STRUCTURE OF THE CONCHIOLIN CASES OF THE PRISMS IN *MYTILUS EDULIS* LINNE

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ABSTRACT

The prisms in the shell of *Mytilus edulis* Linné are calcite needles. Their small size and their thin conchiolin cases distinguish them from the prisms of many other species of mollusks. These *Mytilus* prisms have been studied with the electron microscope. The material consisted of positive replicas of surfaces of the prismatic layer, etched with chelating agents, and of preparations of tubular cases from decalcified prisms which were compared with the conchiolin from decalcified mother-of-pearl of the same species. In the replicas, the cases appear as thin pellicles in the intervals between the prism crystals. Both the prism cases and the nacreous conchiolin, disintegrated by exposure to ultrasonic waves and sedimented on supporting films, appear in the form of tightly meshed, reticulated sheets, described as "tight pelecypod pattern" in former studies on nacreous conchiolin of *Mytilus*. The results show that in the shell of this species the same conchiolin structure is associated with aragonite in mother-of-pearl and with calcite in the prismatic layer.

It has been suggested (1, 2) that the scleroprotidic substance (conchiolin) might play a part in determining the two forms of crystallization of calcium carbonate, calcite and aragonite, with which it is associated in the mollusk shells. Under these conditions, there might be two kinds of conchiolin which would differ from each other, either by their chemical composition or their structure, or by both characteristics.

Information about this problem is fragmentary. On the one hand, in *Mytilus* (3) prism conchiolin contains more carbon, in *Meleagrina margaritifera*, *Pinna nigra*, and *Pinna nobilis* (2) more glycine and tyrosine, in *Pinctada martensii* (4) more phenylalanine, proline and less alanine than nacreous conchiolin. On the other hand, the amino acid content of wholly calcitic (*e.g. Gryphaea angulata*, *Ostrea edulis*, *Pecten maximus*, 2) or aragonitic (*Turbo marmoratus*, 2) shells did not give any evidence of a specific relationship between a particular crystalline form of calcium carbonate and a well defined chemical form of conchiolin.

Lace-like reticulated structures have been observed in nacreous conchiolin by electron microscopy (5–7). Little is known about the submicroscopic structure of prism conchiolin which forms the cases surrounding the prism crystals. The micrographs so far published of replicas of outer edges of the prismatic region of *Pinctada martensii* (8) and of *Crassostrea virginica* (9) show the prism conchiolin to be in the form of bands with a fine grained and pebbled structure but without evidence of fibrous material (9).

In an electron microscopic study (unpublished) of the prismatic layer of twenty-two species of pelecypods, the prisms of *Mytilus edulis* Linné were found to be an especially appropriate material, because of the extreme thinness of their conchiolin cases, for investigating the relationships between the ultrastructure of conchiolin and the type of crystallization of calcium carbonate.

The present note reports some results of an electron microscopic study of the blue-violet substance (*blaue Schicht*) which forms the prismatic layer in *Mytilus edulis* in which the prisms are extremely thin calcite needles (10–14). These prisms are smaller in size than those of many other species of mollusks (Fig. 1).

PROCEDURES

The preparations consist of: (*A*) positive carbon replicas (double stage method) (15), shadow cast with palladium, of (*a*) the margin of the inner surfaces of shells and of adjacent portions of periostracum (growth region of the prisms), (*b*) regions of fracture of the prismatic layer, (*c*) polished surfaces of the same layer etched with chelating agents (sequestrene NA 2, Alrose Chemical Corporation, Providence, Rhode Island; titriplex III, Merck, Darmstadt, Germany); (*B*) Prisms, separated mechanically from periostracum and mother-of-pearl, decalcified by chelation, and exposed (in distilled water) to ultrasonic waves, in order to unfold and break their tubular cases; (*C*) Mother-of-pearl, prepared in the same way as the prisms. Drops of suspensions of the disintegrated materials were deposited on standard screens coated with films of formvar and of carbon. After drying in air, the preparations were shadow cast with palladium. About 250 preparations were examined and 230 micrographs recorded.

