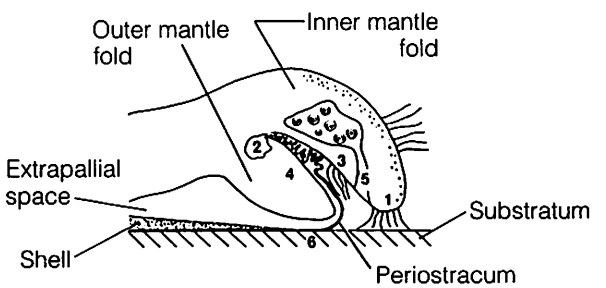


Figure 38. Diagram of a radial section of left mantle lobe margin in early dissoconch oyster depicting release of periostracum and attachment to hard substratum. Numbers refer to processes described in text. Length of mantle folds about 20 μm. From Tomaszewski (1981).

in others as chalky shell with laths arranged in a spongy pattern. This ordered pattern of metamorphic events occurring in rapid succession in young oysters is a manifestation of complicated biochemical changes taking place at cellular levels — a major aspect of shell construction of great interest, but yet to receive experimental study (Wilbur and Saleuddin 1983).

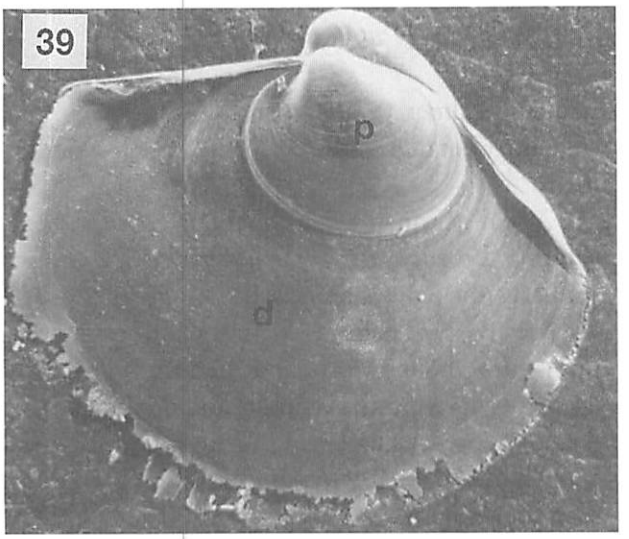
Instructive in this context would be a study of possible changes in metabolic gradients along the umbo-commisural axis of the mantle lobes (Rosenberg et al. 1989) during and after secretion of prodissoconch I, prodissoconch II, and early adult valves. The process of rapid transition from larval to juvenile shell has existed among bivalves for a long geologic period. Lutz and Jablonski (1978), as a case in point, reported the presence of a distinct prodissoconch-dissoconch boundary in valves of late Cretaceous juvenile bivalves. Kobayashi (1980) suggests that ancestral microstructure of the shell of bivalves could have been an aggregation of the small, simple crystallites seen in modern larval shells. J. Carter (University of North Carolina, Chapel Hill, pers. comm.), on the other hand, notes that bivalves appear to have evolved from either prismatic or nacroprismatic monoplacophorans; the Cambrian species *Pojetaia runnegari*, for example, is internally laminated (nacreous of an unusual type) and prismatic. Waller (1978 and pers. comm.) reported that foliated calcite can be traced as far back as the Devonian, and prismatic calcite has been present in the Eupteriomorphia since even earlier times.

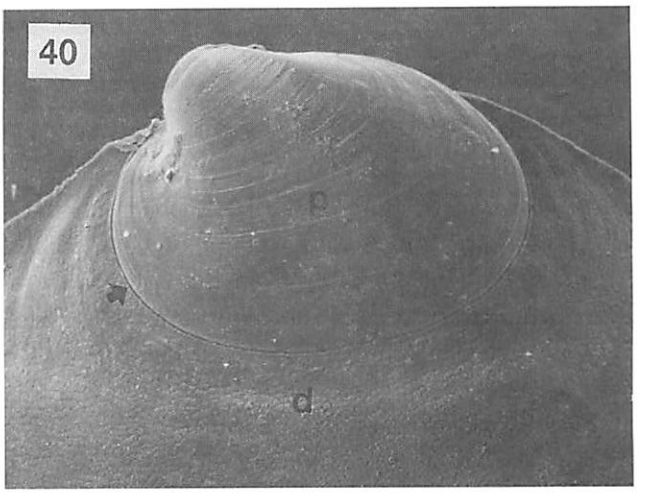
Shell Units

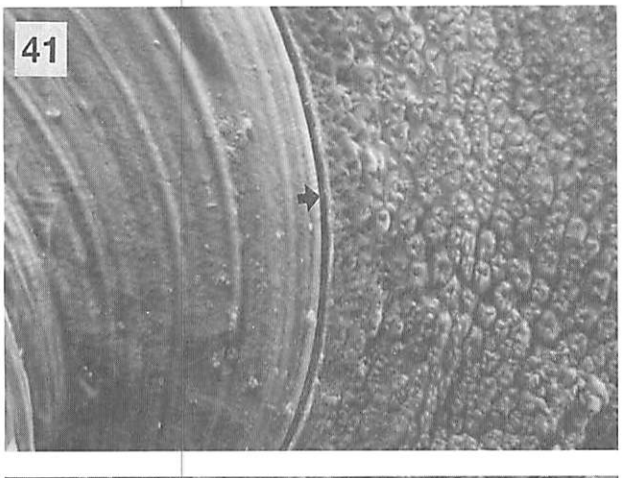
As the early spat grows, there occurs a downward flaring of both valves. This makes possible adhesion of the left valve to the substratum, bringing margins in contact with the substratal surface. Calcitic prisms of the outer layer of the right valve immediately past the metamorphic line are small and slightly misshapen (Figs. 41, 42) but soon assume a more characteristic shape with further shell development (Fig. 43), reaching 9 to 11 μm in maximal surface dimension in spat 31 d old and about 1 mm shell height. The angular three-dimensional form of the prisms is evident at a fractured margin of a valve (Fig. 44) (Carriker and Palmer 1979a).

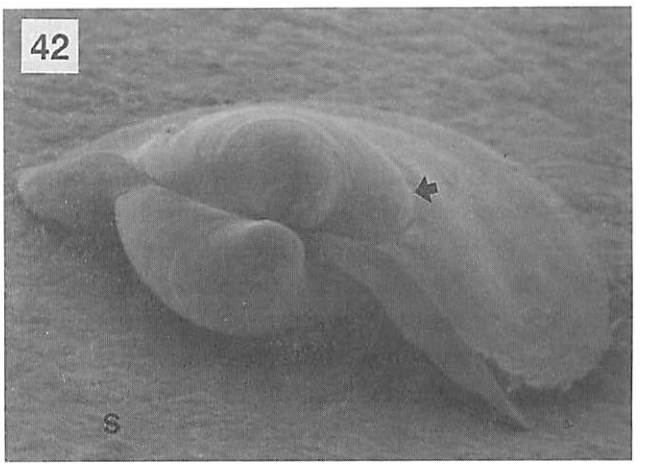
On the interior surface of the margin of the right valve, the mantle forms minute precursors of calcitic laths, overgrowing and shadowing the characteristic pattern of the prismatic layer laid down earlier (Fig. 45); upon further shell development, laths become more characteristically lath-like (Fig. 46).

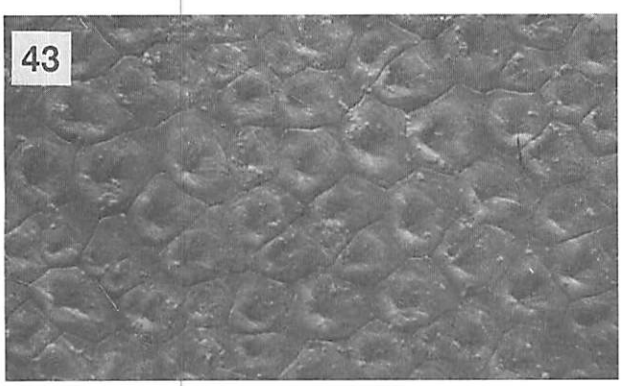
Except for a thin outer layer of prismatic calcite and the aragonitic scars of the myostracal and Quenstedt muscles (Stenzel 1971), the left valve is composed primarily of foliated calcite. At low magnifications the interior surface of the valve appears smooth, and the original prodissoconch valve is often clearly outlined beneath the foliated calcite (Fig. 47) deposited over both the prodissoconch II valve and the dissoconch valve. The anterior adductor muscle, formed in the early veliger, has been resorbed, and the posterior adductor muscle, which appears in the early umboned larva, has already migrated across the metamorphic juncture toward its adult position ([a] in Fig. 47). As the adductor muscle continues to move posteriorly keeping pace with the growth of the valves, the mantle deposits a thin myostracal layer of shell over the surface of the foliated calcite in its path (Fig. 48). This myostracal surface is exceedingly smooth. The surface of myostracum left behind the migrating scar becomes covered with new layers of foliated calcite (Fig. 47) obscuring it from view. The foliated calcite typical of the interior surface of the valves consists of successive terrace-like layers of overlapping folia that in turn are composed of long thin laths whose boundaries are sometimes visible at the surface (Fig. 49). The thin external layer of the left valve is prismatic calcite (Fig. 50) (see also Waller 1978), contrary to the report of Taylor et al. (1969) that in all probability in all species of oysters the outermost layer of only the right valve consists of simple calcitic prisms. Prisms are polyangularly cylindrical in shape, and comparable in appearance, but smaller in diameter, than those in the right valve (Figs. 43, 44). Foliated calcite is deposited directly upon the prismatic layer (Fig. 46), leaving only a narrow exposed prismatic zone at valve margins. Classification and microstructure of shell units will be elaborated in the section on the adult shell of *C. virginica.*

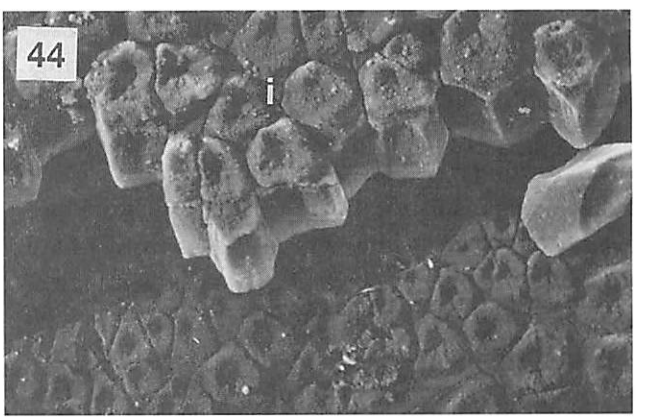












Figures 39-44. (39) Early dissoconch set on mylar film. Prodissoconch [p] 320 µm high, spat [dissoconch, d] 800 µm high. 5% bleach 30 min. Bleach dissolved the fragile marginal organic matrix causing shell edge to crumble. (40) Prodissoconch [p] (315 µm long) on early dissoconch right valve [d]. Sharp metamorphic line [arrow]. 5% bleach 60 min. (41) Higher magnification of metamorphic boundary [arrow] shown in Fig. 40. Prodissoconch valve (left) homogeneous aragonite; spat (right) prismatic calcite. 5% bleach 30 min. HFW = 120 µm. (42) Early dissoconch. Umbonal (anterior) view to show juncture of prodissoconch II and dissoconch parts of valve [arrow] and graceful downturning of spat valves onto mylar substratum [s]. 5% bleach 30 min. Spat 0.6 mm long. (43) Early dissoconch. Prisms on exterior surface of right valve in Fig. 41, about midway between metamorphic line and shell margin. HFW = 55 µm. (44) Early dissoconch. Prisms on exterior surface of right valve in imbricate scale [i] at margin of spat. 5% bleach 60 min. HFW = 60 µm.

Hinge and Ligament

The button-shaped, inner ligament of larval oysters is characteristic only of the larval shell and the veliger mode of life. A different set of ligamental structures, evolving probably from the larval hinge-ligament apparatus, meet the requirements of the dissoconch shell (Trueman 1951; Carriker and Palmer 1979a).

By a dissoconch height of about 0.8 mm (Fig. 47), the larval hinge apparatus with its teeth has become completely buried beneath dissoconch shell. The pivotal axis of the hinge has shifted from the center of the prodissoconch hinge (location of the inner larval ligament at the time of setting) anteroventrally about 0.1 mm to the future center of the dissoconch hinge (Fig. 20), where the inner dissoconch ligament forms anew and develops into the future adult ligamental resilium. As the shell continues to develop, both the ligamental system and the pivotal axis migrate posteriorly within the hinge area and between the valve ends. Simultaneously the interumbonal space enlarges, resulting in additional space between the umbones. The dissoconch hinge lacks teeth.

By a shell height of 0.8 mm, the inner dissoconch ligament of a spat has grown to a tapering, block-shaped, fibrous structure, the resilium (Galtsoff 1964; Stenzel 1971; Carriker and Palmer 1979a), about 30 µm wide (dorsoventral axis), supported in shallow resilifers of the hinge (Fig. 20). Ventral and dorsal extensions — the lamellar ligaments — have begun to form at this stage, probably representing extensions of the outer ligament (Trueman 1951). The internal supportive function of the hinge apparatus, served by the larval ligament in prodissoconch II, is assumed by the dissoconch resilium. The inner larval ligament is a separate structure and, like the prodissoconch provinculum, functions only during the planktonic stage of the veliger (Trueman 1951; Carriker and Palmer 1979a), then to be resorbed or possibly incorporated in the early dissoconch inner ligament.

At a dissoconch height of about 1.8 mm, the resilium is broadly triangular in shape, fitting into a similarly shaped resilifer in the hinge area of each valve (Fig. 51). The inner ventral width of the resilium is now about 75 µm, and the sheet-like lamellar ligaments have formed ventrally and dorsally between the opposing flat surfaces of the hinge bourrelets (Fig. 52). Lamellar ligaments are considerably thinner than the resilium (Galtsoff 1964).

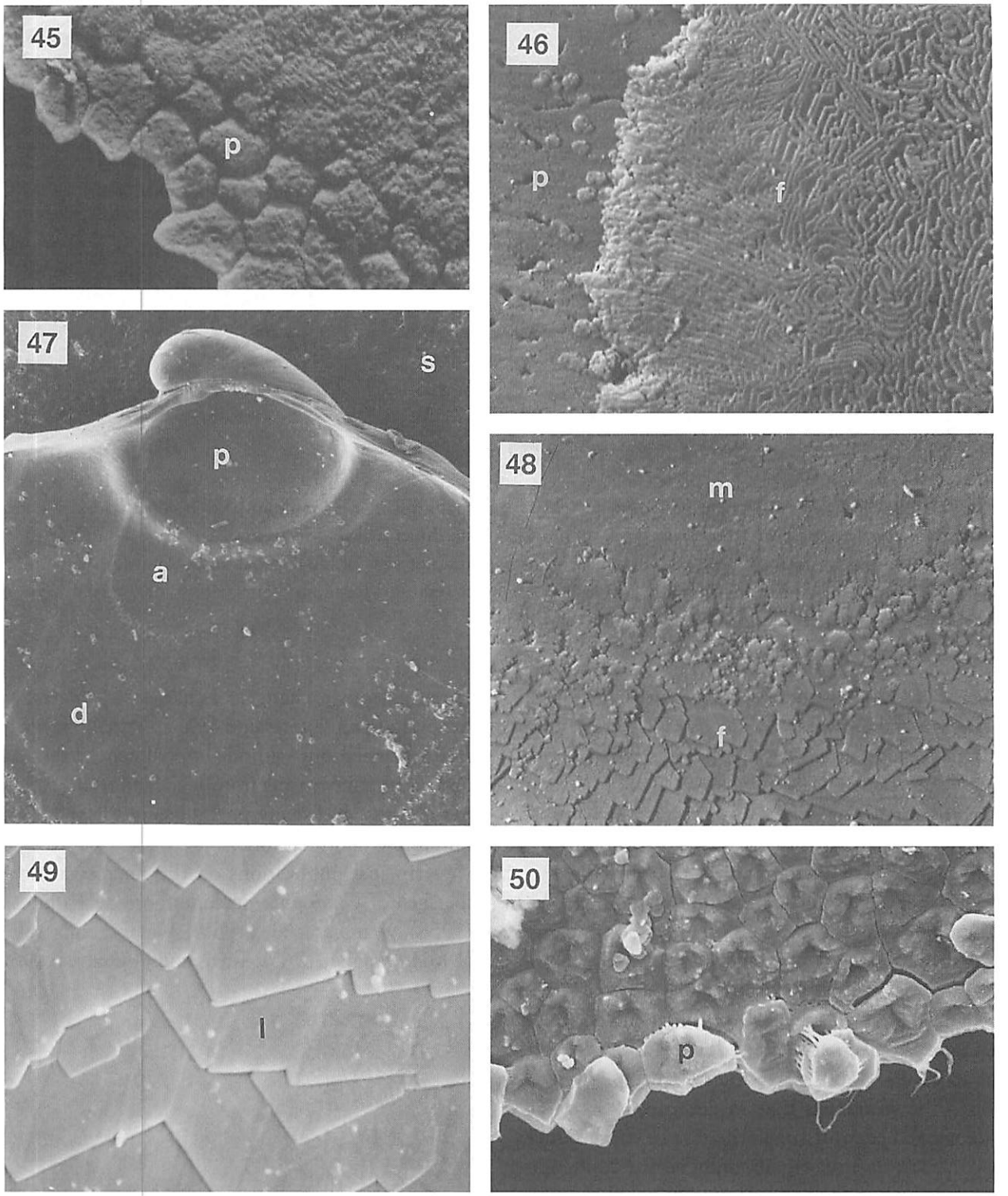
The resilium contains aragonitic fibrils (Stenzel 1962; Taylor et al. 1969; Carriker and Palmer 1979a), ranging in diameter from 0.3 to 0.4 µm. These will be described in the section on the adult shell. Lamellar ligaments lack the fibrils. Under compressive stress the resilium is strong, but under tension it is weak, whereas lamellae are strong under bending stress (Stenzel 1971), facilitating the opening-closing function of the valves at the hinge.

The ventral shift of the pivotal axis of the hinge, among other functional advantages, could without doubt permit wider gaping of the valves than would be possible were the pivotal point to remain at the closely apposed umbones. Ventral migration of the axis could have evolved as a result of the close juxtaposition of the large asymmetric umbones; or conversely, evolution of the umbones to become prominent features of the shell could have come about from a shifting of the axis.

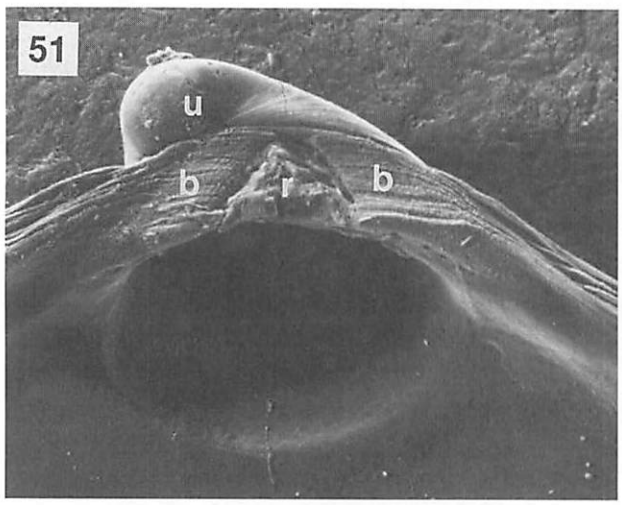
ADULT SHELL

Scaly, drably colored, encrusted, roughly shaped, asymmetric valves unquestionably place adult specimens of *C. virginica* among the least aesthetically appealing in the Class Bivalvia (Fig. 53). Yet, the internal microstructure of their monomyarian valves, each char-

Figures 45-50 (opposite page). (45) Early dissoconch. Prisms [p] partially covered with foliated calcite of the chalky variety on interior surface near posterior margin of right valve. Prismatic edge has been broken off. 5% bleach 60 min. HFW = 60 µm. (46) Early dissoconch. Interior view of posterior margin of left valve where foliated structure [f] overlies prismatic structure [p]. 5% bleach 60 min. HFW = 30 µm. (47) Early dissoconch. Interior view of left valve on mylar film [s]. Ad-



ductor muscle scar [a] straddles prodissoconch II [p] and dissoconch [d] valves. Adductor muscle scar 165 µm wide. 5% bleach 30 min. HFW = 650 µm. (48) Early dissoconch (same specimen as in Fig. 47). Ventral boundary of adductor muscle scar [smooth myostracum, m] and foliated calcite (overlapping folial laths [f]). 5% bleach 60 min. HFW = 50 µm. (49) Early dissoconch. Foliated calcite on interior surface of left valve. Lath [l]. 5% bleach 30 min. HFW = 17 µm. (50) Early dissoconch. Prisms [p], some partially dislodged, on interior surface of ventral margin of left valve. 5% bleach 30 min. HFW = 50 µm.



Figures 51-52. (51) Early dissoconch. Hinge area in left valve of spat showing torn resilium [r] in resilifer, traces of lamellar ligament still attached to bourrelets [b], umbo [u] of prodissoconch. Inner width (dorsoventral axis) of resilium 75 µm. 5% bleach 60 min. (52) Early dissoconch of *Crassostrea rhizophorae.* Interior view of hinge apparatus, resilium [r] in center between valves and lamellar ligaments [arrow] to left and right of resilium. Resilium 5 µm wide (dorsoventral axis). No bleach. From Newball and Carriker (1983).

acterized by four distinctive microstructural groups, singles out these oysters as uncommonly interesting malacologically (Yonge 1960; Galtsoff 1964; Carriker and Palmer 1979a; Carter 1979, 1980b). From basic microstructural units, their transitional forms, and the supporting periostracal and conchiolinal organic matrix, the oyster mantle fashions the microstructurally complex architecture of its shell. Development of the valves reflects changes accompanying increase in size from planktonic larva to the mature sessile individual (Carriker and Palmer 1979a), mechanical stress resulting from antagonistic functioning of the ligament and adductor muscle (Wainwright 1969), and environmental variables (Seed 1980). The wonder is that so deceptively simple a histological sheet of tissue as the mantle can produce the complex, diverse, organic-mineralized configurations expressed in shell ontogeny from prodissoconch to early spat and adult valves.

Because the oyster lacks an internal skeleton, its valves serve as an exoskeleton, providing support for the soft internal organs and preventing collapse of the mantle cavity. Consequently, vital physiological functions of the mantle cavity, i.e., circulation of seawater, gaseous exchange, discharge of gametes, and removal of pseudofeces and fecal and catabolic wastes, can be carried out without impediment. Valves also protect internal organs from mechanical impacts, osmotic stress, and many predators, and from desiccation in the intertidal zone.

Principal publications on the microstructure of the shell of *C. virginica* include those by Tsujii et al. (1958), Watabe et al. (1958), Watabe and Wilbur (1961), Watabe (1965), Travis and Gonsalves (1969),

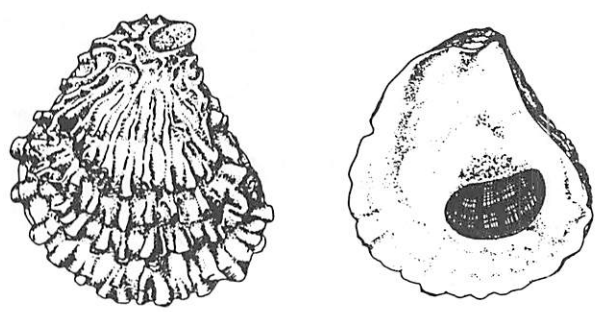


Figure 53. Oyster valves from Wellfleet Harbor, Massachusetts. Left, exterior view of left valve; right, interior view of right valve. Height of valves 9.5 cm, length 8 cm. From Galtsoff (1964).

Margolis and Carver (1974), Carriker and Palmer (1979a), and Carriker et al. (1980a). Reviews on oyster shell are contained in Taylor et al. (1969), Grégoire (1972), and Wilbur (1972).

Shell Macroform

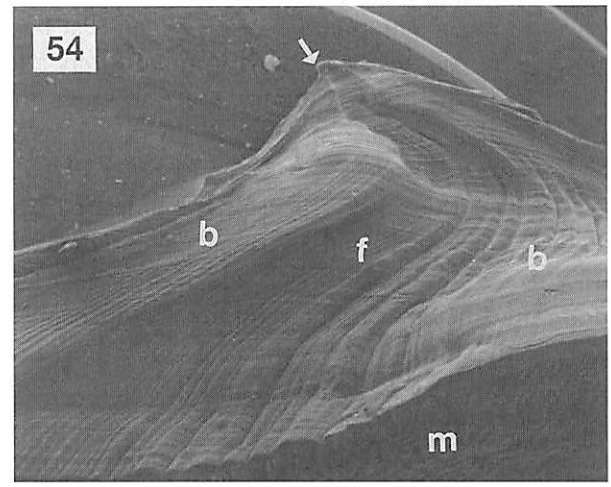
Appearance and Axes

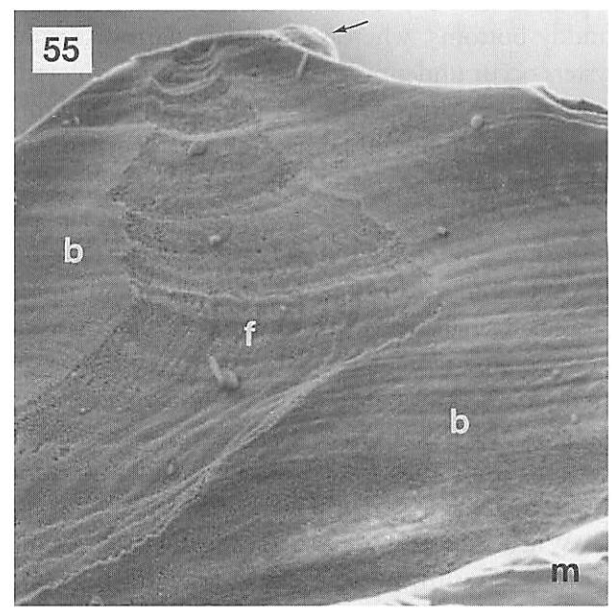
The macromorphology of the shell (including both valves and their parts) of adult *C. virginica* was clearly described and illustrated by Galtsoff (1964). The soft body (Latin, "molluscus" = soft) of the bivalve is supported between two calcareous valves joined by a resilient ligament at the anterior margin. The umbonal end of each valve where growth commenced is more pointed than the opposite posterior side (Fig. 53).

Valves are asymmetrical, the left being larger and more concave than the right. Normally the oyster is affixed to a hard substratum (is pleurothetic) by its left valve. If free of attachment and dropped into the water, it tends to land left valve down on the bottom because of the greater thickness and weight of that valve (Gunter and Burke 1978). Externally the right valve is relatively flat, and the left is cup- or saucer-shaped. The soft body of the oyster is nearly symmetrical with reference to a plane of symmetry passing between the valves parallel to their margins.

The mid-anteroposterior segment of the hinge in each valve consists of a central gulley, the resilifer (called the chondrophore by Galtsoff 1964), flanked ventrally and dorsally by elevated shelves, the ventral and dorsal bourrelets (the nymphae of Galtsoff 1964). (Some of the terms for parts of the hinge structure have been changed in this chapter from those used by Galtsoff [1964] to conform to current usage [Stenzel 1971; Harry 1985; Waller, pers. comm.]). As young oysters grow, each resilifer increases in size forming an often serpentine widening depression leading posteriorly from the umbo to the mantle isthmus between broadening bourrelet plateaus (Figs. 54, 55) (Carriker and Palmer 1979b; Carriker et al. 1980a). With increasing age of an oyster the resiliferal valley in the right valve becomes elevated onto a supporting buttress, the associated bourrelets being depressed below it on anterior and posterior sides; the left resilifer remains sunken between its bourrelets.

The central part of the ligament, the resilium, is carried between the depressed resilifer of the left hinge





Figures 54-55. (54) Hinge surface of left valve of young dissoconch oyster, ventral to the right. Resilifer [f], bourrelets [b], contact with mantle isthmus [m], umbo [arrow]. Ligament removed by dissolution in full strength bleach, freeze dried. HFW = 7 mm. (55) Hinge surface of right valve of young dissoconch oyster, ventral to the left. Resilifer [f], bourrelets [b], contact with mantle isthmus [m], umbo [arrows]. Ligament removed by dissolution in 100% bleach. HFW = 6 mm.

and the elevated resilifer of the right hinge. Lamellar ligaments (tensilia of Galtsoff 1964) are supported between the bourrelets of the hinge. The surfaces of the hinge thus comprise the growth tracks of the ligament: the resilifer, which is the track of the resilium; and the anterior and posterior bourrelets, which are the tracks of the anterior and posterior lamellar ligaments. The tongue and grooving of resilifers and bourrelets in the hinge minimize sheer, in much the same way that articulating teeth do in the hinge of prodissoconch II valves (Carriker and Palmer 1979a).

Beaks of the valves represent the oldest part of the dissoconch shell, and are usually slightly curved and directed toward the dorsal side of the valves; less commonly they can point ventrally. Prodissoconch valves at the anterior tip of the beaks are soon worn away in *C. virginica*. Direction and degree of curvature of the beaks, as well as their relative proportions, can vary widely. Narrow, straight, or slightly curved beaks are usually formed by oysters growing on soft, muddy bottoms, whereas extremely narrow, slender oysters occur under overcrowded conditions in oyster reefs. Other forms of beaks, unrelated to environmental conditions and perhaps of genetic origin, also exist. The pointed end of the right (flat) valve is always shorter than that of the opposite left (cupped) valve. The angle between the two beaks determines the maximal extent to which valves can open. Chomata, small ridgelets and pits opposing them on the inner margins of the valves near the hinge, are absent in species of the genus *Crassostrea* (Stenzel 1971; Harry 1985) whose valve margins are smooth (various kinds of chomata are useful identification aids in oyster systematics, Harry 1985).

When a closed oyster shell is held with the hinge pointing upward, both valves visible and beaks directed toward the observer, the flat valve with the shorter convex resilifer is the right one, and the cup-shaped valve with the longer concave resilifer is the left one (Figs. 56, 57). The anterior margin of the shell is the hinge (or beak) side and the posterior margin is opposite. With the exterior surface of the flat (right) valve facing the observer, and the beak directed upward, the ventral end is at the right, and the dorsal is at the left of the valves. Ventral and dorsal sides of an oyster shell can also be identified by position of the adductor muscle scar on the inner face of each valve. The scar, oval-shaped and generally highly pigmented, is asymmetrically located closer to the dorsal than to the ventral end of the valve (Fig. 57).

The three principal dimensions of oyster valves include (Fig. 57): height, distance between umbones and posterior margins of the valves; length, maximal distance between ventral and dorsal margins parallel to the hinge axis; width, maximal distance between the outside surface of closed valves measured at right angles to the plane of closure of the valves.

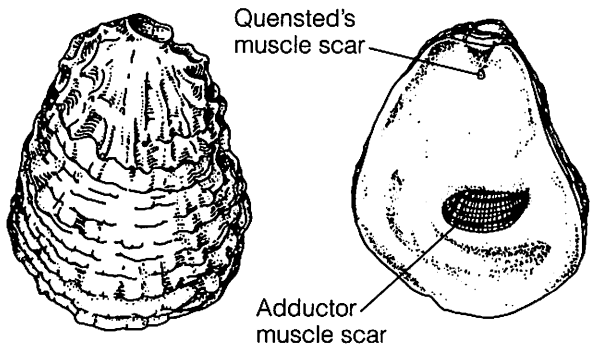


Figure 56. Oyster valves from Great South Bay, New York, 5 years old. Dimensions, 10.0 × 6.6 cm. Left, exterior view of left valve; right, inner surface of right valve. From Galtsoff (1964).

