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ULTRASTRUCTURAL MORPHOGENESIS OF PRODISSOCONCH AND EARLY DISSOCONCH VALVES OF THE OYSTER *CRASSOSTREA VIRGINICA*

Melbourne R. Carriker and Robert E. Palmer

COLLEGE OF MARINE STUDIES, UNIVERSITY OF DELAWARE, LEWES 19958

ABSTRACT

Results are reported on an investigation with the scanning electron microscope on the ultrastructure of valves of the American oyster, Crassostrea virginica *(Gmelin), emphasizing normal developmental ultramorphology of prodissoconchs I and II and newly set dissoconchs raised under favorable hatchery conditions. Developmental anatomical features described by early malacologists by light microscopy are reviewed, and structures visible only by scanning electron microscopy are described for the first time. Terms for larval stages are defined. Mineralogical determinations confirmed earlier reports that prodissoconch II valves of this species are aragonitic, and showed for the first time that valves of prodissoconch I are also aragonitic. Development of the following structures was examined: prodissoconch I and II valves, punctuate-stellate pattern on exterior of prodissoconch I valves, valve edges, prodissoconch I-II transitional band, provinculum, terminal and denticular hinge teeth, larval and spat ligament, resilium, fasciole and notch, prodissoconch II — dissoconch metamorphic juncture, adductor muscle scar, granular homogeneous shell, prismatic calcite of both dissoconch valves, and terraced and chalky foliated calcite. Transition from homogeneous aragonite of prodissoconch to foliated and prismatic structure of dissoconch is sharp. Larval teeth of prodissoconch II are obliterated by deposition of dissoconch shell, and the pivotal axis shifts anterior to larval umbones where a prominent inner ligament forms during prodissoconch stage and subsequently develops into ligamental resilium of spat.*

INTRODUCTION

Several investigators have described the shell of early stages of the American oyster, *Crassostrea virginica* (Gmelin), by light microscopy. This research is reviewed by Rees (1950), Carriker (1951), Stenzel (1971), and Dinamani (1976). With the exception of one micrograph by Dinamani (1976) of the hinge of a larva 250 μm long, there are no published reports on the ultrastructure of prodissoconch and early dissoconch valves of this species. Ultrastructural investigations of valves of early stages of other species of oysters are similarly limited (Dinamani, 1976). This is surprising in view of the economic importance of oysters and the proliferation of papers on the ultrastructure of valves of other molluscan larvae (see, for example, Robertson, 1971; Thiriot-Quievreaux, 1972; Giusti, 1973; Turner and Boyle, 1974; Richter and Thorson, 1975; Boyle and Turner, 1976; LePennec and Masson, 1976; Waller, 1976; and Togo, 1977).

The current emphasis on culture of commercial species of oysters in various types of controlled systems (Price et al., 1976), and the importance of information on normal shell ultramorphology as a basis for comparison with anatomical and functional anomalies which may occur in mariculture, prompted us to undertake the present study. There were also other considerations. For example, the shape, thickness, strength, coloration, and ornamentation of oyster dissoconch valves reflect environmental conditions (Galtsoff, 1964; Palmer and Carriker, 1979). Whether the ultrastructure of valves of prodissoconchs and early dissoconchs also reflects growing conditions has yet to be determined; the present study provides a background and stimulus for such investigations in the future. Furthermore, the remarkable capacity of bivalves to concentrate chemical elements in their soft and calcareous tissues is well documented (Galtsoff, 1964; Milliman, 1974; Frazier, 1975, 1976). Among others, factors involved in incorporation of chemical elements in shell include conditions of crystal growth, types of crystals, and differences in shell layers and other structures (Wilbur, 1972; 1976). It is clear that analysis of these factors also depends upon a knowledge of shell ultrastructure.

The present publication is the first reporting results of a comprehensive investigation with the scanning electron microscope (SEM) of the ultrastructure of the valves of *Crassostrea virginica* (Gmelin), and concentrates on the normal developmental ultramorphology of the valves of prodissoconchs I and II and newly set dissoconchs raised under favorable hatchery conditions. Developmental features described by early malacologists by light microscopy are reviewed and new structures not visible with the light microscope are described for the first time.

MATERIALS AND METHODS

Oysters.

Larvae and spat of *Crassostrea virginica* were raised in the maricultural facility of the College of Marine Studies, University of Delaware in Lewes, Delaware (Pruder et al., 1976). Brood stock were collected in the Broadkill River near Lewes, and conditioned and spawned (Maurer and Price, 1967) in the hatchery of the maricultural facility. Four different larval broods, from mixed parents, were reared in 400-ℓ conical tanks from November 1977 to June 1978. Water in the larval tanks was changed every second day with seawater of an approximate salinity of 30 ‰ collected at high tide at Indian River Inlet, Delaware. Temperature of the seawater in larval tanks ranged from 27 to 28°C. Larvae were fed an algal mixture of approximately 60% *Thalassiosira pseudonana* 3H Hasle et Heimdal and 40% *Isochrysis galbana* Parke at an initial concentration of roughly 50,000 cells ml^{-1}. Pediveligers were allowed to set on sheets of mylar film to facilitate study of the relationship of the attached left valve to the substratum.

Preparation of valves for scanning electron microscopy (SEM).

(a) Cleaning. Live prodissoconch I larvae were placed in 2% clorox (0.1% sodium hypochlorite; clorox = 5.25% sodium hypochlorite) alkalized with NaOH to a pH of approximately 10 (S.E. Siddall and R.A. Lutz, personal communication) and left in the solution for 10 min. During this time soft tissues were dissolved and valves were cleaned. However, about 30 min were required in the solution before the larval ligament dissolved sufficiently to allow valves to separate. Prodissoconch II and early dissoconch valves were more difficult to clean and separate and required immersion in a 5% alkalized solution of clorox (0.26% sodium hypochlorite) for 40 min to 2 hr. Sonication in the clorox solution and raising the temperature of the solution to about 60°C accelerated dissolution of soft tissues and the larval ligament, but also tended to erode the valves. Larvae and spat were flushed onto a fine stainless steel screen which was dipped directly into solutions. The screen, immersed in a finger bowl of sodium hypochlorite solution, was placed under a binocular microscope, where progress of digestion of soft tissues and separation of valves was followed. Freezing live larvae and spat in seawater for several days not only maintained the valves in good condition for later study, but also killed the larvae in a gaping condition which hastened digestion of soft tissues by sodium hypochlorite. After cleaning, frozen larvae gaped more widely than those not frozen.

For study of the larval ligament, larvae which

had been frozen in seawater were left in fresh seawater at room temperature to allow microbiological activity to clean out soft tissues. This procedure removed soft tissues, but did not appreciably injure the hinge and ligament. Loose mucoid films from the valves were removed by dipping the valves in 2% clorox for about 15 sec and then rinsing them thoroughly in tap water (weakly acidic).

After rinsing, valves were pipetted into small concave dishes, excess water was drawn off, and the dishes were placed in an oven at 60°C to dry for several days. Valves were subsequently stored in vacuum desiccators in the small dishes.

Initial optical search for the ligament of pediveligers was done by staining valves, which had been cleaned microbiologically, with full strength Magalhaes' (1948) stain (equal parts 1% aqueous crystal violet and 0.3% aqueous basic fuchsin) for about 15 min. The ligament stained intensely, whereas valves themselves took up the stain only slightly. Intact gaping valves of the microbiologically cleaned pediveligers were then rinsed and dried for mounting.

(b) Mounting on SEM stubs. SEM aluminum pin stubs were cleaned in acetone, and valves of early stage larvae were placed directly on the surface of the stub in a drop of tap water. Excess water was blotted off with absorbent paper, and residual water was evaporated under the lamp of the microscope. The stub preparation was then thoroughly dried in an oven at 60°C for at least a day.

Late stage larval valves, dried in an oven, were scattered onto the surface of a 1:1 mixture of silver paint and clear fingernail polish applied to a SEM stub. The paint-polish mixture was slightly tacky but not soft enough to let the valves sink into the conductive adhesive. Progress of hardening of the mixture was followed under the binocular microscope by touching the surface with the tip of a fine needle. Nail polish reduced the tendency of the silver paint to flow onto the surface of specimens and did not contaminate the SEM column.

For the study of the ligament, larvae were mounted on double adhesive tape on aluminum pin stubs.

Small pieces of mylar film containing cleaned attached spat were cut from the setting sheet and glued to stubs with silver paint. Paint was also applied to the mylar film around each spat to reduce charging. Some right valves were separated from attached left valves and mounted exterior side down.

Mounting oyster valves on double adhesive tape was considerably easier than on the paint-polish mixture, but unfortunately the tape tends to contaminate the SEM column and impairs resolution so it was used only when other mounting media were unsuitable.

Fractured sections of larval valves were prepared in two ways. In the first, valves were crushed slightly between a microscope slide and cover slip and then the valve fragments were scattered onto the hardening surface of silver paint-nail polish. In the second method, a fragment of cover slip was pressed gently onto the surface of a stub on which valves had been mounted until the desired amount of breakage had been achieved.

Before examination in the SEM, specimens and stubs wers coated in vacuum with two or more coats of carbon and gold (400-600A). Heavy coats were necessary to reduce charging, especially with prodissoconch I valves, which appear to contain a high proportion of organic material.

(c) Identification of crystal type. Cleaned valves of prodissoconch I and II were analyzed by X-ray diffraction by mounting entire valves on the revolving spindle of a Gandolfi camera, or by staining valves with Feigel's solution (Milliman, 1974). Aragonite turns black in the solution, and calcite remains colorless.

ULTRASTRUCTURE AND MINERALOGY OF VALVES OF LARVAL AND EARLY DISSOCONCH STAGES

Dimensions and Terminology.

Sibling oyster larvae maintained under identical conditions of culture grow at widely different rates and matamorphose at different times (Loosanoff and Davis, 1963; Newkirk et al., 1977; observations in maricultural facility, College of Marine Studies). For this reason, in the following account we will identify larval stages primarily by form and size rather than by age. At normal summer temperatures in the mid-Atlantic area oyster larvae generally start setting about two weeks

after fertilization, although specific rate of development may vary with temperature.

Considerable variation has been reported on the maximal larval size of *Crassostrea virginica* along the Atlantic seaboard, there being a tendency for larvae to set at a larger size in northern than southern latitudes. A range of maximal length of 248 to 400 μm has been recorded by various investigators (Carriker, 1951).

Dimensions of larval valves of *Crassostrea virginica* during development from the first shelled stage to setting have been recorded by Chanley and Andrews (1971) for larvae from Virginian estuaries as follows: length, 60 to 350 μm; height, initially 10 μm less than length, increasing to equal length at 90-100 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 50μm long. Metamorphosis occurs at a length ranging from 310 to 350 μm. Valves are round at a length of 80 to 100 μm, and become knobby at 85 to 125 μm (see also careful work of Loosanoff and Davis, 1963; Galtsoff, 1964; Forbes, 1967).

Some confusion exists on terms used for larval stages of bivalves (Carriker, 1961; Chanley and Andrews, 1971; Stenzel, 1971; and others). Because of this we describe briefly terms employed in this paper with reference to those used by other investigators.

Dimensions of height, length, and width of shelled larvae are defined as follows (Galtsoff, 1964): *height*, distance between umbo and ventrum; *length*, anteroposterior distance; *width*, maximal distance between exterior surfaces of the right and left valves (Chanley and Andrews, 1971, used the term depth for width).

Prodissoconch I. First shelled stage developing from nonshelled veliger. It bears thin, uniform, smooth, transparent valves secreted by the shell gland and mantle epithelium (Kume and Dan, 1968). The D-shaped or straight-hinged veliger refers to the shape at both prodissoconch I and early prodissoconch II valves. Stenzel (1971) refers to prodissoconch I as the phylembryo or protostracum veliger.

Prodissoconch II. New shell is added by the mantle both around the edges of and inside prodissoconch I valves. The new shell bears concentric growth striae which clearly distinguish it from shell of prodissoconch I. From a round shape, the valves soon become umboned and asymmetrical, the left larger than the right, and the anterior end of the valves becomes more pointed than the posterior end. The umbones point posteriorly. Stenzel (1971) refers to this stage as the prodissoconch veliger, and omits the terms prodissoconch I and II.

Pediveliger. This is the prodissoconch II stage in which velum, foot, and eyes are fully developed, and the bivalve is preparing to set.

Dissoconch. As soon as settlement and matamorphosis occur, the mantle of the spat (juvenile oyster) initiates secretion of the adult form of shell structure. A sharp transitional line of demarcation, the *metamorphic line*, is clearly evident at the boundary between prodissoconch II and the early dissoconch.

Confusion also exists on the terminology for regions and ultrastructural units of the shell of oysters and other bivalves (Tsujii et al., 1958; Watabe et al., 1958; Watabe and Wilbur, 1961; Wada, 1963; Watabe, 1965; Taylor et al., 1969; Kobayashi, 1971; Waller, 1975; Wilbur, 1976). We have adopted the terminology proposed by Taylor et al. (1969) for *Crassostrea virginica* as follows:

The exterior of dissoconch valves is covered by a very thin organic *periostracum*. This lies over the thin layer of *prismatic calcite* visible externally near valve margins (primarily of the right valve) as overlapping layers of *imbricated scales* ("spurs" of Nakahara and Bevelander, 1971). On older portions of valves the prismatic structure is generally worn off. Prismatic calcite is composed of individual *prisms*, each a mineral core within an organic envelope. The bulk of the valves is composed of *foliated calcite* (the calcitostracum, subnacreous, or nacreous layer of several authors). This region consists of fine sheets, or *folia* (or laminae), grouped into larger *lenticular folia*. Individual folia are composed of small elongate *laths* (the tablets or lamellae of other authors), joined together by organic matrix, and on the interior surface of valves frequently resemble tiles on a roof. Lenses of *chalky shell*, consisting of laths ar-