OBSERVATIONS

At the inner marginal surface of the shell valves, the prismatic layer reveals the free ends of the prisms, disposed side by side (Figs. 3 and 4). The prism extremities coincide with the crystal planes and, when not blunted by natural or artificially induced corrosion, appear as truncated pyramids (Figs. 3 and 4). In replicas of shells of the living animal, these prism extremities frequently appear embedded in an amorphous substance (Fig. 4). This material is a freshly deposited secretion product of the prismogenic palleal epithelial cells. In the growth areas of the prisms, round crystal seeds scattered at random

FIGURE 1

Dust, decalcified by chelation, from filed prismatic substance of *Mytilus edulis*. About forty tubular prism cases, alined side by side, are shown by phase contrast microscopy. At the same magnification, a single prism case from *Pteria* or *Pinna* would exhibit a diameter larger than that of the whole group shown in this picture. × 600.

FIGURE 2

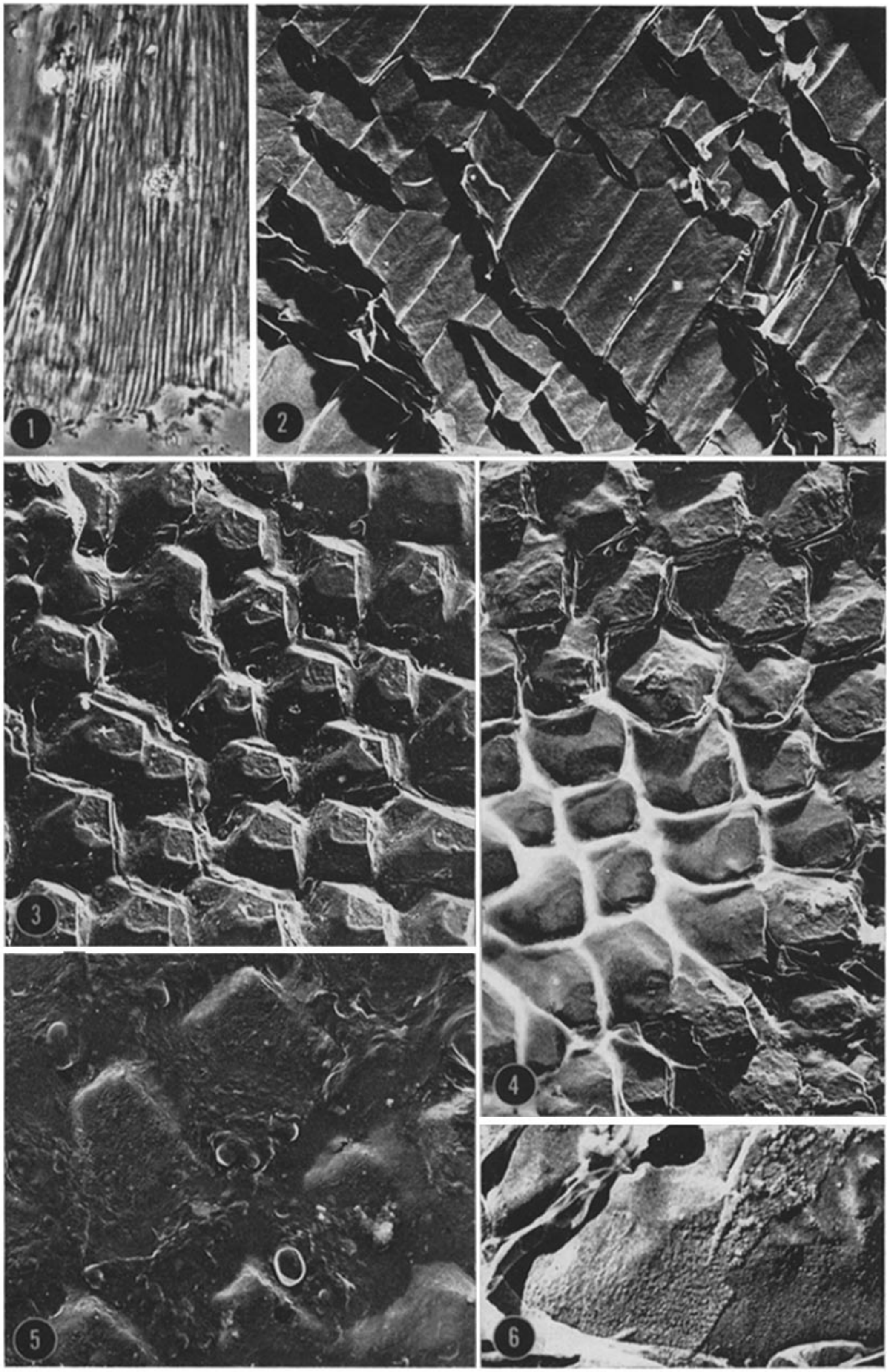
Electron micrograph of a positive metallic replica of the surface of a fracture in the prismatic layer. About seven superimposed rows of broken prisms are shown. Between the surfaces of contact delimiting the prisms, the organic cases appear as thin white filaments. × 6,000.