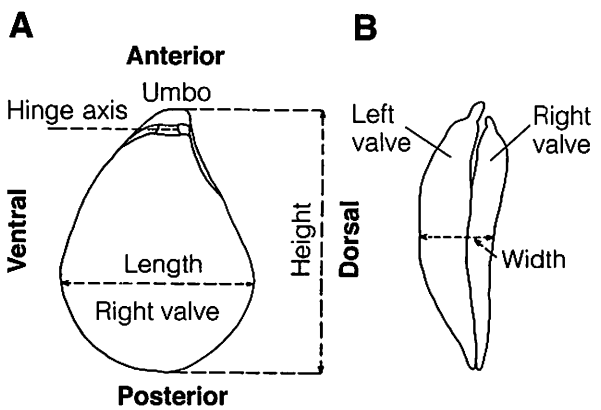


Figure 57. Diagram showing method of measuring the height, length, and width of oyster valves. A, interior view of right valve; B, side view of valves.

The terminology employed by earlier workers (see Galtsoff 1964) for orientation of the valves of *C. virginica* was based on the hinge line on the dorsal side and the gills under the anterior side. In the present volume, for the sake of consistency, we employ terms based as closely as possible on the orientation of the internal organs, as follows (terms used by Galtsoff [1964] are given in brackets):

Anterior: region over the mouth and hinge line [dorsal];

Posteror: region opposite the hinge line [ventral];

Dorsal: region over opening of the promyal chamber and anus [posterior];

Ventral: region over the gills [anterior].

Environmental Effects

The effect of the environment upon valve morphology can be pronounced (Seilacher et al. 1985). Shell form is thus not a reliable character for identification of ostreid species (Harry 1985). In fact, the shape of an oyster shell is so modifiable that it cannot be expressed in precise geometrical terms (Lison 1942; Galtsoff 1964).

Even an incomplete list of factors suspected of modifying shell morphology is a long one: bottom types, salinity, temperature, current velocity, turbidity of the seawater, direct sunlight, calcium concentration, and chemical pollution (Galtsoff 1964; Medcof and Kerswill 1965; Frazier 1976; Palmer and Carriker 1979; Seilacher et al. 1985).

Environmental effects on the shell morphology of *C. virginica* are varied. Oysters growing singly on firm bottom tend to develop rounded valves sculptured with radial ridges and foliated processes; those living on soft, muddy bottoms or in clusters and reefs as a rule possess long, slender, sparsely ornamented valves. Shells of oysters grown under unfavorable conditions are often thin and fragile. "Coon" oysters from overcrowded reefs in the Carolinas and Georgia tend to be narrow and characterized by light shells. Heavy, strong shells are not typical of any particular region, occurring on hard, natural bottoms throughout the range of distribution of the species from the Gulf of St. Lawrence in Canada to Florida, Yucatan and the West Indies (Newball and Carriker 1983), and are indistinguishable from one another (but see Shell Pigmentation, p. 147).

Oysters grown under anomalous conditions often produce shells that differ morphologically from those in "more normal" habitats. For example, compared with control oysters, thicker, more rounded valves developed in 1-year-old *C. virginica* held for one year in a combination of elevated temperatures (9.6°C over that of control oysters), swift currents, and an approximate concentration of chlorine of 0.5 ppm discharged into the canal of a fossil fuel plant in Connecticut (Ruddy et al. 1975). And in a tributary of Chesapeake Bay contaminated by manganese, iron, zinc, copper, and cadmium, young oysters developed significantly thinner valves than control oysters over the period of a year (Frazier 1976). Determining the cause of the structural differences in the shell is difficult because of the multiple factors involved.

Cultural experiments in Delaware suggest that environmental factors (and possibly also nutrition) influence pigmentation of the exterior of the shell, especially of the right valve (Palmer and Carriker 1979). The two laboratory culture systems differed from the natural habitat (Broadkill estuary) in having lower, more constant temperatures, lower salinities, and lower calcium concentrations; temperature, salinity, and pH were similar. In oysters (2.1 to 4.7 cm mean heights) raised in the estuary, right valves were brightly colored with blotches of yellow, purple, and blue on a dark brown background overlain with radiating stripes, the one along the principal axis of growth generally a wide yellow band. Oysters in recycled laboratory seawater (calcium concentration, which was normally well above 250 ppm in the estuary, dipped below 150 ppm in the recycled water during 5 of 11 weeks of culture) had shells that were off-white to pale yellow in color, often with a purple-brown band along the principal axis of growth. Shells of oysters in laboratory seawater changed every second day (calcium concentration was low, but not as low as in the recycled water) were also off-white to

pale yellow, but lacked radiating colored bands. The myostracum of the adductor muscle scar was brownish in laboratory oysters and purplish in oysters from the estuary. The laboratory oysters were not exposed to high intensity light. Oysters in all three habitats were derived from a single spawning and attached to a similar kind of substratum.

Shells in oysters from the three systems were substantial and solid, as indicated by density values, but those of oysters in the recycled seawater proved to be significantly denser than those in the two other systems. Density values demonstrated that oysters raised in the estuary and in seawater changed every second day possessed shells that were about 25% chalky, whereas nearly all oysters in the recycled seawater had mostly foliated shell. Unexpectedly, oysters grown in recycled seawater where they were exposed to fluctuating and often depressed levels of calcium, produced thick, strong shells with a minimum of chalky material. Valves of oysters grown in the two laboratory systems possessed prominent scales, usually over 1 mm in length, with flutings and gentle ruffes at the edges. Oysters in the estuary were relatively unornamented (Palmer and Carriker 1979).

Oyster biologists have known in a general way for a long time that environmental factors can have a pronounced influence on the morphology of oyster shell. However, single-variable, controlled studies, like that of Medcof and Kerswill (1965), for example, are required in order to understand and attempt experimental modification of bivalve shell form, sculpture, and pigmentation. The following variables (assuming nutritional requirements have been standardized) could be examined in initial experimentation: nature of the substratum, spectrum and intensity of light, and calcium levels.

Medcof and Kerswill's (1965) study demonstrated that intense daylight does affect the color and morphology of eastern oyster shell. Paired lots of young oysters were grown in light-exposed and shaded areas of intertidal beaches in Prince Edward Island and Nova Scotia, Canada. Shading increased linear shell growth about 1.5 times, but reduced the thickness-to-length ratio. Volume of space between valves generally was greater in exposed than in shaded bivalves, and specific gravity of the shells was higher in light-exposed than in shaded oysters. Light-exposed oysters appeared more symmetrical and more ornamented than shaded ones. Exposed oysters were often brightened by colored radial markings, mostly green and brown — something of a parallel with the color of oysters grown in the estuary by Palmer and Carriker (1979). Medcof and Kerswill (1965) concluded that daylight can retard linear growth of shells and seems to reduce the capacity of the mantle edge for marginal shell production, but to increase its capacity to produce pigments.

Dimensions

Individuals of *C. virginica* of marketable size usually range in height from 10 to 15 cm; depending on the place of origin, an oyster of this size could vary in age from 3 to 5 years, older oysters coming from cooler, more temperate regions.

Oysters generally continue to grow throughout the life cycle, though more slowly with age, and can attain impressive sizes. Old individuals usually occur on undisturbed bottoms in the absence of commercial shellfishing. The largest oyster found by Galtsoff (1964) came from the vicinity of Boothbay Harbor, Maine. It was 20.6 cm high, 9.7 cm long, and 6.5 cm wide; total weight of the live mollusc was 1,230 g, of which 1,175 g was shell and 35.8 g was meat. The remaining 19.2 g was weight of seawater retained in the mantle cavity. Porter (1975) recorded the largest *C. virginica* found in North Carolina as having a height of 21.2 cm. Ingersoll (1881) reported a large specimen from Damariscotta River, Maine, which had a shell height of 35.5 cm and a shell length of 11 cm.

Shift in Axes

Nearly circular shells occur only in very young oysters (Fig. 58, left), especially in those growing singly on flat surfaces. Within a few weeks after setting, valves become elliptical, newly deposited shell at the margin of the valves forming a band wider at the midposterior area than at the sides. Pigmented bands on the surface of valves (Fig. 58) superimposed

on growth annuli (rings, striae) illustrate the differential rate of growth along the periphery of the valves. The principal axis (normal axis, Owen 1953) of growth is often clearly marked by these intensely pigmented bands, especially on young oysters. Additional pigmented radial bands are located to either side of the principal band (Fig. 58). As an oyster continues to grow, the principal vector of growth can shift from the center to one side, curving as it does, with the result that the oyster becomes slightly oval and asymmetric (Fig. 58, right). Change in direction of the principal axis of growth apparently is not associated with any environmental factor as it does not take place in all oysters growing under identical conditions (Galtsoff 1964). Occasionally, pigmentation of the principal axis is so pronounced as to appear artificial; in some of these, secondary axial bands remain unpigmented.

The principal axis of valves of oysters usually curves slightly dorsally (Fig. 58, right) but can frequently shift ventrally. These "inverted" individuals are normal with a typically cupped left valve and well developed, grooved beaks. A mechanical obstruction or an unusual position on the bottom does not seem to alter the direction of the growth axis. Inversion seems to be expressed only in the form of the valves. Once established, the axis of growth can change abruptly as much as 90°, even in oysters as old as 6 or 7 years. In an extreme case, clearly indicated by pigmented bands on the surface of the valves, an oyster changed direction of growth at the end of each growing period (Galtsoff 1964).

Culture of *C. virginica* in the laboratory in closed systems does appear to have an effect on direction of the principal axis, contrary to Galtsoff's (1964) observation that it is not influenced by environmental conditions. In experiments by Palmer and Carriker (1979), over 95% of the valves of oysters grown in closed systems curved ventrally, yet in a nearby estuary, shell of one half of the oysters curved dorsally and another half ventrally; all oysters were derived from a single spawning and were attached to the same kind of substratum. Perhaps, then, axial direction is a genetic expression that under some circumstances can be influenced by environment.

Dimensional Relationships

Form of a bivalve shell can be expressed as an index of shape, determined as a ratio of the sum of height and width of a shell to its length (Galtsoff 1964). In the entire range of distribution of *C. virginica* along the Atlantic and Gulf states, the index of shape varied from 0.5 to 1.3 (Fig. 59). No significant

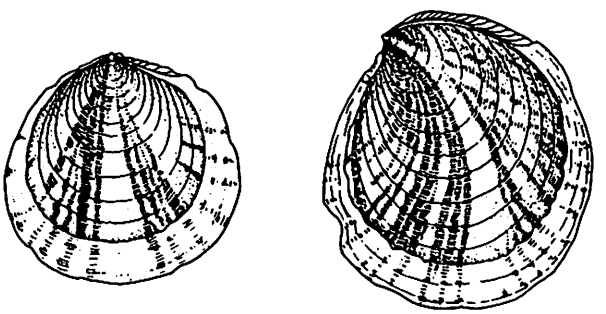


Figure 58. Exterior surface of right valves of two small oysters growing attached to tar paper. Height of left oyster 0.85 cm, of right oyster 1.0 cm. In the larger oyster the principal axis curves dorsally. From Galtsoff (1964).

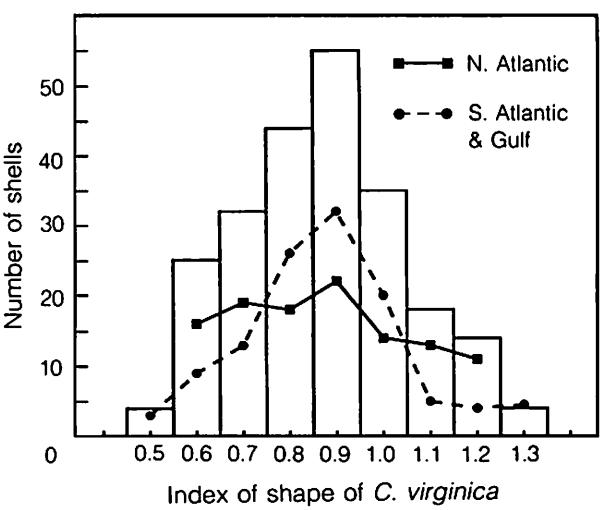


Figure 59. Histogram of distribution of index of shape (height+width/length) of shells of oysters from the Atlantic Coast. Solid squares, frequency distribution of the index of North Atlantic oysters; solid circles, of South Atlantic and Gulf of Mexico oysters. From Galtsoff (1964).

differences were found in the index of shape of northern and southern populations of oysters that were examined separately. In contrast to the index of shape of other bivalves, that of the oyster is highly variable (Galtsoff 1964).

Shell Area

The relationship between height and area of oyster shells can be represented by a curve (Fig. 60). The data for this curve were taken by Galtsoff (1964) from a random sample of live oysters collected in coastal areas between Prince Edward Island, Canada, and the eastern end of Long Island Sound.

Chalky Deposits

The semi-glossy inner surface of oyster valves is frequently marked by irregularly shaped chalky-white areas, or "chalky deposits" (Fig. 61). These islands consist of relatively soft, porous shell material contrasting with the appearance and texture of the surrounding smooth semi-glossy shell surface (Korringa 1951a; Galtsoff 1964). Biologists have yet to find the cause of these deposits, though several hypotheses (no one any more convincing than the others) have been offered (Korringa 1951a; Galtsoff 1964): i.e., deposits (a) are formed in places where the mantle loses contact with the shell; (b) result from secondary solution of calcium salts of the shell; (c) are related to the abundance of calcareous material in the substratum; (d) maintain efficient functioning in older oysters; (e) serve as padding when a large quantity of shell volume is produced; or (f) preserve a volume relationship between meats and shell cavity and regulate the contour of the inner surface of the valves. The formation of chalky shell will be discussed in more detail later.

Chalky patches often become covered by the semi-glossy shell material as the shell grows, and become incorporated within the valves; new chalky patches can then be deposited anew on the surface in other regions of the valves. Galtsoff (1964) examined 472 shells collected at random from bottoms along the Atlantic Coast from Long Island to Georgia. Nearly one half of the total number of valves examined was free of chalky patches; in about 25% of the valves, deposits covered less than one quarter of the valve area, and in less than 3% they covered more than three quarters of the valve surface. There was no part of the valve surface where deposits occurred most often.

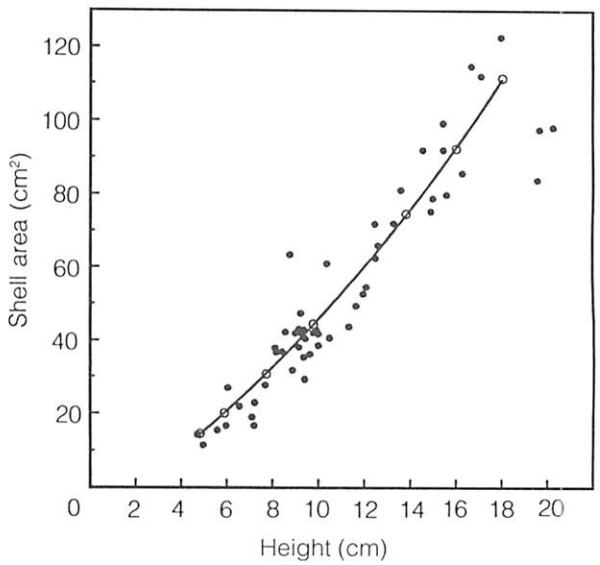


Figure 60. Area of valves plotted against height of valves of oysters collected at random from Prince Edward Island, Canada to the eastern end of Long Island Sound, New York. From Galtsoff (1964).

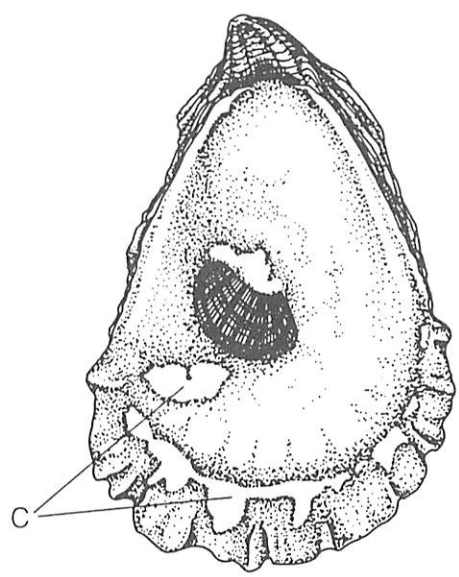


Figure 61. Chalky deposits [C] in newly formed shell at the interior edge of left valve and adjacent to muscle scar. Valve 12 cm high. From Galtsoff (1964).

The fact that chalky deposits appear in valves during the first week of life of *C. virginica* (Palmer and Carriker 1979) suggests that these patches are normal parts of the shell (Galtsoff 1964).

Chambers

These are irregularly shaped cavities, or "blisters," that occur within the valves of *C. virginica.* Chambers are sometimes subdivided into series of subchambers by thin sheets or layers of shell (partitions, or "paper shell" of Okoshi et al. 1987). The cause of these chambers is unknown. In some cases they could result from invasion of the shell cavity as by annelid worms (species of *Polydora*) or by perforations of the shell by boring sponges (*Cliona* spp.) and boring clams (*Diplothyra smithii*) (Galtsoff 1964; Stenzel 1971). Uninfested chambers contain seawater; infested ones, mud. Chambers are present throughout the Bivalvia; the first phase of formation is an organic layer, followed by mineralization (Waller 1990, pers. comm.). Formation of partitions in the chambers of *C. gigas* was examined electron-microscopically by Okoshi et al. (1987). In some bivalves, the sequence of shell layers repeats that formed at the mantle edge, and the initial organic deposit thus resembles periostracum (Wilbur 1972; Waller 1990 and pers. comm.).

Chambers can also be induced artificially by inserting a foreign object, such as a small piece of plastic sheeting, between the mantle and a valve (Galtsoff 1964). The plastic material in some oysters brings about a pathological condition that results in the formation of leathery capsules similar to chambers frequently found on the inside of shells near the adductor muscle, and is accompanied by deposition of shell material at a rate greatly in excess of that under normal conditions. Galtsoff (1964) found no evidence to suggest that chambers are associated with shrinkage or other body changes in C. *virginica* (see also Stenzel 1971).

Adductor Muscle Scars

The place of attachment of the adductor muscle, the muscle scar or imprint, is the most conspicuous area on the interior surface of the valves of *C. virginica.* It is pigmented, the color ranging in different individuals from light lavender-white to dark purple.

The scar is located slightly off center toward the posterodorsal side of each valve (Fig. 57). In part, shape of the scar reflects the shape of the valve, being almost round in broad round valves, and elongated in long narrow valves. The outline of the scar is slightly concave on the side facing the hinge, and convex on the ventral side. Slightly curved growth annuli, following the curvature of the ventral edge of the valves, are evident on the surface and most pronounced on the ventral side of the imprint.

Size and shape of scars are variable (Fig. 62) (Galtsoff 1964). Outlines in this figure were drawn from replicas of scars from 169 shells taken at random from several oyster bottoms on the Atlantic and Gulf Coasts. As would be expected, the larger the valve, the greater the area of the scar; the relationship (Fig. 63) is rectilinear, although the scatter of points increases with increase of valve and scar areas. The ratio of scar area to shell surface area varies from 8 to 32, the peak of the frequency distribution being at 16 to 18 (Galtsoff 1964).

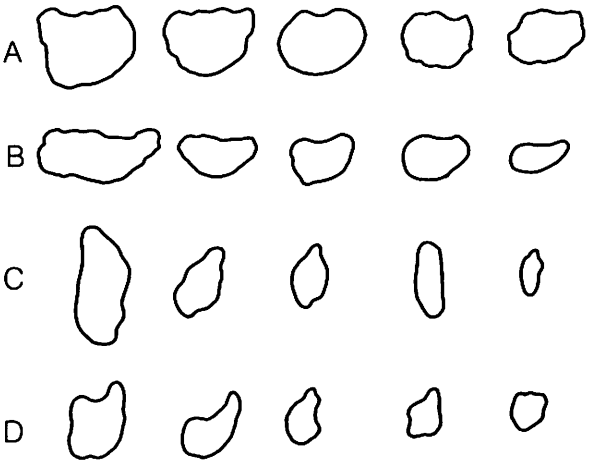


Figure 62. Replicas of adductor muscle scars from oyster shells collected at random. Rows A and B, types of scars normally found on broad and rounded valves; length of scars almost equals or exceeds the height. Rows C and D, scars often occurring on long and narrow valves; height of scar exceeds the length. Replicas were selected from shells taken from oyster beds along the Atlantic and Gulf Coasts. From Galtsoff (1964).

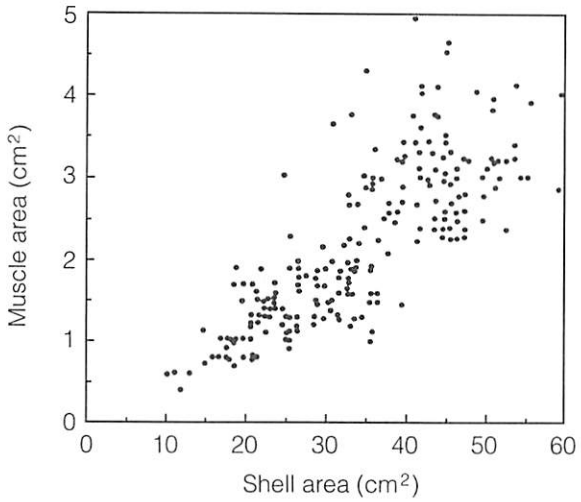


Figure 63. Relationship between area of adductor muscle and area of the valve of oysters. From Galtsoff (1964).

A small, unpigmented, oval area on the dorsal half of the interior surface of each valve of *C. virginica* is the Quenstedt scar (Fig. 57), attachment site for a gill protractor muscle (Harry 1985). Galtsoff (1964) considered the pair of Quenstedt muscles nonfunctional and observed that in his collection of living oysters the scar is scarcely visible. In live oysters, slight adhesion of the mantle to the valve surface indicates the location of the scar. Quenstedt muscles were mistakenly thought by Stenzel (1971) to be modified anterior pedal muscles.

Ligament

The ligament-hinge complex of dissoconch bivalves is superbly structured, not only to remain functional throughout ontogenetic growth of the valves, but also to support the purely mechanical function of opening and closing the valves (Trueman 1954; Shadwick and Gosline 1983).

The ligament of adult *C. virginica* consists of a relatively thin band of dark, elastic, organic material within the opening-closing, dorsoventrally directed pivotal axis of the hinge below the interumbonal region, and somewhat hidden from outside view. The ligament is non-living, resilient material that forms a bridge between the anterior margin of the valves, forcing the valves apart when tension of the adductor muscle is released, and being compressed when contraction of the adductor muscle closes the valves. The central part of the ligament, the resilium, creates the opening moment, whereas the ventral and dorsal lamellar ligaments take the place of hinge teeth and ensure valve alignment (Yonge 1982). The once actively functioning articulating teeth of prodissoconch valves have been buried in the hinge region, and articulation is now supported entirely by the complex microstructure and configuration of the ligament and ligament-supporting surfaces.

The most recently secreted layers of the ligament lie in contact with the subligamental epithelial ridge of the mantle isthmus (a continuation of mantle margins of right and left mantle lobes) on the inside of the valves (Owen et al. 1953). Oldest strata of the ligament face the outside between the beaks; as these layers become exposed to seawater, microbial action, and elastic fatigue failure, they crack and crumble and become nonfunctional (Trueman 1951, 1964; Galtsoff 1964; Stenzel 1971; Dungan and Elston 1988). Splitting of older regions of the ligament is characteristic of many bivalves and is the result of increase in size of the interumbonal space with growth, which separates the umbones beyond the limit of stretchability of the ligament (Stasek 1963). When valves are forcibly broken apart, the ligament breaks approximately along the pivotal axis of the valves (Fig. 64), the two halves of the ligament remaining attached securely to their respective valves.

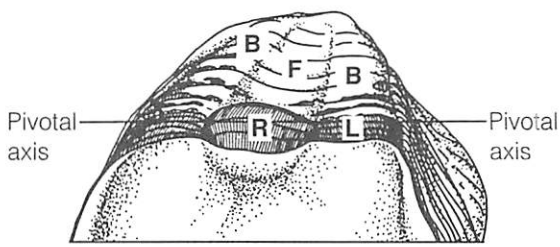


Figure 64. Interior view of ligament on right valve of large oyster. The resilium [R] occupies the central position and on both sides is flanked by lamellar ligament [L]. Resilifer [F], bourrelets [B]. Resilium is 1.2 cm long (dorsoventral dimension). From Galtsoff (1964).

In a live oyster, the normal gaping of valves never attains the potential maximum limited by the angle and length of the beaks because even at periods of greatest relaxation the adductor muscle exerts a slight pull against the elastic tension of the ligament. The extent to which valves can gape maximally depends largely on the shape and size of the beaks and the amount of interumbonal shell growth that has occurred (Stasek 1963). For example, in oysters with a proportionately large space outside the ligament between the beaks, valves can gape widely, whereas in oysters with close-set beaks, movement of the valves on the hinge is severely limited regardless of the degree of relaxation of the adductor muscle.

The dissoconch ligament is composed of three distinct anatomical parts: (a) the light brown, central, mineralized resilium (or inner ligament of Yonge 1982) lying posterior to the pivotal axis of the hinge and secured to the resilifer of each valve, and (b) ventral and (c) dorsal, dark green lamellar ligaments (lamellar, or outer ligaments of Yonge 1982) located ventral and dorsal to the resilium (Figs. 64, 65, 66) and attached to the bourrelets on each valve hinge (Fig. 64). Growth of new ligament takes place only on its internal ventral surface facing the mantle isthmus. Periostracum on the outside surface of the ligament is present only in young dissoconch oysters before erosion of umbones commences. The ligament effectively seals the space between the anterior edges of the valves, forming an elastic watertight joint that prevents entry of seawater and organisms that otherwise could invade the mantle cavity (Fig. 65) (Galtsoff 1964). Because beaks of the oyster are asymmetric, the distance between resilifers and bourrelets, and thus the thickness of the ligament, is greater at the ventral than at the dorsal end (Fig. 66), suggesting

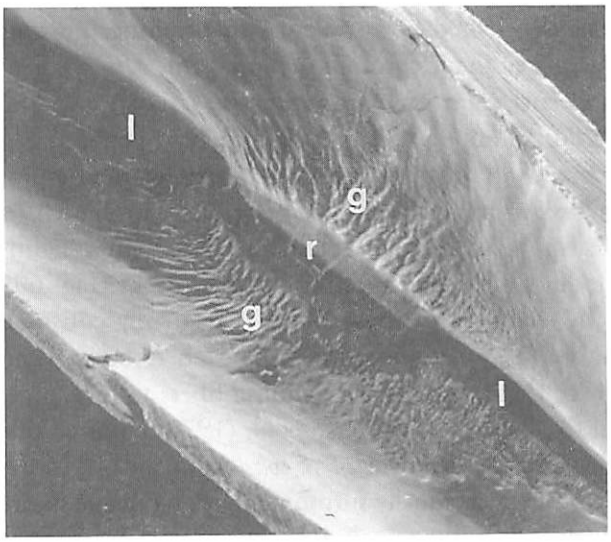


Figure 65. Interior view of ligament in place between left valve (lower left) and right valve (upper right). Resilium [r], lamellar ligaments [l], rugose zone of foliated structure [g]. 20% bleach 10 sec. HFW = 3.8 mm.

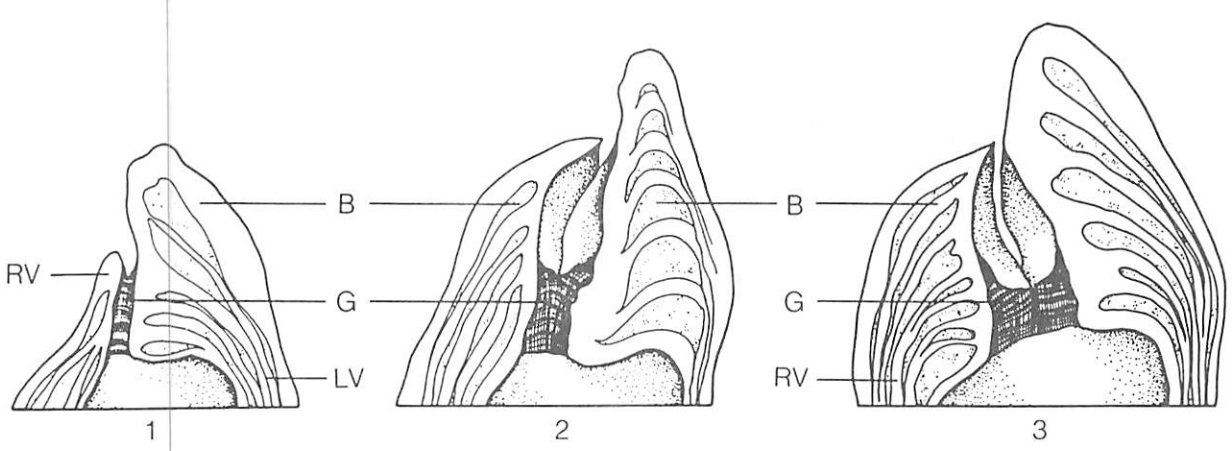


Figure 66. Three sections through hinge and ligament of the shell: (1) dorsal section, (2) central section, including resilium, (3) ventral section. Beak [B], ligament [G], left valve [LV], right valve [RV]. Note arched lines in resilium in section (2). Central section 3.5 cm wide at bottom. From Galtsoff (1964).

more bending outward of the resilium in the central region as a result of compression.

Aragonitic Fibers

The organic material of the resilium is reinforced with white aragonitic fibers or needles (Stenzel 1963; Galtsoff 1964; Carriker et al. 1980a) embedded and dorsoventrally oriented within the lamellae (Figs. 67, 68). The morphologically long (crystallographic c) axis is oriented perpendicular to the inner growing margin of the resilium in the soft clam, *Mya arenaria*, and surf clam, *Spisula solidissima* (Marsh and Sass 1980), although no similar studies have yet been performed on *C. virginica*. Dissolution of the organic matrix of the resilium of C. *virginica* with bleach reveals long, slender, crystalline needles, with a slightly nodular surface suggesting growth marks (Fig. 69). The diameter of these fibers is relatively constant, about 0.15 µm. Thickness of each fiber is about twice that of the matrix binding fibers to each other in fixed specimens. Marsh et al. (1978) reported that fibers in the ligament of *S. solidissima* and *M. mercenaria* are individually surrounded by an organic sheath distinct from the remainder of the matrix; Marsh et al. (1978) were able to dissolve the matrix, but not the fiber sheaths, with papain. In these species, sheath proteins contain smaller proportions of glycine and methionine than those of the ligamental matrix. Furthermore, aragonitic fibers are twinned crystals and in general the twin forms a thin organic membrane through the center of each crystal (Marsh and Sass 1980). Micrographs by Carriker et al. (1980a) suggest the presence of organic sheaths around each fiber in the resilium of *C. virginica*.

