TISSUE & CELL 1978 **10** (4) 619–627
Published by Longman Group Ltd. Printed in Great Britain

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MORPHOLOGICAL STUDIES ON THE MANTLE OF THE FRESH-WATER MUSSEL *AMBLEMA* (UNIONIDAE): SCANNING ELECTRON MICROSCOPY

ABSTRACT. The scanning electron microscope has been used to describe the surface morphology of the mantle in mantle-shell preparations from the fresh-water mussel *Amblema*. In some regions (adductor muscle insertions), the mantle is firmly attached to the shell. In other areas (along the main course of the mantle), transient adhesions between the outer mantle epithelial cells and the nacre appear to temporally further compartmentalize the extrapallial fluid possibly as a prerequisite for the initial crystallization phenomenon. At the mantle edge, as well as at the isthmus, the periostracum was seen to extrude from the periostracal groove. At the siphonal edge, peculiar finger-like processes were identified; these may represent primitive photoreceptors. The epithelial cells of the outer mantle epithelium are all microvillated whereas those of the inner mantle epithelium are both microvillated and ciliated. Specific differences in surface morphology are described for various regions of the outer mantle epithelium. These may be related to precise regionalized functional differences of this tissue. Several functions of the mantle, in addition to shell formation, and based on its various morphologies, are also discussed.

Introduction

THE mantle is believed to be the shell-forming organ in mollusks (Wilbur, 1964, 1972). The light microscopic and transmission electron microscopic structure of this tissue has been previously described (Kawaguti and Ikemoto 1962; Nakahara and Bevelander, 1967; Saleuddin, 1970; Timmermans, 1969). To date, however, detailed regionalized scanning electron microscopic descriptions of the mantle have not been reported. Additionally, the previous morphologic studies have utilized mantle tissues which have been forcibly cleaved from its end product, the shell. From our initial studies (Petit, 1977a, b), we noted that the relationships between the mantle and the shell were both complex and variable, going from regions of permanent attachment to areas of temporary transient adhesion. Thus, we designed experiments which would preserve these mantle–shell relationships and their morphology in the various functional states: muscular attachment, nacreous apposition, periostracum elaboration, etc.

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Received 14 February 1978.
Revised 22 June 1978.

Materials and Methods

Mussels were collected from a local fresh-water lake. They were subsequently identified as *Amblema plicata perplicata*, Conrad. Animals were maintained in stock aquaria with constant regulation of light, temperature, pH, and medium ion content.

For this study, several different procedures were utilized to induce the initial adductor muscle relaxation. First, an experimental anesthetic (Sandoz MS 222), 1 g/liter of aquarium fluid, was used. Adductor relaxation generally occurred within 30–45 min. In a second group of experiments, cooling, by the overnight refrigeration (4°C) of the entire animal, also produced muscle relaxation.

Finally, a few specimens were frozen by placing them overnight in a freezer (−20°C). As a general consequence of these methods, a slight opening of the valves occurred which allowed the operator to surgically section both adductor muscles without disturbing the fragile mantle edge attachment and thus preserve the mantle–shell relationships.

In some instances, whole animals, prepared as above, were placed in either methanol (100%, for light microscopic orientation) or glutaraldehyde (2·5%) or formaldehyde (4%), buffered with 0·1 M cacodylate (pH 7·4), for scanning (SEM) and transmission electron microscopy. For SEM, animals were repeatedly washed in buffer, post-osmicated, dehydrated in ethanol and embedded intact in Spurr medium (Spurr, 1969). Following curing for several days at 60°C, the embedded animals were sectioned with a lapidary saw (Cab-Mate, Graves Co., Del Ray Beach, Florida). With this procedure, 24 2-mm sections ('slices') were obtained from a 5-year-old clam. This technique permitted us to accurately visualize and grossly identify areas of mantle attachment and adhesion for subsequent precise microscopic (SEM) sampling.