FIGURES 3, 4, AND 5

Positive metallic replicas of the surfaces of the prismatic layer at the inner margin of the shell valves. Fig. 5 shows the growth region of the prisms, near the periostracum. The figures show representative aspects of large areas of the surfaces in the living animal. The free extremities of the prisms, in the shape of truncated pyramids, coincide with the crystalline planes (Schmidt, 14). In Fig. 3, the sharp edges (brightly illuminated) of the prisms form angles of 110°. In an area of Fig. 4 (left) an amorphous substance, which is associated with the cases, and is easily removed by natural and induced corrosion (top and bottom), embeds some of the prisms. In Fig. 5, a sheet of conchiolin overlaps and blunts the extremities of about seven prisms. Numerous round crystal seeds, either single or coalescent (center), are scattered on the conchiolin membrane. Fig. 3, × 6,000; Fig. 4, × 9,000; Fig. 5, × 10,000.

FIGURE 6

Prismatic layer embedded in palatal, polished obliquely to the long axis of the prisms, and etched with titriplex for 5 minutes. The picture shows a portion of a case (compare with Figs. 8 and 10) freed by etching and collapsed onto the mineral background. × 18,000.



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(Fig. 5) increase in size and compress the substance into the conchiolin cases wrapping the prisms. These cases, freed from the mineral by corrosion, appear as thin pellicles or filaments in the intervals between the prisms (Figs. 3, 4) and are also visible around the prisms in the plane of fracture (Fig. 2) and in polished and etched preparations (Figs. 6 and 7).

Decalcified prisms disintegrated by exposure to ultrasonic waves reveal tubular debris of cases (Fig. 8) in the form of tightly meshed, reticulated sheets, in which the small openings are frequently obliterated by shrinkage (Fig. 10). These sheets have the same structure as that already observed in nacreous conchiolin of the same (5) and of other species of *Mytilidae* (7), and described as "tight pelecypod pattern" (Fig. 9).

In former studies (5, 7) on the substructure of the reticulated sheets of nacreous conchiolin, it has been assumed that the trabeculae of the reticulum consist of a core of fibrils coated with muffs of substances appearing as protuberances and bulging, round bodies of various sizes. As in nacreous reticulated sheets (7, Figs. 9, 10, 18), fibrils (not shown) were detected in the reticulum of prism cases. Such fibrils frequently protruded at the edge of broken cases. In Figs. 9 and 10 of the present paper, fibrils are detected by the arrangement in rows of granulations which coat them.