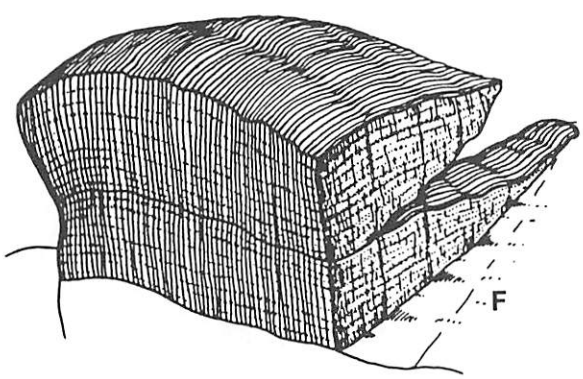
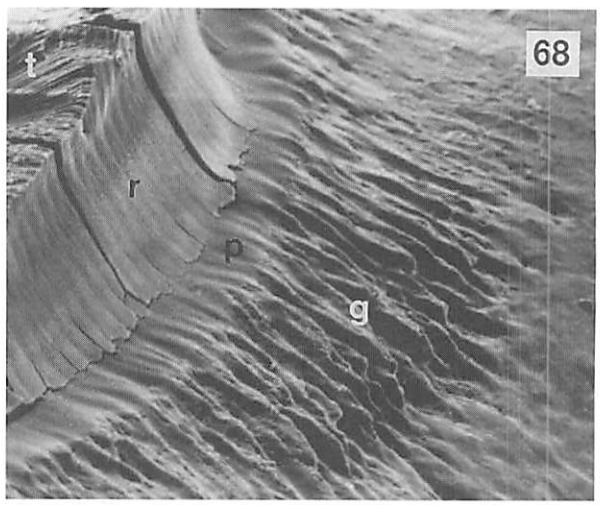
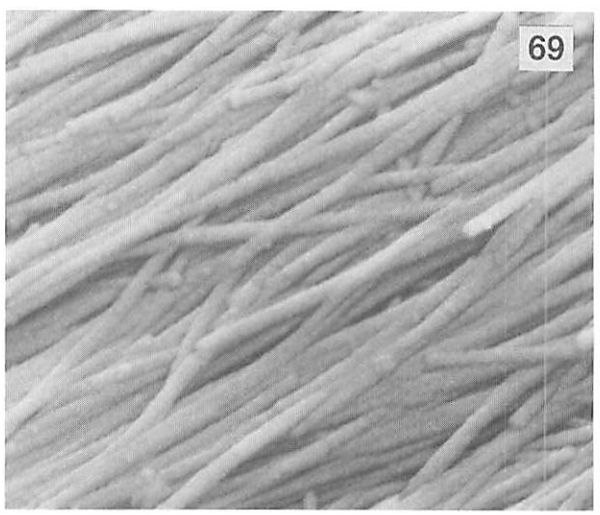


Figure 67. Block of resilium of oyster attached to resilifer [F]. Maximum height of resilial block, 4 mm. Oldest part of resilifer extending anteriorly between beaks of valves, upper right. From Galtsoff (1964).





Figures 68-69. (68) Resilium broken in half along the pivotal axis, one half still attached to resilifer of one valve, fracture surface at upper left [t]. Resilium [r], ligostracal prisms deposited in advance of formation of resilium [p], rugose zone of foliated structure [g]. 20% bleach 10 sec. HFW = 1.5 mm. (69) Aragonitic fibers of resilium, organic part of resilium removed with 100% bleach. HFW = 3.8 µm.

Lamellar Ligaments

Dorsal and ventral lamellae (Figs. 64, 65) consist of tenacious organic material (conchiolin) that withstands considerable stretching without breaking. Like the resilium, lamellar ligaments are composed of lamellae (or strata) secreted by the ventral and dorsal parts of the subligamental epithelium. Galtsoff (1964) observed two types of organic structures in unstained, nondecalcified lamellae teased apart in glycerine: heavy, well defined bundles of fibers within the anteroposterior plane of the lamellae, and short slender fibrils at right angles to the fibers. The only mineralized structures present were scattered patches of calcium carbonate crystals. At light microscopic magnifications, lamellar ligaments appear smooth and homogeneous, and the boundary between lamellar ligaments and the resilium is sharp.

Physical Properties

Using oyster shells from which the soft organs of the body had been removed without injury to the ligament, Galtsoff (1964) determined the elastic force of the ligament of *C. virginica* in a complex apparatus of his own construction. He found that the elastic property of ligaments of oysters from seven different geographic areas along the eastern and Gulf coasts of the United States varied widely between and within each geographic group, especially in oysters from Peconic and Apalachicola Bays (Fig. 70). These oysters came from a variety of habitats with differing salinities and annual temperatures. Whether differences in the elastic force of the ligament are the result of ecological dissimilarities remains to be investigated.

With exposure to air, the elasticity of the ligament changes, and resiliency is gradually lost (Galtsoff 1964). As drying progresses, greater force must be applied to bring the valves together (Fig. 71) and the ligament becomes harder and more brittle until it finally breaks along its pivotal axis. Change in elasticity begins after the first 90 min of drying of shells in which tissue has been removed. Obviously, oysters that are still alive can be held in air for many days and the ligament retains its elasticity.

Chemical Composition

In analyses of dry ligaments of 5 and 6 year old oysters, Galtsoff (1964) observed that the calcium carbonate of the resilium varies from 30 to 67% by weight; that of lamellar ligaments ranges from 5.3 to 8.5%. Kahler et al. (1976) reported a calcium carbonate concentration by weight in powdered dry resilium in the same species of approximately 92%. This may be an overestimate as the sum total of this protein and calcium carbonate content analysis was 126%.

The chemical composition of the ligament approaches that of the periostracum of the shell in its alanine and glycine content, but differs from that of shell layers (Brown 1975). Staining reactions and various tests by Galtsoff (1964) indicated that proteins comprising the resilium and lamellar ligaments of *C. virginica* are not identical.

The resilium of *C. virginica* consists of 33.9% protein and 0.12% carbohydrate by weight (Kahler et al. 1976). Its principal amino acid, glycine, is present in amounts almost triple those of the next most abundant amino acids, aspartic acid and serine. Glycine concentration is directly correlated with resilience, which corresponds to the strength of the resilium under compression (Wainwright 1969) and inversely correlates with calcium carbonate (Kahler et al. 1976).

Lamellar ligaments consist principally of a quinone-tanned protein, which contributes to their tensile quality. Recovery from compression by the resilium probably is mediated through easing of steric stress induced during compression, and the crystalline fibers appear to provide mechanical "firmness" similar to that provided by reinforcing rods in concrete (Kahler et al. 1976).

Ligament-hinge Interface

A thin, distinct, mineralized layer, the ligostracum, interfaces the shell surface of the hinge with that of the ligament (Carriker and Palmer 1979b). This stratum is microstructurally and generally mineralogically different from the underlying calcitic foliated structure. Ligostracum on the hinge of the right

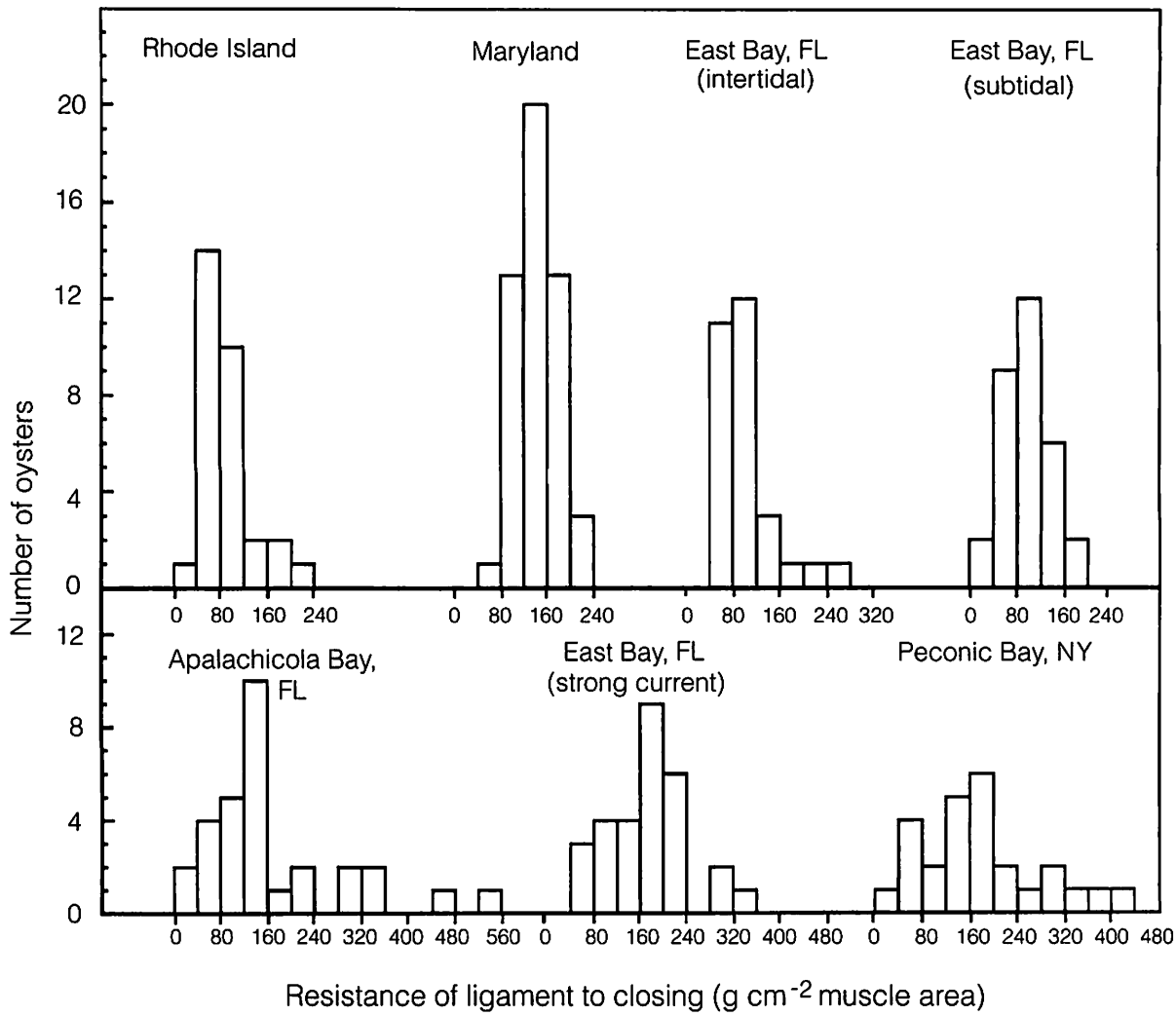


Figure 70. Frequency distribution of elastic property of ligaments in seven groups of adult oysters. Elastic property expressed as the pulling force of the adductor muscle (in g cm^{-2} of muscle area) required to counteract the action of the ligament. From Galtsoff (1964).

valve is aragonitic; that of the left valve, calcitic with traces of aragonite. The ligostracal surface on both the resilifer and bourrelets of the left valve is conspicuously annulated (Fig. 72), growth lines being more obvious on the bourrelets. The ligostracum on the right hinge is also annulated, but less prominently. The acicular, aragonitic "basal part" of the ligament described by Mano (1980) in *Mercenaria mercenaria* and the scorched mussel *Brachiodontes exustus* is suggestive of the ligostracal layers in *C. virginica*. Analysis of microstructures and microgrowth increments in the ligostracum of the hinge of fossil oyster shells can yield useful information on environmental conditions, growth rates, and age and season of the year when death occurred (Kent 1988; Custer and Doms 1990).

Ligostracal prisms on the resilifer of the left valve tend to lie obliquely to the surface, range in diameter from a fraction of a micrometer to about 6 μm, and are interrupted periodically by growth lines (Fig. 73). The layer averages 8 to 16 μm in thickness, and prisms are spaced apart and bear deep pits.

Ligostracal prisms on the resilifer of the right valve and on bourrelets of left and right valves are

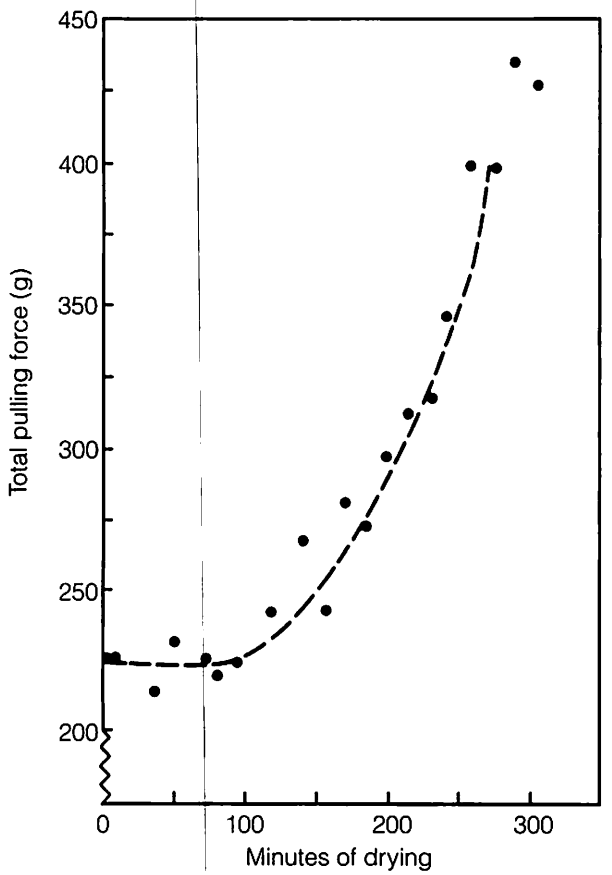


Figure 71. Effect of drying at 20° C on elasticity of the ligament of adult oysters from New York. From Galtsoff (1964).

generally oriented at nearly right angles to the surface of the underlying foliated structure, and form a distinct layer removable by fracturing (Fig. 74). Prisms of the bourrelets of the left valve are considerably more pitted and irregular than those of the right valve. In fracture sections, bourreletal strata average 15 μm in thickness and taper to half this thickness at edges. The central part of the right resiliferal ligostracum increases to 30 μm.

The finely tuberculate, pitted, spaced arrangement of the distal ends of prisms of the ligostracum provides a strong functional surface for bonding ligament and hinge shell surfaces. Because muscle attachment sites in molluscs are invariably aragonitic, it would appear that aragonitic or partially aragonitic ligostracal prisms also enhance attachment of the ligament to hinge surfaces (Carriker et al. 1980a).

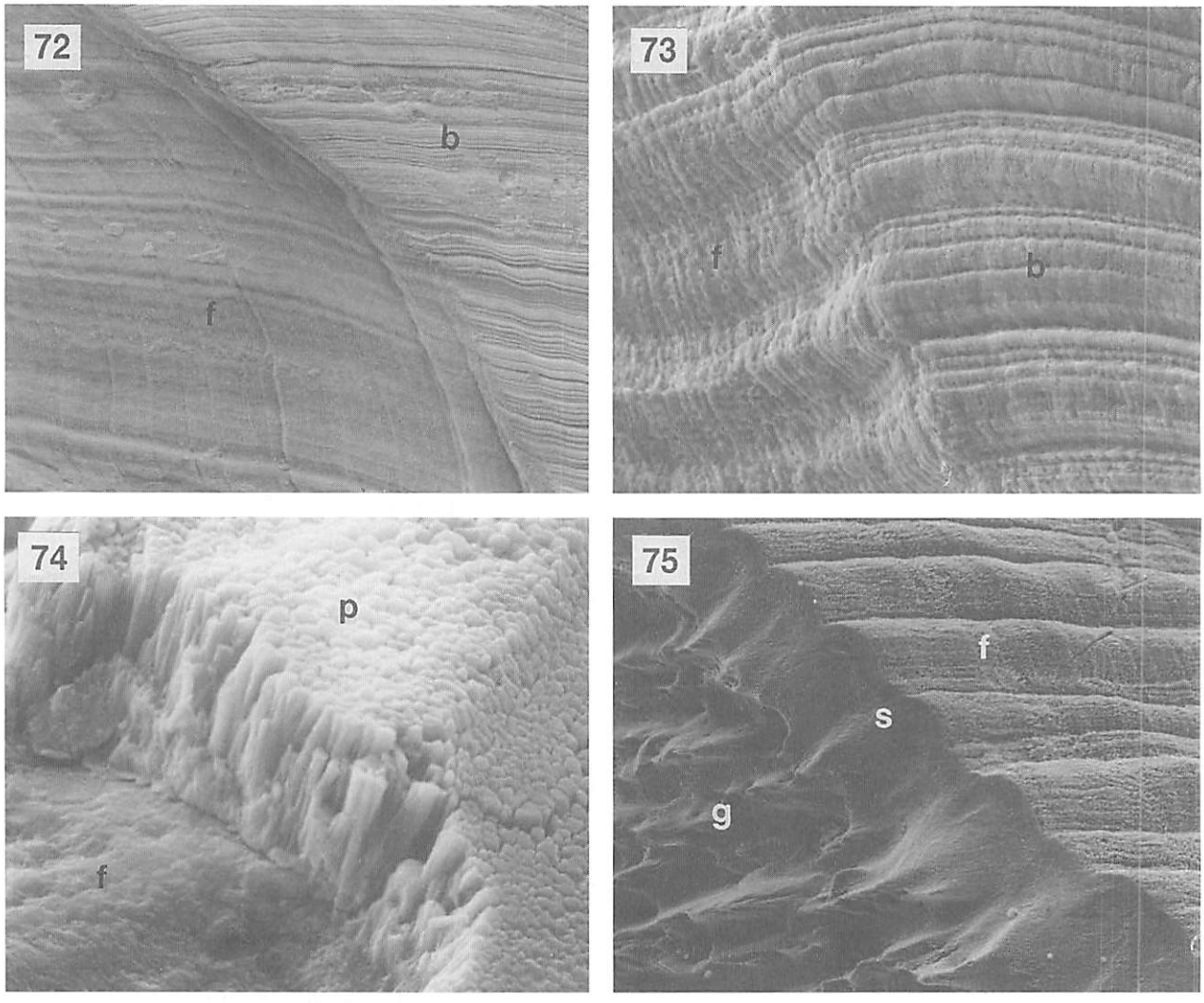
Although orientation of ligostracal prisms is at right angles to the resiliferal surface of the right valve and that of bourrelets on both right and left valves, it is oblique only on the resiliferal surface of the left valve. This orientation could be related to the pull of the ligament on hinge surfaces during opening and closing of the valves at the time of shell deposition; research on this matter is worth pursuing from a mechanical point of view.

Resiliferal and bourreletal ligostraca form a sharp boundary at the ligament-mantle isthmus interface (Figs. 54, 75). The resiliferal surface is frequently thrown into wave-like folds that parallel the dorsoventral plane of the resilifer (Fig. 75, upper right). Below the ventral boundary of the resilifer, the shell surface is relatively smooth for a short distance (Fig. 75) and represents ligostracal structure forming in advance of the growing ligament. Ventral to this zone lies a wider area, the rugose zone, in which the shell surface becomes conspicuously pitted with deep valleys and sharply pointed crests (Fig. 54).

Subligamental Epithelium and Ligament Formation

Continuity of right and left mantle lobes across their anterior margins takes the form of a ridge of simple, tall, columnar epithelium whose dorsoventral length corresponds to that of the ligament. This epithelium secretes the resilium and lamellar ligaments and resiliferal-bourreletal ligostraca to which they attach. The ridge in cross-section is semi-cylindrical. The epithelial surface facing the ligament is slightly undulating (Fig. 76), the wave pattern possibly corresponding to the undulating surface of the resilifer (Figs. 54, 75).

The subligamental epithelium is darker in color than epithelia of adjacent mantle lobes, and consists of a single layer of extremely tall, narrow cells arranged in fanlike groups set on a well developed basal membrane (Fig. 76). Height of cells varies from 50 to 200 μm. Distally, they become darker, presumably because of the presence of secretory granules, precursors of ligamental material. The ridge is well supplied with



Figures 72-75. (72) Resilifer [f] and bourrelet [b] of hinge of left valve. Ligament dissolved in strong bleach. HFW = 1.3 mm. (73) Higher magnification of surface of resilifer [f] and bourrelet [b] shown in Fig. 72. HFW = 130 μm. (74) Ligostracal prisms [p] of bourrelet of right valve, fracture, underlying foliated structure [f]. Ligament dissolved in strong bleach. HFW = 80 μm. (75) Resiliferal-mantle isthmus juncture. Resilifer [f], smooth surface of juncture [s], rugose foliated structure [g]. Ligament dissolved in strong bleach. HFW = 0.6 mm.

blood vessels and muscle fibers arranged parallel to the length of the ridge (Galtsoff 1964).

One can only surmise the degree of complexity of the physiological-biochemical processes taking place in this seemingly histologically-simple subligamental epithelium that in a continuously expanding growth phase secretes and forms the micro-structurally intricate ligostraca, lamellar ligaments, resilium, and aragonitic fibers.

Periostracum

The external surface of the shell of bivalves is covered by a relatively thin, pliable, firmly attached, unmineralized periostracal sheet (Galtsoff 1954). This is thought to protect the mineralized parts of the valves from corrosion (see p. 152), and serves as the initial matrix for deposition of shell biomineral crystals at valve margins (Bottjer and Carter 1980).

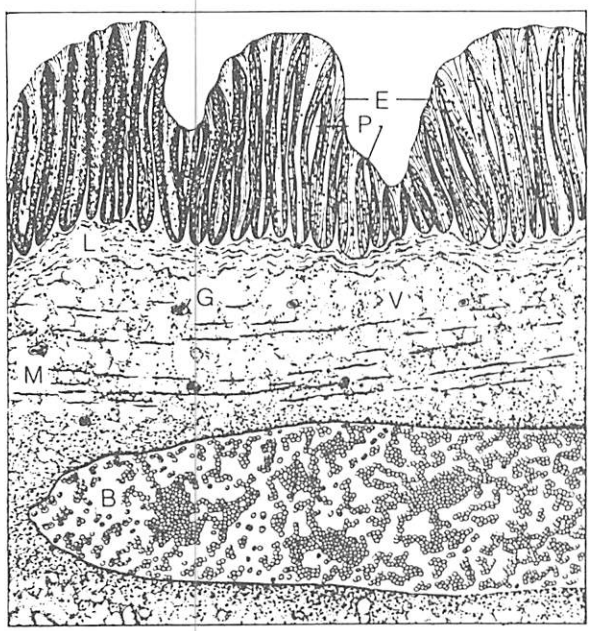


Figure 76. Dorsoventral section through the subligamental epithelium and ridge bridging the right and left mantle lobes. Large hemolymph vessel [B], basal elastic membrane [L], epithelium [E], muscle fibers [M], pigment cells [G], pockets between epithelial cells [P], vesicular cells [V]. HFW = 0.8 mm. From Galtsoff (1964).

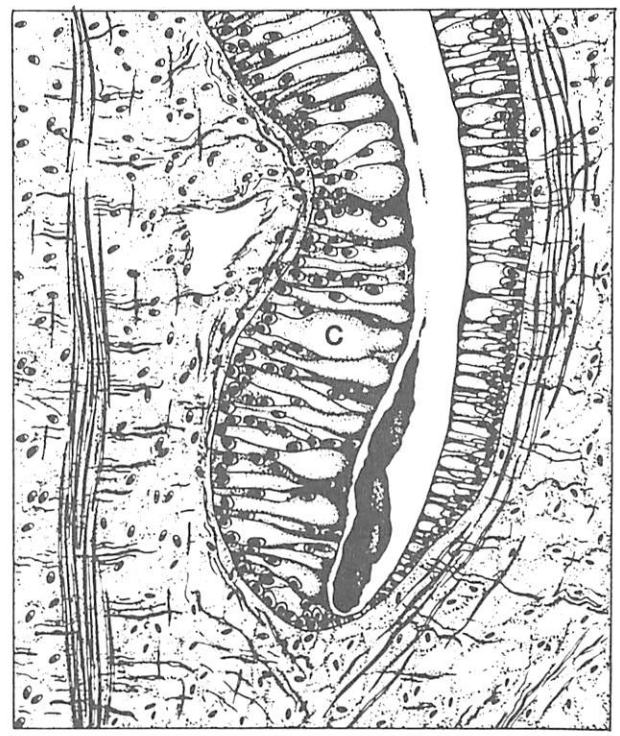


Figure 77. Histological section at right angles to margin of mantle (antimarginal) through mantle margin and periostracal groove, outer fold to the left, inner fold to the right. Cells [C] on the inner surface of the outer fold are distended with secretion. Black mass at bottom of the groove is conchiolin. HFW = 250 μm. From Galtsoff (1964).

New periostracum is secreted within the periostracal groove at mantle margins, and emerges from the groove to extend the formed periostracal layer on the surface of the valves as valves grow (Saleuddin and Petit 1983; Waite 1983; Wilbur and Saleuddin 1983).

Periostracal Groove

In adult *C. virginica*, the periostracal groove is a narrow space between epithelia of the middle and outer folds of both right and left mantle lobe margins, and is lined with a single layer of epithelial cells (Fig. 77). The cells in the outer part of the groove are tall and ciliated, whereas those in the inner region are secretory, nonciliated, and differ histologically from the ciliated cells (Galtsoff 1964).

There is a conspicuous difference in histological appearance of secretory cells on the outer and inner sides of the groove. Those lining the outer side (inner surface of the outer fold) are distended distally and taper toward the base. Typical goblet cells containing eosinophilic granules, round mucous cells, and amebocytes are present in epithelia on both sides of the groove. At the extreme base of the groove, the tall secretory cells are abruptly replaced by short cubical cells that extend outward a short distance on the inner wall of the groove and then increase in height outward along the inner groove wall. Material released by the secretory cells accumulates at the bottom of the groove in close contact with epithelia on both sides of the groove (Galtsoff 1964).

Formation and Maturation

The formation of the periostracum has not been examined in any detail in adult *C. virginica*, but a number of studies summarized by Saleuddin and Pe-

tit (1983) on other species of bivalves, and the study by Tomaszewski (1981) on the early dissoconch of *C. virginica*, suggest some details.

The basal secretory cells at the bottom of the periostracal groove possess long microvilli, and their cytoplasm contains numerous Golgi complexes, mitochondria, and membrane bound vesicles whose contents are thought to be precursors of periostracum. Newly formed periostracum is advanced out of the groove by continuous secretion of additional material, receiving supplementary coatings and treatments as it moves out of the groove. The process of maturation and tanning of periostracum is not fully understood. In bivalves it is probably quinone-autotanning in which a L-DOPA-containing protein is oxidized by polyphenoloxidase to a quinone-containing protein. The latter reacts with itself and other proteins to produce cross-linked periostracal structure (Waite et al. 1979; Waite 1983, and pers. comm.). Two enzymes, localized in the mantle of various species of molluscs, could have roles in the processing of periostracum: acid phosphatase, possibly modifying periostracum; and phenoloxidase, in tanning periostracal proteins. Quinone tanning of proteins requires the presence of three components, all of which are present in periostracum: o-diphenols, fibrous proteins, and phenoloxidases (Samata et al. 1980).

In many species of bivalves, especially those producing a relatively thick periostracum (e.g., mytilids), the forming sheet of free periostracum newly released from the periostracal groove loops outward from the mantle edge to the shell margin to which it remains fixed, and in so doing encloses the extrapallial space within which subsequent shell formation occurs (Saleuddin and Petit 1983). In *C. virginica*, however, the mantle edge does not remain more or less fixed in position relative to the valve margins, but instead moves freely in and out during opening and closing of the valves. During periods of active shell growth, the mantle is extended a considerable distance beyond the edge of the shell (Carriker et al. 1980a).

Secretion of periostracal material from the periostracal groove of live adult *C. virginica* was observed by Galtsoff (1964) under low optical magnification. He removed a small piece of the edge of the right valve, exposing the intact opposite left valve and its mantle, and saw that periostracal material oozed out of the groove as a clear, viscous substance. While secreting, the mantle edge was extremely active, extending and retracting as successive layers of periostracal material were laid down (Fig. 78). As a shell layer was deposited beyond the edge and in the plane of the valve, the margin of the mantle rolled upward, its outer fold parallel to the plane of the valve and the middle and inner folds facing upward. Tentacles of the inner fold bent downward, while those of the middle fold contracted slightly. The outer fold, which lacks tentacles, underlay the sheet of new viscous periostracum that oozed from the periostracal groove between the outer and middle folds. During secretion, the edge of the mantle frequently extended out and then withdrew to the position shown in Fig. 78. At the time of extension, the outer fold temporarily supported the semi-liquid periostracum, and by moving in and out spread it over the previously deposited shell layer. As a result, the proximal part of the newly forming valve received a larger quantity of periostracum and became thicker than

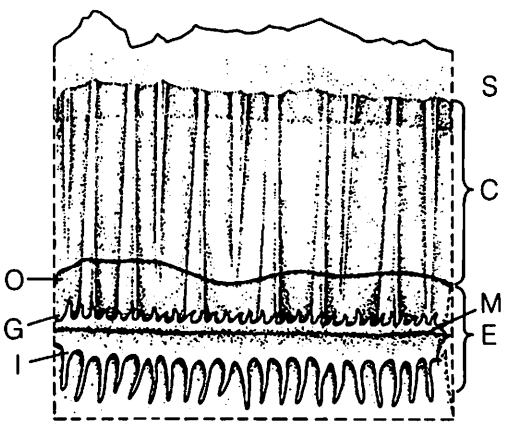


Figure 78. View from above of small area of left mantle edge and newly secreted shell. The mantle was exposed by cutting off a piece of the right valve, and the live oyster was placed in seawater. New shell [S], conchiolin sheet [C], outer mantle fold [O], middle mantle fold [M], inner mantle fold [I], periostracal groove [G], edge of mantle [E]. Drawn from life. New shell area outlined by broken line is in plane of the drawing. HFW = 10.5 mm. From Galtsoff (1964).

the distal part. When secretion ceased, the periostracum became incorporated into the newly forming shell. As Waller (1980) points out, newly generated periostracum in most bivalves has little mechanical strength and is greatly extendible before tanning.

Chemical Properties

Protein is the major constituent of periostracum. Minor components include carbohydrates, semiquinone-containing pigments, lipids, polyphenols, chitin, and metals (Waite 1983). The proteins are characterized by rather high glycine levels and low cystine levels.

Saleuddin and Petit (1983) found in the three ridge clam *Amblema plicata* that amino acid composition of the periostracum within the periostracal groove is different from that of the outer periostracal cover of the valves. For example, periostracum within the groove has a lower ratio of glycine, but higher ratios of alanine, aspartic acid, glutamic acid, and arginine; and free periostracum, periostracum of the outer shell edge, and that of the outer surface of the umbo are characterized by different amino acid ratios. Saleuddin and Petit (1983) proposed that amino acid composition changes before each of three orders of biomineral organization, namely, (1) initial formation of needle crystals, (2) spherical association of needles, and (3) needles stacked in columns to form prisms. It is after this final organization of crystals that the organic matrix becomes fixed in the mineral structures.

Manganese and iron are concentrated in the periostraca of freshwater bivalves, but are not detectable in the California mussel *Mytilus californianus* (Swinehart and Smith 1979); similar studies have not been made on the periostracum of *C. virginica.*

Surface Microstructure

The periostracal layer bonded to the external surface of the right valve of *C. virginica* is thin, nonmineralized, and remains only over the more recent unworn parts of the valve surface. Even on unworn surfaces, some periostracum (Fig. 79) is so thin that the organic envelope of shell prisms and the overlying periostracal layer are frequently difficult to differentiate (Fig. 80). Thickness of the periostracum is highly variable and ranges from only one to several micrometers (Carriker et al. 1980a).

Elevation of the periostracum into folds and wrinkles is common. The soft, newly secreted organic sheet at the valve margin is easily wrinkled and folded as the mantle margin moves, and this could account for this type of surface sculpture. Ridges are more frequent where prismatic growth has been interrupted than where normal development occurs (Fig. 80). Periostracal ornamentation varies in the same right valve, as well as in right valves of different individuals, from one or two creases per prism (Fig. 81) to crowded folding and crumpling (Fig. 82).

Extreme thinness of the periostracum in this species could account for the fact that no studies on its chemical characteristics (other than those of Tomaszewski [1980, 1981] on early spat) have been reported.