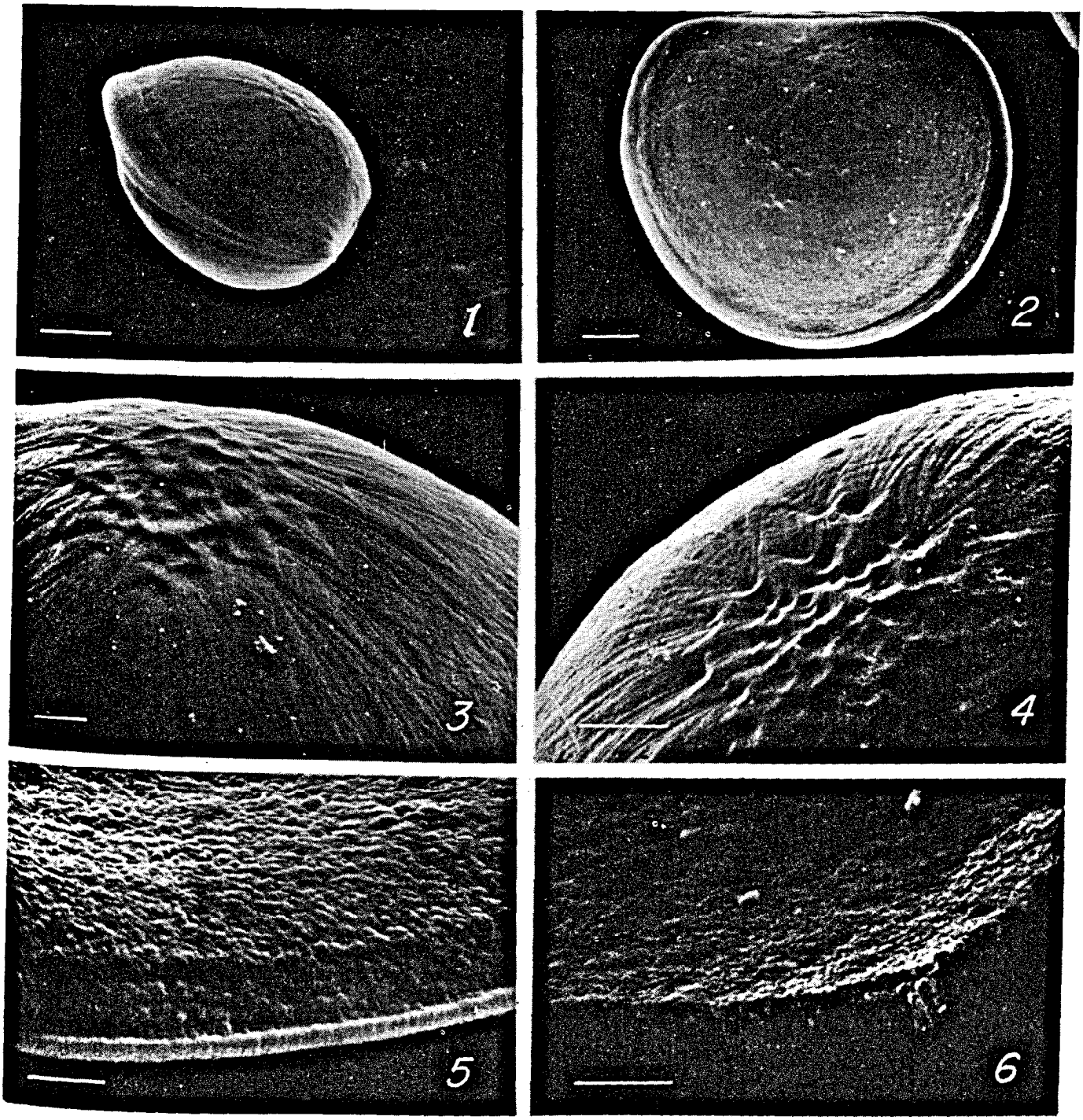


FIGURE 1. *Prodissoconch I, ventrolateral view. Rim of prodissoconch II forming. 2% clorox 6 min. Scale bar = 20 μm.*

FIGURE 2. *Prodissoconch I. Valve view. Narrow rim of prodissoconch II shell forming. Treated in 2% clorox for 10 min. Scale bar = 10 μm.*

FIGURE 3. *Prodissoconch I. Punctate- stellate pattern on one valve. 2% clorox 75 min. Scale bar = 5 μm.*

FIGURE 4. *Prodissoconch I. Punctate- stellate pattern on one valve, higher magnification than Figure 3. 5% clorox 23 min, and 3 min sonication. Scale bar = 5 μm.*

FIGURE 5. *Prodissoconch I-II. Interior view of edge of valve showing thin outer layer (0.5 μm thick) of prodissoconch I valve; inner surface of granular shell units of prodissoconch II valve. 2% clorox 10 min. Scale bar = 2 μm.*

FIGURE 6. *Prodissoconch I-II. Fractured section of valve and interior of valve. Fracture 1 μm thick. Scale bar = 2 μm.*

ranged irregularly in a spongy pattern, occur frequently in foliated structure.

Mineralogy.
Mineralogical determinations confirmed Stenzel's (1964) report that prodissoconch II valves of larvae of *Crassostrea virginica* are aragonitic, and demonstrated for the first time that valves of prodissoconch I are also aragonitic.

Prodissoconch I.
The D-shaped, straight-hinged outline of prodissoconch I valves of *Crassostrea virginica*, when seen in side view by scanning electron microscopy (Figure 2), resembles the optical illustrations of this stage prepared by several investigators (Carriker, 1951). Not so evident in optical illustrations, however, is the considerable width of larvae even at this early stage. Rather than a thin, flattened wafer, the closed valves resemble an oblong, flattened globe (Figure 1).

Shortly after prodissoconch I valves are formed, mantle edges and mantle surfaces begin secretion of prodissoconch II. Along the margin of the valves the new shell takes the form at first of a narrow, transitional band, easily distinguished from prodissoconch I shell by its smooth exterior surface (Figures 1 and 2). This is followed by shell which is thicker and begins to show fine concentric growth striae (Figure 1).

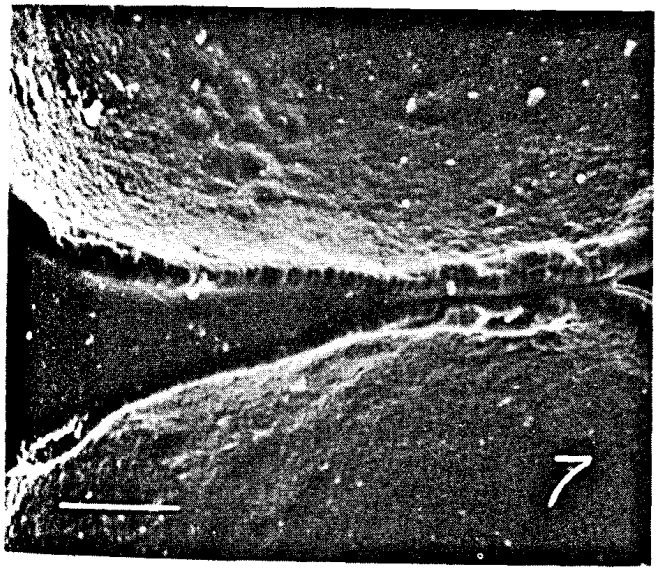
Most conspicuous, however, and not described before in oysters, is the striking punctate-stellate pattern on the surface of the center of each prodissoconch I valve (Figure 1-4). The center of the pattern consists of shallow punctate marks ranging in diameter from about 0.5 to 3 μm (Fig. 3, 4). These merge peripherally with acutely pointed triangles which radiate and overlap over most of each valve. Concentric striae, about 0.3 μm apart, blend with the radial lines from the apexes of the triangles (Figure 4). The punctate portion of the pattern probably overlies the embryonic shell gland and represents initial mineralization of conchiolin (Trueman, 1951; Kume and Dan, 1968). The radiating triangles probably reflect activity of that part of the shell-secreting mantle epithelium peripheral to the shell gland.

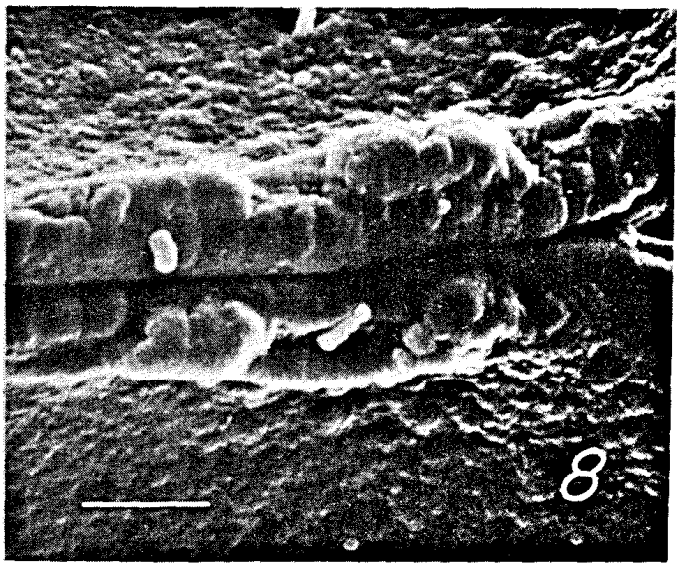
The edge of the valve of a larva 16 hours old seen from the interior shows a distinct outer layer about 0.5 μm thick (Figure 5). Inside this the shell consists of granular shell units ranging in diameter from 0.1 to 0.5 μm in surface view. A fractured section of a valve of a larva 19 hours old (Figure 6) is about 1 μm thick and is composed of an exterior layer approximately 0.7 μm thick (probably prodissoconch I) and an inner layer (probably prodissoconch II) of granular shell units, about 0.3 μm thick, probably partly exposed by action of the clorox. Granular units in the fractured section range in diameter from about 0.05 to 0.1 μm.

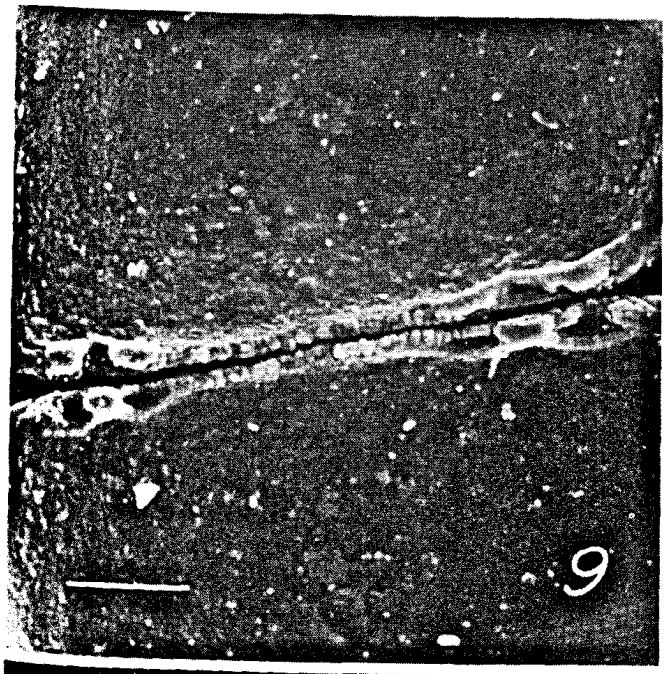
As valves of prodissoconch I grow, the provinculum (the primitive hinge apparatus, Rees, 1950; Galtsoff, 1964) thickens, and rudimentary swellings on the anterior and posterior ends develop into small, well-defined interlocking provincular teeth (Figures 7-10). By very early prodissoconch II stages, these have developed into one large taxodont tooth on each end of the provinculum of one valve, and two large taxodont teeth on each end of the other valve, with corresponding interlocking sockets in opposing valves (Figure 10). Length of the provinculum is about 45 μm (Figures 7 and 9). The space between the large side teeth is at first covered with shallow corrugations (Figures 7 and 9); these soon develop into minute denticles. Dentition is thus essentially heterodont (Dinamani, 1976). Length of each side tooth ranges from 4 to 5 μm. The granular nature of the shell units in the valves, and especially around the provinculum, is clearly shown in Figure 10. Largest granules measure about 0.4 μm.

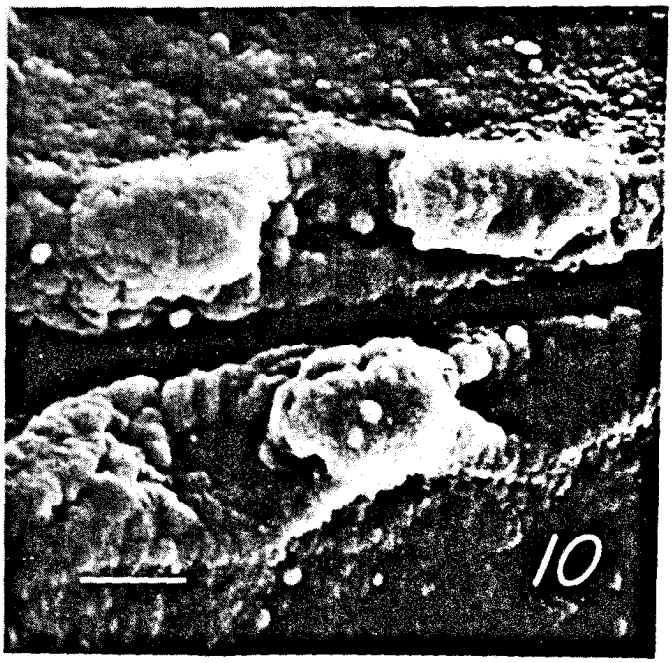
Prodissoconch II.
As larvae increase in size, their valves become unequal, the left valve (the future attached valve of the spat) growing considerably wider than the right, and the left umbo projecting farther from the provinculum than the right one (Figures 14 and 17). Umbones grow and point posteriorly as valves develop (Figures 16 and 17). The prodissoconch I-II transitional band becomes conspicuous, particularly in side view (Figures 14 and 15) and ranges in width from about 7 to 8 μm. The punctate-stellate pattern on the exterior surface of the valves of prodissoconch I persists through prodissoconch II (Figure 13).