DISCUSSION

Interpenetration of prismatic and nacreous layers occurs consistently in the mollusk shells as a result of movement over the same region of a shell of portions of the palleal epithelium respectively specializing in the formation of prisms and of mother-of-pearl. Collection of fragments consisting of prismatic layer devoid of nacreous substance is therefore always questionable. Some sheets of conchiolin might be, at least in part, nacreous conchiolin folded into tube-like formations during sedimentation and desiccation. In fact, routine control by x-ray diffraction of the degree of homogeneity of the samples used revealed weak traces of nacreous aragonite in the calcite pattern of the prisms. However, sheets of conchiolin identical in their texture with the fragments of cases disintegrated by exposure to ultrasonic waves, wrap the prisms in regions where mother-of-pearl is unquestionably absent (Fig. 6).

In the shell of *Mytilus edulis*, the same lace-like conchiolin structure (tight pelecypod pattern) is, therefore, associated with aragonite in mother-of-pearl and with calcite in the prismatic layer. Different conditions were recorded in twenty other species of mollusks, as will be reported elsewhere (16).

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Figure 7

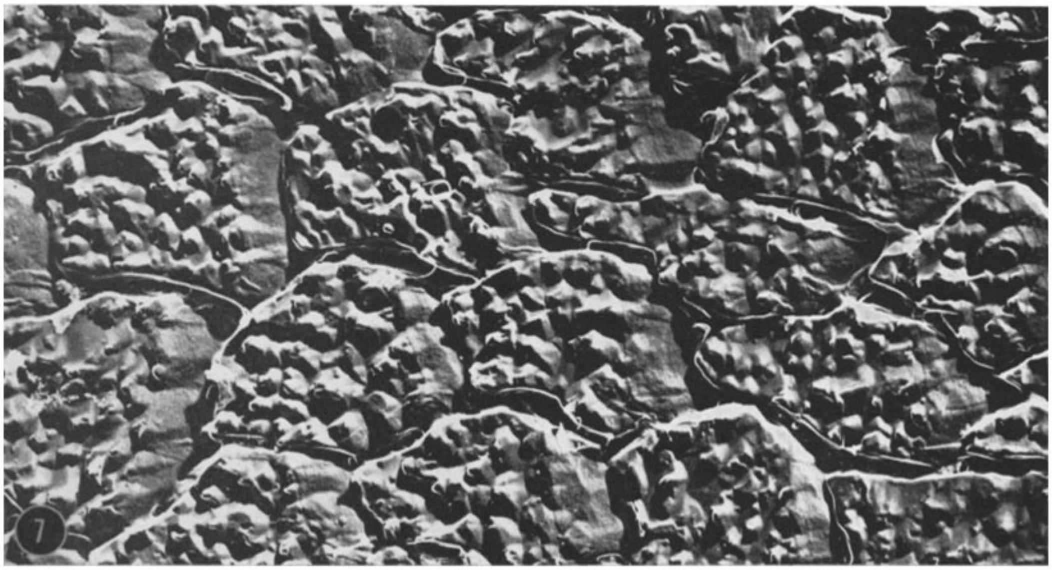
Prismatic layer embedded in palatal, polished obliquely to the long axis of the prisms, and etched with sequestrene for 4 minutes. Some degree of orientation in rows is exhibited by the pyramidal hillocks and pits which characterize the etch figures of calcite. Portions of the cases appear collapsed onto the mineral substance on the right side of several prisms. × 7,000.

Figures 8 and 10

Fragments of organic cases from decalcified prisms lying on carbon (Fig. 8) and on formvar (Fig. 10) films. The reticulated sheets forming the walls of the cases appear as granular membranes after shrinkage. In Fig. 10, the presence of fibrils is indicated by arrangement in rows of the granulations which coat these fibrils. Identical structures were recorded in the organic sheets of decalcified mother-of-pearl from *Mytilidae* (see Fig. 9; see also reference 5, Pl. XV, Fig. 1 and reference 7, Figs. 3 and 4). Fig. 8, × 16,500; Fig. 10, × 40,000.