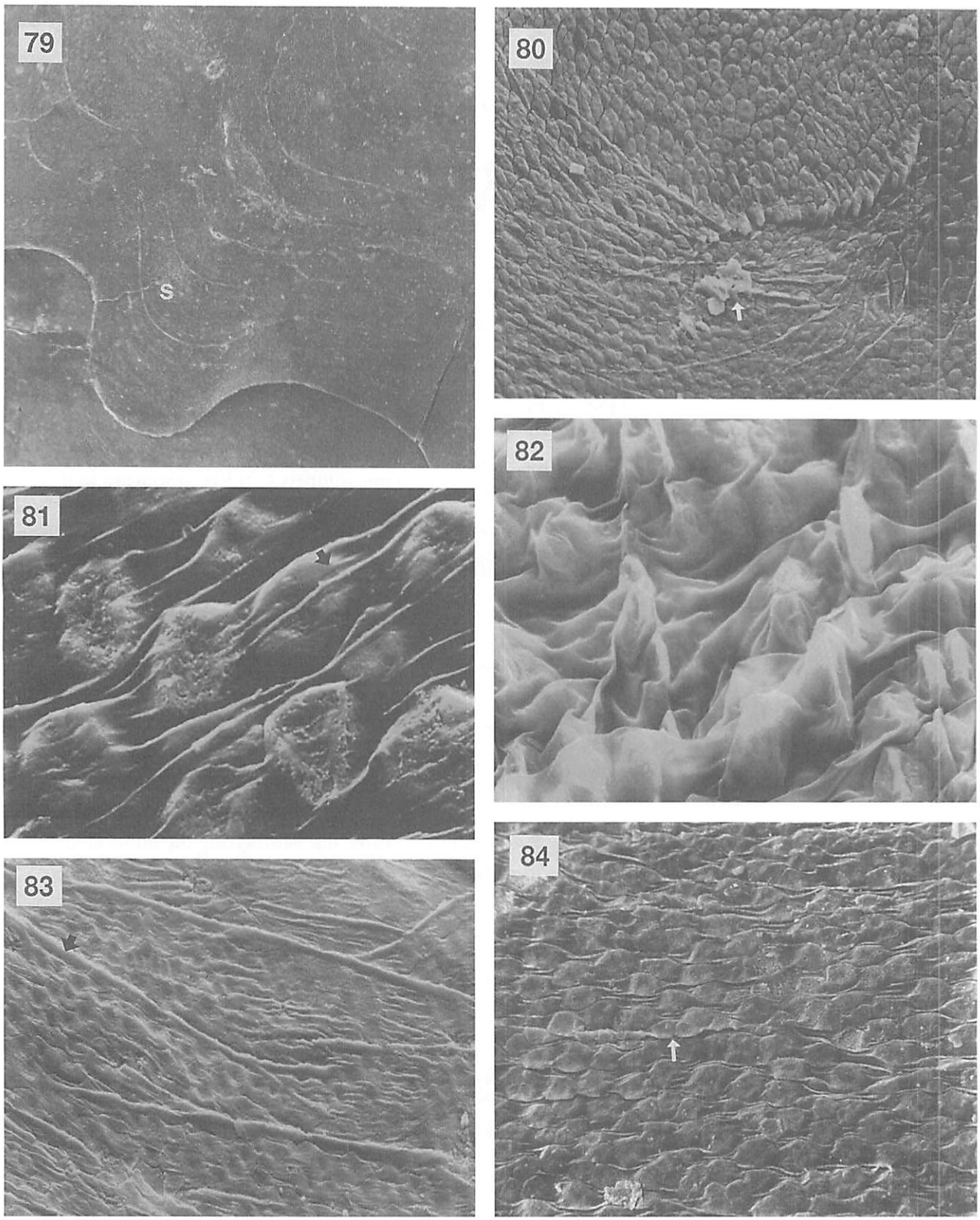
The periostracum of the unworn surface of the left valve (Fig. 83) is likewise a thin organic veneer, thinner even than that of the right valve. The underlying shell microstructure shows clearly through the periostracum, whose surface varies from smooth, to minutely creased, to prominently folded and creased (Fig. 83). In places the periostracal surface is thrown into microscopically conspicuous ridges and grooves between rows of prisms, giving the impression of tiny scales (Fig. 84).

Valves

Terminology

Valves of *C. virginica* are composed of four major types of mineralized microstructural units, and their intergrades, arranged in a variety of layers and groupings. Each microstructural unit consists of a shell biocrystal of $CaCO_3$ with traces of other minerals, encased within a thin sheath, or envelope, of organic matrix. The four types of microstructures are present in both valves, but in different proportions (Carriker et al. 1980a; Watabe 1988).

Simple prismatic microstructure constitutes the thin outer layer of both valves, being more prominent on the right than on the left valve (Fig. 85). Simple prismatic structure consists of compact, closely joined



Figures 79-84. (79) Adult oyster, prismatic scale [s], right valve. 100% bleach 15 sec. HFW = 2.5 mm. (80) Periostracum, exterior surface, right valve. Periostracal folds [arrow] overlying prisms at spot where prismatic growth was interrupted and then resumed. 100% bleach 1 min. HFW = 325 μm. (81) Periostracum, exterior surface, right valve. Periostracal creases [arrow] overlying prisms. 20% bleach 10 sec. HFW = 40 μm. (82) Periostracum, exterior surface, right valve. Dense convo-

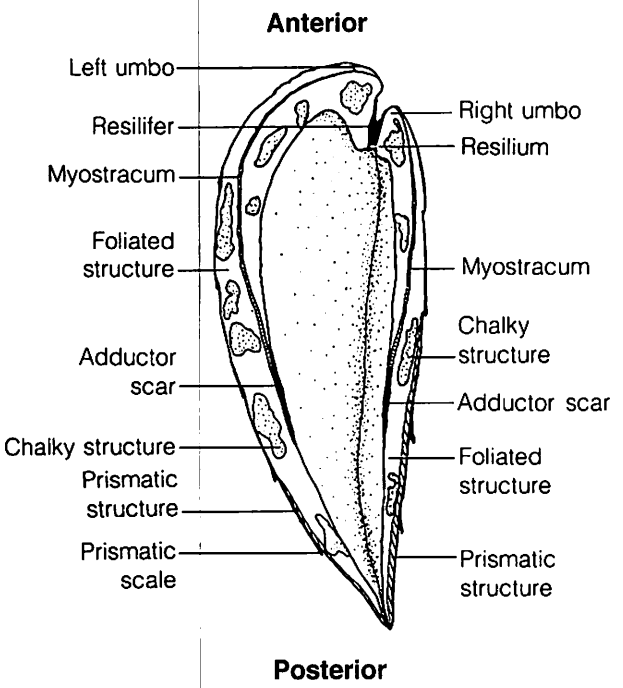


Figure 85. Diagrammatic drawing of ventral half of valves of oyster, 5 cm high, sectioned anteroposteriorly through middle of hinge. Thickness of valves exaggerated to illustrate major shell regions.

layers of regular, calcitic, single prisms (Carter 1980b). On the external surface, primarily of the right valve, the ventral rim of many individual strata of prisms tends to flare away from the valve surface to form overlapping, imbricated scales (Fig. 79).

On older surfaces of valves, prismatic structure generally becomes eroded, and with it, the scales, exposing the underlying foliated structure. Foliated structure constitutes the bulk of both valves. It consists of fine sheets (folia, or laminae) grouped into larger structures, the lenticular folia. Individual folia are composed of small elongated laths (tablets, blades, lamellae of earlier authors) joined side-to-side by organic matrix in a configuration that leaves the surface resembling a tiled roof (Runnegar 1984). Foliated calcite in oysters has been called calcitostracum, subnacre, or nacre by earlier writers. However, the term "nacre" refers to a specific set of aragonitic structures not present in any true oyster (Waller 1985).

Lenses of chalky structure, consisting of blade-like microstructures arranged irregularly in a spongy pattern, occur commonly throughout foliated structure, and are perhaps a structural modification of foliated structure (Carter 1980 a, b).

The adductor muscle scar is the surface of the myostracal support for attachment of the adductor muscle and is composed of aragonitic, irregular, simple prisms.

Occasionally, patches of organic material are deposited over the interior surface of the foliated structure. Prominent umbonal plicae, or folds, extend from the left umbo posteriorly on either side of the valve, and are especially conspicuous in young dissoconch valves. Growth annuli (growth rings, bands, or lines) of interrupted growth or changes in pattern of structure, are present in the prismatic structure of both valves (as well as in the ligostraca of resilifers and bourrelets already discussed).

Boundaries between the major different kinds of microstructures are marked by gradual to sharp transition zones (Watabe and Wilbur 1961; Carriker et al. 1980a; Watabe 1988). As a general rule, aragonitic structures can intergrade with one another and calcitic structures can also intergrade, but aragonitic structures do not intergrade with calcitic structures, probably for crystallographic reasons (Waller, pers. comm.).

Shell Formation

Investigations of the structure and formation of molluscan shell have burgeoned during the past three decades (for example, Wilbur 1964, 1972, 1976, 1980; Taylor et al. 1969; Kobayashi 1971; Grégoire 1972; Watabe and Wilbur 1976; Crenshaw 1980;

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luted periostracal creasing. 15% H_2O_2 1 min. HFW = 85 μm. (83) Periostracum, exterior surface, left valve. Periostracal ridges [arrow] overlying prisms. 100% bleach 1 min. HFW = 180 μm. (84) Periostracal folds [arrow], exterior surface, left valve. 100% bleach 5 sec. HFW = 210 μm.

Omori and Watabe 1980; Samata et al. 1980; Samata 1982; Saleuddin and Petit 1983; Weiner et al. 1983; Wilbur and Saleuddin 1983; Watabe 1984; Weiner and Traub 1984; Wheeler et al. 1988a, b; Simkiss and Wilbur 1989), but many biological aspects of biomineralization remain unexplored (Crenshaw 1989; Simkiss and Wilbur 1989). There is, for example, much to be learned about control of the polymorphic type, form, size, and orientation of shell microstructural units, thickness and configuration of microstructural layers (lamina), and the processes of formation of soluble and insoluble organic matrix and of $CaCO_3$ crystals from the extrapallial fluid secreted by the mantle epithelium (Carter 1980a; Wheeler et al. 1981; Wilbur and Bernhardt 1984; Elliott 1985; Crenshaw 1989; Simkiss and Wilbur 1989). It is thought that formation of an organic matrix probably precedes formation of biocrystals. The matrix in all likelihood is involved in the nucleation, orientation, type, and size of biocrystals, and in providing the surface to which soluble matrix attaches and functions as a nucleating surface. It has been suggested that once crystal nuclei are formed, biocrystals grow inorganically. As a stratum (lamina) of biocrystals attains a certain thickness, apparently a layer of insoluble matrix is added and then sclerotized *in situ*, stopping further thickening of the layer. As a consequence, the inner surface of valves thickens incrementally. The mechanism for this stratification, involving mantle secretory cells and the complex of secretions in the extrapallial space, is unknown (Watabe 1984; Weiner and Traub 1984; Addadi and Weiner 1985; Crenshaw 1989; Simkiss and Wilbur 1989; Wheeler and Sikes 1989).

Formation of the multilayered bivalve shell is thus an intricate, continuously repeating process. Suggested steps in this process, with reference to *C. virginica*, are given in abbreviated sequential order in the following four paragraphs (based on Wilbur 1976; Wilbur and Saleuddin 1983; Watabe 1984; Crenshaw 1989; Simkiss and Wilbur 1989) as a guide for descriptions of the ultramorphogenesis of the shell of *C. virginica* outlined in the subsequent paragraphs numbered 1 to 3 (pages 123 to 125):

• Each basic shell microstructural unit generally consists of a polycrystalline aggregate of $CaCO_3$ within a thin insoluble organic sheath (exception, laths of foliated calcite thought to be single crystals, Runnegar 1984; Waller, pers. comm.). Biocrystals develop in the extrapallial space within the soluble matrix (between the inner shell surface and the outer mantle epithelium) from mineral and organic material passed through or originating in the mantle epithelium.

• Each biocrystal aggregate starts as crystal seeds (or spherulites) that grow, orient, and coalesce within the soluble organic matrix. As the biocrystal develops, it presses organic matrix between it and adjacent forming biocrystals, each of a given general size and shape consistent with the microstructural and mineralogical (aragonitic or calcitic) type of the group.

• The periostracal groove releases successive layers of tanned periostracal material upon which the mantle margin secretes and forms layers of prismatic microstructure in an "open," transitory extrapallial space. On the interior surfaces of the valves, strata of foliated microstructures form on the matrix surface of previous layers of microstructures, within the fixed extrapallial space in the region between the adductor muscle and umbones, and within the transient extrapallial space in the region between the adductor muscle and posterior margin of the valves.

• Microstructural layers form consecutively on the inside of the valves atop previous layers of insoluble organic matrix serving to separate individual biocrystals and to bind individual biocrystals and biocrystal layers into the unified cohesive structure of the shell.

The bulk of the valves of *C. virginica*, primarily foliated calcite, is thus formed layer by layer within the extrapallial space on the interior of the valves, whereas prismatic calcitic layers and their pigmentation are developed at mantle margins. Cells of the folial secreting epithelium are nonciliated, range in

shape from cylindrical to cuboidal, and are set on a basal elastic membrane (Galtsoff 1964) (Fig. 86).

Shell formation occurs in two major steps: (a) ion transport, protein synthesis, and secretion by mantle epithelial cells, and (b) physicochemical processes in the extrapallial space in which mineral crystals are nucleated, oriented, and grow in intimate association with the secreted organic matrix (Wilbur and Saleuddin 1983; Crenshaw 1989). Insoluble organic matrix (conchiolin) is the hard, structural framework of the shell, and the soluble matrix coats the insoluble matrix and interacts with mineral crystals (Wheeler et al. 1988b; Simkiss and Wilbur 1989).

1. Extrapallial Fluid. This fluid is a complex mixture of a large number of inorganic and organic substances controlled by the metabolic activity of the outer secretory mantle epithelium. Inorganic ions pass from the mantle hemolymph, diffusing or being actively transported across the epithelium into the extrapallial space; organic compounds are secreted into the space by cells of the epithelium (Crenshaw

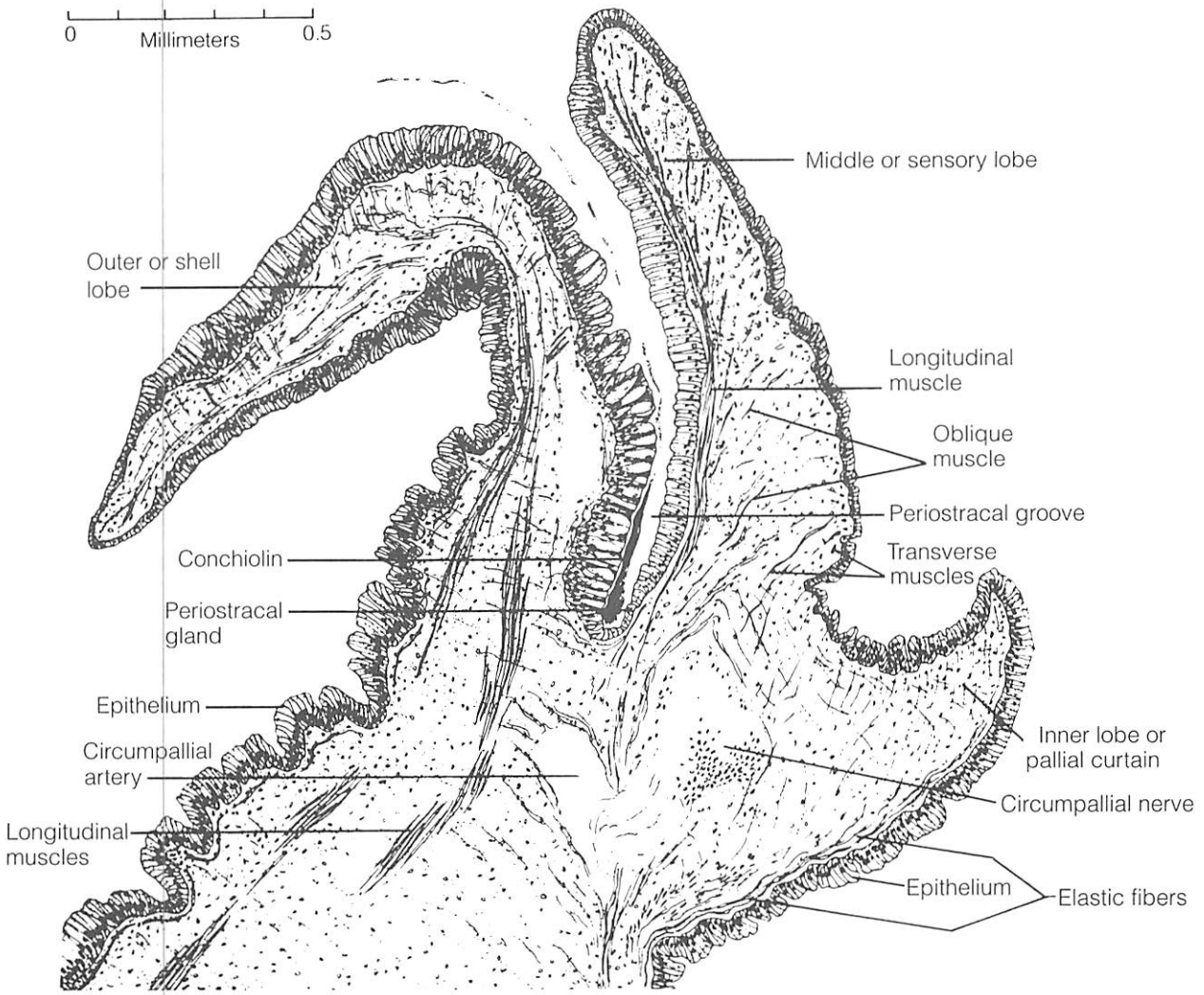


Figure 86. Radial histological section of edge of mantle lobe of adult oyster. Outer fold at left (faces valve which is not shown) is bent as a result of fixation. Section passes between tentacles of inner and middle folds, tentacles thus not shown. HFW = 2 mm. From Galtsoff (1964).

1980; Wilbur and Saleuddin 1983). Wheeler (1975) suggests that the pH of the extrapallial fluid during active shell formation in *C. virginica* is regulated by carbonic anhydrase in the mantle.

In *C. virginica*, the region of the mantle lobes between the adductor muscle and the posterior margin of the valves is frequently withdrawn so that the exposed part of the inner shell surface in this area is flooded with seawater. How the mantle resumes its position relative to the shell microstructural units that were being formed prior to withdrawal of the mantle has not been determined.

Major cations in the extrapallial fluid of bivalves include Na, K, Ca, and Mg, and the major anions HCO_3, Cl and SO_4 (Wilbur and Saleuddin 1983). Crenshaw (1972) determined the following millimolar (mM) concentrations of ions in the extrapallial fluid of *C. virginica*: Na 441, K 9.4, Ca 10.8, Mg 57, total CO_2 5.0, Cl 480, and SO_4 48.3 (see also Wada and Fujinuki 1974 and Watabe 1981). Differences in concentration of inorganic ions in seawater and extrapallial fluid are small (Crenshaw 1972; Wada and Fujinuki 1976). However, in the extrapallial fluid of *C. gigas*, concentrations of the metals Cu, Zn, Fe, and Mn are much higher than in environmental seawater, a ratio that could hold for those of *C. virginica* as well (Carriker et al. 1982).

Organic compounds in extrapallial fluids of molluscs include amino acids, proteins, mucopolysaccharides, organic acids, and probably lipids (Wilbur and Saleuddin 1983; Crenshaw 1989). Organic compounds in the extrapallial fluid of *C. virginica* have not been analyzed. Mantle epithelial cells lining the extrapallial space in *Mytilus edulis* differ along the length of the mantle, showing a progressive reduction in the protein synthetic apparatus and an increase in glycogen away from the mantle margin, possibly indicative of a change in secreting activity of these cells (Bubel 1973). The same could hold true for *C. virginica*, but this has not been reported.

2. Crystal Formation. Biocrystals develop within the extrapallial space from ion clusters of critical size when the fluid is supersaturated with $CaCO_3$. The soluble fraction of the organic matrix, a sulfated, high molecular mass glycoprotein of 170,000 Daltons in *C. virginica*, selectively binds calcium and is proposed to have a primary role in crystal nucleation (Samata et al. 1980; Crenshaw 1989; Wheeler and Sikes 1989). Ion clusters grow into critical nuclei that develop on organic granules or matrix. According to Wada (1980), a specific organic granule appears to provide the active surface that acts as a template for crystal nuclear formation; the active site could be the negative charge on sulfate groups of acid mucopolysaccharide linked to aspartic acid and serine residues in systematic sequences in the glycoprotein. Crystal nuclei develop into small crystals that combine to form a shell "biocrystal." Insoluble organic matrix thus provides the organizational structure for the shell, and acidic macromolecules associated with this organic network nucleate and regulate mineral deposition (Crenshaw 1989; Simkiss and Wilbur 1989). The fundamental process by which the matrix controls crystal growth appears to be through adsorption to growing crystal surfaces (Wheeler et al. 1988a).

The size of a given shell microstructural unit in *C. virginica*, and thus of the thickness of the layer of which it is a part, is undoubtedly determined by such little understood factors as: concentration and distribution of Ca^+ and HCO_3^-, kinds of soluble organic and inorganic substances in the extrapallial fluid, nature of the matrix on which crystals grow, and external environmental conditions (Wilbur 1964, 1976; Crenshaw 1982; Krampitz 1982; Wilbur and Saleuddin 1983; Crenshaw 1989). Little of this has been examined in *C. virginica*, though Wheeler et al. (1981) noted *in vitro* that soluble shell matrix in this species is inhibitory to initiation of $CaCO_3$ crystal formation and growth. Insoluble shell matrix, as well as endocrine activity, could also be involved in microstructural regulation (Doderer 1983; Wilbur and Saleuddin 1983; Simkiss and Wilbur 1989).

Biological control (Carter 1980a) of the chemical composition, structure, form, and orientation of strikingly different types of shell microstructures by specific groups of cells in the shell-secreting epithelium has yet to be investigated experimentally (Wilbur and Saleuddin 1983).

3. Rate of Shell Formation. Shell growth does not occur uniformly over the entire surface of the valves. In *C. virginica*, for example, rate of formation of new shell is maximal along the sector from the umbo to the posterior margin of each valve, and is faster in the left valve than in the right one.

Galtsoff (1964) removed recently formed marginal shell from adult living oysters, and some two months later broke off and weighed the new shell added beyond the break. The quantity of new shell deposited per unit area during this time was 2.2 to 6.2 times greater on the left than on the right valve. He then decalcified the valves of adult oysters and determined that the content of total conchiolin (presumably insoluble organic matrix) in these valves varied from 0.3 to 1.1%. Living oysters with one valve removed and edges of the mantle cut away behind the periostracal groove secreted a new conchiolinal layer over the surface of the exposed mantle lobe in 5 d, but the layer remained uncalcified for over 3 weeks.

Unfed *C. virginica* continue to deposit shell for 4 to 6 weeks in artificial seawater supplemented with $CaCO_3$ (Conger et al. 1978). Galtsoff (1937) found that oysters in Long Island Sound deposit shell throughout the winter.

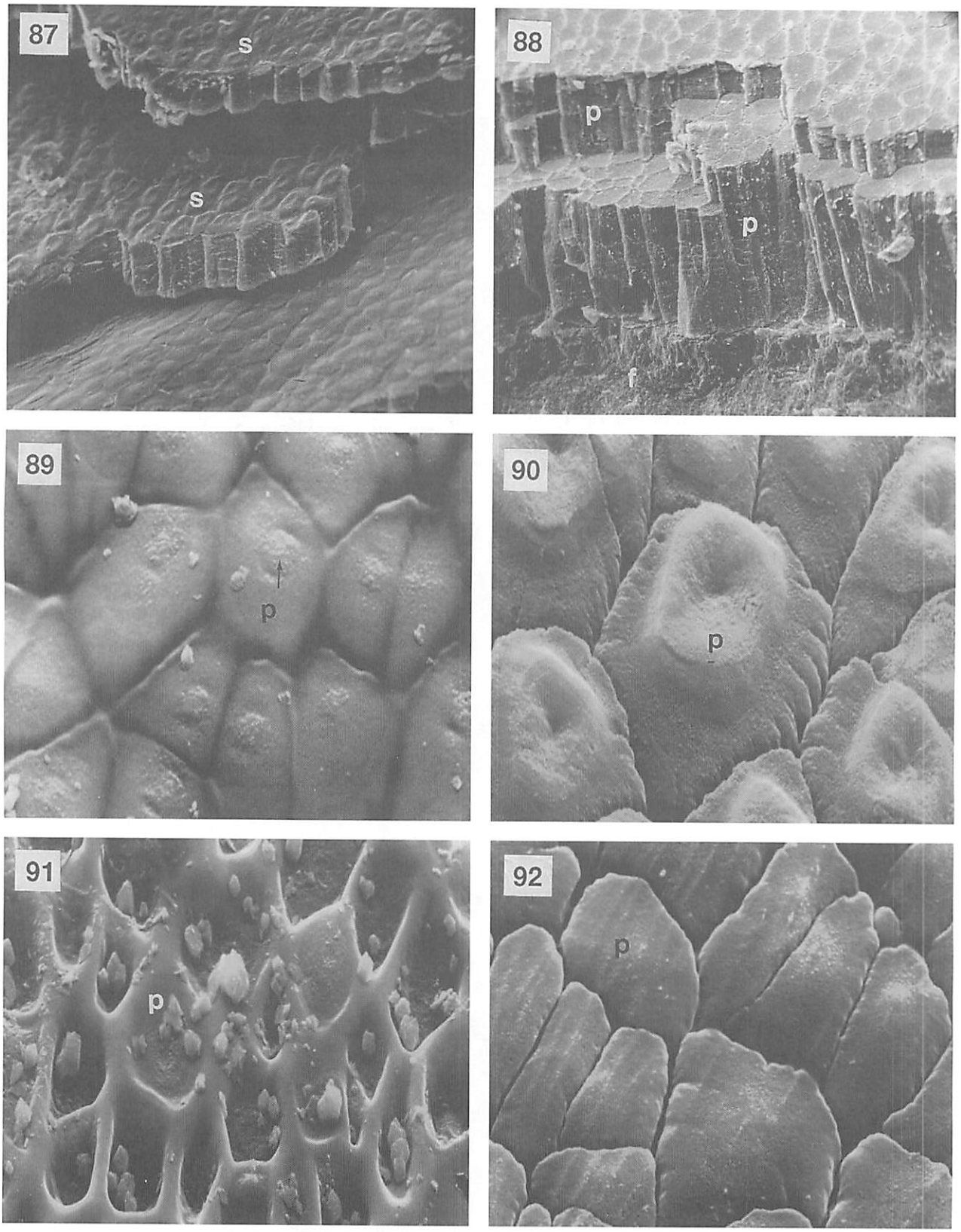
Shell Microstructures

Major variations in shell mineralogy, microstructure, and architecture among bivalve taxa probably are largely biologically controlled, and have evolved with a variety of advantages, including resistance to breakage on impact, localization of breakage, resistance to abrasion and predation, flexibility, low density, and volumetric or energy economy of secretion (Carter 1980a). Three of the types of microstructures (prismatic, foliated, and chalky) in the valves of *C. virginica* are calcitic, and the fourth (myostracal) is aragonitic. Foliated layers have entirely replaced ancestral middle and inner aragonitic layers, resulting in valves that are almost entirely calcitic (exceptions: mineral fibers in the ligament, muscle scars, and larval valves) (Carter 1980a). The four major types of microstructures in the valves of *C. virginica* are described in the following sections (Carriker et al. 1980a; Watabe 1988).

Simple Prismatic Structure: Right Valve. The sculpture of prismatic scales of this valve in *C. virginica* ranges from flutings and ruffles to relatively smooth, gracefully undulating, overlapping terraces. Growth annuli in scales are clearly evident, especially near the edge of each scale (Fig. 79). Successive layering of prismatic strata occurs primarily on the right valve. Scales seem to form in the manner described by Nakahara and Bevelander (1971) for pearl oysters; that is, after establishment of a single layer of short prisms (Fig. 87), the mantle margin retracts, then reextends beyond the first scale to form a new scale under and separated from the first. The reason for formation of scales of a single layer of prisms, in contrast to thick prismatic shell composed of several layers of prisms (Fig. 88) deeper in the valves, is unknown.

The prismatic structure of the right valve consists of generally discrete, parallel, columnar, closely packed units, or prisms, of variable size, polygonal in cross-section, and delineated from each other by relatively thick, nonmineralized, generally simple, conchiolinal walls or sheaths (Stenzel 1971; Carriker et al. 1980a).

1. Exterior Surface. The central part of the exterior surface of most prisms is elevated in the form of a shallow boss (Fig. 89). The shape of the boss varies from that of a relatively smooth dome to an elevation with a conspicuous disk in the center (Fig. 89). The disk can be raised (Fig. 89) or depressed (Fig. 90). When the surface of prisms is partially demineralized, organic prismatic walls become clearly visible (Fig. 91). Some prisms assume an overlapping tongue-like form (Fig. 92), with shallow striae running parallel to the long axis of each prism. The central disk of each prism can be relatively smooth (Fig. 90), conspicuously punctate (Fig. 89), or characterized by a series of concentric circles. At higher magnifications this pattern of circles appears distinctly granular. Taylor et al. (1969) observed that the central boss appears to



Figures 87-92. (87) Prisms, fracture of two scales [s], right valve. Surface of valve brushed clean. HFW = 215 µm. (88) Prisms [p], multilayered fracture, over foliated structure [f], right valve. HFW = 210 µm. (89) Prisms [p], exterior surface, each prism with punctate central disk [arrow], right valve. Brushed clean. HFW = 40 µm. (90) Single prism in center [p],

represent the original spherulite that developed on the periostracal substratum (Fig. 90). It is also possible that some of the punctate marks in the disk are a part of the periostracum (Fig. 89), and that treatment with bleach partially removes them with the periostracum (Fig. 90).

Organic walls separating prisms are often raised above the general surface of the valve as crests that divide most, but not all, of the prisms (Fig. 89). Tsujii et al. (1958) explained that this is the form to be expected if approaching crystal edges squeeze the matrix; yet this is not always the case. When the surface of a valve is treated with bleach to dissolve the conchiolin, deep, distinct grooves are exposed in place of the organic sheaths and interprismatic matrix (Fig. 90). The outer surface of the mineral core of prisms often displays vertical furrows, some so pronounced as to give the appearance of crenulations (Fig. 90). These could have formed as a result of lateral growth of the mineral core against the organic matrix. Grooves probably serve as an anchorage for the matrix wall, contributing to the strength of the structure.

2. Interior Surface. Mineral crystals form in the sheet of periostracal material deposited by the mantle margin along the edge of the valve from minute, randomly distributed, crystalline bodies. These crystallites coalesce, increase in size, and make contact with other crystals, forming characteristic polygonal outlines in the new stratum of prisms. The surface area of individual prisms increases away from the margin of the shell (Fig. 93). Indentations occur in the sides of some prisms, some penetrating deeply in graceful curves into the prisms (Fig. 94). Increase in size away from the growing shell margin could reflect geometric selection (Taylor et al. 1969), a case in which prisms grow at irregular rates, and slow growing ones are crowded out for lack of space (Fig. 93). It is more likely, however, that as the prismatic layer thickens, fusion occurs between prisms. Conchiolin spurs extending part way across prisms (Fig. 94) are consistent with the idea of coalescence of adjacent prisms (see also Palmer and Carriker [1979] and Tsujii et al. [1958]).

New layers of prisms on the interior surface of valves are sometimes incomplete (Fig. 95), suggesting that lateral growth of prisms could take place by sideways extension of existing prisms. Additional layers of prisms can start on these surfaces with minute crystallites that coalesce and form polygonal figures, much as takes place at the mantle margin. The proportion of sheath thickness to diameter of mineral core among prisms decreases as they grow in size (Fig. 93). Interprismatic walls are generally less conspicuous on the interior than on the exterior surface of prismatic structure. The surface of prisms adjacent to the mantle is finely rugose with roughly parallel minute grooves (see also Tsujii et al. 1958), a feature emphasized by treatment with bleach (Fig. 96).

3. Fracture Surfaces. The free margin of prismatic scales consists of a single layer of short prisms (Fig. 87) whose interprismatic sheaths fracture in a scale-like design (Fig. 97). Away from the posterior margin of the valve, beneath the scales, prismatic structure becomes tightly multilayered and prisms are many times longer than those at the margin (Fig. 88). Adjacent horizontal strata of prisms are joined by a sheet of conchiolin which fractures cleanly at interfaces (Fig. 88).

Fractures of prismatic scales viewed from the interior surface of the valve emphasize the varied polygonal shape of prisms (Fig. 98). Individual prisms are composed of tranverse bands clearly visible after the organic sheath has been chemically removed, and probably represent growth increments (Fig. 98: also see Taylor et al. 1969).