As development proceeds, umbones come close together medially (Figure 17), preventing valves from opening widely. A laterodorsoanterior view









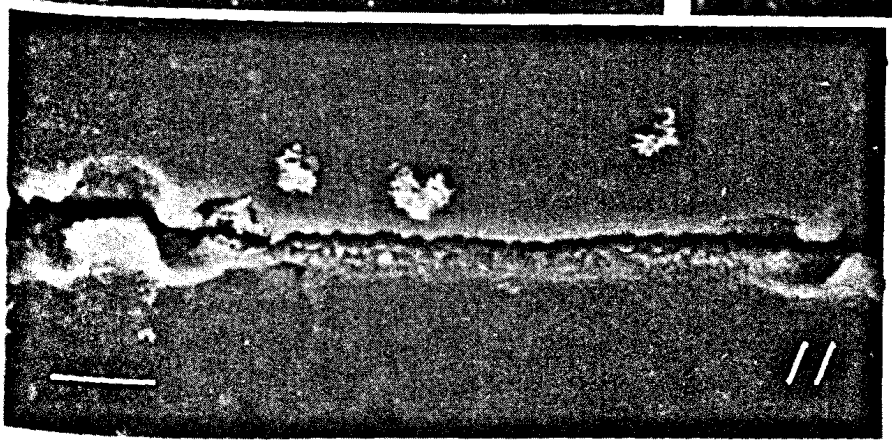


FIGURE 7. *Prodissoconch II. Provinculum, 45 μm long, of matching pair of valves. Taxodont terminal teeth forming at right end. 2% clorox 10 min. Scale bar = 10 μm.*

FIGURE 8. *Higher magnification of terminal teeth in provinculum of valves in Figure 7. Tooth on upper valve fits in socket of lower valve. Scale bar = 3 μm.*

FIGURE 9. *Prodissoconch II. Provinculum slightly more developed than that in Figures 7 and 8. Denticles starting to form between terminal teeth. 2% clorox 10 min. Scale bar = 10 μm.*

FIGURE 10. *Large terminal teeth of left end of provinculum shown in Figure 9. Scale bar = 2 μm.*

FIGURE 11. *Prodissoconch II. Provinculum. Cleaned microbiologically. No larval ligament evident. Scale bar = 5 μm.*

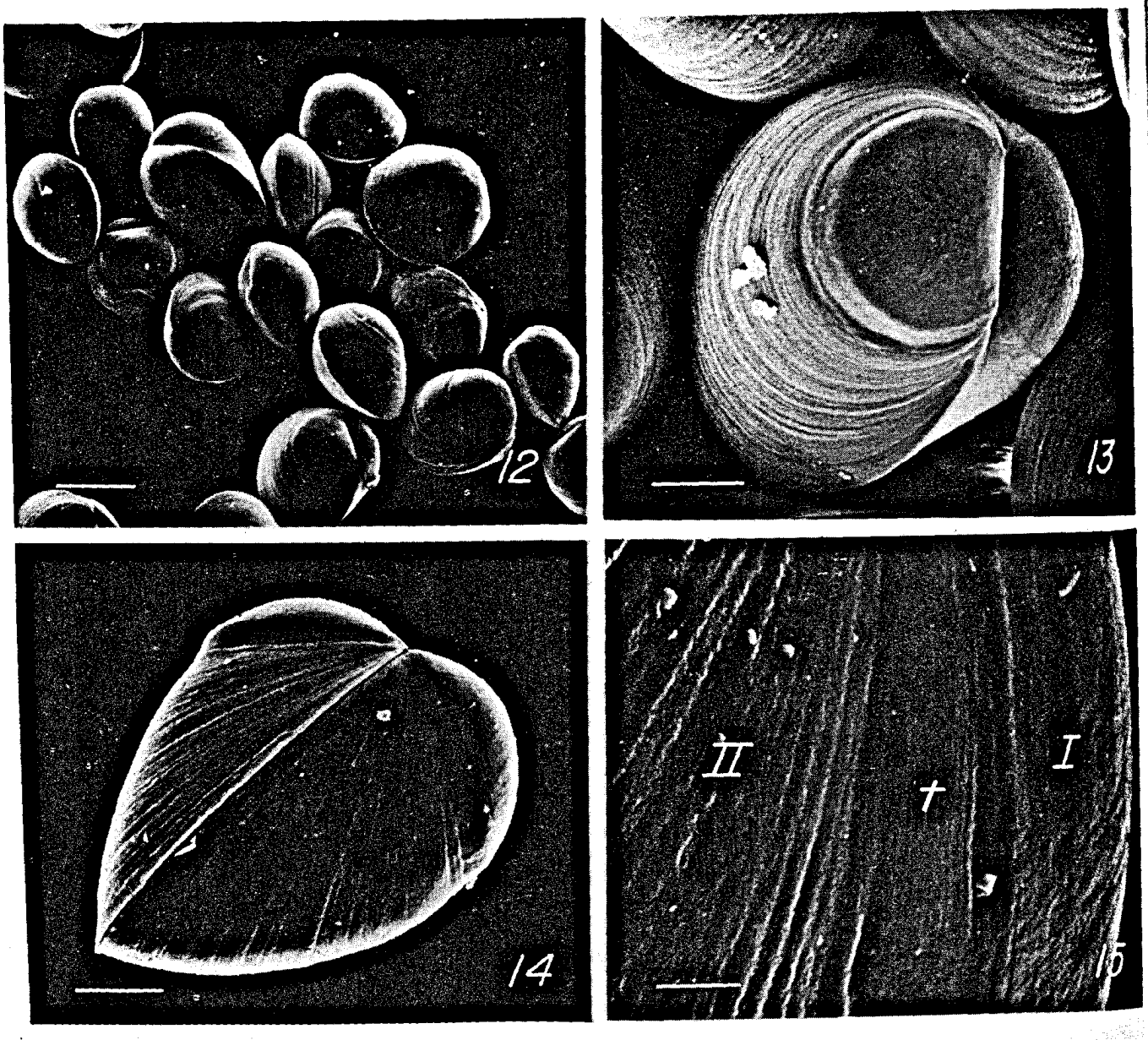


FIGURE 12. *Prodissoconch II. Larva to left of center has notch in right valve caused by injury during culture. 5% clorox 10 min. Scale bar = 100 μm.*

FIGURE 13. *Prodissoconch II. Umbonal (dorsal) view of larval valves, and punctate-stellate pattern of Prodissoconch I. 5% clorox 10 min. Scale bar = 20 μm.*

FIGURE 14. *Prodissoconch II. Side view of larval valves. 5% clorox 23 min, 3 min sonication. Scale bar = 20 μm.*

FIGURE 15. *Higher magnification of Figure 14 showing transitional band (t) between prodissoconch I (I) and II (II). Scale bar = 4 μm.*

of the valves (Figure 16) shows the marked flaring of the anterior and posterior sides that occurs with growth.

Prominent features of cloroxed exterior surfaces of prodissoconch II valves are close-set, conspicuous, concentric annulations (Figures 13, 14, 17, and 18). The finest of these growth striae are about 0.8 μm apart (Figure 18). Removal of the periostracum from the exterior surface with 5% clorox for 45 min exposed shell units on the exterior surface of the valves (Figure 19). The units are granular in nature and vary in size from 0.1 to 0.2 μm. Rod-like forms on the surface of the shell are probably remnants of periostracum left after treatment with clorox. At low magnifications, the interior of the valves appear smooth (Figure 21).

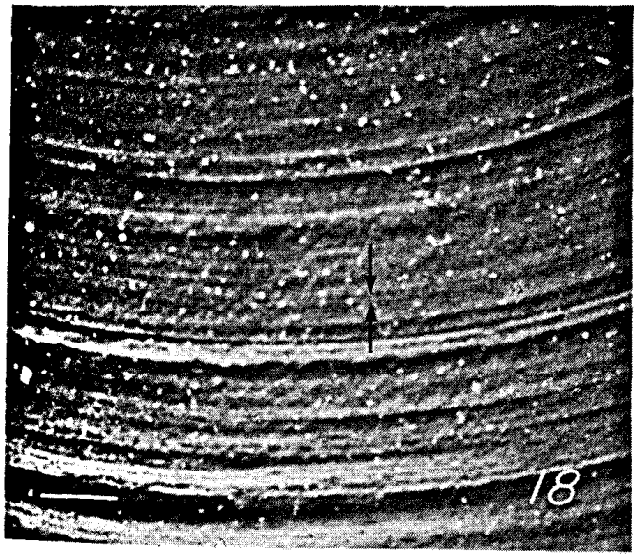


FIGURE 16. *Prodissoconch II. Laterodorsoanterior view of left valve of larva. 5% clorox 25 min, 6 min solication. Scale bar = 30 μm.*

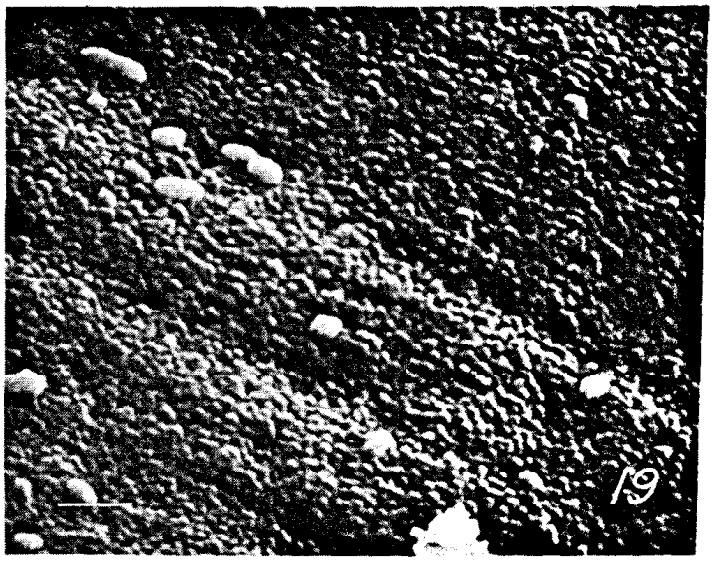


FIGURE 17. *Prodissoconch II. Umbonal view. 5% clorox 45 min. Scale bar = 30 μm.*

Contact of valve edges is tight when the shell is closed (Figures 12, 14, 16, and 20). As valves increase in size, rims in contact with each other become terraced internally (Figures 22 and 23), resulting in a step which runs around each valve up to the provinculum. The terracing is revealed by treatment of the shell with 5% clorox for about 45 min, which removes the periostracum. Shell units on the valve edge are granular and range in diameter from about 0.1 to 0.5 μm (Figure 23).

By the early prodissoconch II stage, the provinculum is well-developed (Figure 24); the terminal rectangular teeth, about equally developed on both sides of the provinculum, are large; and den-

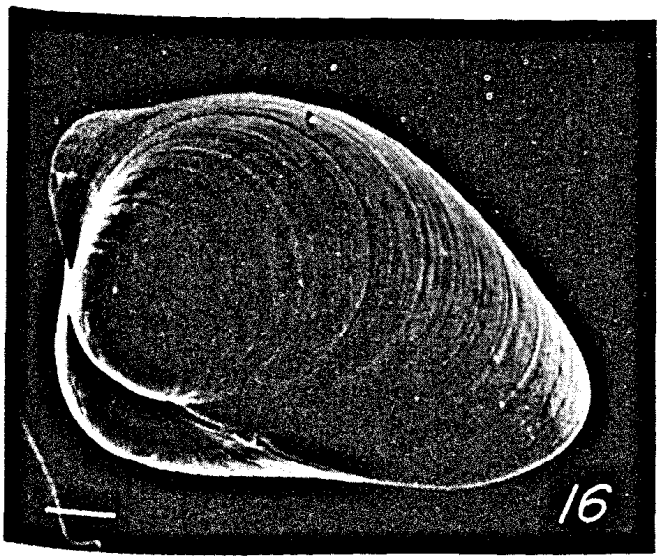


FIGURE 18. *Prodissoconch II, pediveliger. Exterior surface of valve. Closest growth striae are 0.8 μm apart (see arrows). 5% clorox 45 min. Scale bar = 10 μm.*

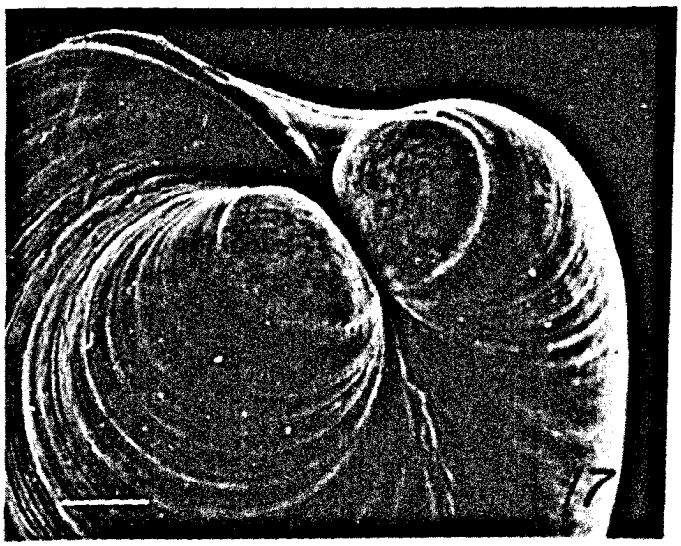
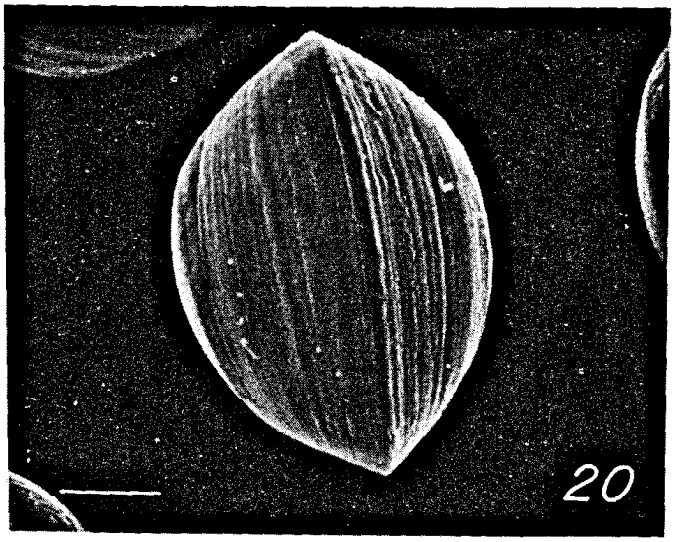
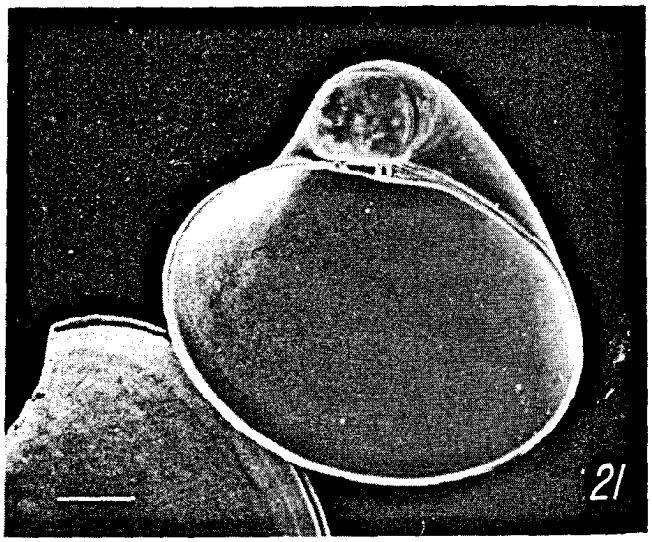
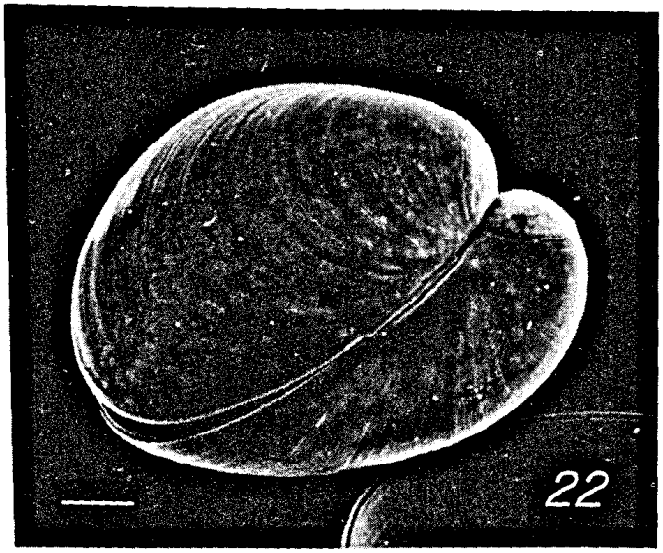


FIGURE 19. *High magnification of Figure 18 to show granular structure of exterior shell surface. Granules about 0.1 μm in diameter. 5% clorox 45 min. Scale bar = 1 μm.*