Figure 9

Fragment of an organic sheet of decalcified mother-of-pearl (nacreous conchiolin). The structure of nacreous conchiolin in *Mytilidae* is characterized by a condensed reticulum, appearing as a granular membrane and consisting of thinly fibrillar trabeculae studded with protuberances of various sizes. × 40,000.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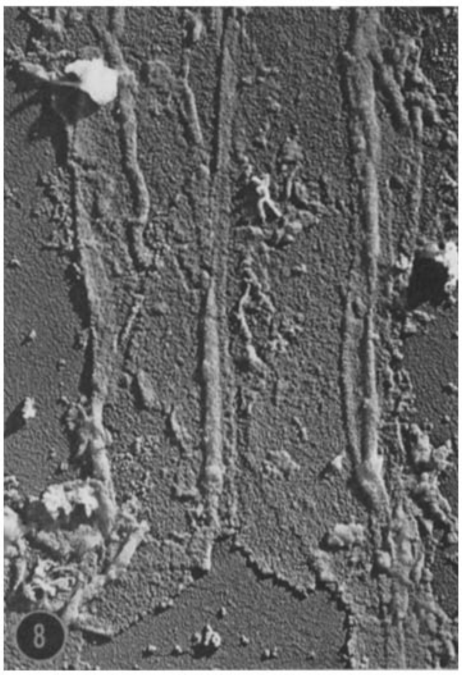
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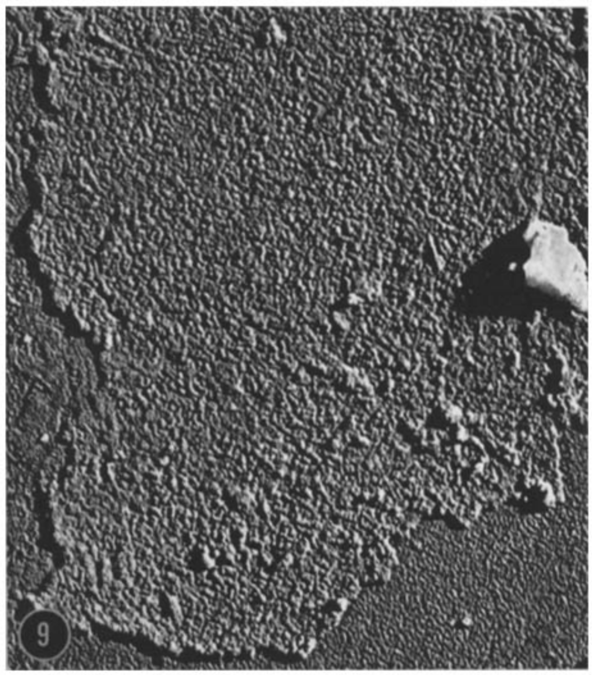
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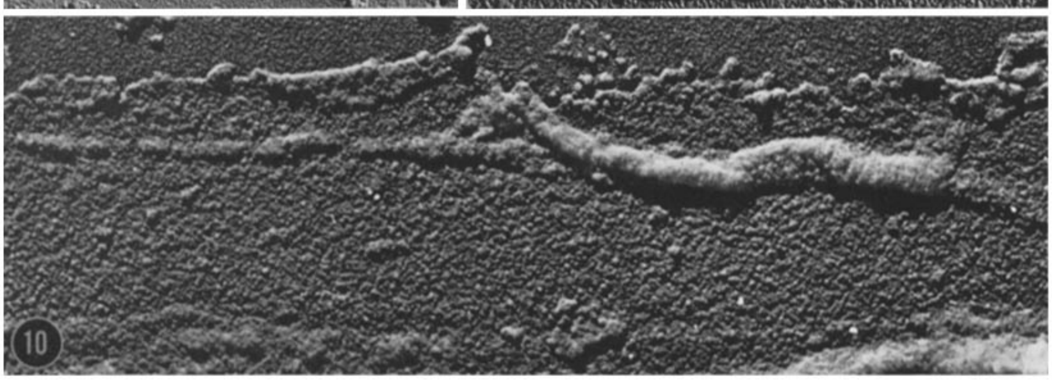
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