Thick prismatic layers are characterized by long columnar-shaped prisms (Fig. 88). In scales, the long axis of prisms tends to be inclined toward the shell margin in a direction from exterior to interior of the valve (Stenzel 1971).

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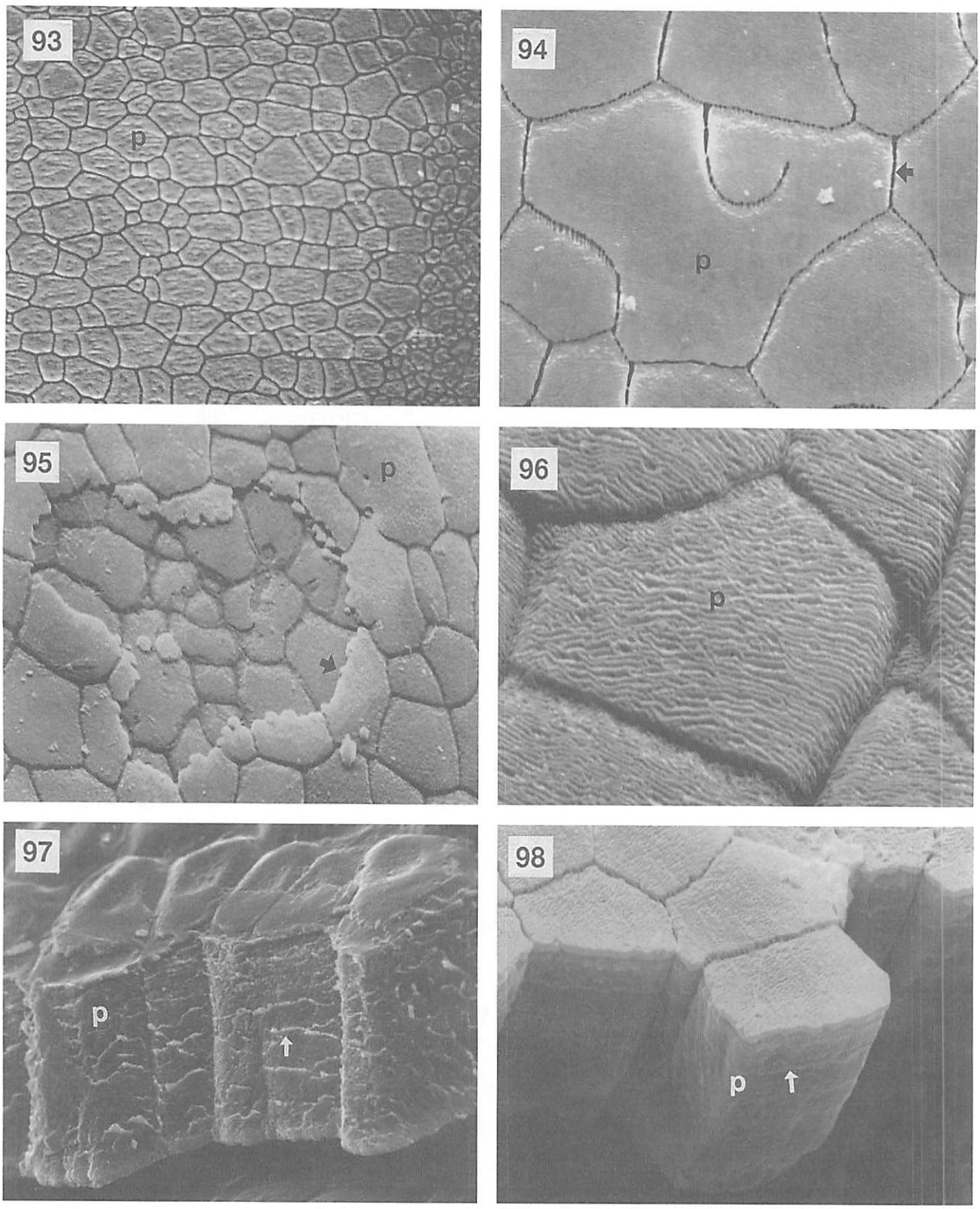
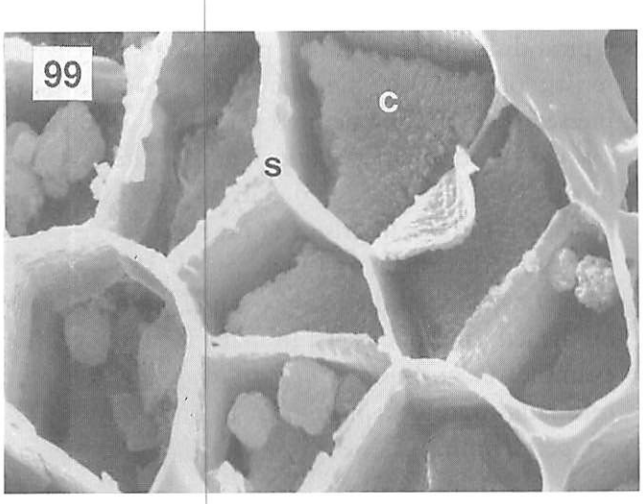
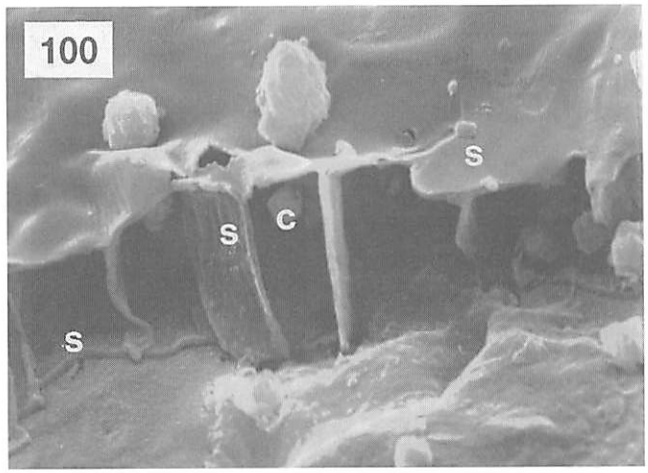
exterior surface, right valve. 100% bleach 1 min. HFW = 45 μm. (91) Depressed prisms [p], exterior surface, right valve. Brushed clean, normal microbial decomposition. HFW = 80 μm. (92) Prisms [p], exterior surface, overlapping, tongue-shaped. Right valve. 100% bleach 1 min. HFW = 40 μm.



Figures 93-98. (93) Prisms [p], interior surface of right valve, shell margin to left. 5% bleach 1 min. HFW = 160 µm. (94) Prisms [p], interior surface of right valve, prism junctures [arrow]. 100% bleach 1 min. HFW = 40 µm. (95) Prisms [p], interior surface of right valve, incompletely formed layer of prisms [arrow]. Interprismatic organic matrix present. 20% bleach 10 sec. HFW = 80 µm. (96) Prisms [p], finely rugose interior surface of right valve. 20% bleach 10 sec. HFW = 18 µm.





Figures 99-100. (99) Prismatic organic sheaths [s], exterior surface of left valve. Mineral core [c]. Etched in standing seawater, brushed clean. HFW = 45 µm. (100) Organic sheaths [s] of prisms from which mineral cores [c] have been dissolved in standing seawater, fracture of exterior prismatic scale, left valve. Brushed clean. HFW = 45 µm.

4. Organic Sheaths. The polygonal shape of organic sheaths mirrors that of the enclosed mineral core (Fig. 99), and an organic layer bounds both their exterior and interior ends (Fig. 100).

5. Dimensions. Crystallites at the forming margin of prismatic shell range in diameter from 0.01 to 8 µm; farther in they average 9.1 µm, and near the advancing edge of foliated structure on the interior, 44.6 µm (Tsujii et al. 1958). Thickness of organic sheaths ranges from 0.16 to 0.75 µm, and averages 0.5 µm (Tsujii et al. 1958; Travis and Gonsalves 1969). In rapidly growing oysters the maximal surface dimension of prisms in a transect from near the edge of the valve to the foliated structure on the anterior surface of the right valve ranges between 14 and 23 µm (Palmer and Carriker 1979). In fracture sections, length of prisms can range from 32 to 84 µm (Fig. 88, also see Taylor et al. 1969; Travis and Gonsalves 1969; Palmer and Carriker 1979).

Within the mineral core of each calcitic prism of the shell of *C. virginica*, according to Travis and Gonsalves (1969), there is enclosed an intraprismatic organic matrix organized into small elongated compartments with walls about 65 nm thick. These walls are evident in ultrathin sections (Fig. 101). Each compartment encloses a mineral crystallite. The length of each compartment is about 600 nm and parallels the long axis of the prism; the width of each compartment is about 100 nm. The surface of the partially dissolved mineral core in Fig. 96 suggests the protrusion of crystallites from a surface in which the intraprismatic organic matrix has also been partially dissolved. Intraprismatic organic matrix has also been described in shell prisms of five species of pteriid bivalves (Nakahara et al. 1980) and in *C. gigas* (Suzuki and Uozumi 1981) (also see Simkiss and Wilbur 1989). However, Towe and Thompson (1972), studying the calcitic prismatic and aragonitic nacreous layers of *Mytilus californianus* and the aragonitic prismatic layer of *Mercenaria mercenaria*, denied the existence of a coherent intracrystalline matrix. It is clear that the intracrystalline structure of microstructural units requires further investigation (Watabe 1988).

(97) Prisms [p], fracture of single scale showing interprismatic scale-like conchiolin [arrow]. Right valve, brushed clean. HFW = 60 µm. (98) Few prisms [p] of prismatic scale of right valve, interior surface, fractured after treatment with 100% bleach 1 min, showing stratification [arrow] of internal structure of mineral core. HFW = 45 µm.

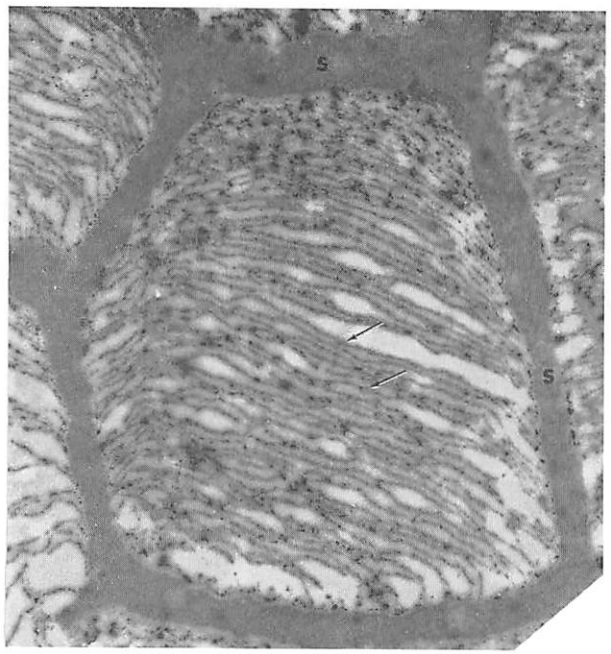


Figure 101. Transmission electron micrograph of cross-section of prism of oyster shell demineralized on the grid showing cross walls of intraprismatic organic matrix [arrow], and prismatic organic sheath[s]. HFW = 8 μm. From Travis and Gonsalves (1969).

Simple Prismatic Structure: Left Valve. Prisms of the outer calcified layer of the left valve possess the same characteristic honeycomb-like sculpture as those on the right valve. However, interprismatic conchiolinal walls and prismatic strata are thinner, and prisms are shorter in the left than in the right valve. Prismatic scales are small or absent altogether.

1. Exterior and Fracture Surfaces. In contrast to the generally bossed exterior of prisms of the right valve, that of the left valve tends to be concave and flat (Fig. 102), or slightly domed (Fig. 103). Ornamentation in the center of each prism varies from the typical disk with concentric, punctate marks characteristic of prisms of the right valve, to slightly depressed (Fig. 102) or elevated (Fig. 103) granular areas. Corrugations are present along the exterior edges of prisms (Figs. 102, 103), but are not as deep as analogous furrows in prisms of the right valve.

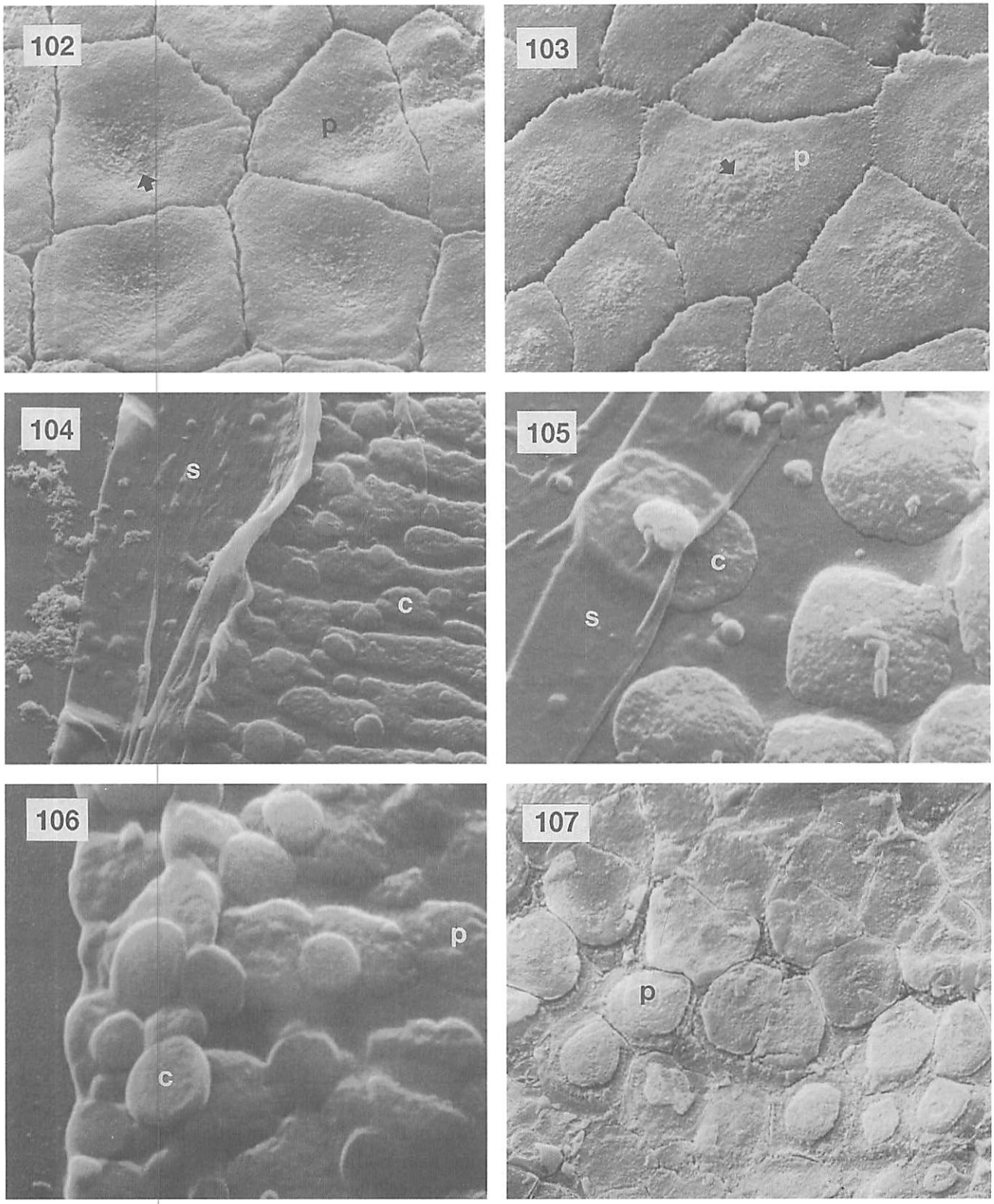
Earlier investigators (Taylor et al. 1969, for example) overlooked the inconspicuous prismatic layer on the left valve of oysters. In many fossil oysters (for example, *Flemingostrea subspatulata*), however, the prismatic stratum is prominent in both valves (Carter, pers. comm.).

Prism Formation

The process of prism formation (compare with adhesion of spat to substratum after setting, Fig. 38) was observed by Galtsoff (1964). He inserted small glass cover slips between the edge of the mantle and the valve, and removed them at regular intervals for examination under the light microscope. In the earliest stages of mineralization, minute "granules of calcium salts" became visible in polarized light. Within 24 to 48 h, typical hexagonal crystals of calcite appeared and gradually increased in size; they presented a "picture of great brilliance and beauty," but did not yet show orientation relative to the growth axis of the shell. Within the next 48 h, the crystals increased further in size and became arranged in a distinct pattern characteristic of prismatic shell. The form of individual prisms varied widely. The thin-walled capsule of conchiolin surrounding each prism became visible as the mineral core was dissolved in weak hydrochloric acid (Galtsoff 1964).

Formation of prismatic structure at the mantle-shell margin can also be examined with the scanning electron microscope using small spat of *C. virginica* about 2 mm high growing on a glass surface (Carriker et al. 1980a). The right valve and flesh are removed, leaving the left valve attached to the glass sur-

Figures 102-107 (opposite page). (102) Prisms [p], exterior surface, central concave granular structure [arrow], left valve. 100% bleach 1 min. HFW = 25 μm. (103) Prisms [p], exterior surface, elevated central granular structure [arrow], left valve. 100% bleach 1 min. HFW = 25 μm. (104) Interior margin of forming left valve of spat (3.0 mm high) set on glass. Partially wrinkled organic sheet [s] at left and developing crystallites [c] in sheet at right. No cleaning treatment. HFW = 25 μm. (105) Interior margin of forming left valve of spat (1.7 mm high) set on glass, edge of organic sheet [s] folded over



a large crystallite [c] with a newly forming crystallite atop it. No cleaning treatment. HFW = 12 µm. (106) Interior margin of forming left valve of spat (3.6 mm high) set on glass. Prisms [p] well formed in membrane to the edge, margin folded inward. Crystallite [c]. No cleaning treatment. HFW = 12 µm. (107) Margin of left valve, exterior surface, thin wafer-like prisms [p] in incomplete prismatic layer. 100% bleach 1 min. HFW = 75 µm.

face. An extremely thin sheet of organic matrix is laid down by the mantle edge over (that is, inside) and beyond the previously formed organic layer and its developing prisms (Fig. 104), and this remains attached to the glass surface. Thus the newly extended sheet retains its identity during preparation for microscopy. Minute crystallites embedded within the marginal organic sheet take form in the zone extending beyond the previously calcified layer (Figs. 104, 105), as well as over the calcified layer (Fig. 105). Shape of the developing prisms varies roughly from rounded to oval. As prisms continue to grow laterally, apparently squeezing organic matrix between them, they assume their definitive form, varying from elongated to polygonal with a typical central disk (Fig. 106). The extreme thinness of prismatic strata in the left valve is illustrated in Fig. 107, in which an external layer of forming prisms was left incomplete. Partial dissolution of the mineral content of prisms in this layer illustrates the internal configuration of the matrix sheaths (Fig. 108) with occasional projections (spurs) of each sheath into the mineral core (see also Fig. 94).

The organic layer between mineral cores of prisms (Fig. 108) probably combines both the external periostracal layer and inter- and intracrystalline matrix. In older oysters beyond the recently settled stage, whose valve margins project freely off the substratum into the seawater, increments of newly formed organic sheets must be sufficiently firm to retain their form on the edge of the outer mantle fold long enough for mineralization and solidification to take place. Both left and right mantle margins extend beyond valve edges during shell formation. However, there is little likelihood that the two marginal sheets ever adhere to each other because during the process of shell formation the valves are open (apart) and the oyster is pumping seawater.

Mantle lobes are muscularly active and highly contractile. They can extend beyond the edge of the valves, withdraw some distance inside the shell, roll into a tube, or form ridges that serve as temporary channels for discarding mucus and foreign particles. These movements can involve the entire surface of the mantle, or only a small part of it, depending upon the intensity of stimulation received by tentacles of the middle and inner folds. In a closed oyster, mantle edges are withdrawn to about midway between the distal margin of the gills and edges of valves (also see Eble, Chapter 2).

The pronounced muscular activity of the mantle adds a spatial complication to interpretation of shell microstructural formation in *C. virginica*. If mantle lobes were to remain in a fixed position relative to the inner surface of the valves, intimate micro-association between mantle epithelial cells and developing shell microstructural units could be hypothesized; however, because this is not the case, some alternatives are possible: (1) mantle lobes return to a precise position relative to the growing microstructural units of each valve after each excursion or change in form, and accretion of microstructural units is thus under close cellular control; (2) there is no close spatial association between cells and microstructural units: in this case after nucleation of mineral crystallites and formation of the first layer of microstructures, shell formation could proceed simply by addition of chemical substances from the extrapallial fluid to the forming microstructural units, much as inorganic crystals grow, the imprint of forming microstructural units providing the control; or (3) mantle cells, chemically stimulated by forming microstructural units, could continue to secrete the type of microstructural unit over which they happen to lie at the time of re-extension of the mantle lobes.

The case of prismatic scales of the right valve, in which the inner end of the long axis of columns of prisms (Fig. 88) is inclined toward the valve margin, could be explained as follows. Proliferation of shell-secreting epithelial cells in the mantle as new shell is formed (Stasek and McWilliams 1973) probably results in an antimarginal advance (i.e., toward the edge of the shell) of the mantle margin. Thus according to hypothesis (1) in the previous paragraph, secretory cells associated spatially with prisms they secrete would be moved radially with them, resulting in the observed bending of the long axis of forming prisms. Whatever the explanation, the spatial association of shell-secreting epithelial cells and shell microstructural units remains unresolved.

Change from prismatic to foliated structure occurs gradually (Fig. 109); precursors of foliated laths form over prisms. The presence of a layer of organic matrix on the surface of prisms is thought to be necessary for initiation of the new layer of microstructures (Watabe 1984). What initiates the change in formation from one type of microstructure to another is still a perplexing question.

Foliated Structure, Both Valves. The inner massive part of each valve of *C. virginica* consists of calcitic foliated shell, a primary feature of the Ostreoida, possibly derived from simple prismatic calcite such as is found in scallops (Waller 1978), but perhaps from nacre in oysters (Carter 1980a, 1989b). Of all the regions of oyster shell, this type of structure has received the most attention from micromorphologists. A detailed description was given by Taylor et al. (1969), with additional data in Tsujii et al. (1958), Watabe et al. (1958), Watabe and Wilbur (1961), Watabe (1965), Grégoire (1972), Carriker et al. (1980a), Runnegar (1984), and Watabe (1988).

On much of the inner surface of the valves of *C. virginica*, especially on the ventral side, single folia are inclined slightly (less than 10°) to the inner surface of the valve (Runnegar 1984) and laths successively and regularly overlap in parallel in a design resembling a tiled roof. Thin walls of intercrystalline conchiolin delineate laths from each other.

1. Interior (or Mantle-facing) Surface. Primarily in the region of the valves between the posterior margin and the adductor muscle, laths are oriented parallel to each other, and the growing front of each faces the posterior margin of the valves (Fig. 110). In the area between the adductor muscle and the umbones, different clusters of laths are generally oriented in various directions relative to each other; in some spots, for example, in sharply angular designs (Fig. 111), in others in exquisite rosette patterns (Fig. 112), and in still others in a disorderly array of branching, bending laths (Fig. 113). Both width and exposed length of laths vary greatly among adjacent laths, between folia, between different areas of shell, and between different individual oysters. In some places, laths are closely joined to their neighbors, whereas in others, gaps of varying width can occur between them. The surface of some laths appears smooth; in others, gently dimpled. Some laths bear slight ridges that run parallel to the long axis; and in others, the former positions of crystal fronts (possibly growth halts) are visible (Fig. 112).

Exposed rows of folia near the margin of the valves are generally straight, whereas toward the umbones, adjacent laths are increasingly angled to each other. The growing front of folia can be sharply truncated, or bevelled. In some cases, the front can be in close contact with underlying folia, whereas in others it can be elevated to varying degrees, leaving a clean space between folia.

Intercrystalline conchiolin is not conspicuous in micrographs of most folia, but can be inferred from an occasional fracture section (Fig. 114). It is less abundant than in prismatic structure, and in *C. virginica* consists of an oriented network with mesh openings of 6 to 8 nm (Watabe and Wilbur 1961).

2. Fracture Surfaces. Foliated structure does not fracture as cleanly at intercrystalline conchiolinal boundaries as does prismatic structure, but fractures do provide valuable information on the form of laths. The layering of folia is illustrated in Figs. 115 and 116.

The types of foliated microstructures facing the mantle described in the previous section are illustrated in fractures in Figs. 115 to 117. Laths and folia are generally tightly joined to each other. Surface texture ranges from smooth, to slightly dimpled, to parallel lined, to chevron marked (Fig. 116). Chevron marks, representing growth halts of crystal fronts, point in the direction of the shell margin (see Fig. 110). Thickness of most laths is relatively uniform (Fig. 116). Extreme disorganization of laths is not uncommon, nor is the meeting of laths at diverging angles (Fig. 117). Carter (1980b) refers to these as complex cross-foliated structure. Only rarely will a fracture clearly expose intercrystalline organic matrix, outlining boundaries of adjacent laths by distinct conchiolinal ridges (Fig. 114). In fractures treated with bleach to remove organic matrix and some of the mineral, individual laths stand out clearly (Fig. 118); what appear to be consecutive series of thin

chevron-shaped increments on the long axis of each lath are evident.

3. Dimensions. Observed apparent interfacial angles of the growing edges of foliated calcitic laths are highly variable; in *C. virginica*, alpha averages about 103°, and beta and gamma are each about 131° (Watabe et al. 1958; Runnegar 1984).

On the average, thickness of each lath is about 0.2 μm. The length is difficult to determine because of indefinite orientation of long axes of laths relative to fracture surfaces; the length of the longest lath observed free is 9 μm (Watabe and Wilbur 1961). A thin layer of conchiolin is present about every 30 layers of folia (Watabe and Wilbur 1961; Carriker et al. 1980a), whereas a separate layer of matrix is absent between individual folia (Watabe 1965).

According to Watabe (1965) each lath is composed of intralath subunits 10 to 40 nm wide and 15 to 20 nm long arranged in parallel across the width of the lath. This substructure appears to be reflected in the pattern of dissolution of laths treated with bleach (Fig. 118). Watabe (1965) found that a layer of matrix about 12 to 20 nm thick separates individual laths, and an intracrystalline matrix surrounds each intralath subunit. The latter substructure is reminiscent of the subunits in prisms (see p. 129; also Travis and Gonsalves [1969]), but recall the objections of Towe and Thompson (1972). Waller (pers. comm.) cautions that interpretations of intralath microstructure should be guarded because laths are thought to be single crystals: in the typical physical growth of crystals, halts and starts are commonplace; etching, working on the atomic crystal structure, could be accentuating the halts and starts within the laths.

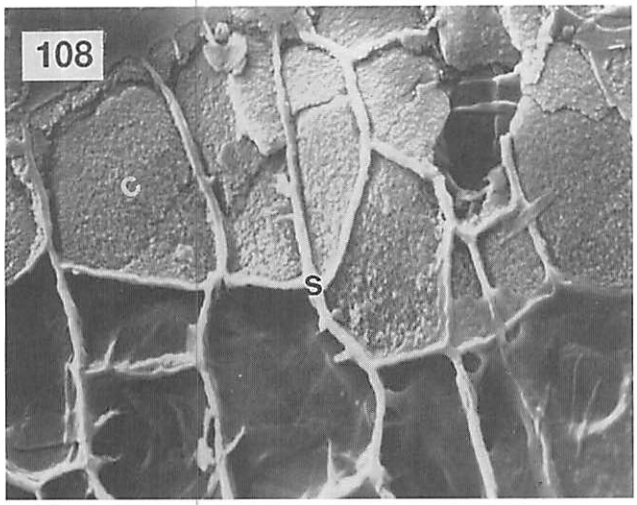
Striking major differences between prismatic and foliated shell are the conspicuously greater quantity of organic matter in prismatic than in foliated microstructures, the larger size of prisms than laths, and orientational difference of these units, prisms being nearly perpendicular and laths nearly parallel to the growing surface.

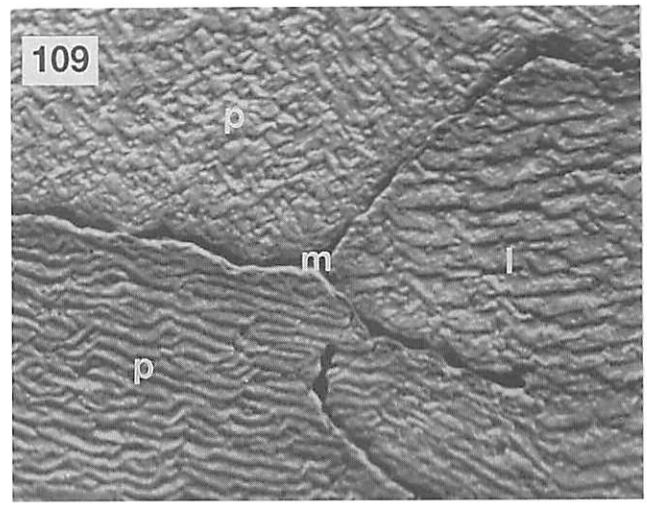
4. Lath Formation. All shell laid down initially by *C. virginica* at margins of both valves is prismatic. Foliated structure, deposited next inside the valves, overlies the prismatic, and therefore originates on the organic matrix of prismatic structure. Foliated growth is initiated in a thin transitional layer of minute aggregating crystallites (Fig. 111, and Watabe and Wilbur 1961), which merge to form a thin sheet of granular blocks each about 0.8 μm in width. These develop further (coalesce?) into folia of laths (or chalky shell, as the case may be). That a layer of prismatic shell is a prerequisite for development of foliated shell is not the case in all bivalves (Pectinacea, for example), is indicated by the fact that prismatic shell has been lost in some of them (Waller 1976). The relationship between organic membranes and crystallites in microstructural transition zones has not been elucidated.

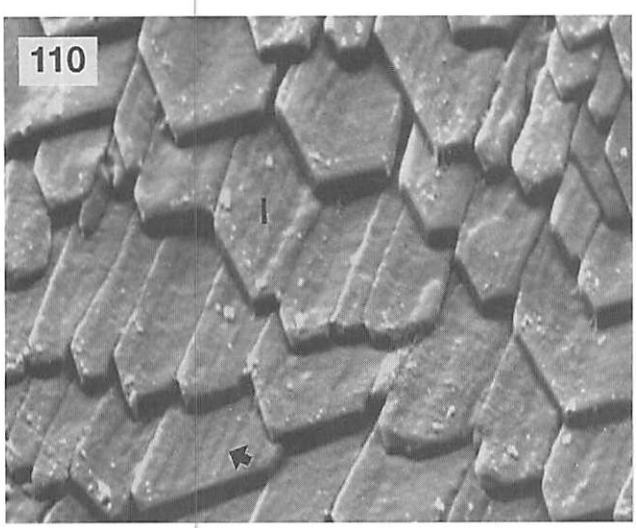
Krampitz et al. (1983) concluded that the organic matrix could have a dominant role in bio-mineralization. Whether nucleating crystallites form within the matrix, coalesce as they grow, and push the surrounding matrix against neighboring growing biocrystals to form incipient laths in *C. virginica* is probably questionable, because random development of laths would more likely yield round or polygonal, rather than rectangular, shapes.