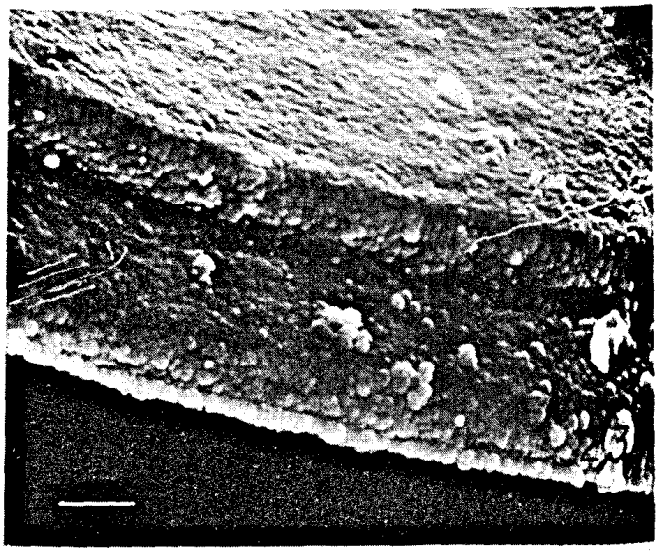


FIGURE 20. *Prodissoconch II. Ventral view of larval valves. 5% clorox 23 min, 3 min sonication. Scale bar = 20 μm.*

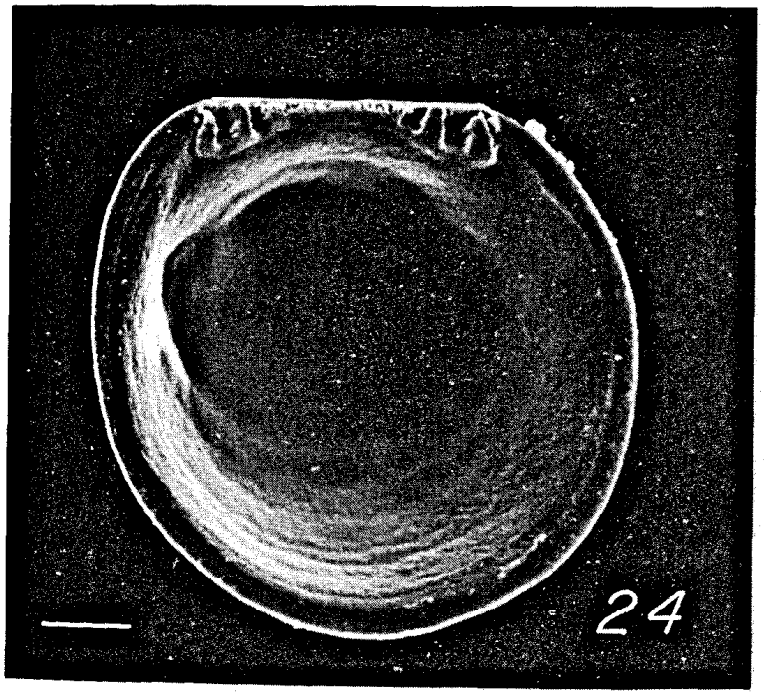
FIGURE 21. *Prodissoconch II, late pediveliger. Interior view of left valve. 5% clorox 45 min. Scale bar = 50 μm.*

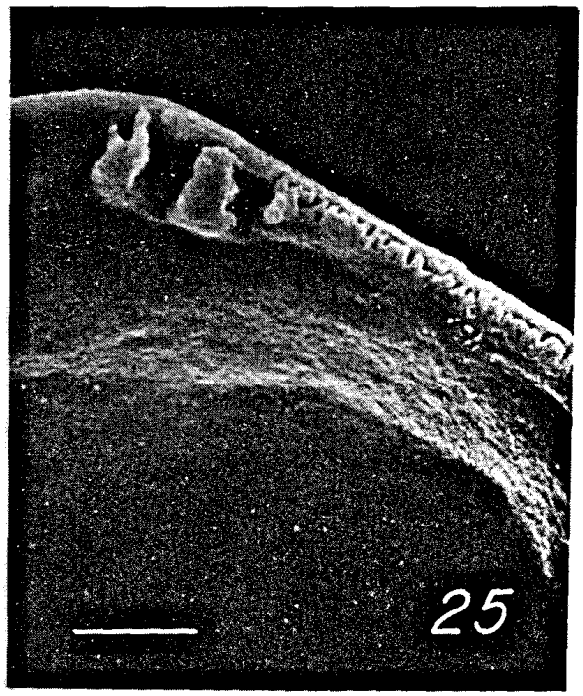
FIGURE 22. *Prodissoconch II, pediveliger. Side view of valves showing terraced edge exposed by immersion in 5% clorox for 45 min. Scale bar = 50 μm.*

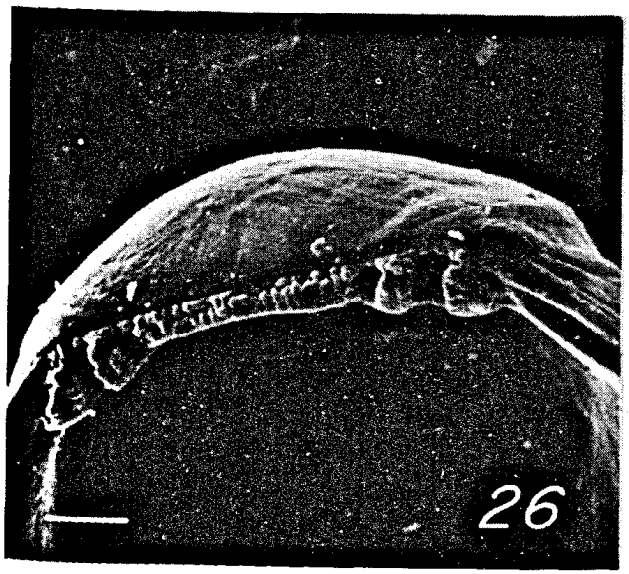
FIGURE 23. *Prodissoconch II, pediveliger. Edge of valve to show terracing after treatment with 5% clorox for 40 min. Scale bar = 2 μm.*

ticles between them take the form of small rounded structures (Figures 25 and 26). As Dinamani (1976) also reported, terminal teeth are slanted toward the median at an acute angle. The length of the provinculum remains approximately the same as larvae grow; 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 (Rees, 1950; Galtsoff, 1964), the terminal teeth serving to support the hinged valves. A view of the interior of the provinculum of intact valves shows how closely the teeth articulate (Figures 27 and 28).

By the mid-prodissoconch II stage, terminal teeth and intermediate denticles are well formed (Figures 29, 30, and 31). Two terminal teeth form at each end of the provinculum of the right valve (Figure 32), and three at each end of the provinculum of the left valve (Figures 29-31). Number and placement of teeth in *Crassostrea virginica* correspond to that in other species of larval oysters which have been examined (Imai, 1977).







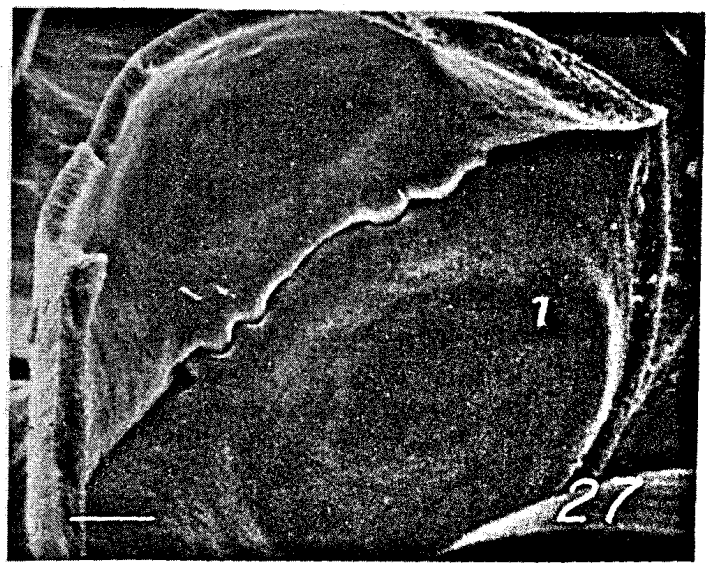


FIGURE 24. *Prodissoconch II. Left valve. 5% clorox 23 min. 3 min sonication. Scale bar = 20 μm.*

FIGURE 25. *Prodissoconch II. Left side of provinculum of left valve, similar to that in Figure 24. 2% clorox 20 min, 3 min. sonication. Scale bar = 8 μm.*

FIGURE 26. *Prodissoconch II. Provinculum of left valve. 5% clorox 40 min. Scale bar = 10 μm.*

FIGURE 27. *Prodissoconch II, middle stage. Interior of provinculum showing closeness of articulation of terminal teeth. 5% clorox 23 min. 3 min sonication. Scale bar = 10 μm.*

Terminal teeth bear conspicuous transverse grooves on their sides (Figures 30-32). These grooves and ridges minimize sheer on the hinge of shells which normally gape widely (Stanley, 1979; Waller, personal communication). By the early pediveliger stage, substantial amounts of shell have been added beyond ends of the provinculum (Figures 29 and 32) and a cardinal plateau, or ridge, about 20 μm wide, has been deposited between the terminal teeth on the right valve (Figure 32); this plateau interlaock with a socket between terminal teeth on the left valve (Figures 29 and 32).

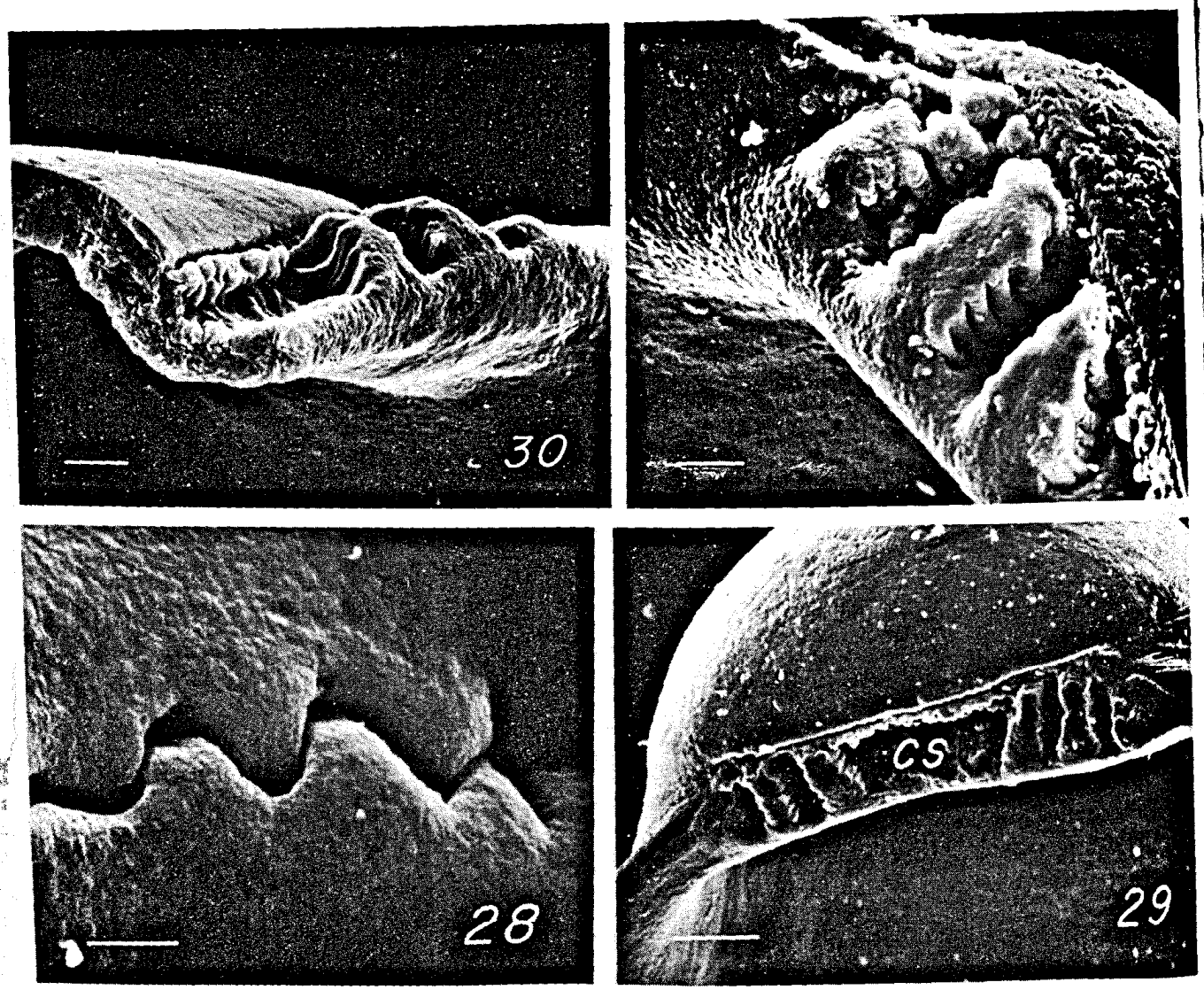


FIGURE 28. *Close view of terminal teeth in Figure 27. Scale bar = 3 μm.*

FIGURE 29. *Prodissoconch II, early pediveliger. Provinculum of left valve, showing cardinal socket (CS) between teeth ventral to denticles. 5% clorox 25 min, 6 min sonication. Scale bar = 10 μm.*

FIGURE 30. *Prodissoconch II, early pediveliger. Denticles and terminal teeth on anterior of provinculum of left valve. Hinge apparatus fractured in half after 5% clorox 25 min, and 6 min sonication. Scale bar = 5 μm.*

FIGURE 31. *Prodissoconch II, early pediveliger. Terminal teeth on posterior side of provinculum showing deep sockets between teeth and grooves on sides of teeth. 5% clorox 25 min, 6 min sonication. Scale bar = 3 μm.*

Denticles are still present, confined to the outer rim of the cardinal socket (Figure 33) and plateau. With continued growth, the provinculum of the late pediveliger begins to fill with shell, gradually obliterating the terminal teeth and denticles (Figure 33). By settlement, or early afterwards, the process of obliteration is completed. Deposition of shell begins at the posterior end of the provinculum and gradually advances anteriorly. The only published scanning electron micrograph of the larval shell of *Crassostrea virginica* is one of the provinculum of the left valve of a late prodissoconch II larva by Dinamani (1976, Figure 6a) which shows essentially what we have found. Pascual (1971) shows similar structures in light micrographs of the hinge of *C. angulata*.