With this information, anesthetized, chilled, or frozen animals were sectioned with the diamond saw. All sectioning was done in the cold room (4°C). The saw blade, as well as the tissues, was kept constantly irrigated by the flow of chilled fixative (either glutaraldehyde or formaldehyde). Tissue slices were then placed in the appropriate fixative. The precise areas identified in the embedded specimens were visualized, dissected and further diced for SEM. Following several buffer washes and post-osmication, tissues were dehydrated in acetone and prepared for SEM by the critical point–CO_2 procedure (Anderson, 1951). Similar tissues were also prepared for transmission electron microscopy. However, the latter will be the subject of forthcoming communication.

Following critical point drying, tissue samples were mounted on copper studs, coated with gold–palladium in a vacuum evaporator equipped with a rotary coating module, and examined in an AMR 1000 scanning electron microscope (Advanced Metals Research Corp., Burlington, Mass.) operated at 20 kV.

Results

Fig. 1 is a diagram of the mantle morphology in *Amblema* showing the specific areas described in this study. These regions are: the distal mantle (2), including the siphons; middle mantle (3); isthmus (IC); the periostracal borders (4); and the mantle edge (1).

The body side (Figs. 1–3) showed a consistent morphologic arrangement throughout the course of the inner mantle epithelium.

Fig. 1. Schematic diagrammatically illustrating the course and arrangement of the mantle in *Amblema*. 1, mantle edge; 2, distal mantle; 3, middle mantle; 4, periostracal border; IC, isthmus, common lamina: IL, isthmus lyre; I, isthmus gutter; Pe, periostracum; PeL, periostracum, ligamentous; A, anterior adductor; P, posterior adductor; In, incurrent siphon; Ex, excurrent siphon; PL, pallial line.

Fig. 2. Scanning electron micrograph (SEM) of the inner mantle epithelium, body side, middle mantle. Both ciliated and microvillated cells are seen. Note that the former occur in branching rows. Numerous orifices of mucocytes are present (arrows). Many mucus droplets are evident. Magnification ×1000.

Fig. 3. High magnification SEM of the inner mantle epithelium, middle mantle. Note the mucocyte orifice (arrow), ciliated cells, and microvillated cells. Magnification ×5000.

Fig. 4. SEM of the outer mantle epithelium, shell side, middle mantle. Only microvillated cells, arranged in a cobblestone-like appearance, are seen. Grooves delineating cell boundaries are clearly evident. Note the conspicuous absence of mucus droplets in this region. Magnification ×1000.

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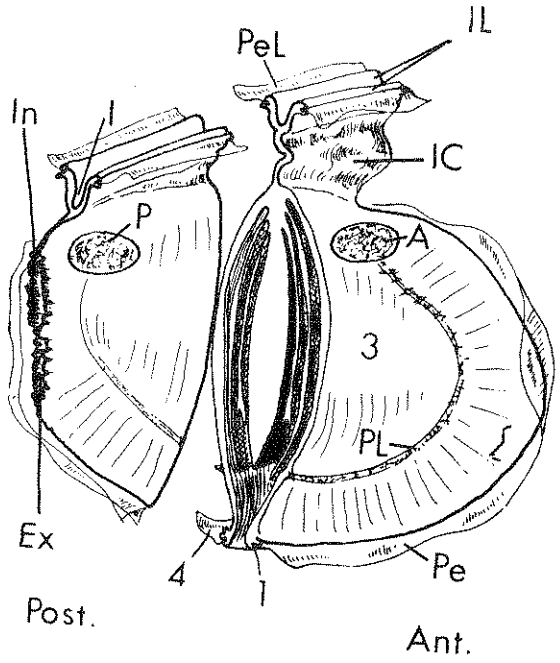