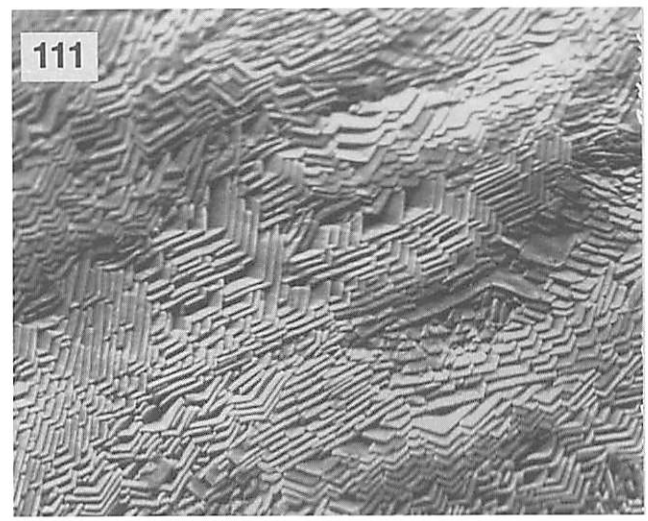
Once formed, individual laths continue to grow at free ends adjacent to the secreting mantle epithelium. Direction of growth generally occurs at a slight angle to the mantle surface and tightly over the underlying laths, but can range to nearly right angles to the surface with increasing space between laths as, for example, the microstructure of chalky shell is approached. Because formation of laths is primarily by incorporation of mineral material, possibly with no, or only small amounts of intracrystalline matrix and small quantities of interlath matrix, foliated shell would

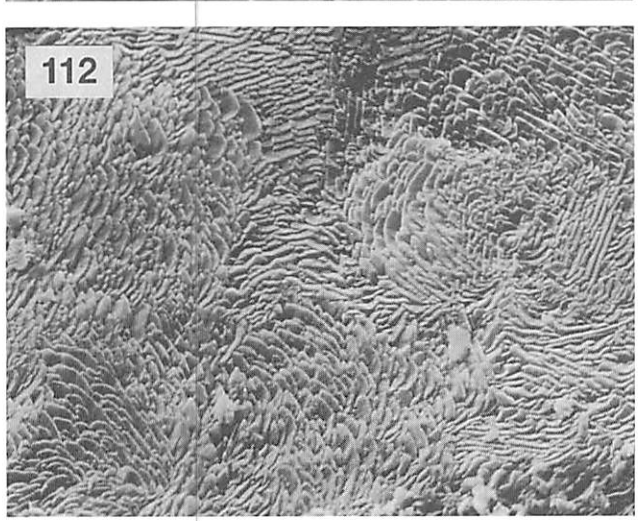
Figures 108-113 (opposite page). (108) Prism sheaths [s] in thin prismatic layer of left valve, mineral cores [c] dissolved in standing seawater. Brushed clean. HFW = 40 μm. (109) Prisms [p] overlaid by initial layer of laths [l], interior surface, near valve margin; boundaries of prisms outlined by dark matrix [m]. Brushed clean. HFW = 15 μm. (110) Laths [l] with ridges

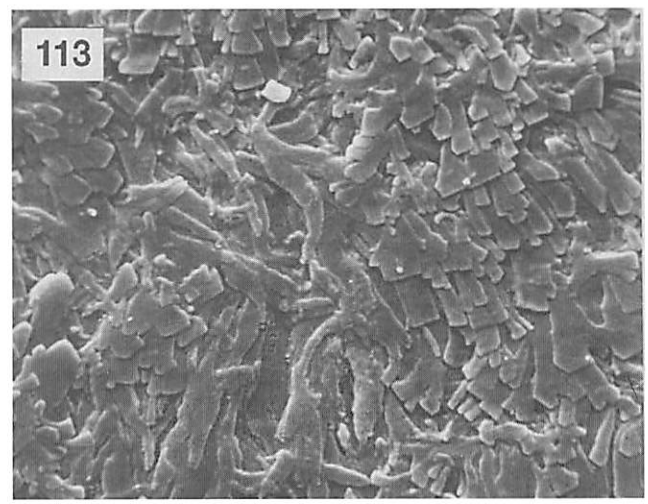




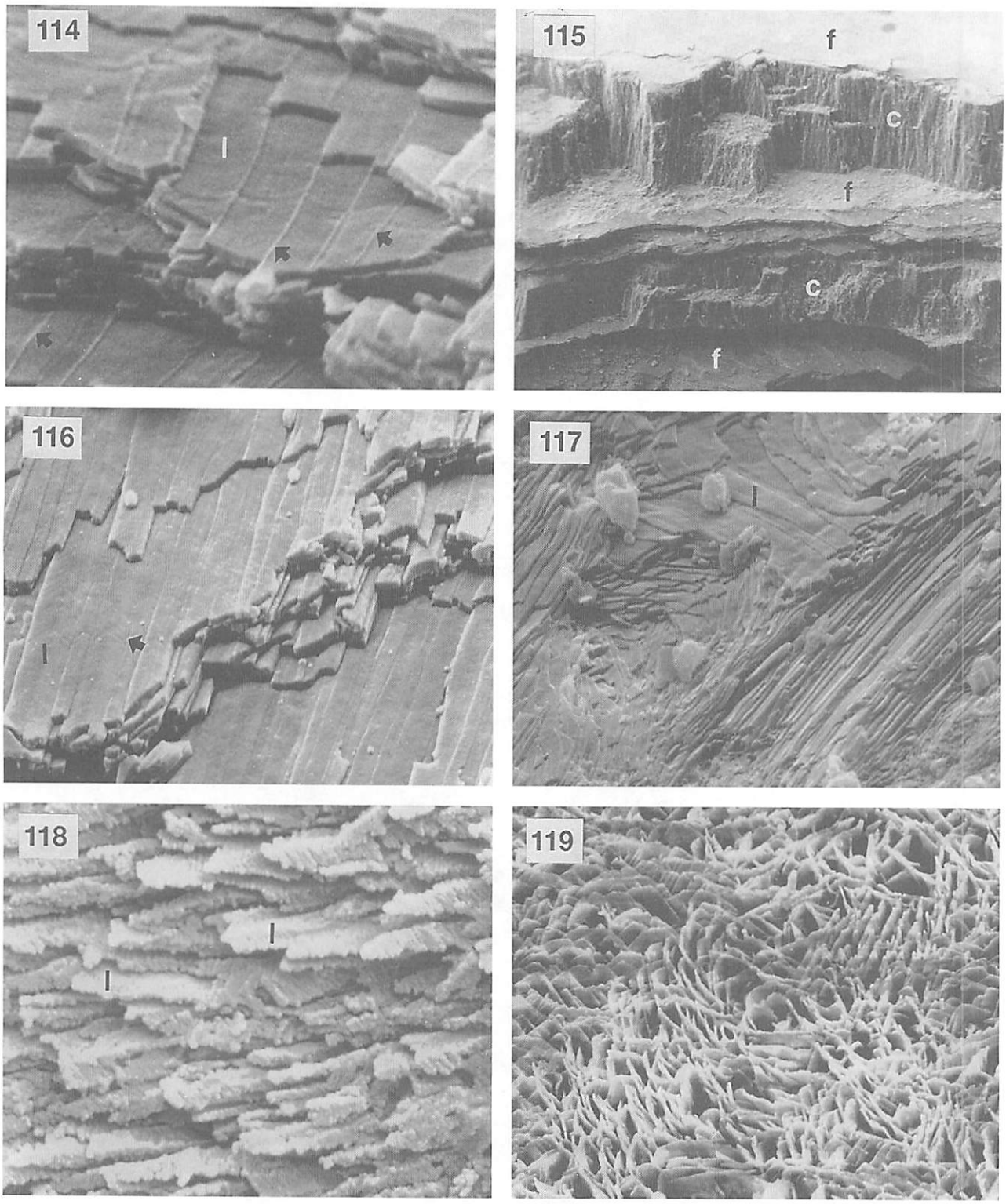








[arrow], interior surface of valve between adductor muscle and valve margin. 5% bleach 15 sec. HFW = 10 µm. (111) Laths, sharply angular pattern, interior surface of valve between adductor muscle and umbo. Brushed clean. HFW = 45 µm. (112) Laths, rosette pattern, interior surface of valve between adductor muscle and umbo. 100% bleach 1 min. HFW = 40 µm. (113) Laths, branching, bending pattern, interior surface of valve between adductor muscle and umbo. 100% bleach 5 sec. HFW = 55 µm.



Figures 114-119. (114) Laths [l], intercrystalline organic matrix [arrow] present where crystals removed by fracturing. No cleaning treatment. HFW = 15 µm. (115) Stratification of foliated and chalky structure. Interior surface of valve at top. Regularly foliated structure [f], chalky structure [c]. No cleaning treatment. HFW = 4 mm. (116) Laths [l], chevron marked [arrow], fracture. No cleaning treatment. HFW = 20 µm. (117). Laths [l], heterogeneously oriented, fracture. No

appear to be organically economical (Watabe 1965; Taylor et al. 1969).

The precise relationship of matrix to minerals at the growing edge of each lath has not been explored. The chevron-shaped increments visible on ends of some laths (Figs. 110, 116) suggest, as do fractures of laths that have been etched (Fig. 118), that development could be by deposition of discrete layers, the mineral possibly packaged between thin sheets of intracrystalline matrix (Watabe 1965; Taylor et al. 1969). This idea is contrary to Waller's suggestion (pers. comm.) that lath patterns reveal the crystallographic structure of laths and are reminiscent of patterns present in purely physical systems (see also Runnegar [1984] and Taylor et al. [1969]).

The following several hypotheses have been offered to explain the direction of growth of laths relative to the interior surface of the valves: posterior direction of growth of the mantle, fibrillar arrangement of the matrix, direction of currents of extrapallial fluids (Grégoire 1972), local concentration differences of $CaCO_3$, adsorption of impurities, and presence of neighboring laths (Watabe et al. 1958; Watabe and Wilbur 1961). Galtsoff's (1964) observations of a living oyster suggest that back and forth movements of mantle lobes could also influence axial direction, whereas relative absence of mantle movements could allow other factors free play (perhaps some of those listed by Watabe et al. 1958; Watabe and Wilbur 1961; Grégoire 1972) resulting in disordered orientation typical of laths in the region between adductor muscle and umbones. The fact that bivalve shell develops tensile stresses in its outer layers and compressive stresses in inner layers between the adductor muscle and the hinge (Wainwright 1969) may also cause the variable orientation of laths.

Chalky Structure, Both Valves. Islands of relatively soft, porous, chalky white, calcitic shell occur randomly on the mantle-facing surface and within the valves of *C. virginica*. This type of shell, at least in limited quantities, appears to be a normal component of the valves. It generally becomes buried as new foliated shell is deposited over the surface of the valves and valves thicken. Deposits of chalky shell are more common in the left than in the right valve, and increase in number and size in older oysters (Korringa 1951a; Galtsoff 1964; Stenzel 1971; Carriker et al. 1980a). When dried, pieces of chalky shell will actually float (specific gravity of about 0.5). Because pure calcite has a specific gravity of about 2.8, density of dried shell gives an accurate measure of the extent of chalky deposits in a valve. In general, high densities (above 2.3) indicate solid, strong valves, whereas low densities (below 2.0) indicate weak, friable chalky shells (Palmer and Carriker 1979).

Chalky shell consists of smooth, blade-shaped microstructures (blades) of various sizes, oriented perpendicular to the inner surface of the valves. Leaflike structures (leaflets) branch at several angles from the central blades, enclosing spaces among the blades and leaflets, and giving chalky shell its characteristic "spongy" appearance (Margolis and Carver 1974). Weiner and Hood (1975) reported 0.91% soluble and insoluble nondialyzable organic matter in chalky layers and a lesser quantity, 0.58%, in foliated regions. In this regard, surfaces of fractured sections of chalky shell treated with 100% domestic bleach for 30 sec, or with 15% hydrogen peroxide for 60 min, exhibit no obvious dissolutional effects when magnified between 2,000 and 5,000 times, whereas when exposed to 0.1N HCl for 30 sec, these surfaces display clear differential dissolution in a nodular pattern (pers. obs.). The microstructural relationship of organic matrix to mineral microstructure of chalky shell has not been determined, and is not evident in electron micrographs.

1. Interior (Mantle-Facing) Surface. The surface of well developed chalky structure touching the mantle epithelium is typically spongy in appearance, the free ends of blades being the most prominent fea-

cleaning treatment. HFW = 50 μm. (118) Laths [l], polished surface treated with 100% bleach 30 sec. HFW = 10 μm. (119) Chalky structure, floral pattern (surface against mantle). 5% bleach 1 min. HFW = 80 μm.

tures (Fig. 119). Blades occur in parallel rows, in floral designs, or at various angles to each other. Pore spaces among the blades and leaflets are distinct and extend deep among the microstructures (Fig. 120).

The transitional zone between foliated and chalky structure is micromorphologically variable. Where chalky shell develops over the ends of laths, space among the laths increases as new shell is deposited (Fig. 121). As foliated structure forms over chalky structure, ends of blades become covered with a granular shell (Fig. 122) which develops into foliated laths. Formation of chalky shell on the sides of foliated laths or over conchiolinal patches likewise is preceded by deposition of finely granular particles.

2. Fracture Surfaces. The often parallel arrangement of blades of chalky shell is most evident in large lenses fractured parallel to the long axis of blades (Fig. 123). Some leaflets contact opposing blades forming an irregular honeycomb pattern (Fig. 124), whereas others project only part way between blades (Fig. 125). The angle of leaflets to blades is variable (Figs. 122 to 125). Distance between rows of blades is also variable; in some strata, blades are close together with little pore space among them, whereas in others, blades are longer, and pore space is larger (Figs. 124 to 126).

Granular shell material deposited over ends of blades in transition to foliated structure adheres closely to the ends of blades and soon fills pores among the blades (Figs. 126 to 127). Fracture surfaces of chalky shell etched lightly with dilute bleach or acid are similar, except that a granular substructure is more conspicuous after treatment with bleach. Etching does not reveal the relationship of conchiolin to the blade-leaflet structure.

3. Chalky Shell Formation. The various hypotheses offered to explain chalky shell formation (see page 108) have been questioned by Galtsoff (1964). For one thing, he points out that chalky shell does not result from solulibilization of calcium salts of foliated shell because dissolution resulting from anaerobic conditions within closed valves is not localized but occurs over the entire inner surface of valves. However, dissolution of some areas of the valve surface of closed oysters can occur (pers. obs., see page 152). The same objection applies to the suggestion of Margolis and Carver (1974) that chalky shell is deposited during periods of maximal ventilation and reduction of $CaCO_3$ saturation. Furthermore, detachment of the mantle from the inner shell surface does not stimulate deposition of chalky material. Galtsoff (1964) lifted the mantle from the valve surface by inserting shallow plastic cups and found that foliated calcite was deposited on the cups, but not on the surface of the valves. At the same time prominent chalky areas formed on surfaces where the mantle was in close contact with the valve surface.

Some environmental factors have been shown to affect chalky shell formation. For example, in experimental cultures there was nearly complete absence of chalky calcite in shells of young *C. virginica* (2.1 to 4.7 cm mean height) grown in recycled seawater, whereas it formed normally in valves of oysters grown in the field and in tanks in which seawater was changed every second day (Palmer and Carriker 1979). Calcium concentration was lower and more highly fluctuating in the recycle system than in the other two systems and was the major difference between the systems. In field experiments, shells of young *C. virginica* grown in direct sunlight possessed fewer chalky deposits than oysters raised in the shade (Medcof and Kerswill 1965), as did *C. gigas* subjected to higher than normal concentrations of salt in seawater (Tanaka 1943). The relation of reduction of chalky deposits to low calcium concentration, direct sunlight, and high salinity is puzzling; perhaps stress imposed by extremes of these factors can influence chalky shell formation. Although no logical explana-

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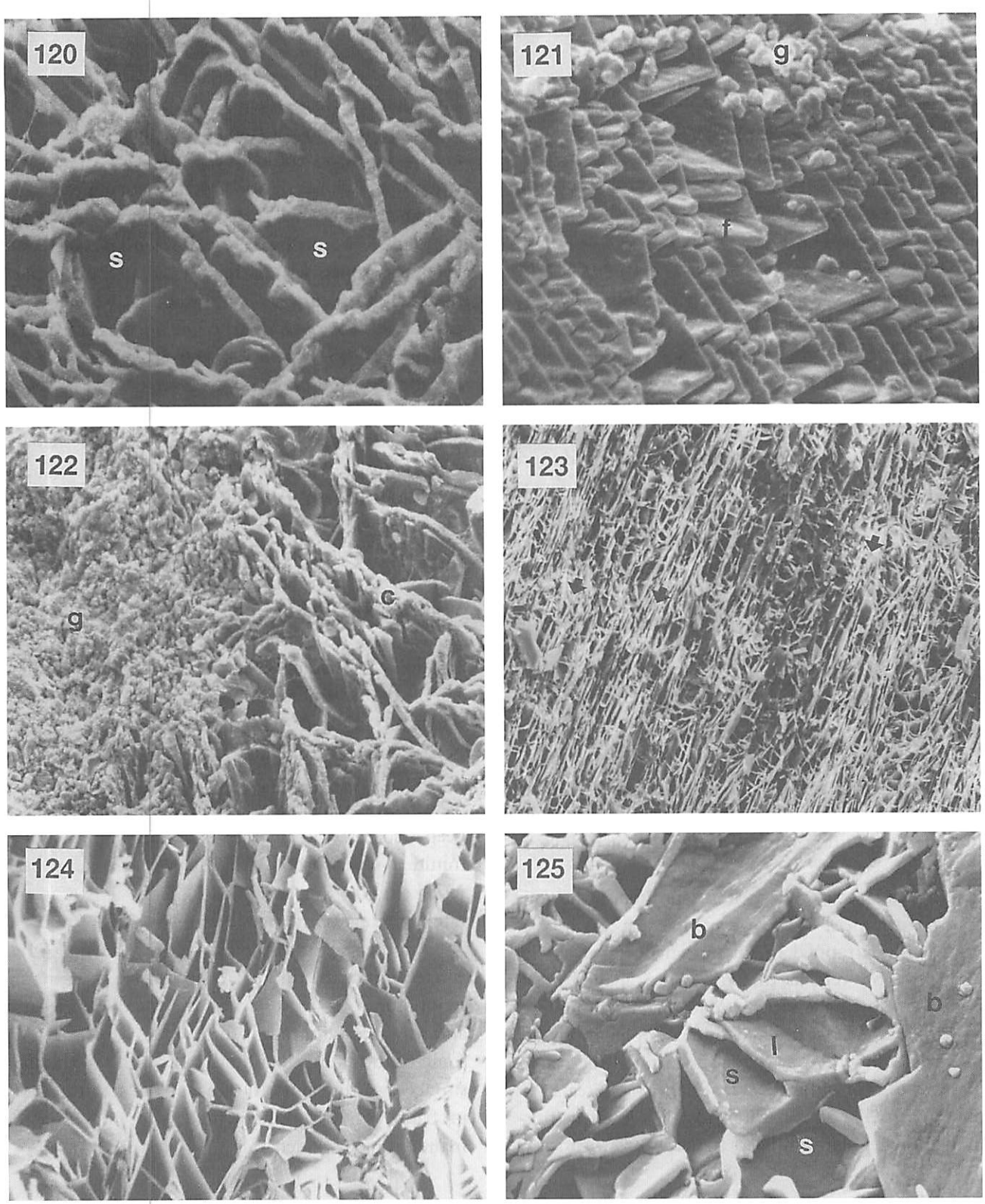
Figures 120-125 (opposite page) (120) Chalky structure, showing deep pore spaces [s] (surface against mantle). 5% bleach 1 min. HFW = 20 μm. (121) Initiation of granular material [g] on open foliated [f] structure (surface against mantle). No cleaning treatment. HFW = 20 μm. (122) Boundary between typical chalky structure [c] and granular material [g] in tran-



sition to foliated structure (surface against mantle). 5% bleach 1 min. HFW = 40 µm. (123) Chalky structure, fractured parallel to long axis [arrow] of blades. No cleaning treatment. HFW = 130 µm. (124) Chalky structure, irregular honeycomb pattern, fracture. No cleaning treatment. HFW = 40 µm. (125) Chalky structure, blades [b] and leaflets [l], pore space [s], fracture. No cleaning treatment. HFW = 20 µm.

tion has yet been offered for formation of chalky foliated deposits, its major advantage would seem to be economy of mineral shell material.

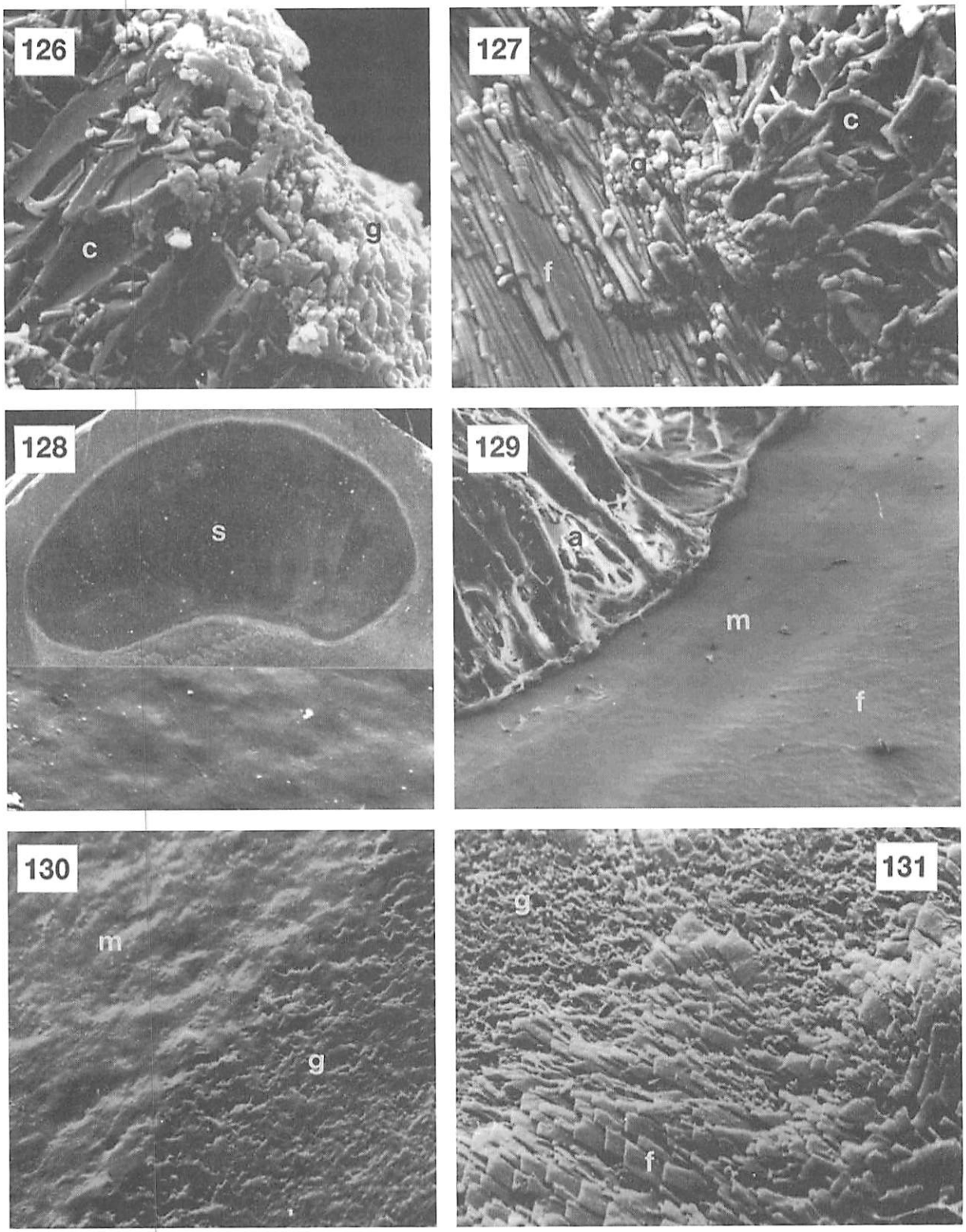
Primarily because of the presence of interstitial spaces among blades and leaflets, current theories on shell formation do not neatly account for formation of chalky deposits. Chalky microstructure originates in transitional zones as crystallites (Figs. 126, 127) bathed in extrapallial fluid. From these emerge the blades, and from the sides of the blades, the leaflets. Blades (Fig. 125) appear to show strata of deposition, suggesting that layers of new mineral are laid down following the form established during initial stages of growth. Although there is approximately a third more conchiolin in chalky shell than in regularly foliated calcite (Korringa 1951a; Weiner and Hood 1975), scanning electron micrographs do not show whether this matrix is primarily on the exterior or within blades and leaflets. Nucleation of leaflets presumably takes place on the surface of the matrix of blades, but what determines the site of nucleation, the direction of growth of leaflets, and why some leaflets contact opposing microstructures while others end in space is perplexing. Even if the mantle surface could be in close apposition to chalky microstructure that is forming, it is problematical whether individual clusters of epithelial cells could extend and control the spatially restricted deposition of shell material that characterizes chalky shell.

Inasmuch as chalky shell is deposited in the aqueous environment of the extrapallial space, composition of fluid trapped in interstitial pores of chalky shell probably reflects that of the extrapallial fluid. However, Korringa's (1951a) data suggest otherwise. Korringa (1951a) extracted pure samples of chalky shell of *Ostrea edulis* in water and titrated the resulting solution for chloride. The quantity of "sea salts" obtained (6.5% as compared to 0.1% each in prismatic and regularly foliated structure) is surprising. Pore fluid in chalky deposits in the region of valves between the adductor muscle and the posterior margin of the valves could be exchanged with seawater when mantle lobes of open oysters retract or curl, exposing the inner shell surface to seawater. As new layers of shell are deposited by the mantle, salts could become trapped within chalky pores. This is much less likely to occur between the adductor muscle and the hinge. It is possible (though not indicated) that Korringa's (1951a) samples were taken posteriorly to the adductor muscle.

In this regard, it is relevant to consider whether density and viscosity of pore fluid in chalky shell exceed that of seawater and what happens to extrapallial fluid when mantle lobes move. In extrapallial fluid of *Mercenaria mercenaria*, mucopolysaccharides account for about one-fifth and protein about four-fifths of the nondialyzable material (Crenshaw, in Wilbur 1972). Concentration of this material is greatest in blood, intermediate in extrapallial fluid, and lowest in mantle cavity seawater (Crenshaw 1972). Biomineralization in bivalves probably occurs under conditions of saturation or low supersaturation of shell minerals (Crenshaw 1972; Wada and Fujinuki 1976); because extrapallial fluid is thus denser than seawater, exchange of chalky pore fluid (especially in deeper spaces) with mantle cavity seawater could be minimal. Viscosity of extrapallial fluid has not been reported, and this could also affect the extent and rate of mixing of the two fluids.

Another factor in the possible exchange of fluids is retention of the film of extrapallial fluid on the interior surface of valves when the mantle is elevated and seawater admitted. If sufficiently viscous, at least some of the extrapallial fluid could cling to the shell surface awaiting repositioning of the mantle; more probably, a new supply of fluid is secreted after the mantle is repositioned.

Figures 126-131 (opposite page). (126) Chalky structure, fracture at right angle to mantle surface; chalky structure [c], transitional granular material at mantle surface [g]. No cleaning treatment. HFW = 35 μm. (127) Transition from foliated laths [f] through granular material [g] to chalky structure [c], fracture. No cleaning treatment. HFW = 45 μm. (128) Adductor muscle scar [s] of young oyster, right valve. Top, full scar: ventral, right; dorsal, left; posterior, top; anterior, bottom. 20% bleach 10 sec. HFW = 8 mm. Bottom, enlargement of area in center of scar. HFW = 35 μm. (129) Transitional



myostracum [m] between adductor muscle [a] and foliated structure [f] on posterior side of adductor muscle scar, left valve. Brushed lightly, 5% bleach 15 sec, critical-point dried. HFW = 1.4 mm. (130) Enlargement of transition between myostracum [m] and granular structure [g] of Fig. 129. HFW = 40 µm. (131) Transition between granular [g] and foliated lath structure [f] of Fig. 129. HFW = 40 µm.

Myostracal Structure, Both Valves. Myostracal shell (hypostracum of earlier authors) secreted at attachment areas of muscles (Oberling 1964) is limited in *C. virginica* to sites of the large single adductor muscle and the small Quenstedt muscles just posterior to the hinge. Dissoconch oysters lack pedal muscles. Pallial and pedal myostraca are also absent; the mantle is attached securely to the shell only around the periphery of the adductor muscle-shell juncture.

Adductor myostracum is a thin, but sturdy, well-developed, aragonitic, irregular, simple, prismatic layer (Carter 1980b). As the post-set oyster grows, the adductor muscle increases in size and simultaneously migrates posteriorly. This makes possible its retention in a functional position relative to the shell cavity (Stenzel 1971).

1. Adductor Muscle-Scar Interface. The surface of the scar from which the adductor muscle has been removed with bleach is extremely smooth and shallowly wavy (Fig. 128). Although the adductor muscle is comprised of two distinct parts (approximately an anterior, two-thirds, translucent area, and a posterior, one-third, milky-white area in living oysters), no microstructural differences are evident in the myostracum in these two areas (Stenzel 1971; Carriker et al. 1980a).

At the front of the posteriorly advancing edge of the growing adductor muscle there is present a narrow transitional zone of newly deposited myostracum about 0.5 to 1.0 mm wide (Fig. 129). The surface of this zone consists of a smooth, typically myostracal inner part adjacent to the base of the adductor muscle, and an outer zone that ranges from smoothly to coarsely granular where it overlaps foliated structure (Figs. 130, 131). In some individuals, on the posterior side of the scar the structure of encroaching myostracum is finely granular and crystallites intermingle with, fill around, and eventually cover foliated laths (Fig. 131). In other individuals, the transitional area is a gradient of granular, discrete, muffin-like microstructures that are deposited over folia and finally merge into a solid sheet of smooth myostracum (Fig. 132). In a third type, the transitional zone consists of mulberry-like microstructure ranging in complexity from single to multiple granular mounds (Fig. 133). These coalesce to form myostracum during migration of the muscle.

The transitional zone between myostracum and foliated structure on the ventral and dorsal sides of the adductor scar is usually much narrower than that on the posterior side and is finely granular. The distinctly granular nature of these transitional surfaces is characteristic.

On the anterior side of the adductor scar, where foliated structure grows over and buries abandoned myostracum, there is also a granular transitional zone (Fig. 134). This zone is narrow in some individuals, and in others can be much wider and consists of several terraces. Growth of foliated calcite over myostracum extends posteriorly on ventral and dorsal sides of the scar to about the midline of the scar, where a reversal occurs and myostracum overgrows the foliated calcite.

2. Fracture Surface. Proof that fully formed myostracum precedes the advancing edge of the adductor muscle is clear in an anteroposterior fracture of a valve through the posterior side of the scar (Fig. 135). Myostracal prisms are present in the entire zone of smooth myostracum, and a granular stratum, homologous with the outer granular zone of transitional myostracum, lies between myostracal and foliated layers.

The thinness of the myostracal stratum is evident in fracture sections from which adductor muscle has been dissolved with bleach (Fig. 136). The irregular, simple prisms are oriented normal to the surface of the layer, and differ strikingly from the regular, simple prisms of the external prismatic structure of both valves. Myostracal prism outlines are highly irregular, with re-entrant angles. Although each of these prisms is bounded by thin, often discontinuous bands of organic matrix in bivalves (Taylor et al. 1969), these walls were not visible in scanning micrographs of myostracum of *C. virginica* even after treatment with bleach (Fig. 136).

The layer of myostracum that becomes buried in foliated structure between the adductor muscle scar and the hinge region is clearly visible in fracture sections (Fig. 137). Transitional granular shell material

is present on both outer and inner surfaces of the myostracal layer sandwiched between strata of foliated structure.

3. Adductor Muscle-Myostracal Interface. Union of the adductor muscle and exterior myostracal surface in *C. virginica* is a powerful one. This muscle in an adult oyster can withstand a pulling force as great as 10 kg; beyond this, the muscle breaks midway between the muscle scars, rather than tearing free from one or the other scar when valves are forced apart (Galtsoff 1964).