The exterior of the larval hinge ligament is the thin nonmineralized layer laid down initially by

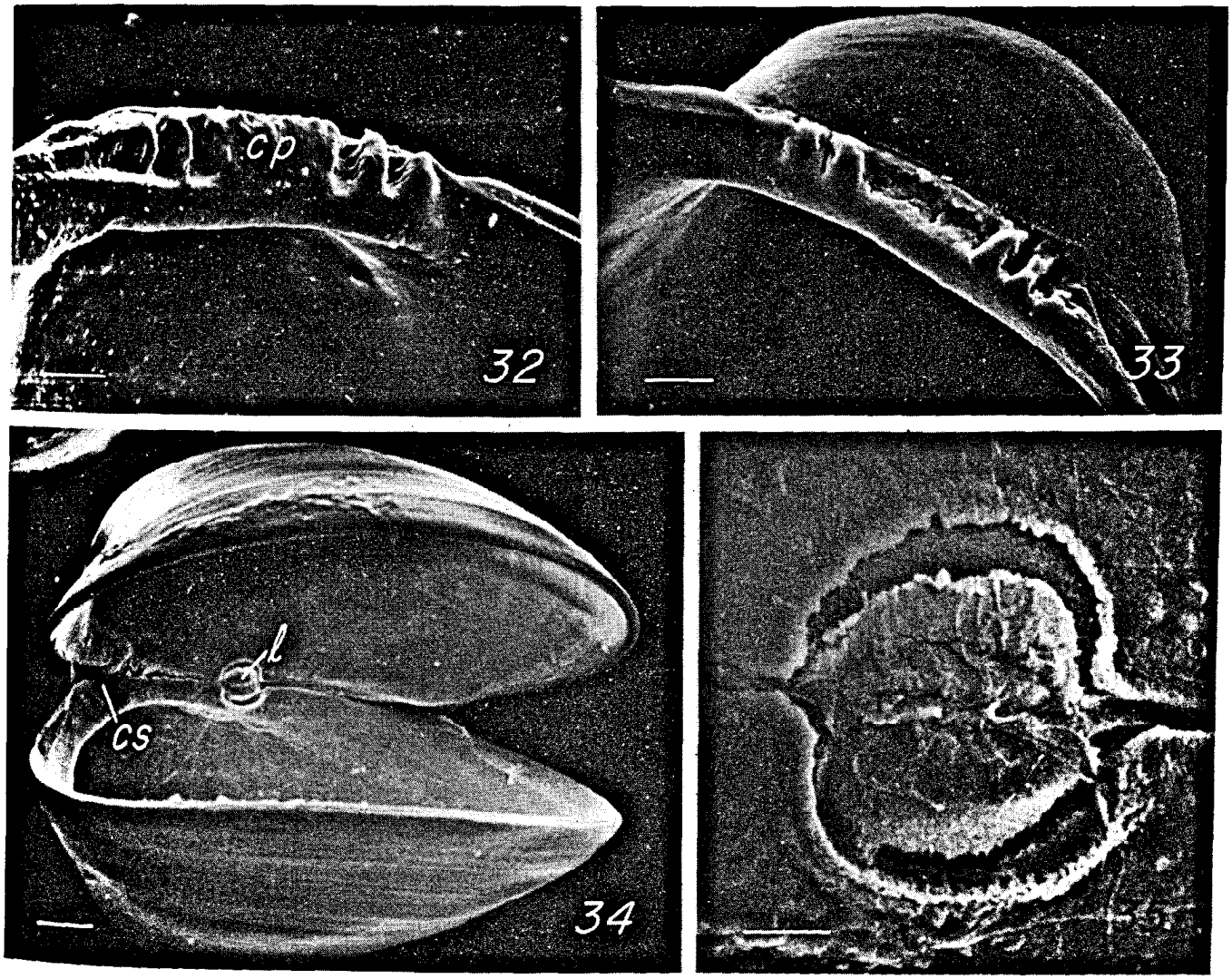


FIGURE 32. *Prodissoconch II, early pediveliger. Provinculum of right valve showing cardinal plateau (CP) between terminal teeth. Umbo is hidden behind hinge line. 5% clorox 40 min. Scale bar = 10 μm.*

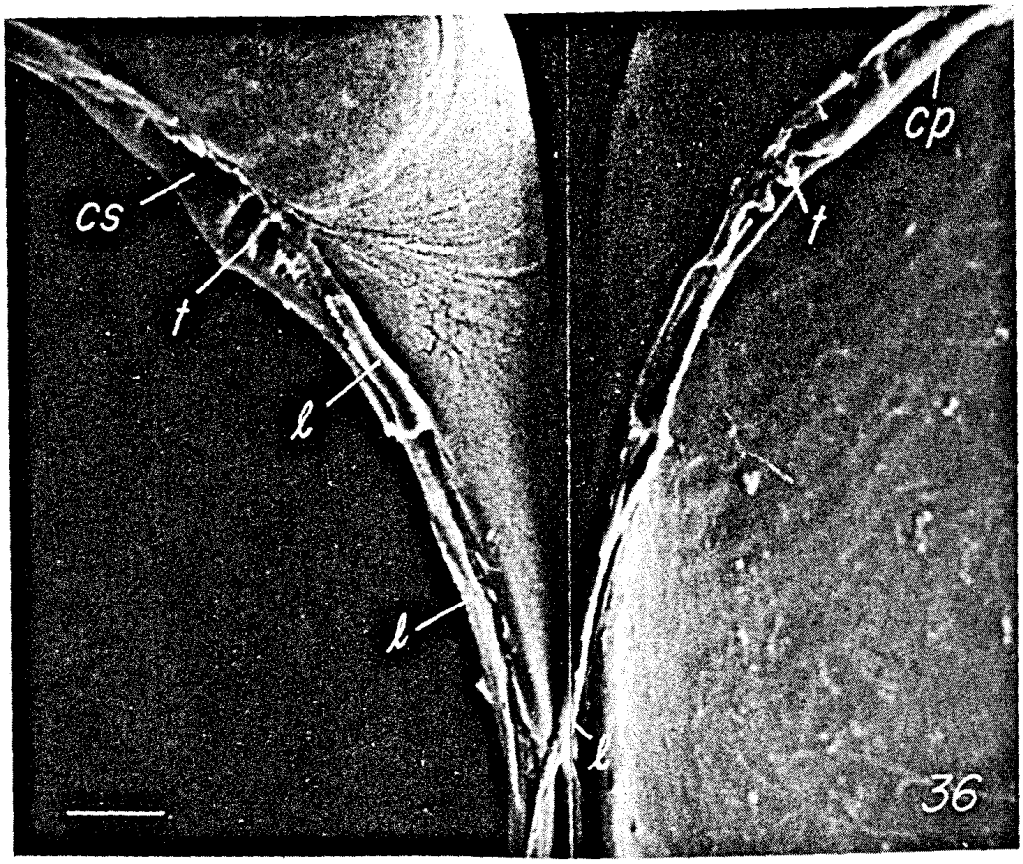
FIGURE 33. *Prodissoconch II, late pediveliger. Provinculum of left valve being obliterated by deposition of shell. 5% clorox 25 min, 6 min sonication. Scale bar = 10 μm.*

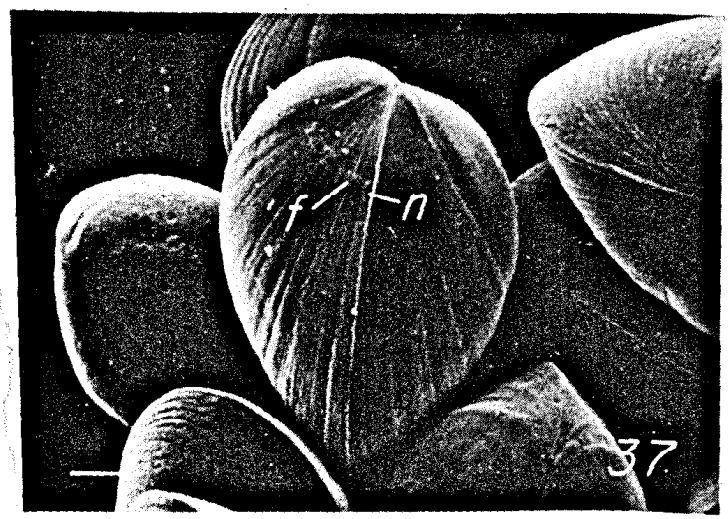
FIGURE 34. *Prodissoconch II, late pediveliger. Interior of provinculum in normally gaping valves to show inner larval ligament (l) and cardinal socket (CS). Cleaned microbiologically. Scale bar = 30 μm.*

FIGURE 35. *Prodissoconch II, inner larval ligament of late pediveliger in Figure 34, interior view. Scale bar = 5μm.*

the mantle epithelium. At first, valves are joined only by this membrane. The interior of the hinge of early prodissoconch II valves which were not treated with clorox show no evidence of an inner larval ligament (Figure 11).

At some stage of development of prodissoconch II (not determined precisely in this study) a small button-shaped inner larval ligament develops about 50 μm anterior to the anterior terminal teeth on the inside of the hinge. By the late pediveliger stage, the inner ligament, now about 20 μm in diameter, is a prominent feature of the inside of the hinge. The inner ligament was readily visible with the light microscope when stained with crystal violet and basic fuchsin after removal of soft tissues by microbiological activity. Scanning electron microscopy showed it even more clearly (Figures 34 and 35). The inner ligament appears to be continuous with the external ligament which has grown anteriorly to cover the dorsal





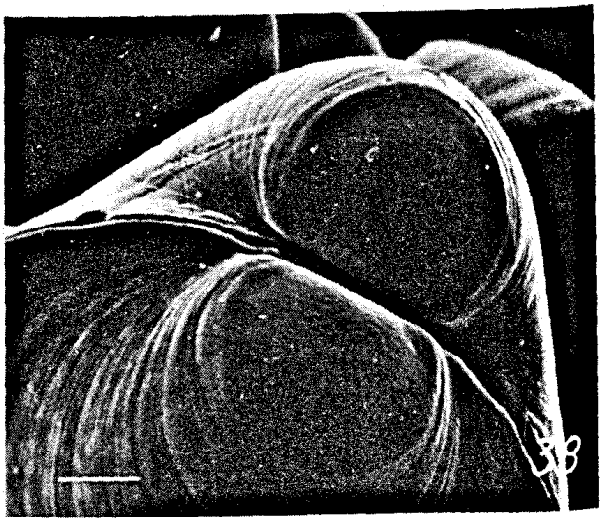


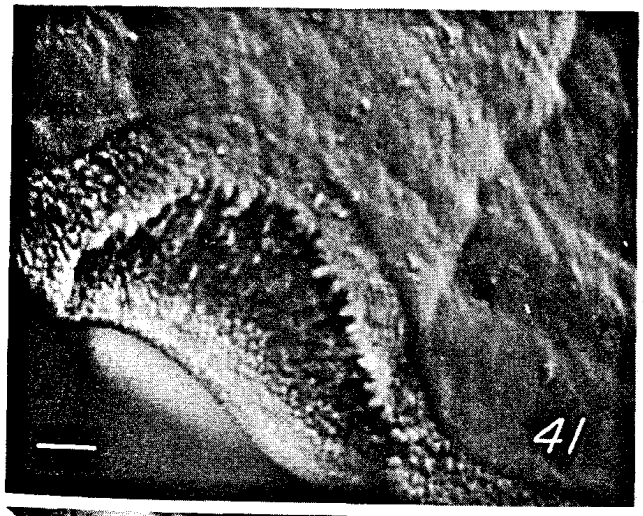
FIGURE 36. *Prodissoconch II, late pediveliger. Provinculum of left and right valves spread open showing anterior terminal teeth (t), outer ligament (l), cardinal socket (CS), and cardinal plateau (CP); inner larval ligament obscured by break. Scale bar = 20 μm.*

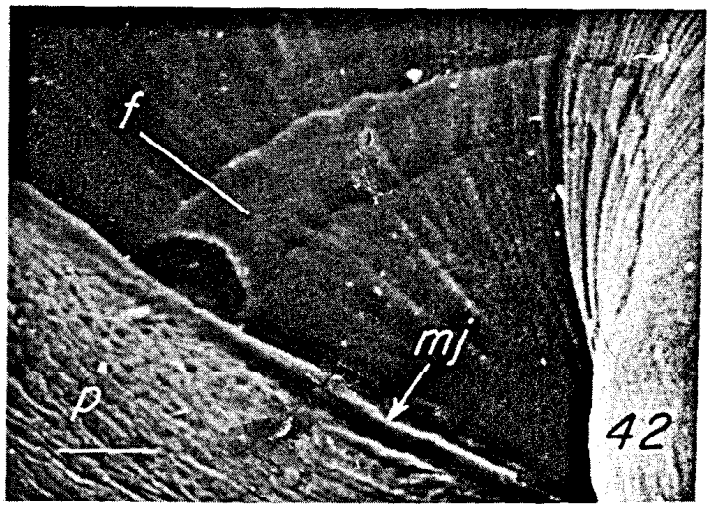
FIGURE 37. *Prodissoconch II, early stage. Posterior end of valves, fasciole (f) and notch (n) on left valve. 5% clorox 10 min. Scale bar = 20 μm.*

FIGURE 38. *Prodissoconch II, early pediveliger. Umbonal view of fasciole and notch on posterior side of left valve. 5% clorox 25 min, 6 min sonication. Scale bar = 20 μm.*

hinged area of the pivotal axis of the hinge (Figure 36).

An external shell structure, the fasciole, first described by Tanaka (1960), is located on the left valve (Figures 37 and 38) of prodissoconch II of *Crassostrea virginica*. The fasciole is a flattened, somewhat cornucopially shaped elevation of the surface of the shell (Figure 39). The fasciole arcs gracefully from the outer boundary of the prodissoconch I-II transitional band ventromedially





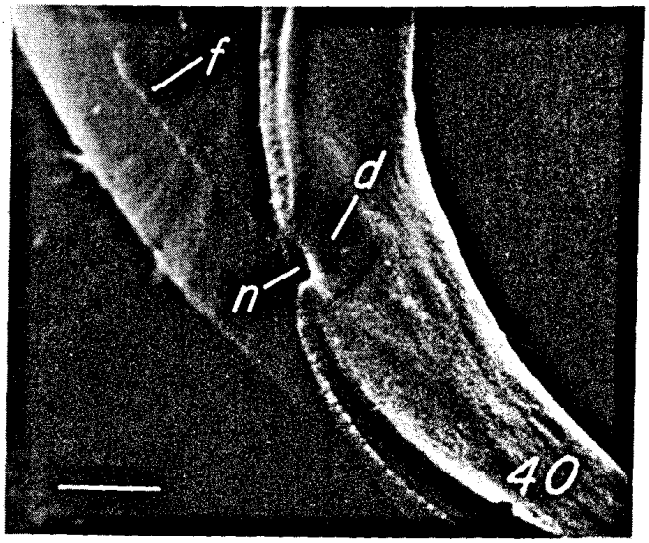


FIGURE 39. *Prodissoconch II, late pediveliger. Fasciole and notch. 5% clorox 45 min. Scale bar = 10 μm.*

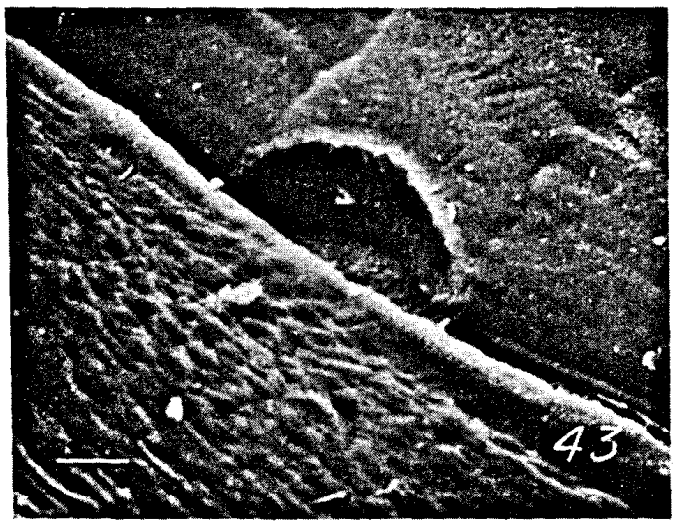
FIGURE 40. *Prodissoconch II, late pediveliger. Valve edge view of notch (n) (fasciole in background, f) and notch depression (d) on interior of valve. 5% clorox 45 min. Scale bar = 10 μm.*

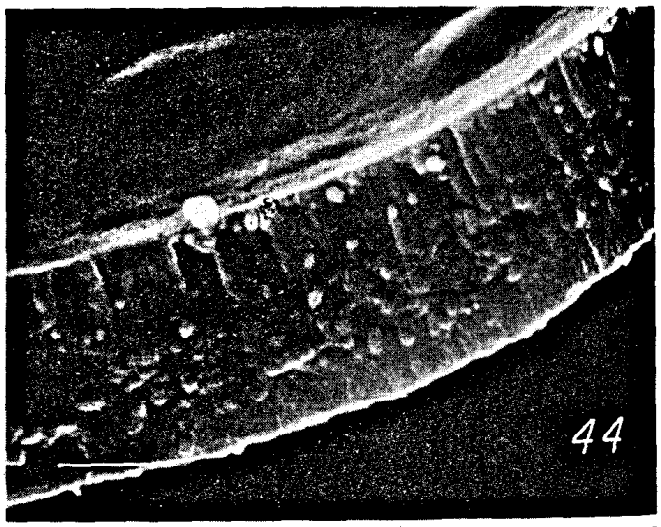
FIGURE 41. *Prodissoconch II, late pediveliger. High magnification of fasciolar notch, end view. 5% clorox 45 min. Scale bar = 2 μm.*

FIGURE 42. *Dissoconch, early stage. Fasciole (f) and notch terminating at metamorphic juncture (mj) and beginning of prismatic (p) dissoconch shell. 5% clorox 30 min. Scale bar = 10 μm.*

to end abruptly at the metamorphic line between prodissoconch II and the spat (Figure 42). Aperiodic lateral extensions of the crest of the fasciole onto the surface of the shell are not closely correlated with the growth striae (Figures 39 and 42). The fasciole is solid and terminates at the prodissoconch II valve margin in a curved notch (Figures 39 and 41). A slight depression is reflected on the interior of the left valve opposite the fasciole and notch (Figure 40). Treatment of valves with 5% clorox for 45 min may have removed periostracum from the rim of the valve revealing the typically granular form of the shell units (0.2 to 0.4μm in diameter). Most of the periostracum on the remainder of the valve appears relatively untouched (Figure 41). The mature fasciolar notch (Figures 42 and 43), relatively resistent to the 5% clorox (30 min), is more smoothly curved than that in earlier stages of development (Figure 41). The ultrastructure of the new dissoconch shell adjacent to the notch is prismatic (Figure 43).

Valves of prodissoconch II larvae are surprisingly thin, ranging from 4 μm in the mid-pro-





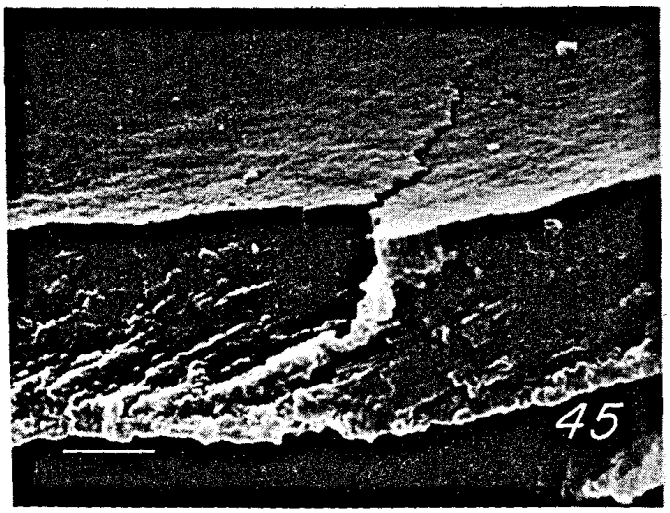


FIGURE 43. *Higher magnification of fasciolar notch shown in Figure 42. Scale bar = 4 μm.*

FIGURE 44. *Prodissoconch II, mid-stage. Fractured section of middle of valve, anteroposterior plane. Cleaned in 5% clorox 23 min, 3 min sonication before fracturing. Scale bar = 2 μm.*

FIGURE 45. *Prodissoconch II, pediveliger. Fractured section of middle of valve in dorsoventral plane. Cleaned in 5% clorox 25 min, 6 min sonication before fracturing. Scale bar = 3 μm.*

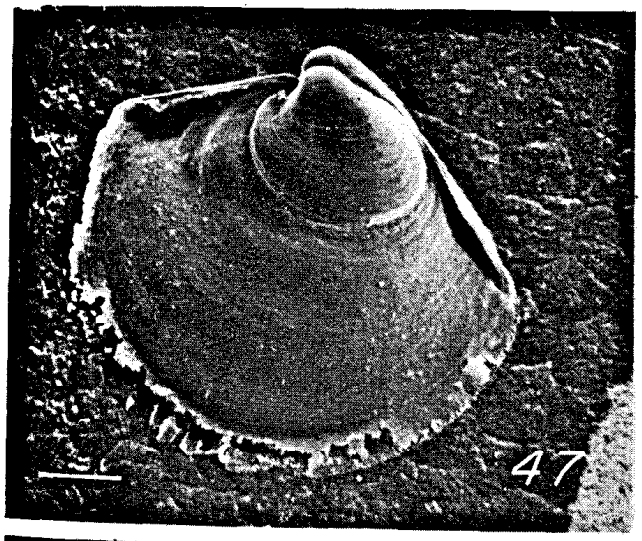
FIGURE 46. *Prodissoconch II, pediveliger. Fractured oblique section in dorsoventral plane, outer part of valve. Granules 0.2 to 0.5 μm in diameter. Cleaned in 5% clorox 45 min prior to fracturing. Scale bar = 2 μm.*

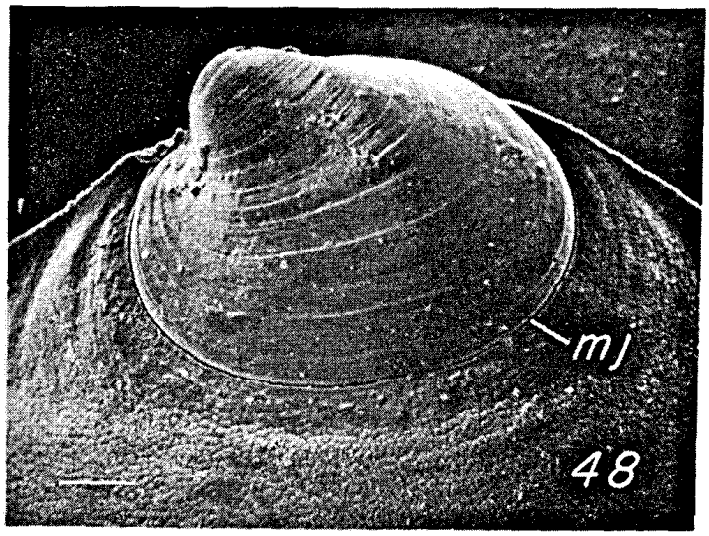
dissoconch stage (Figure 44) to 6 μm in a late pediveliger (Figure 45). The structure of the shell is homogeneous aragonite (Stenzel, 1964; Taylor et al., 1969), and it is composed of small granules ranging in diameter from 0.1 to 0.5 μm (Figures 44-46). There is a tendency for granules to be formed in columns (Figure 44), particularly in the inside layer. Granules are most conspicuous in the central part of the section (Figures 44 and 45), and different sizes of grains appear randomly mixed (Figure 46).

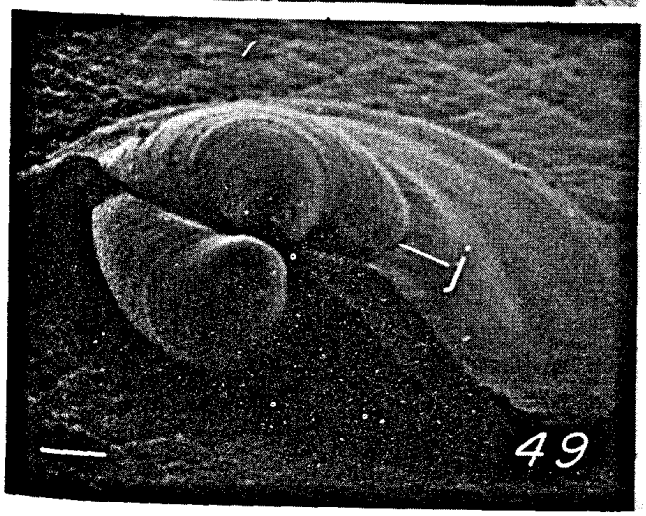
Early Dissoconch.

The abrupt change in the ultrastructure of valves from the homogeneous aragonite of the prodissoconch to the prismatic and foliated calcite of the dissoconch is striking (Figures 47, 48, and 50). Also prominent is the downward flaring of the left valve (Figure 49), facilitating its cementation to the substratum (Cranfield, 1973; 1974).

The outer layer of the right dissoconch valve is composed of prismatic calcite (Figures 50-52). Prisms increase in size from the metamorphic







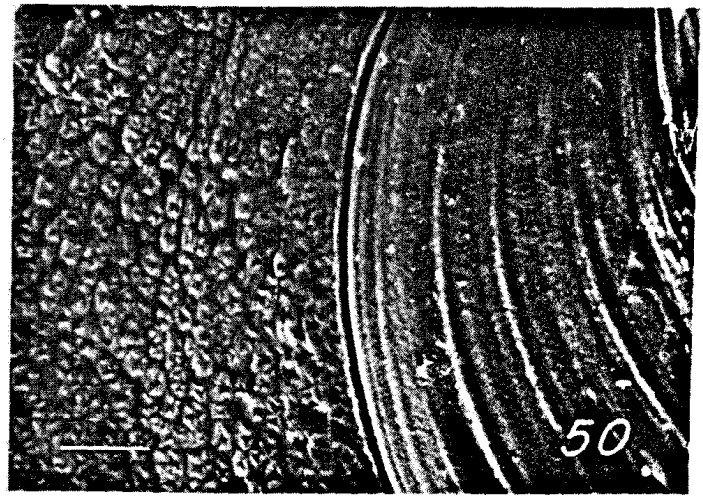


FIGURE 47. *Early dissoconch set on mylar film. 5% clorox 30 min. The clorox dissolved the fragile marginal matrix causing edge to crumble. Scale bar = 200 μm.*

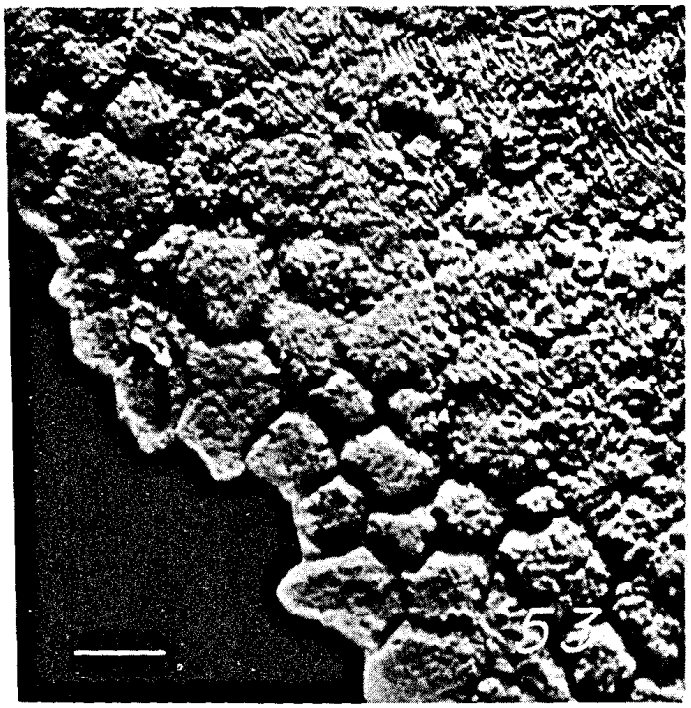
FIGURE 48. *Prodissoconch on early dissoconch valve. Sharp metamorphic line (mj). 5% clorox 60 min. Scale bar = 50 μm.*

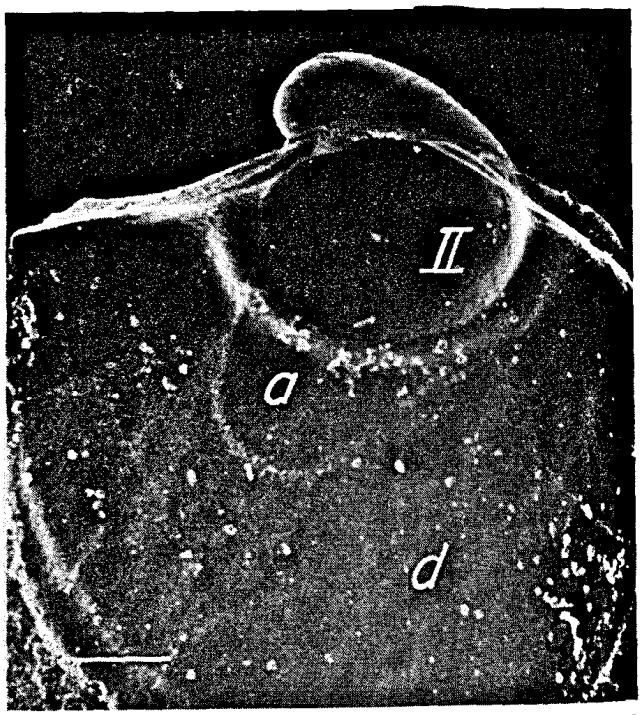
FIGURE 49. *Early dissoconch. Umbonal (dorsal) view to show juncture of prodissoconch II and dissoconch valves (j) and graceful downturning of spat valves onto mylar substratum. 5% clorox 30 min. Scale bar = 75 μm.*

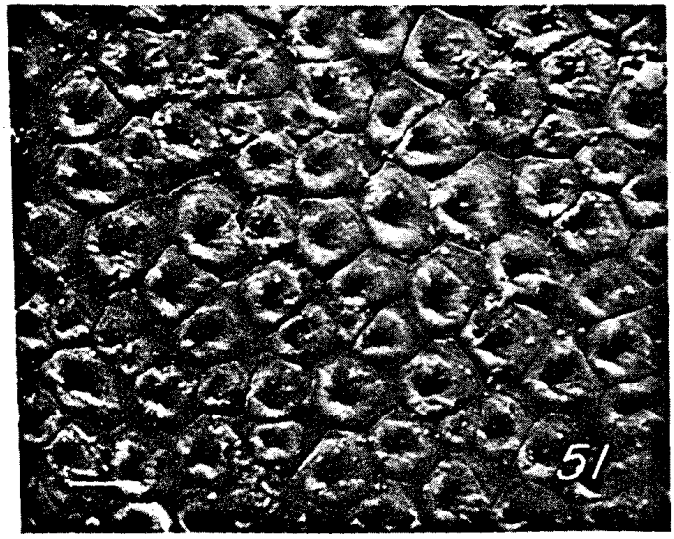
FIGURE 50. *Higher magnification of metamorphic juncture shown in Figure 48. Homogeneous aragonite, prodissoconch valve (right); prismatic calcite, spat (left). 5% clorox 30 min. Scale bar = 15 μm.*

juncture toward the margin, reaching 9 to 11 μm in maximum surface dimension (Figures 50-52) in spat 31 days old and about 1 mm high. Their angular shapes are evident at the margin of the shell after treatment with clorox and gentle fracturing (Figure 52). Foliated calcite overgrows the interior surface of the right valve near the edge of the shell (Figure 53), partially eclipsing the characteristic pattern of the prisms. In this specimen the foliated calcite is of the chalky variety.