A thin organic sheet, the adhesive epithelium, lies over the myostracum under the base of the adductor muscle on both valves. A part of it is visible just posterior to the edge of the adductor muscle (Fig. 135). As an oyster increases in size, the adhesive epithelium proliferates in the posterior direction of growth, forming an additional zone for attachment of the migrating muscle (Nakahara and Bevelander 1970; Tompa and Watabe 1976; Waller 1980). This epithelium, in decalcified histological sections of adductor muscle-myostracum preparations of *C. virginica*, is about 2 μm thick (Galtsoff 1964). It is generally more extensive in area than the area of the adductor muscle affixed to it (Tompa and Watabe 1976; Waller 1980). Galtsoff (1964) determined that the adhesive epithelium contains collagen, an important supportive constituent of connective tissues. When Crenshaw and Watabe (1969) demineralized valves of *M. mercenaria*, they found that the adductor muscle retains its attachment to the organic matrix of the myostracum. This connection probably reinforces attachment of muscle to the scar. Waller (1980), in a study of species of the Order Arcoida, also noted that adhesive epithelial cells can form in advance of attachment of muscles to underlying connective tissue.

At moderate magnifications, the adductor muscle scar of *C. virginica* freed of muscle appears extremely smooth (Fig. 128). At higher magnifications, Nakahara and Bevelander (1970) observed minute pores measuring about 3 nm in the myostracal prismatic surface of the pearl oyster *Pinctada radiata*. In arcoid bivalves, a tendon sheath secreted by tendon cells of the adhesive epithelium of the adductor muscle sends fibers into the myostracum (Waller 1980); these are embedded in prisms during mineralization. Whether tendon sheath fibrils are present in the adductor muscle-myostracal interface in *C. virginica*, and whether they extend primarily into the thin interprismatic conchiolin, or into the mineral crystals, or both, as they form, has yet to be determined.

Shell Microstructural Chemistry. Calcium carbonate constitutes more than 95% by weight of the shell of *C. virginica* (Galtsoff 1964). On the basis of a study of the shells of 34 specimens from Massachusetts, Galtsoff (1954) reported that the concentration of organic matter (presumably based on Kjeldahl determinations) in the foliated calcite varied from 0.46 to 1.1%, that there was no significant difference between the organic content of foliated and chalky shell (but Weiner and Hood [1975] found 0.91% nondialyzable organic matter in chalky shell and 0.58% in foliated calcite), and that the organic content of the whole shell ranged from 0.3 to 1.1%. This is low compared to a range of 2.16 to 2.34% organic content in the whole shell of this species cited later by Galtsoff (1964, Table 9) from other sources. Price et al. (1976) reported that the organic content in 50 eastern oyster shells from North Carolina was 3.04 ± 1.16% determined by loss of weight through combustion at 475°C. According to Goulletquer and Wolowicz (1989), however, the ignition method overestimates the quantity of organic matter by 2 to 5 times. It appears the content of organic matter in the shell of the oyster needs reassessment.

Price et al. (1976) discovered that percent organic matter in valves of *C. virginica* tended to be greater in small individuals and least in larger ones. Organic matter in exposed weathered shells was less than that in shells of living bivalves, whereas rate of decomposition of organic matter in shells buried in sediment was extremely slow.

Calcium carbonate and organic compounds are thus the two major constituents in the shell of *C. virginica*; the balance consists of minor and trace inorganic compounds (Galtsoff 1934, 1964; Stenzel 1971; Masuda and Hirano 1980; Lowenstam and Weiner 1983). Some 40 different biogenic minerals are present in molluscan shell in the form of carbon-

ates, phosphates, halides, oxalates, iron oxides, and silica (Lowenstam and Weiner 1983). According to Masuda and Hirano (1980), the divalent elements of alkaline earths are controlled to a minor degree by shell structures, whereas monovalent trace elements (alkali metals) are not controlled by crystal structure and are randomly distributed in shell as inclusions of carbonates, or hydroxides, or both.

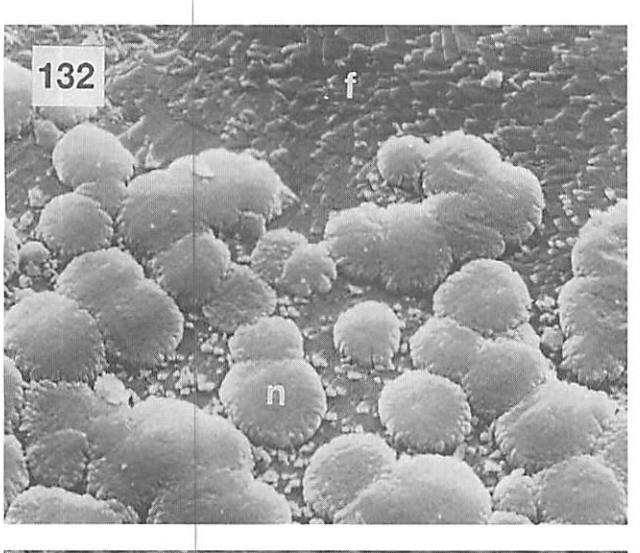
In *Mytilus edulis* shell, selected area electron diffraction revealed the presence of minute quantities of celestite ($SrSO_4$), strontianite ($SrCO_3$), barite ($BaSO_4$), and witherite ($BaCO_3$), possibly as impurities deposited in prismatic crystals during shell formation (Travis 1968). Because chemical composition can vary from one part to another of the shell of a bivalve, among mineral cores and organic envelopes of microstructural units, as well as ontogenetically (Ferrel et al. 1973; Rosenberg 1980; Carriker et al. 1982; Carriker et al. 1991), an analysis of one part of a shell does not necessarily give concentrations of chemicals typical of the whole shell. This is clearly demonstrated by studies of the shell of *C. virginica* by Moberly (1968), Wolfe (1970), Stenzel (1971), Ferrell et al. (1973), and Carriker et al. (1980b, 1982, 1991). For example, Wolfe (1970) recorded a mean value of about 25 ppm of the heavy metal zinc in the shell of *C. virginica*, but Carriker et al. (1991) found that the concentration of this metal in the shell of the same species varied as much as 300 times in different parts of the valves. This wide range of concentration, however, may not be characteristic of all bivalve species. For example, Bourgoin (1990) introduced the use of nacre (the inner white layer) of the shell of *M. edulis* as an alternative to soft tissues for analysis of lead in biological monitoring programs. Although nacre sequestered about one tenth of the lead measured in tissues, levels in nacre and tissues were strongly correlated and the statistical variability of concentrations of lead in nacre was half that in tissues.

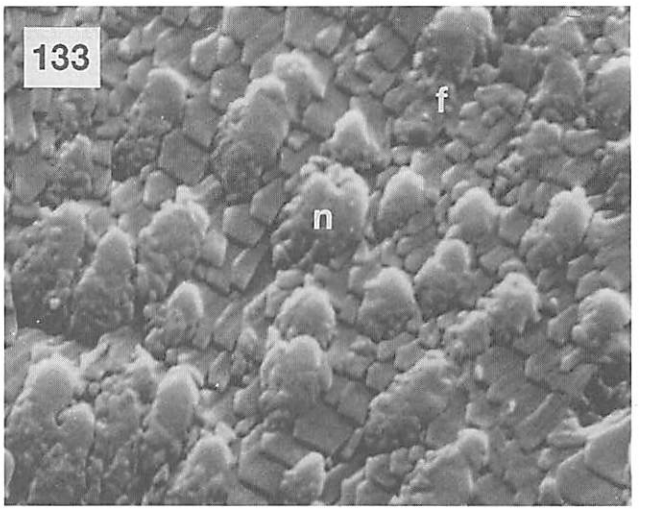
1. Mineral Elements. Carriker et al. (1991) conducted a study with proton induced x-ray emissions of the distribution and concentration of 15 chemical elements (sodium to strontium in the periodic chart of elements) in the four microstructural and two mineralogical regions of shell of rapidly growing adult *C. virginica* from Delaware. The three calcitic (prismatic, foliated, and chalky) and the one aragonitic (myostracal) microstructural groups, as well as the exterior surfaces and margins of the valves adjacent to the inhalant and exhalant mantle openings were analyzed.

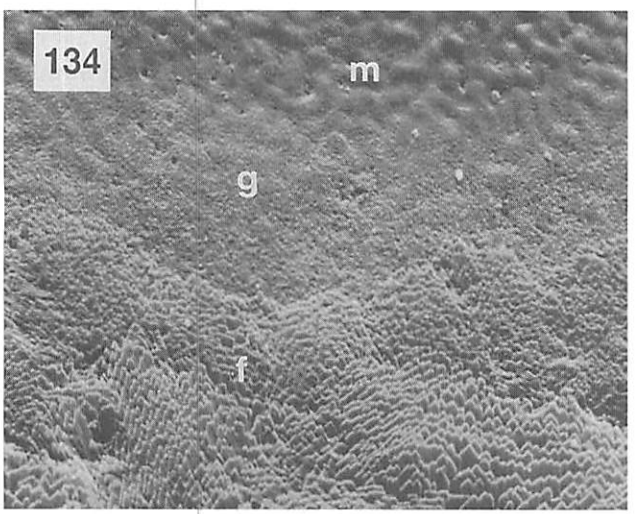
The elemental composition of different regions of the valves varied perceptibly among the three microstructural groups within the single calcitic polymorph, between aragonitic and calcitic regions, and inhalant and exhalant margins of the valves (Carriker et al. 1991). Concentration of elements was calculated as percent by weight (ppt) of the total 15 elements analyzed. Concentration of calcium ranged from 908 ppt (in prismatic shell) to 981 ppt (in foliated shell). Titanium, chromium, manganese, iron, copper, zinc, and bromine varied in concentration in different microstructural groups from less than 0.01 to 4.78 ppt; sodium, magnesium, aluminum, silicon, sulfur, chlorine, and strontium ranged from less than 0.50 to 31.41 ppt. Distribution of elements was not strongly associated with calcitic and aragonitic mineralogical types of shell (see also Wilbur 1972; Masuda and Hirano 1980). Overall, elements were most concentrated in the prismatic region of the right valve.

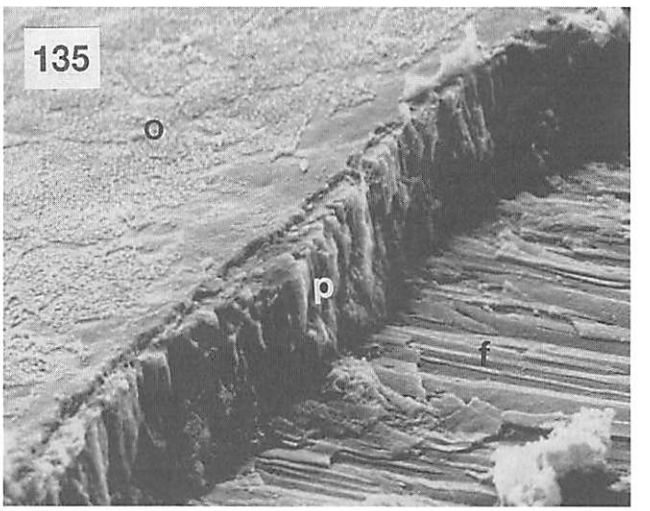
Sodium, magnesium, chlorine, chromium, copper, and zinc were considerably more concentrated in prismatic than in foliated shell (Carriker et al. 1991). Prismatic structure, characterized by thick organic envelopes around individual mineral cores in contrast to thin conchiolinal walls of foliated laths, could explain in part why prismatic structure in addition

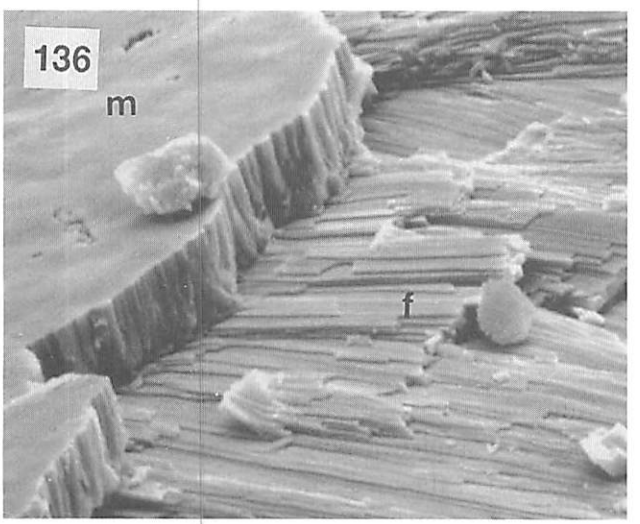
Figures 132-137 (opposite page). (132) Transition between muffin-like microstructures [n] at ventral edge of adductor myostracum and foliated lath structure [f]. 20% bleach 10 sec. HFW = 45 μm. (133) Transition between mulberry-like microstructures [n] at posterior edge of adductor myostracum and foliated lath structure [f]. 20% bleach 10 sec. HFW = 17 μm. (134) Transition between smooth adductor myostracum [m], granular structure [g], and foliated lath structure [f] on anterior side of adductor muscle scar. 20% bleach 10 sec. HFW = 75 μm. (135) Anteroposterior fracture through transi-

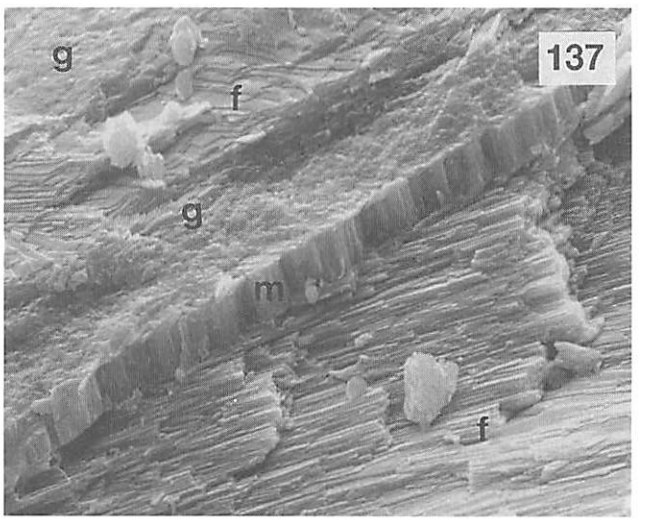












tional myostracum on posterior side of adductor muscle. Organic sheet [o], fracture of myostracal prisms [p], fracture of foliated structure [f]. 5% bleach 15 sec. HFW = 40 µm. (136) Myostracum [m], fracture through center of adductor scar, foliated structure [f]. 20% bleach 10 sec. HFW = 40 µm. (137) Stratum of adductor myostracum [m] buried in foliated structure [f] in region between adductor scar and umbo; granular structure [g]; fracture. 20% bleach 10 sec. HFW = 75 µm.

tended to contain higher levels of such elements as sulfur and possibly also manganese and bromine (Wilbur 1972; Wada and Suga 1976).

Chalky shell contained higher concentrations of sodium than prismatic shell and high concentrations (but lower than prismatic shell) of magnesium, chlorine, titanium, manganese, iron, zinc, and strontium (Carriker et al. 1991). Composition of fluid trapped in interstitial pores of chalky shell during shell deposition could explain the higher concentration of sodium, magnesium, chlorine, titanium, manganese, iron, zinc, and strontium in chalky shell than in foliated shell.

Concentration of elements in aragonitic myostracum was about the same, or lower, than in foliated shell, except for strontium, which was higher than that in any other shell group (Carriker et al. 1991). For crystallographic reasons, strontium frequently substitutes for calcium in the aragonitic lattice but not in the calcitic lattice (Waller, pers. comm.). Bidwell et al. (1986) discovered that strontium appears to be essential for the normal development of the larval molluscan shell, which is generally aragonitic; the possible role of strontium in the formation of myostracal aragonite has yet to be explored.

There was a higher concentration of chromium, manganese, iron, copper, zinc, and strontium on both exterior and interior surfaces of the inhalant margins of the right valve in contrast to lower levels of these elements in the surface of the exhalant margins of the same valve (Carriker et al. 1991). Accumulation of elements from seawater flowing across the mantle margin of the right valve into the mantle cavity of the oyster could explain this difference (Immega 1976; Carriker et al. 1982). On the other hand, in the left valve the higher concentration of many of the elements occurred on ventral exterior margins. These elements could have come, in part, from discharge in the vicinity of the exhalant area of metabolic wastes and fecal materials. Physiological-behavioral explanations for the apparent distributions of these elements have yet to be advanced.

On older parts of prismatic shell surfaces of *C. virginica* weathered under water, some elements (magnesium, silicon, and manganese) increased in concentration, possibly through absorption from seawater, whereas sodium, aluminum, chlorine, titanium, chromium, iron, bromine, and strontium decreased, probably as a result of shell erosion and leaching. There were only slight or variable changes in sulfur, copper, and zinc (Carriker et al. 1991).

Oysters have the capacity to incorporate a number of chemical elements (but not all) in their valves in concentrations considerably greater than those in ambient seawater (Carriker et al. 1980b). Ferrell et al. (1973), for example, analyzed the shells of freshly killed *C. virginica* from three regions in the Gulf and East Coast of the United States, using atomic absorption spectrophotometry for the trace metals lead, mercury, cadmium, zinc, copper, and chromium. All metals were present in quantities greater than the average for the element in seawater, the degree of concentration being more than a thousand fold. Lead, copper, and cadmium exhibited maximal concentration and mercury the least. As Carriker et al. (1991) found, within-sample variation was the greatest source of difference in the amounts of trace metals present. Frazier (1975), following seasonal changes in concentration of trace metals manganese, iron, zinc, copper, and cadmium in the valves of *C. virginica* maintained in plastic trays in Chesapeake Bay, observed no significant seasonal changes, but level of incorporation of manganese was roughly 100 times greater than that of the other metals. In other experiments in which oysters were exposed for a season over a metal-contaminated sediment, their shells were significantly thinner than those of controls, and incorporation of manganese in the shells was suppressed while that of iron, zinc, and copper was increased slightly (Frazier 1976).

2. Organic Matter. The organic matrix of shell of *C. virginica* is composed of two fractions: a protein with a high content of glycine and dicarboxylic amino acids, and sulphated polysaccharide (Simkiss 1965). One third of the total amino acid residues of insoluble organic matrix (conchiolin) consists of glycine, and from 60 to 56% of glycine, aspartic acid, and serine combined. Amino acid composition of conchiolin in prismatic and foliated shell is closely similar. Both prismatic and foliated strata of the shell contain collagen fibrils, but these constitute only a minor

structural component of the insoluble matrix (Travis et al. 1967).

About 50% of the organic matrix of foliated calcite of *C. virginica* is soluble, and about one third of the residues from this fraction are either aspartic acid or asparagine (Weiner and Hood 1975; Runnegar 1984). An analysis of both soluble and insoluble matrix from *C. virginica* yields about 40% glycine, 20% serine, and 10% aspartic acid (Simkiss 1965). If the soluble fraction represents about 50% of the total, the insoluble part probably contains few or no aspartyl residues and more than 40% glycine (Runnegar 1984). A significant proportion of the soluble matrix, probably composed of a repeating sequence of aspartic acid separated by either glycine or serine, could function as a template upon which mineralization occurs. Thus, relatively small changes in either the amino acid sequences or the conformation of matrix proteins could result in very different crystallographies of the mineral phase (Runnegar 1984). In this regard, Samata (1990) reported that the amino acid composition of the different microstructural regions of valves of several species of bivalves (including *C. gigas*) varied primarily according to the microstructure of the shells. An important property of soluble matrix is its capacity to bind calcium. Polyphenol oxidases and polyphenolic compounds in soluble matrix probably play a role in polymerization of the insoluble matrix. The latter consists primarily of a group of proteins with a polysaccharide content; its cross-linked proteins could be quinone-tanned. Polysaccharide components exhibit a structure equivalent to that of chitin (Wilbur and Saleuddin 1983; Watabe 1984).

3. Shell Pigmentation. The exterior, primarily of the right valve, of *C. virginica* is generally pigmented, sometimes intensely so, by light to dark reddish-brown radial bands that arise in the umbonal region and widen toward the posterior margin as valves increase in size. The bands follow the shifting axes of growth, and are most conspicuous in valves of young oysters (Galtsoff 1964; Palmer and Carriker 1979).

Oysters living in warm and tropical regions have darker, more vivid, more varied, and more extensive colorations than those in cooler climates. The inner surfaces of the valves of northern populations of *C. virginica* living north of Cape Cod, for example, are characterized by whitish to grayish-yellow colors, and adductor muscle scars are grayish-red purple to very dusky-red purple. The inner surfaces of valves of the same species living in the northern part of the Gulf of Mexico possess large areas of light brown to grayish purple and only a few patches of whitish color; their adductor muscle scars have approximately the same colors (Stenzel 1971). For species like *C. virginica*, whose populations cover the broad north-south range of some 5,000 miles in habitats varying from cold temperate to tropical (Newball and Carriker 1983), it is thus generally possible to distinguish by pigmentation individuals from at least the extremes of their range.

The chemical composition of pigments in oyster shell has not been determined. Presumably elements could be bound directly to the shell periostracum, the organic matrix, or both, be present in organometallic pigments, substitute for calcium or carbon in the inorganic fraction of the mineral cores, or simply be adsorbed onto the shell surface (Rosenberg 1980). Dark pigments in molluscs, derived from tyrosine and containing an indolic chromophore (Fox 1966), could be responsible, in part, for the dark pigmentation. In this regard, Carriker et al. (1982) observed that strongly pigmented areas on the surface of the right valve of C. *virginica* grown in controlled laboratory conditions contained low concentrations of calcium and high concentrations of magnesium, aluminum, silicon, and titanium. Because not all elements present were analyzed, it is not possible to say which of these elements, or others, produced the color, probably in the organic matrix of the shell.

Neutron radiation can change the color of nacre to black in shells and pearls of freshwater bivalves, but not in marine bivalves (Horiguchi and Tsujii 1967). Normal yellow pigmentation in shell of pearl oysters apparently is secreted by cells in the mantle and pearl sac epithelium (Wada 1969). The degree of the color change from radiation is approximately proportional to the concentration of trace elements present, especially manganese (Horiguchi and Tsujii 1967). The effect of normal ambient solar radiation on the color of periostracum of oysters in the wild

has not been analyzed; that it does have some effect is suggested by the darker, more varied coloration of oysters in the tropics than those in temperate zones (Stenzel 1971) and the results of observations by Medcof and Kerswill (1965) and Palmer and Carriker (1979).

4. Contaminants. Simkiss (1965) discovered that inorganic material left after acid hydrolysis of the organic matrix of shell of *C. virginica* consists of a number of clay minerals, principally pyrophyllite, a clay characteristic of the North Carolina coastal region from whence the oysters came; these clays could have been present in either the matrix or in mineral cores, or both, as contaminants incorporated during shell formation. Whether clays are necessary in the process of shell formation is not known. Later, Immega (1976) likewise found inorganic detritus in acid soluble residues of the foliated calcite of the shell of *C. gigas.*

Whether metals such as titanium, chromium, iron, copper, zinc, and possibly others, represent normal constituents of oyster shell (at least in minimal quantities), or are adventitious contaminants incorporated during mineralization, has not been determined (also see Bourgoin [1990] and page 144). The fact that their concentrations in shell tend to follow those in ambient seawater suggests the latter (Carriker et al. 1982).

Conchiolinal Patches. From time to time thin, greenish-brownish conchiolin sheets are deposited, apparently randomly, by the mantle on the inner surface of foliated structure. In fracture surfaces, these organic patches appear as thin lines interleaved among strata of folia.

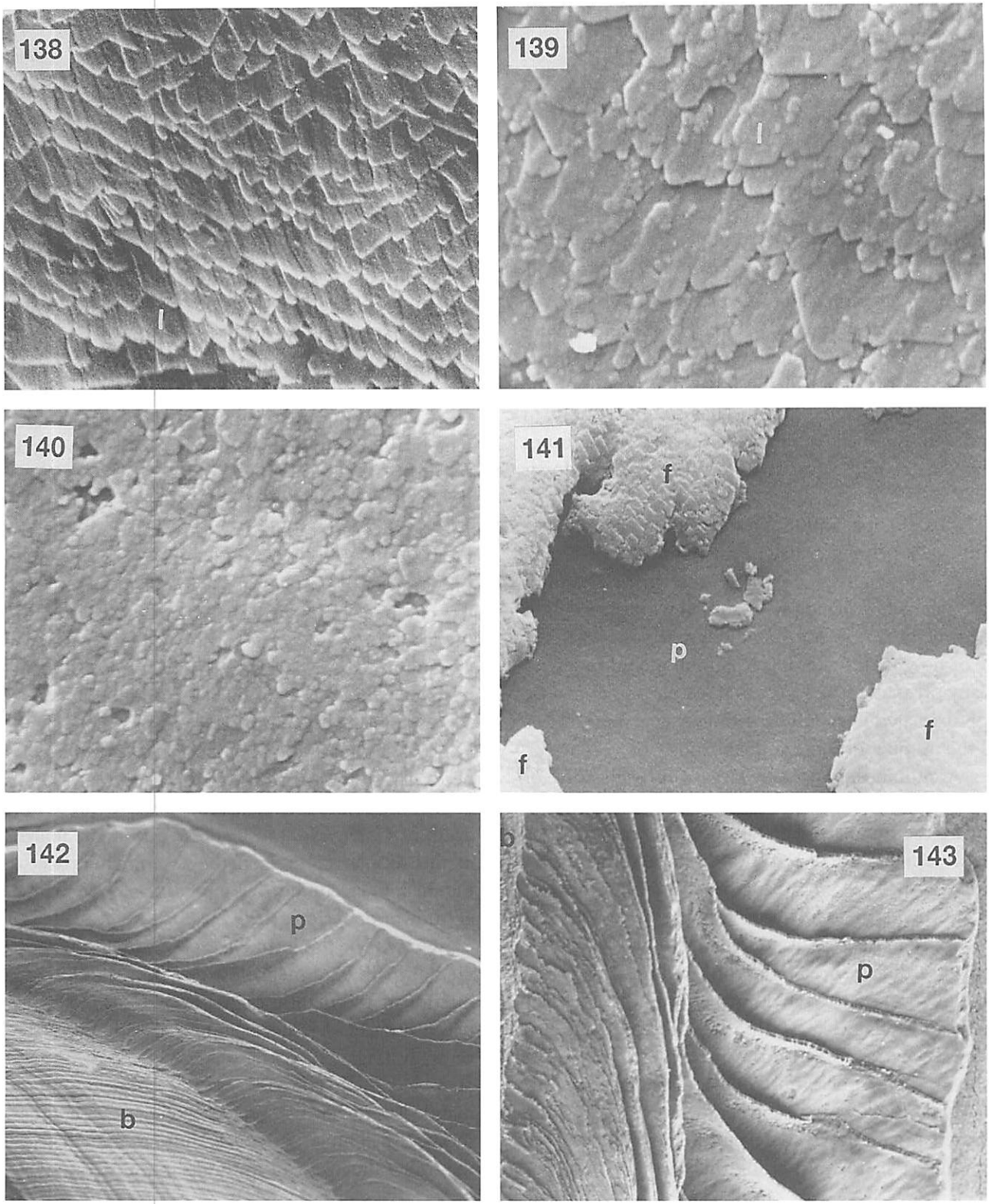
Patch conchiolin is deposited first as an extremely thin layer of finely granular material that completely covers laths, blurring their outlines (Fig. 138). As the patch thickens, more granules are added, and outlines of laths become increasingly indistinct (Fig. 139). After further deposition, laths become totally obscured and a solid pavement of granular material forms (Fig. 140). Sometimes the surface of the patch becomes exceedingly smooth (Fig. 141). The new layer of folia deposited by the mantle forms directly on the surface of the patch, in most cases, apparently, with little intervention of transitional granular shell material (Fig. 141).

Korringa (1951a) hypothesized that conchiolin patches are secreted by *O. edulis* as a defense against intrusion by burrowing worms, or "for no obvious reason." Taylor et al. (1969) suggested that these layers in the Seychelles oyster *Ostrea cuccullata* could be a reaction to excessive corrosion of older parts of its shell. Clearly, the matter requires experimental study.

Umbonal Plicae. Umbonal folds of shell that extend posterior-laterally from bourrelets along both sides of the left valve are especially noticeable in young *C. virginica* up to about 15 mm in height growing attached to a smooth substratum (Figs. 58, 142). The inner row of these plicae, adjacent to the bourrelets, are close-set, tend to follow the contour of the edge of the valve, and appear like extensions of bourreletal growth bands. Plicae of the outer row are higher and more widely separated, and flare outward, forming keels over the basement sheet of shell deposited upon the substratum (Fig. 143). In older oysters, these plicae tend to disappear, or only a few remain, sometimes as exaggerated folds, along the sides of the valve.

Microstructurally, the center layer of each plica is composed of prisms, extensions of prismatic scales on the exterior of the valve (Fig. 144). Plical prisms are exposed on the underside (that facing the substratum) of plicae; on the upper side (exterior), each plical prismatic layer is covered by a thin deposit of foliated structure (Fig. 144).

Figures 138-143 (opposite page). (138) Thin layer of conchiolin over foliar laths [l] on interior surface of valve. 5% bleach 1 min. HFW = 20 μm. (139) Thin granular layer of conchiolin over foliar laths [l]. No cleaning treatment. HFW = 20 μm. (140) Thick granular layer of conchiolin over foliated structure. No cleaning treament. HFW = 20 μm. (141) Conchiolin



patch [p] and initial deposition of foliated structure [f]. 5% bleach 1 min. HFW = 20 µm. (142) Umbonal plicae [p] of young oyster, bourrelet [b]. Ligament dissolved in bleach. HFW = 2.4 mm. (143) Umbonal plicae [p], outer row bourrelet [b]; higher magnification of Fig. 142. HFW = 1.2 mm.

The presence of plicae on only the left valve suggests that they support the anterior region of the valve and facilitate its attachment to the substratum.

Shell Annuli. Interruption of the normal structural configuration of molluscan valves at the growing margin as a result of physiological, environmental, and other factors is marked subsequently in the shell by macroscopic and microscopic growth lines or bands. A careful search by Lutz (1976) and Palmer and Carriker (1979) failed to reveal discernible internal annuli preserved in the foliated structure of the shell of *C. virginica.* The mechanism that results in annuli in the shells of other species of molluscs is unclear (Lutz and Rhoads 1980; Neves and Moyer 1988).

Macroscopic annuli, however, are frequently present in the prismatic shell of the right valve of *C. virginica.* Most conspicuous are gross surficial annuli that generally coincide with annual winter periods at the time of year in north temperate regions when little or no growth takes place. Annuli include a few to many prismatic scales. Age in years can sometimes be approximated in rapidly growing oysters on the basis of the number of these annual bands.