Except for a thin outer layer of prismatic calcite, and the aragonitic myostraca of muscle scars (Stenzel, 1971), the left dissoconch valve is composed of foliated calcite. At low magnifications the interior surface of the valve appears smooth, and the prodissoconch valve is clearly outlined beneath the foliated calcite (Figure 54), which is deposited over both the prodissoconch II valve and the dissoconch valve. The anterior adductor muscle develops first in the early veliger, and the posterior adductor appears in the early umboned







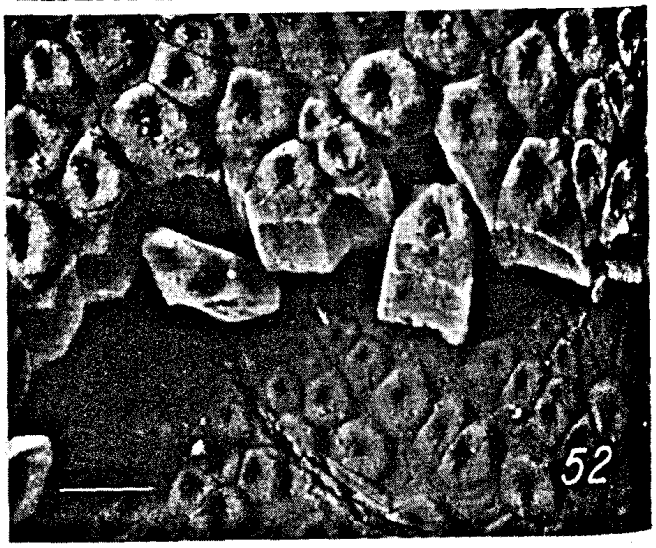


FIGURE 51. *Early dissoconch. Prisms on exterior surface of right valve in Figure 50, about midway between metamorphic line and ventrum. Scale bar = 6 μm.*

FIGURE 52. Early dissoconch. Prisms on exterior surface of right valve in imbricate scale at ventral edge of spat. Prisms in underlying scale smaller. 5% clorox 60 min. Scale bar = 8 μm.

FIGURE 53. Early dissoconch. Prisms partially covered with foliated calcite of the chalky variety on interior surface near ventral margin of right valve. Prismatic edge has been knocked off. 5% clorox 60 min. Scale bar = 8 μm.

FIGURE 54. *Early dissoconch. Interior view of left valve on mylar film. Adductor muscle scar (a) straddles prodissoconch II (II) and dissoconch (d) shell. 5% clorox 30 min. Scale bar = 100 μm.*

larva. Following settlement, the anterior muscle is resorbed, and the posterior one moves anteroventrally to occupy its definitive position (Galtsoff, 1964; Stenzel, 1971). In the juvenile stage illustrated in Figure 54, the anterior adductor muscle scar has disappeared, and the posterior one has moved across the metamorphic juncture to its adult position.

Migration of the adductor muscle scar is accomplished by deposition of a thin myostracal

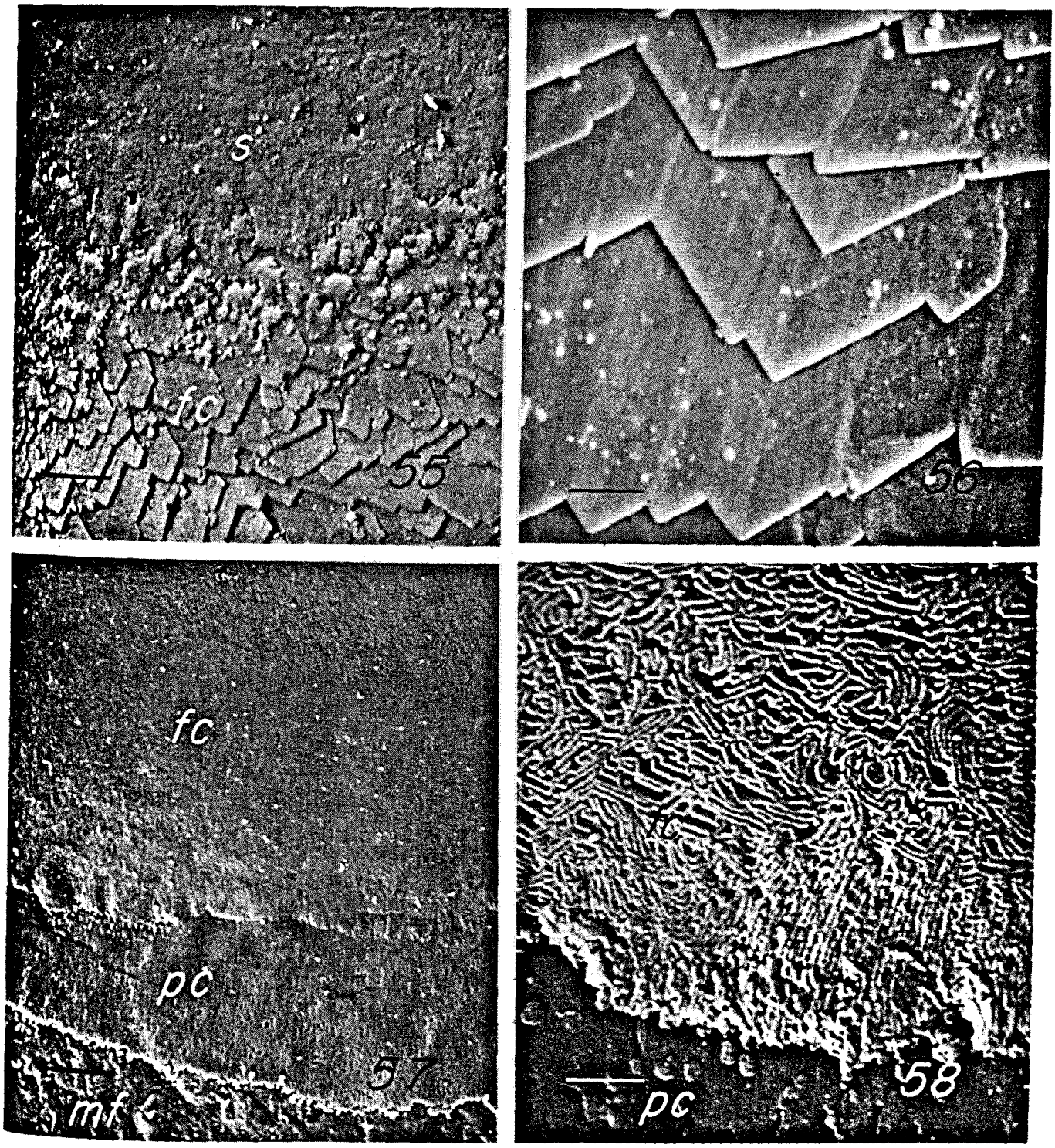
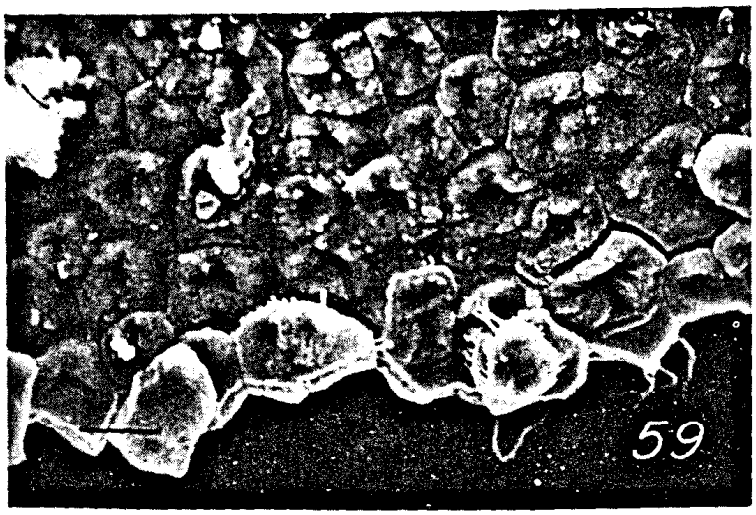


FIGURE 55. *Early dissoconch. Ventral boundary of adductor muscle scar (smooth myostracum, s) and foliated calcite (overlapping folia, fc). Same specimen as in Figure 54. 5% clorox 60 min. Scale bar = 7 μm.*

FIGURE 56. *Early dissoconch. Foliated calcite on interior surface of left valve. 5% clorox 30 min. Scale bar = 2 μm.*

FIGURE 57. *Early dissoconch. Ventral margin of left valve on mylar film. Outermost zone on mylar film (mf) is prismatic calcite (pc), and inner layer deposited over it is foliated calcite (fc). 5% clorox 60 min. Scale bar = 40 μm.*

FIGURE 58. *Higher magnification of Figure 57 at boundary of prismatic (pc) and foliated (chalky) (fc) structure. Scale bar = 4 μm.*



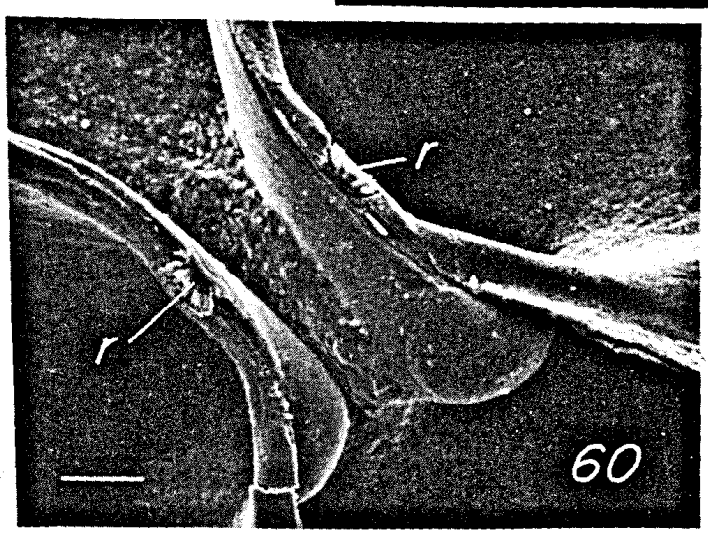


FIGURE 59. *Early dissoconch. Prisms, some partially dislodged, on interior surface of ventral margin of left valve. 5% clorox 30 min. Scale bar = 5 μm.*

FIGURE 60. *Early dissoconch. Opened matching pair of valves of spat showing resilium (r), one half in each chondrophore in hinge apparatus. Scale bar = 40 μm.*

FIGURE 61. *Enlargement of resilium in right valve in Figure 60. Scale bar = 8 μm.*

layer of shell over the surface of the foliated calcite in its path. The pattern of myostracal deposition is illustrated in Figure 55. This micrograph also illustrates the extreme smoothness of the surface of the myostracum to which the adductor muscle attaches. The rear (or dorsal) part of the myostracum, left behind as the scar migrates during shell growth, is covered by new layers of foliated calcite (Figure 54), and thus eventually becomes buried from surface view. A high magnification of the foliated calcite typical of the interior surface of valves shows the successive terrace-like layers of overlapping folia which in turn are composed of long thin laths whose boundaries are sometimes visible at the surface (Figure 56).

Shell of the left valve is secreted closely against the substratum (Figures 54 and 57). The outer layer is prismatic calcite (Figure 59), contrary to the report of Taylor et al. (1969) that in probably 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 (Figures 51 and 52). Deposition of foliated calcite follows close upon the prismatic layer (Figures 57 and 58), so that the prismatic zone is rather narrow and easily overlooked (Figure 57). The foliated calcite shown in Figure 58 is of the chalky variety.