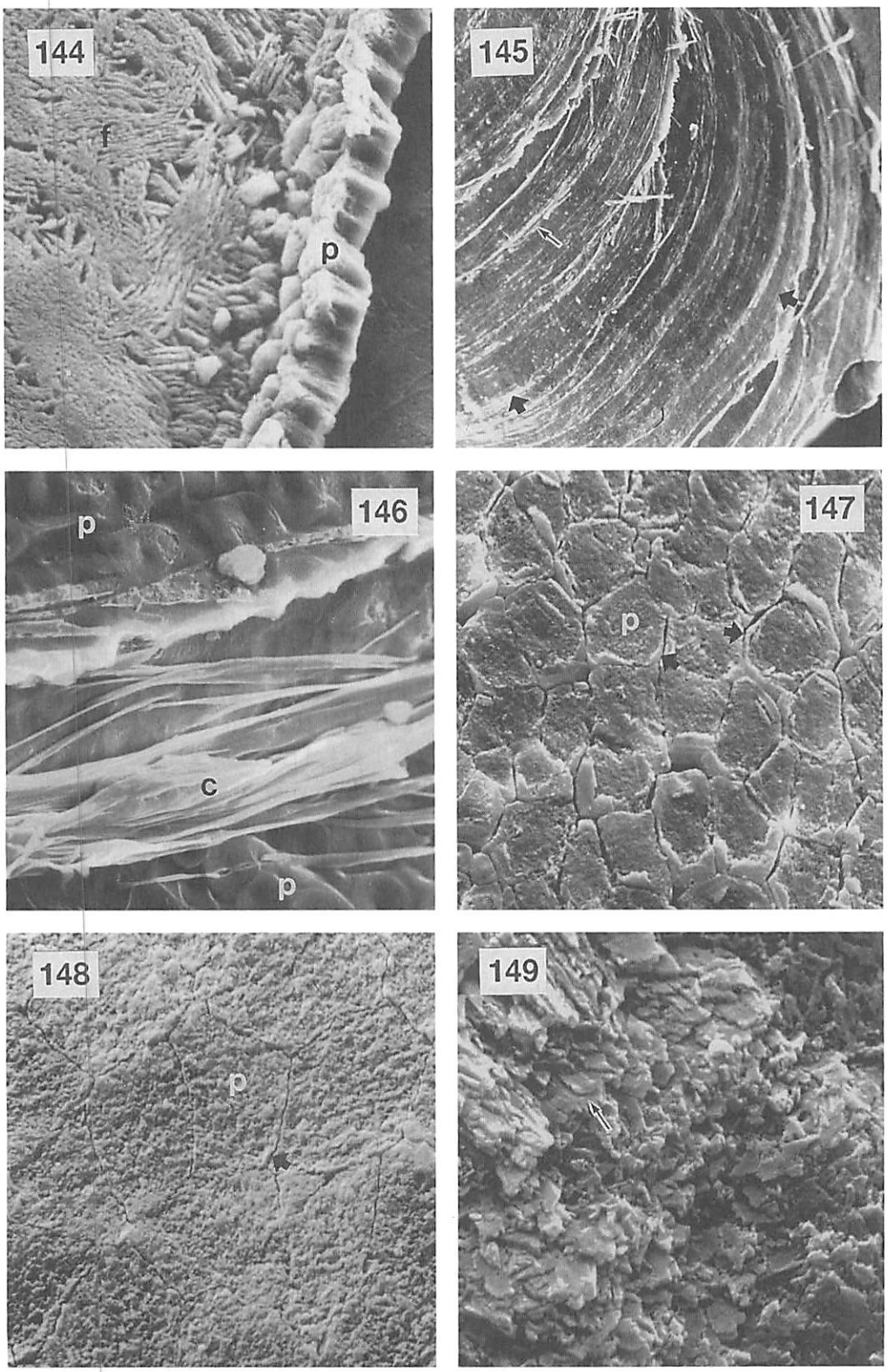
Annuli in the prismatic layer of the right valve of *C. virginica* become closely and irregularly spaced during the middle to late fall in north temperate zones as temperature of seawater drops, and before cessation of shell growth during the winter months (Fig. 145). Microstructurally each annulus is characterized by an accumulation of conchiolin in the form of a folded wrinkled sheet (Fig. 146), reminiscent of the organic sheet secreted in advance of mineralization at the mantle edge (Fig. 104). Structure of the annulus suggests that a sudden drop in temperature can inhibit crystallite nucleation, but not organic secretion, and with a following rise in temperature a new, fully formed prismatic layer develops under it (also see Wheeler et al. 1987). During the warm growing season when rapid shell growth takes place, coarse to fine annuli form on the surface of newly forming prismatic layers (Fig. 79). Their temporal significance has not been determined.

Although not conspicuous in young dissoconchs, adductor myostracum in older oysters is characterized by annuli that range from very fine to coarse broad lines. These are commarginal to the ventral margin of the myostracal scar (Stenzel 1971; Carriker et al. 1980a). The relationship between frequency and size of myostracal annuli and growth rate of oysters has not been investigated.

Conspicuous microstructural annuli are also present in the resilium (Figs. 67, 68) and in the ligostracum of resilifers and bourrelets (Figs. 72, 73) of the shell of *C. virginica* (see also Palmer and Carriker 1979). In oysters raised in the Broadkill estuary, Delaware, USA, about one annulus was present on the surface of bourrelets for each tidal cycle. In oysters cultured simultaneously in controlled systems in the laboratory, an average of four to five bourreletal annuli formed per day. More bands formed on bourrelets per unit area than on the corresponding adjacent resilifer (Palmer and Carriker 1979). The significance of annuli in the hinge structure of *C. virginica* needs clarification. In this regard, Custer and Doms (1990) showed that reliable estimates of the season of death of *C. virginica* can be derived from an analysis of the annular pattern on the anterior bourrelet of the shell; the technique has application in the seasonal study of prehistoric shell samples.

Growth of the ligament of *C. virginica* is incremental in three dimensions: posteriorly toward the interior of the mantle cavity, from right to left between surfaces of resilifers and bourrelets, and dorsoventrally (Figs. 66, 67; also see Galtsoff [1964] and Stenzel [1971]). The subligamental epithelium of the mantle isthmus, which produces the ligament and associated shell hinge structures, thus not only increases the size of the ligament in three directions as the oys-

Figures 144-149 (opposite page). (144) Upper surface of a single plica. Thin layer of foliated structure [f], prismatic scale [p]. High magnification of Fig. 142. HFW = 150 μm. (145) Closely spaced annuli [arrow] on surface of right valve formed during the late autumn. Oyster 4 cm high. No cleaning treatment. HFW = 2 mm. (146) Accumulation of strips of conchiolin [c] at an annulus in valve of Fig. 145. Prisms [p]. HFW = 70 μm. (147) Surface of naturally worn prismatic structure



of right valve. Grooves [arrow] between prisms [p] exaggerated by 100% bleach 1 min. HFW = 70 µm. (148) Surface of naturally worn prismatic structure of left valve. Grooves [arrow] between prisms [p] not as large as in Fig. 147. 100% bleach 1 min. HFW = 35 µm. (149) Surface of naturally worn foliated structure of left valve. Lath [arrow]. No chemical treatment, brushed clean. HFW = 30 µm.

ter grows, but also spreads lamellar ligaments apart dorsoventrally to accommodate the growing resilium.

Ligamental strata in bivalves represent local modification of shell layers, and thus growth bands of the ligament and corresponding bands in valves are generally homologously comparable (Owen et al. 1953; Trueman 1964; Kahler et al. 1976). In *C. virginica*, however, because of the absence of annuli in foliated structure and rapid wear of the thin prismatic layers on the exterior of both valves, correspondence of banding in shell and ligament is not easily determined.

Weathering of Exterior Shell Surfaces

Just as the outer part of the ligament deteriorates with age, the exterior of valves becomes worn upon exposure to destructive mechanical, chemical, and biological factors (Korringa 1951b, 1952; Galtsoff 1964; Stenzel 1971; Carriker 1981; Yatsu 1988). The surface of the imbricated scales of older parts of the valves (that is, in the vicinity of the umbones) is the first to be eroded, exposing foliated structure beneath.

Weathering of shell surface can result from a wide range of factors: abrasion by pelting suspended sedimentary grains, especially sands, in rapidly flowing seawater; dissolution by organic and inorganic acids and chelators released from reducing organic sediments, especially in quiet waters; dissolution by bacterial and fungal secretions and the catabolic and decomposition products of sessile organisms; and burrowing by a broad range of species of shell-penetrating algae, fungi, and small invertebrates (Carriker and Smith 1969; Carriker 1981; Yatsu 1988).

Where rate of erosion of organic matrix exceeds that of mineral prisms, the outlines of the conchiolin envelopes of microstructures tends to be emphasized (Fig. 147). If wear of both organic and mineral components progresses at about the same rate, prism boundaries are less conspicuous (Fig. 148). When mineral cores of prisms dissolve first, the configuration of organic sheaths is clearly revealed (Figs. 99, 108).

Erosion soon obliterates the microstructural outline of folial laths after the prismatic layers are gone (Fig. 149). Dissolution of intercrystalline organic boundaries appears to take place rapidly, probably because the proportion of organic matrix to mineral cores is small and interlath layers are thin. Pits of various sizes and shapes are frequent in the mineral component of eroding microstructures, probably as a consequence of differential dissolution of the various mineral constituents of the crystals (Travis and Gonsalves 1969; Carriker 1978).

The least soluble shell-forming mineral appears to be calcite with a low magnesium content, whereas aragonite has an intermediate solubility (Chave et al. 1962; Chave 1964). In a study in tumbling barrels of the durability of valves of six taxa of molluscs, Chave (1964) found that the shell of species of *Crassostrea* was the second most durable.

Dissolution of Mantle-facing Shell Surfaces

During the growing season, mantle-facing surfaces of both prismatic and foliated regions of valves of *C. virginica* normally are clean and microstructures are clear-cut and sharply outlined (Figs. 89, 110, 111, 112, 119, 121).

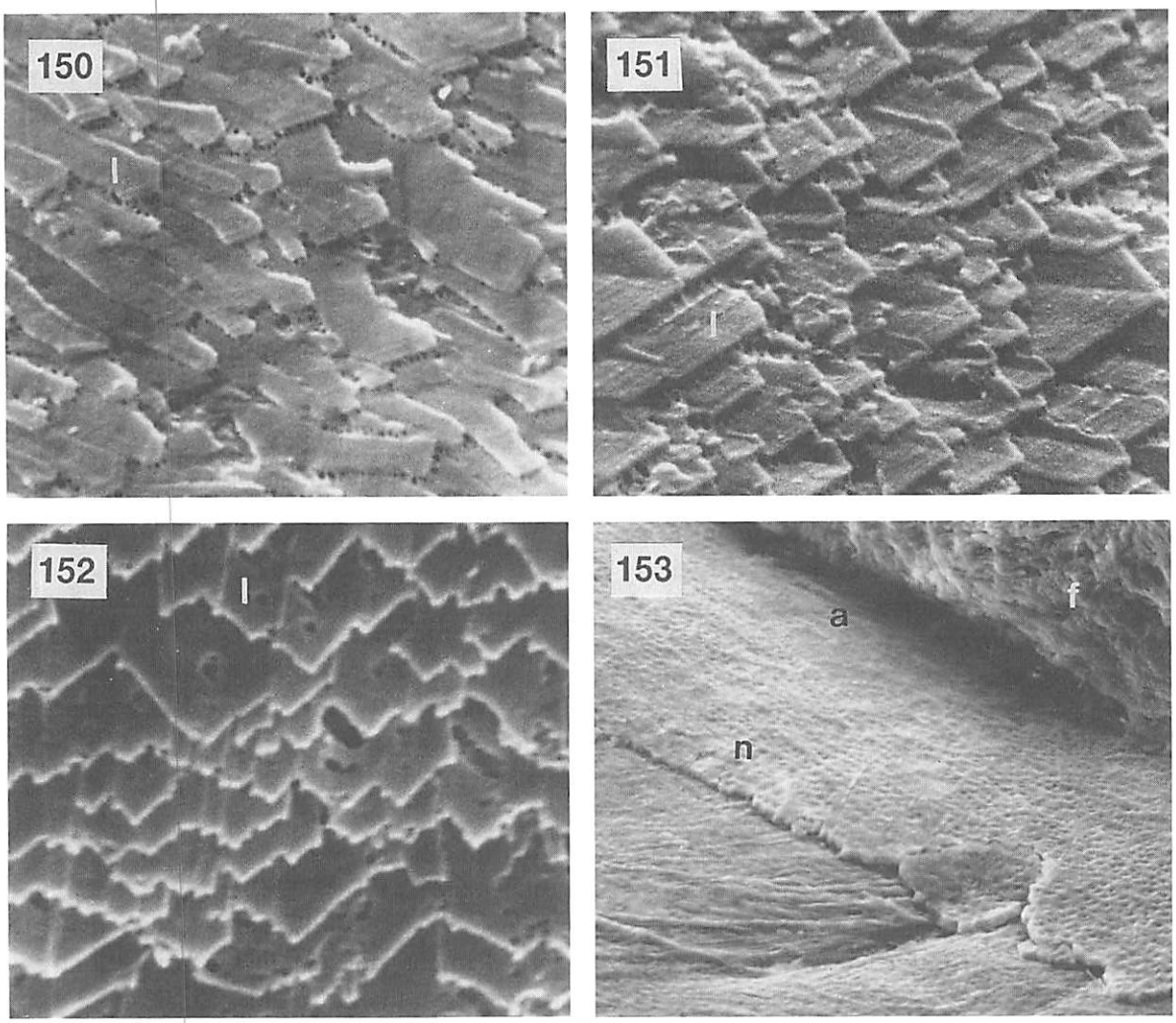
However, when oysters are forced to remain closed at room temperature for about 3 d, for example, noticeable dissolution of microstructural units can result on some areas of the valve surface (pers. obs.) (Figs. 150, 151, 152). Occasionally, minute pits of various sizes are etched. Severe dissolution leaves indistinct, or totally obliterated microstructural units. Curiously, at the same time on other areas of the same valves solubilization can be spotty and scarcely noticeable. Elevated regions of shell surfaces tend to be least affected (Watabe et al. 1958; Carriker et al. 1980a).

Degree of dissolution of interior shell surfaces varies roughly with duration of anaerobiosis; etching appears to be a common rather than a rare event (Watabe et al. 1958), though its relation to the seasons in temperate and tropical regions has not been addressed. During anaerobic metabolism, neutral substances can be converted into organic acids, lowering the pH of the extrapallial fluid (de Zwaan and Wijsman 1976) and causing surficial dissolution of primarily the mineral constituents. Succinic acid has been implicated as a possible solvent (Crenshaw and Neff 1969).

The fundamental cause of the inter- and intracrystalline dissolutional patterns is still an open question (Crenshaw 1980; Lutz and Rhoads 1980). Quite likely, differing solubility of different constituents of the microstructural units (for example, soluble and insoluble organic matrix, minor and trace minerals) could be a part of the answer (Travis and Gonsalves 1969; Carriker 1978).

Evolution of Mechanical Properties

Relatively few species of bivalves secrete principally calcitic shells (Waller 1976, 1978; Carter 1979, 1989a). Those that do are confined to an epifaunal existence and include species like *C. virginica* that are attached to hard substrata (Taylor and Layman 1972). With age, individuals of *C. virginica* secrete increas-



Figures 150-153. (150) Dissolution of folial laths [l] (between muscle scar and umbo) in oyster held closed for 3 d. No chemical treatment. HFW = 20 μm. (151) As in Fig. 150, but dissolution of folial laths [l] between muscle scar and shell margin. (152) As in Fig. 151, folial laths [l] between muscle scar and shell margin. (153) Microstructure of new prismatic shell, external view, formed in left valve after the margin was removed by filing. Oyster 5 cm high. Normal foliated shell, filed surface [f], new abnormal shell [a], new normal shell [n]. 5% bleach 10 sec. HFW = 270 μm.

ingly thick valves characterized in extreme cases by massive quantities of foliated and chalky calcite. Anatomically, predatory muricid gastropods are limited by the length of their proboscis in the depth to which they can bore into shell of epifaunal prey; hence, a thick shell provides some protection against predation by these snails (Carriker and Van Zandt 1972) and could have been a factor in the biological success of the genus *Crassostrea.*

Available paleontological data suggest that primitive bivalves were aragonitic (Carter 1980a), and that simple prismatic aragonite and simple prismatic calcite probably had independent origins (Waller 1990, pers. comm.). Calcitic laths probably evolved from simple calcitic prisms (Carter 1980a), perhaps through intermediate grades of fibrous prismatic or homogeneous structure or both (Carter 1989a). Because of the insular position of most chalky lenses, and their taxonomic distribution restricted to the Ostreacea (Waller, pers. comm.), it is probable that chalky calcite evolved later from foliated calcite.

Foliated shell of oysters is considered the weakest of all shell structures tested (by compression, bending, impact, microhardness, and density) in a wide range of species (Taylor and Layman 1972; Currey 1980, 1988, 1990). Nonetheless, it is a singular fact that Crassostreid oysters have evolved into widely distributed, highly successful species (Seilacher et al. 1985). An extraordinary fecundity certainly could have contributed to this success (see Thompson et al., Chapter 9), as could have the biomechanical properties of the shell (Waller 1972). The relatively large proportion of organic matrix in the prismatic margin of the right valve of *C. virginica,* for example, provides a flexible shell surface (Carter 1980a) for apposition against the thick, more densely mineralized, foliated, left valve margin during closure. The oyster, commonly surrounded by a wide range of concentrations and sizes of suspended particles, including sand (Carriker 1986), actively discharges these from the mantle cavity by forcefully snapping its valves together (Galtsoff 1964). The flexible right margin yields to hard particles when it closes against the less yielding left valve edge, and thereby reduces the possibility of fracture and chipping of the valves.

Granted a poor replacement for nacre because of its suggested inferior mechanical properties (Waller 1976), foliated calcite must still possess some qualities that contribute to the success of oyster species. Various hypotheses have been offered: (1) low expenditure of energy required for secretion and rapid deposition of folia (Taylor and Layman 1972), (2) service as a reservoir for metabolic excretory by-products (Degens 1976), (3) evolutionary advantage of the low density characteristic of folia, (4) volumetric economy of folial calcite (i.e., calcite fills a larger volume per mole than aragonite, Stenzel 1964), and (5) localization of folial fractures resulting from impacts (Carter 1980a). The significance of these possible advantages in evolution may become clearer as shell microstructural biology becomes better understood.

There is no obvious correlation between the content of organic matrix in molluscan valves and shell strength, yet shell possesses a higher microhardness than inorganic calcite or aragonite alone (Taylor and Layman 1972). The composite nature of shell (Wainwright 1969) contributes the qualities of microhardness and pliability not present in nonbiogenic polymorphs of calcium carbonate.

Environmental Microstructural Effects

Although environmental conditions can have a marked influence on the gross morphology of the valves of *C. virginica* (Medcof and Kerswill 1965; Galtsoff 1964; Wilbur 1964, 1972; Stenzel 1971; Ruddy et al. 1975; Palmer and Carriker 1979; Seilacher et al. 1985), little is known about the influence on shell microstructure of environmentally related modifications of shell secretory mechanisms. Palmer and Carriker (1979) discovered that prisms of the right valve are significantly larger in oysters maintained in the summer in a natural estuary where growing conditions are good, than those of oysters held under laboratory cultural conditions. Oysters in the estuary deposited about two micro-growth annuli in the ligostracum of the hinge per day, whereas cultured oysters formed four to five per day. Wada (1961) studying nacre formation in the pearl "oyster," observed seasonal variations in the size of arago-

nitic lamellae. Lutz (1976), Lutz and Rhoads (1977), and Carter (1980a) examined microstructural changes in other bivalve species. The effect of changing seasons on microstructures of the shell of *C. virginica* has not been investigated.

Environmentally associated microstructural studies of bivalves have emphasized primarily the effect of the total composite environment (Galtsoff 1964) or of seasonal influences (Wada 1961; Lutz 1976). The effect of single isolated factors (while other factors in the environment are held more or less constant) on the size, shape, proportion of organic matrix to mineral crystals, substructure, and chemistry of microstructures has not been attempted. Prisms of the right valve of *C. virginica*, because of their relatively large size and thick organic envelope, would be good subjects for study. Among physical factors, the influence of temperature, salinity, and light are obvious initial variables for examination. Important chemical factors to be explored could include a range of concentrations of calcium, magnesium, manganese, and strontium, taking advantage of the fact that oysters have the capacity to concentrate many elements in their shells from seawater (Carriker et al. 1980b, 1982). Because the concentration of a number of different elements differs in the different major microstructural regions of the valves (Carriker et al. 1991), studies should be limited to one type of microstructure at a time.

Genetic Modification

Though formation of microstructures (previous section and Prezant et al. 1988) and macrostructures of the shell of *C. virginica* can be modified by environmental factors, primary control of the mineralogy, micromorphology, and macromorphology of the shell is undoubtedly under genetic control (Kennedy et al. 1969; Carter 1980a; Bougrier et al. 1985). The fact that this species has perhaps 10,000 genes on its 10 chromosomal pairs, that recombination of genes occurs at crossing over during meioses in both males and females, that there is random assortment of chromosomes in gametogenesis, and that the species is characterized by wide physiological variation in its lengthy geographic distribution from the Gulf of St. Lawrence into the tropics, makes it a viable animal for selective inbreeding, hybridization (Longwell 1976), and probably genetic engineering (Pimentel et al. 1989). Newkirk (1980b) noted that there is a clear potential for rapid response to carefully controlled selection among populations of commercial species of bivalves. For additional discussion of these matters, see Gaffney in Chapter 11, Longwell and Stiles in Chapter 12, and Newkirk in Chapter 18.

Probably because the shell of *C. virginica* is already of commercial value in its present form, and has never been considered to possess marketable esthetic characteristics, no serious attempts have been made to improve its shape or pigmentation by genetic experimentation. From what has been said, it is likely that the potential for modification of the valves by genetic manipulation for the purpose of enhancing cultivation, and facilitating handling and commercial distribution is good, and seriously worth considering.

In this context, Bougrier et al. (1985) crossed individuals of *C. gigas* possessing smooth valves with those characterized by wavy valves; regardless of the male type of shell, the resulting progeny exhibited the mother's valve type. As a result of extensive investigations aimed at genetically improving shell and pearls of *Pinctada fucata*, Wada (1984, 1986) proposed that breeding programs be instituted for the stable production of superior pearls. And Newkirk (1980a) confirmed, through a number of crosses, that variation in shell color of *Mytilus edulis* is determined predominantly by a simple genetic mechanism.

Breakage and Regeneration

Individuals of *C. virginica* whose valve edges have been broken off compensate for this loss by forming new shell at a rate at least as rapid as that of undamaged oysters living under identical conditions (Loosanoff and Nomejko 1955). Stimuli caused by breakage of the shell result in contraction of the muscles of the mantle. Of the possible stimuli initiating the sequence of regeneration of the shell, mechanical irrita-

tion of mantle cells in the region of the broken shell is probably the most obvious (Wilbur 1964, 1973). In this regard, Bahr and Hillman (1967) observed in experiments on effects on gametogenesis of shell damage in *C. virginica* that individuals with repeatedly filed valve edges exhibited a slightly higher degree of development of gonads and were more likely to be males than those individuals whose valves went unfiled. This interesting observation has not been pursued further.

Wilbur (1964) showed by x-ray diffraction that the structure of the organic matrix of regenerated shell of *C. virginica* is of the β-keratin type in contrast to the α-keratin type in normally formed shell, and that the shell margin, which normally consists only of calcite, contains both calcite and aragonite during regeneration. Divergence from normal calcite could be due to the different composition of the matrix.

Recently, Carriker and Prezant (unpublished) examined ultrastructurally the effect of filing about 5 to 8 mm of shell from the ventral margin of young oysters (5 to 6 cm high) growing rapidly in a local estuary. Radial sections (umbo to posterior margin) were sawed through normal and new shell; some polished sections were etched with dilute HCl, and others with dilute bleach. New prismatic shell laid down in both right and left valves after filing was within the range of form and size of prisms normally occurring in shell margins (Palmer and Carriker 1979) and appeared normal after a narrow zone of disturbance. This zone in both valves consisted of a layer of four to five distorted, fragile prisms of irregular form and size, preceded by a layer of approximately similar width of variably sized minute prisms immediately adjacent to the file line. Beyond the zone of disturbance, which contained a larger quantity of organic matter than normal prismatic shell (as shown by dissolution with bleach), prisms soon normalized (Fig. 153), assuming the typical honeycomb-like pattern with thick interprismatic conchiolinal walls (Carriker et al. 1980a). X-ray diffraction (Carriker and Palmer 1979b) of representative pieces of the new shell showed the polymorph to be calcite. The absence of aragonite as observed by Wilbur (1964) is unexplained.

Shell Anomalies

Morphologically, shells of *C. virginica* are highly plastic, varying in shape, thickness, and sculpture. Individuals readily adapt to a variety of solid substrata, often faithfully reproducing the sculpture of irregularly shaped solid objects to which they happen to attach.

Galtsoff (1969) defined anomalies as deviations from a common type in excess of normal variations; they are not harmful and could be adaptive. Anomalies discovered by Galtsoff in his study of some 8,000 oyster shells from coastal waters of the United States included: (1) changes in direction of the principal axis of growth, (2) bifurcation of the valves in which two principal axes of growth appear, (3) stair-shaped valves resulting from periodic interruptions in rate of growth of shell along its periphery and continuous mineralization of older parts, and (4) excessive mineralization of the right valve, resulting in right valves considerably thicker than left ones.

Pearl-like formations on the interior surfaces of valves, which Galtsoff (1969) termed "malformations" because normal physiological activities can be hindered by excessively large formations, rarely occur in *C. virginica*. As a matter of fact, edible oysters produce no true pearls at all, but only globular, lusterless nodules or concretions of calcite. The largest nodule Galtsoff (1969) ever found measured 2.5 × 3.0 × 1.8 cm and occupied an extensive area of the valve near the muscle scar in an oyster only 8 cm high. The reason that true pearls are not formed in Ostreids is that species in this genus are incapable of secreting nacre. "Pearls" form, as Galtsoff (1964) reported, but they are composed of foliated calcite, not nacre, and lack the pleochroic quality sought in nacreous pearls (Waller, pers. comm.).

Occasionally notes are published on unusual cases of duplication ("mimicking") of substratal sculpture in the shell. These include replication of the sculpture of a snail shell (*Trochus maculatus*) by *O. edulis* (Smith 1878), grooved surface of a phonograph record on both valves (Gregg (1948), and the pattern of a golf ball (Golf Magazine 1978) by *C. virginia*, and lettering on an automobile tire cultch by *C. rhizophorae* (Littlewood 1984, 1988).

Xenomorphic growth of valves is explained by the fact that the attached valve reproduces the microtopography of the substratum and the free valve conforms to the pattern of the attached valve (Gregg 1948; Waller, pers. comm.). The extent of involvement of mechanoreceptors on the margins of the mantle in the replication process is not known. From a pragmatic point of view, Littlewood's (1984, 1988) observations on imprinting point to a ready method for labelling cultivated oysters.

IN PROSPECT

Synthesis of the literature on the Ostreidae during preparation of this chapter has demonstrated the past and important potential value of the eastern oyster in the investigation of many of the unsolved questions on bivalve shell formation and mineralization — approached interdisciplinarily across micromorphological, mineralogical, crystallographic, physiological, biochemical, genetic, and developmental disciplines. For example, sexually mature individuals readily spawn in the laboratory. Planktonic larval stages are relatively short-lived and, like young dissoconchs, are easily cultured under closed laboratory conditions. Spat can be raised as either attached or as cultchless individuals. Mineralogical and microstructural regions of the valves are distinct and identified without difficulty. The periostracal sheet from the periostracal groove is free from valve margins except, possibly, when shell deposition is taking place. Dissoconchs of all ages tolerate and recover from perforation or removal of parts of the valves for experimental purposes.

Many appealing important problems on the multifaceted biology of the shell of *C. virginica* await deciphering. The following, by way of examples, are a few of the more provocative.

In embryos the process of initial shell formation has not been investigated. Is it the same as that in the few other species of marine bivalves that have been studied? Published reports indicate that valves of all bivalve larvae examined to date are predominantly aragonitic. Might not early mineralization in embryos be a propitious stage at which to experiment on transformation of aragonite to other polymorphs of calcium carbonate? Discovery of the fasciole in the left valve of prodissoconch II larvae has stimulated considerable interest. What is the function of the postanal ciliary tuft, and how does its activity result in the cornucopially-shaped fasciole? Does this tuft have some function in setting activities?

Extreme thinness of the periostracum of prodissoconch valves probably evolved as one of the complex of larval features contributing to lightness-favoring planktonic existence. But formation of periostracum during the veliger stage has not yet been examined; nevertheless, study of the process, especially during metamorphosis, could be instructive. Although it is questionable if the periostracum is required to protect the mineralized valves of the veliger from erosion, inasmuch as duration of the larval stage is so brief, it is conceivable that periostracal development in the veliger serves simply as a progenitor for periostracal formation in the presumptive dissoconch. Determination of change in microstructure and physiology/biochemistry, if any, of epithelial cells in the periostracal groove and of biochemical composition of the periostracal sheet during transition from pediveliger to spat might reveal information on this interesting metamorphic question. Because of the probable vulnerability of the shell formation process in early veligers, prodissoconch valves could be important microstructural, and perhaps chemical indicators, of beyond-the-normal extremes of physical and chemical factors (such as minor and trace heavy metals, hydrocarbons, suspensions of particles, salinity, and temperature).

An uncommon aspect of metamorphosis of prodissoconch II to the early dissoconch spat is the abrupt transformation of the shell from primarily homogeneous-aragonite to prismatic-calcite. Of lively interest is the subtle cellular differentiation that must occur in mantle margins at metamorphosis. Are microstructural and microchemical changes evident at the cellular and molecular level by modern scientific technology? Unfortunately, knowledge of these metamorphic changes is essentially nonexistent.

The shape, sculpture, and pigmentation of dissoconch valves of *C. virginica* can vary conspicuously

within populations and in different habitats, suggesting a high degree of plasticity of the shell-forming function in response to environmental and genetic factors. To what extent macro- and microarchitecture, surface sculpture, type of polymorph, and shell color are environmentally or genetically controlled is still a vexing question. Insights can be had through controlled single-variable ecological studies and genetic manipulation. In this regard, it would be commercially desirable to develop oysters with a proportionately larger shell cavity and a thicker, stronger periostracum (more resistant to erosion and inroads of burrowing organisms) than possessed by existing oysters. Many suggestions have been made in the past of materials to coat oysters for pest prevention, but none of these has ever proved practical.

Periostracal formation has been studied in recently set *C. virginica*, but not in adult individuals. The fact that periostracum of the left valve serves the function of adhesion to hard substrata in juveniles, whereas that of the right valve is freely exposed to the environment, suggests an inquiry on how periostracal formation and its chemistry differ in the periostracal grooves of left and right mantle folds. It is conceivable that the chemistry of the periostracum is altered once the left valve grows away from the substratum.

The type of association between the surface of mantle cells and that of shell microstructural units apposed to them in the extrapallial space is a fascinating, but totally unsolved, complex problem. Of even more interest is the specific spatial relationship, if any, between individual mantle cells and the individual matrix and mineral components of each shell microstructural unit. In species of bivalves like *Mytilus edulis* in which mantle margins remain affixed to valve edges, it is easy to visualize an intimate and possibly fixed spatial association between cells and microstructural units bathed in the extrapallial fluid. But in species like *C. virginica* in which both mantle lobes are mobile and frequently extend beyond and withdraw within valve margins, there can be no such stationary spatial association. Just because of this, the oyster should be an important adjunct experimental bivalve to *M. edulis* in the continuing study of microstructural development in the extrapallial space of bivalves.

Formation and function of chalky shell in the valves of dissoconch oysters is a veritable enigma. Nothing is known about its functional role as an exoskeletonous structure. Knowledge of the manner of formation of the blades and leaflets that constitute this "spongy" type of shell structure would undoubtedly contribute to the explanation of shell formation in molluscs in general.

The biology and chemistry of incorporation of trace and minor elements in the shell of bivalves is an increasingly popular subject of research, heightened by the fact that many bivalve species can concentrate a number of elements in their shells many times the concentration in surrounding ambient seawater. Some of these species, including oysters, thus theoretically and pragmatically are becoming important monitors for heavy metals in the aquatic environment. A fundamental question to be resolved in this regard, though, is whether such elements are incorporated in the valves as adventitious contaminants, or are substituted for calcium in the inorganic fraction of the mineral cores. A further pressing question relates to whether the organic matrix surrounding each mineral core is also taking up these elements.

A wide variety of particles remains suspended in the aquatic habitat of bivalves, especially that of estuarine species like the eastern oyster. In view of this, we should ask whether clays and possibly other particles (both organic and inorganic) are involved in any way in the basic chemical process of shell formation and mineralization. Experimentation using oysters, with their open mantle-margin system, would be a rewarding place to start, comparing shell developed by oysters living in highly filtered seawater and that formed in normally turbid estuarine waters.

Yonge (1960), writing about *O. edulis*, observed that "Probably no marine animals — and certainly no marine invertebrates — have been so intensively studied, and we are no more than at the beginning of intimate knowledge about them." As abundantly demonstrated in this chapter, I can now add that considerable progress has been made beyond "the beginning of intimate knowledge" of the shell of *C. vir-*

ginica. Looking ahead, though, I suggest that much yet remains to be deciphered in such areas of shell formation as microstructure, biomineralogy, crystallography, physiology, biochemistry, genetics, and biomechanics in planktonic, metamorphosing, early and mature individuals, and the effect of environmental factors on the processes involved.

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