At a dissoconch height of about 0.8 mm (Figure 54), the larval hinge apparatus with its heterodont teeth has become completely buried beneath

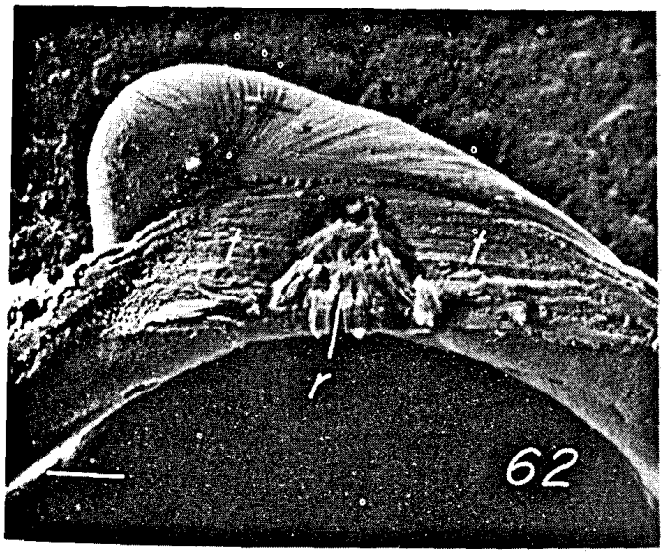


FIGURE 62. *Early dissoconch. Hinge area in left valve of spat showing resilium (r) in chondrophore, tensilia (t), and umbo of prodissoconch. 5% clorox 60 min. Scale bar = 4 μm.*

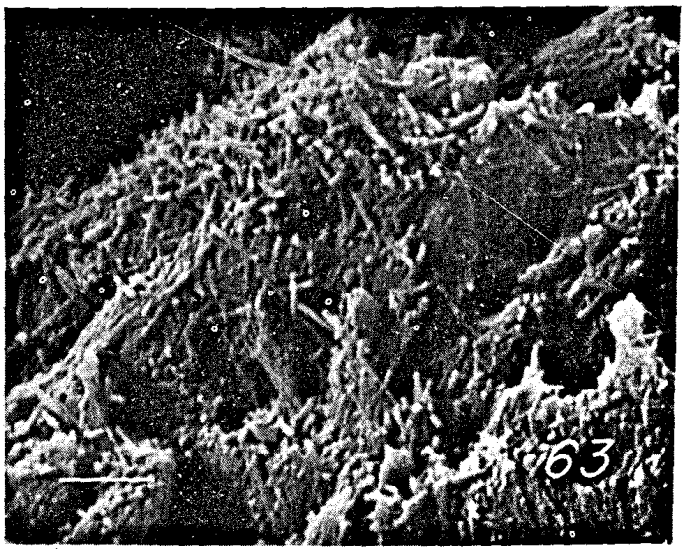


FIGURE 63. *Enlargement of calcareous fibers in resilium shown in Figure 62, exposed by treatment of the ligament with 5% clorox for 60 min, about 5 min of these at 60°C. Scale bar = 5 μm.*

dissoconch shell. The pivotal axis of the hinge has shifted from the center of the prodissoconch hinge anteriorly about 0.1 mm to the future center of the dissoconch hinge (Figure 60), the location of the inner larval ligament at the time of setting. The dissoconch hinge lacks teeth. At a spat height of 0.8 mm the inner ligament has grown to a block-shaped structure (the resilium, Galtsoff, 1964) about 30 μm wide (anteroposterior axis), supported in shallow chondrophores (Figure 60). Lateral extensions of the ligament, the tensilia, (Galtsoff, 1964) have began to form at this stage (Figure 62) and probably represent extensions of the outer ligament (Trueman, 1951). At a dissoconch height of about 1.8 mm the resilium is triangular in shape, fitting into a similarly shaped chondrophore in the hinge area of each valve (Figure 61). The inner width of the resilium is now about 75 μm, and broad flattened sheet-like tensilia have formed anteriorly and posteriorly between the opposing flat surfaces of the hinge nymphae (Figure 61) (Galtsoff, 1964). Tensilia are considerably thinner than the resilium. The resilium contains calcified fibrils (aragonitic according to Stenzel, 1962; Taylor et al., 1969; and confirmed by us), ranging in diameter from 0.3 to 0.4 μm, which are exposed by treatment with clorox (Figure 63). Tensilia lack calcified fibrils. Under compressive stress the resilium is strong, but under tension it is weak; tensilia are strong under bending stresses (Stenzel, 1971). In *Mytilus edulis* the aragonitic crystals of the ligament are enclosed in organic envelopes and are long, needle-shaped, single, and widely dispersed (Bevelander and Nakahara, 1969; Carriker, unpublished), somewhat similar to those in the oyster.

DISCUSSION

Comparison of larvae of *Crassostrea virginica* collected in estuaries (Carriker, 1951; 1959) and cultured in the laboratory (Carriker, 1959), or in a hatchery (College of Marine Studies), indicates that the external morphology is similar, and that confined culture, at least as seen at optical magnifications, does not seem to alter or deform the valves. This is reassuring, and suggests that larvae examined in this study probably had normally formed prodissoconch and early dissoconch valves.

Our finding that the polymorph of calcium carbonate in the valves of all prodissoconchs is aragonite confirmed Stenzel's (1964) report on prodissoconch II. Our results also demonstrated that larvae reared under hatchery conditions were identical mineralogically to those examined by Stenzel from another geographic region (Connecticut waters).

The striking punctate-stellate pattern on the exterior surface of the valves of prodissoconch I is probably associated with the shell-secreting activity of the developing shell gland and the circumferential spread of mineralization activity over the mantle epithelium of the veliger (Raven, 1966; Kume and Dan, 1968; see also LaBarbera, 1974). The process bears examination at cytological and ultrastructural levels. Ansell (1962) described, but did not illustrate, small punctate markings on the prodissoconch I valves of the bivalve *Venus striatula*.

According to Taylor et al. (1969), homogeneous shell structure is always aragonitic, and consists of minute calcium carbonate granules, all with similar crystallographic orientation. The crystallographic orientation of shell units of prodissoconch valves was not determined by us, but the larval shell is aragonitic, and ultrastructurally is composed of granules. In prodissoconch I valves the diameter of the granules ranges from about 0.05 to 0.1 μm, and in older prodissoconch II valves the diameter increases to a maximum of 0.4 μm. Accordingly, prodissoconch valves may be classified as homogeneous aragonite (Taylor et al., 1969). Whether the calcareous granules are formed in an organic matrix has yet to be determined.

As Stenzel (1971) pointed out, taxodont teeth of prodissoconch valves of common species of Ostreidae are larval structures only, and, as illustrated in this paper, are obliterated by the addition of new shell during the late prodissoconch II and early dissoconch stages. With the gradual functional loss of larval teeth, a more rapid process in the posterior ones (as also described for prodissoconchs of *Crassostrea angulata* [Pascual, 1971]), the pivotal axis of the hinge shifts to a position anterior to the umbones. The internal supportive function of the hinge apparatus is assumed by the dissoconch ligamental resilium, held securely in chondrophores, and by the tensilia, located between the broad lateral opposing nymphal surfaces. As illustrated by Ranson (1960), Galtsoff (1964), Stenzel (1971), and by us, the inner larval ligament is a distinct structure located anterior to the provinculum in the hinge apparatus of veligers. Pascual (1971) for *C. angulata* and Dinamani (1973) for *C. glomerata* noted that the "larval ligament" is also anterior to the provinculum in the prodissoconch of these species.

Stenzel (1971) suggested that the ligament, presumably the inner one, forms in the middle of the provinculum in the prodissoconch (at what stage is not indicated), so that the two valves open with opposing umbones remaining close together; as valves become more inequilateral and umbones more prominent, the ligament migrates smoothly and gradually along the hinge toward the anterodorsal valve margins, leaving umbones and larval teeth behind in their original positions. Our observations indicate, however, that the inner ligament forms anew anterior to the umbones where it later develops into the adult ligamental resilium. No inner larval ligament was observed by us inside the hinge between the terminal teeth. Tensilia, probably as extensions of the outer larval ligament, subsequently form to either side of the resilium. We concur with Trueman (1951) that the larval ligament at least in oysters is characteristic of the larval shell and the larval mode of life and that a different set of ligamental structures, evolving from the larval apparatus, are formed to meet the requirements of the post-larval shell.

A shift of the pivotal axis of the hinge, among other possible advantages, could permit wider gaping of the valves than would be possible were the pivotal point to remain at the closely apposed umbones. The point is provocative and raises the question whether, in the course of evolution, migration of the axis resulted because of the close juxtaposition of the large umbones or, conversely, evolution of the umbones to prominent features was made possible by shifting of the axis. A detailed study of the ontogenetic development of the ligament of oysters would shed further information on this interesting question.

No ligamental pit is evident at the site of the inner ligament in late veliger stages after the ligament has been removed with clorox. The absence of a depression is not unusual, as the adductor muscle, for example, attaches to ultrastructurally smooth myostracal surfaces.

The fasciole and notch, so characteristic of the left valve of prodissoconch II shells, were apparently first observed by Tanaka (1960) in *Saccostrea echinata* and seven other species, and recently, and independently, by Waller (1978) in

Ostrea edulis and by us in *Crassostrea virginica*. Pascual's (1971) light micrographs of larval valves of *Crassostrea angulata* suggest the presence of the fasciolar notch, but he makes no mention of it. The presence of this shell structure only in the prodissoconch II stage strongly suggests that it accompanies some function of a specialized part, or organ, of the mantle edge. The prominence of the fasciole and notch, and the depression of the interior surface of the valve in the vicinity of the notch, as well as increase in diameter of the fasciole as the larva grows, suggest that the organ is important to the veliger, possibly in some larval sensory function associated with feeding, swimming, or sensing light intensity. Since the fasciole terminates abruptly at the metamorphic juncture at the time of settlement, it is also possible the function of the organ, chemosensory or thigmosensory, or both, is related to searching by the pediveliger for a suitable settlement site and to subsequent cementation by the mantle edge and foot (Cranfield, 1973; 1974). Scanning electron microscopy of anesthetized, critical-point dried veligers of *Ostrea edulis* suggestsd to Waller (1978) that the notch is associated with a postanal ciliary tuft which propels water out of the mantle cavity. The potential importance of the organ in veliger and setting activities suggests further study of its structure and function.

The reason for the sharp transition at the metamorphic line from aragonitic homogeneous granules to foliated and prismatic calcite with islands of aragonite in myostracal muscle scars and resilial fibers (Stenzel, 1962, 1963, 1964; Galtsoff, 1964; Taylor et al., 1969) is still unclear. Unquestionably, the sudden structural change is associated with rapid metamorphosis from the motile pediveliger to the immobile spat; however, a more gradual transition than the sharp line of demarcation between the two types of shell might be expected.

Transition in shell structure, of course, could not occur without a change in the pattern of secretion of shell materials by the mantle epithelium. This means that epithelial cells, which in the larval stages were forming aragonitic granules, suddenly reconstitute themselves in the spat to secrete calcitic prisms at the rim of the valves. A further reconstitution occurs in the epithelium behind the valve edges where foliated calcite is secreted, in some places in the form of tightly packed laths, and in others as chalky shell with laths arranged in a spongy pattern. The ordered pattern of events which occurs in such rapid succession in young oysters are but manifestations of complicated biochemical processes and changes occurring at the cellular level, and a challenge to students of animal development (Raven, 1966). Lutz and Jablonski (1978) reported on the presence of a distinct prodissoconch-dissoconch boundary in the valves of juvenile late Cretaceous bivalves. Thus the process of rapid transition from larval to juvenile bivalves has been in existence over a long geologic period.

Why all, or nearly all, Bivalvia have aragonitic larval shells (Stenzel, 1964) is still unclear. Stenzel conjectures that aragonitic valves may be more advantageous than calcitic valves to motile veligers because aragonite is harder, has greater strength as a structural material, and is less prone to breakage by cleaving than calcite (see also Waller, 1975). Calcitic valves, on the other hand, may be more advantageous than aragonitic valves to bivalves which are permanently immobilized on the bottom, because calcite is less soluble in seawater and because it is secreted more economically than aragonite (calcite fills a larger volume per mole than aragonite) (Stenzel, 1964). Evolution of extreme thinness of larval valves may have been possible because of the structural characteristics of aragonite. The small mass of the valves facilitates suspension in the water column for the duration of the larval life history, and provides some mechanical protection from small predators and extreme abiotic conditions.

Our study has illustrated something of the ultrastructural complexity of the valves of young *Crassostrea virginica* and the developmental changes that occur in the shell during growth from the straight-hinge stage to the early spat. Results may be helpful to future investigators in the ultrastructural study and comparison of fossil and modern larvae (Lutz and Jablonski, 1978), systematic investigation of bivalve larvae employing fine structural features for diagnosis and identification (Chanley and Andrews, 1971), physiologic and embryologic study of shell formation (Raven, 1966; Kume and Dan, 1968), ex-

amination of erosional effects of laboratory and field environmental conditions on larval valve surfaces, recognition of abnormal ultrastructural shell formation, and investigation of the chemical composition of larval valve regions by such modern techniques as the proton microprobe.

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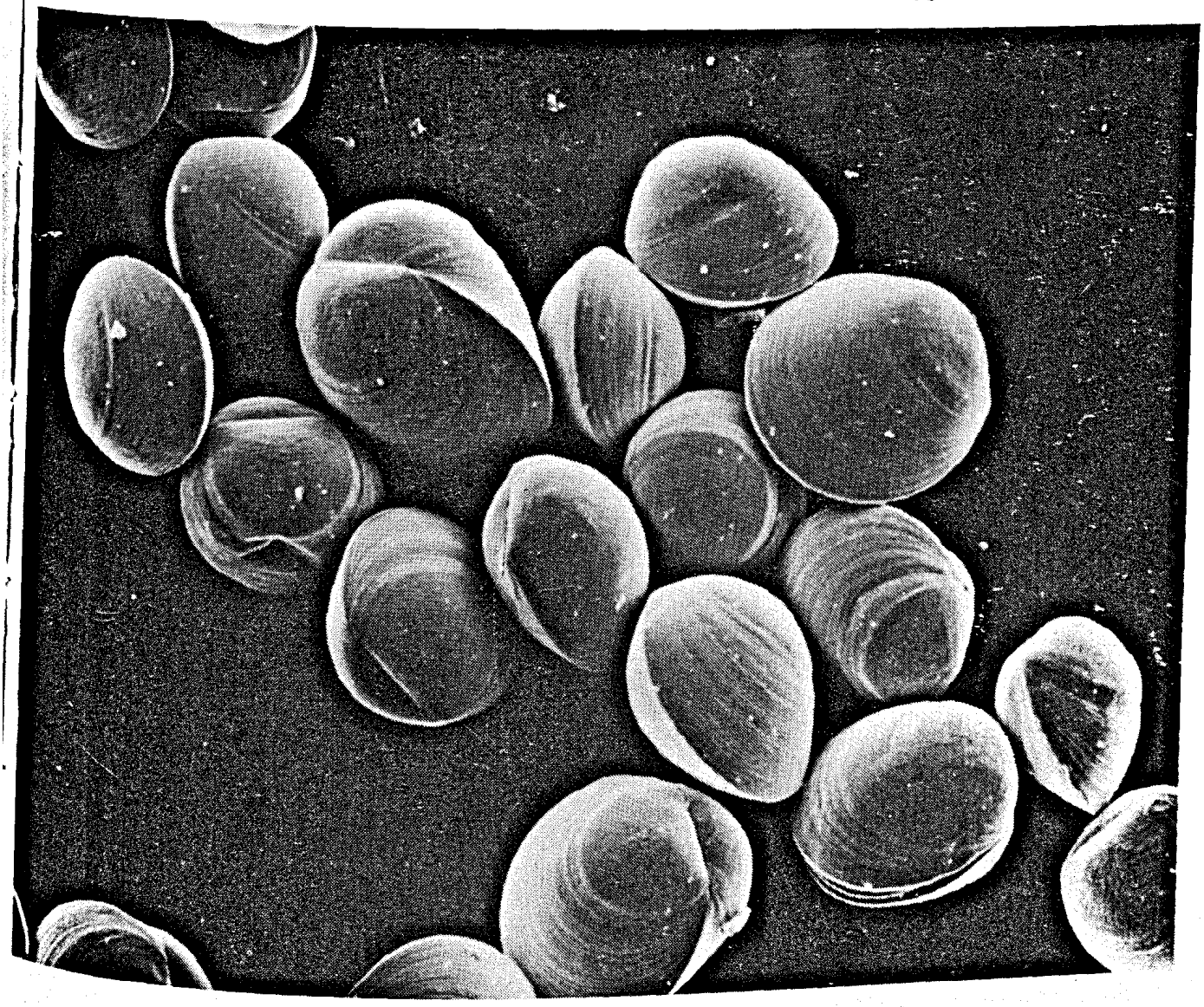

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