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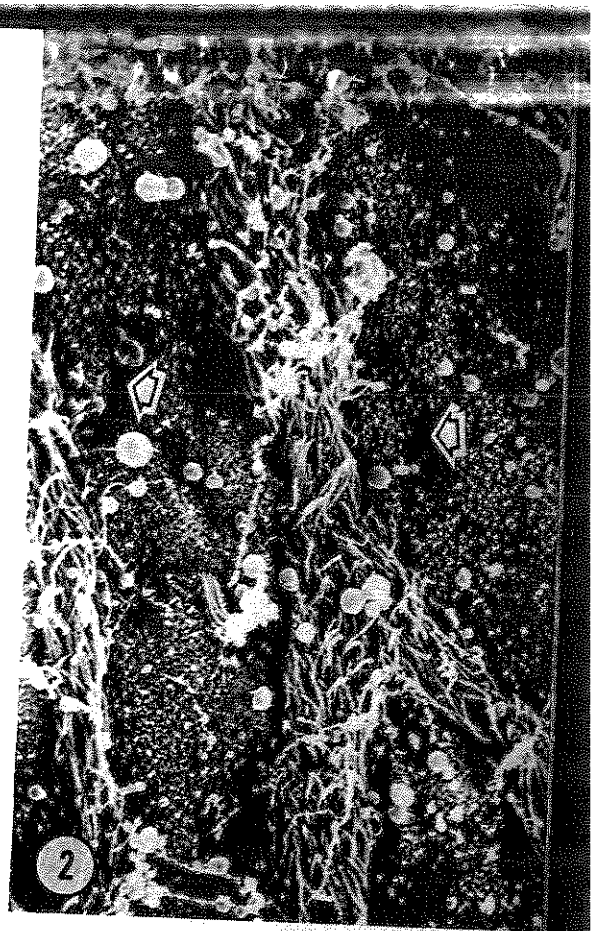

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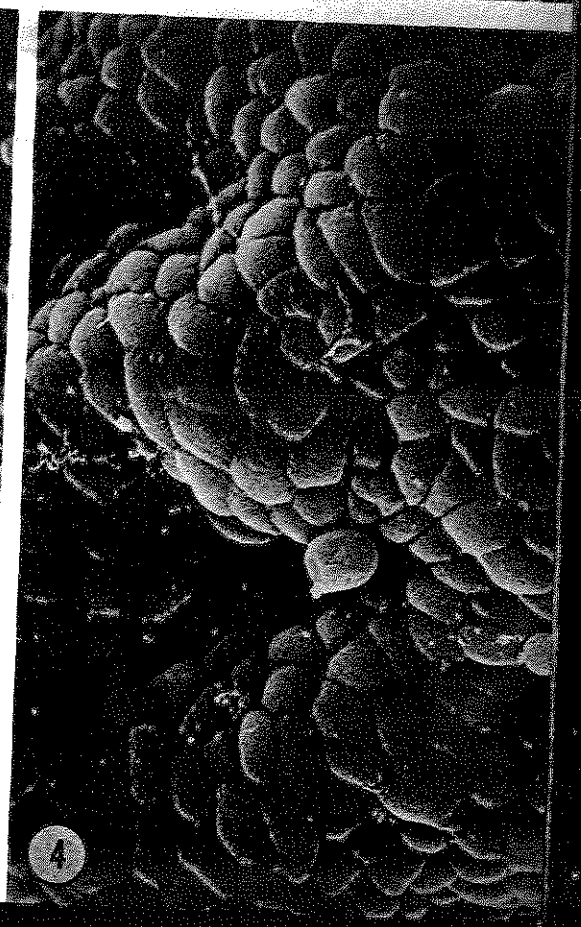

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Branching rows of cilia delineated areas of short microvilli between which are located the orifices of numerous secreting mucocytes (Figs. 2, 3). As a result, high concentrations of mucus droplets (globules) were seen all over this epithelial surface (Figs. 2, 3).

On the shell surface, the outer mantle epithelium (Fig. 1) showed a somewhat similar arrangement throughout the course of this tissue. Subtle differences, however, were seen in specific areas of this organ.

In the middle mantle (Fig. 1), cells with short microvilli were identified (Fig. 4). These cells were arranged in a cobblestone-like network. Grooves (junctions) demarcating adjacent cell borders were clearly visible. No cilia were seen on this aspect of the mantle. Mucus droplets were scarce in this mantle region. With the special techniques described in this paper, we obtained identical images in areas free of any shell attachment or adhesion. However, we identified alternating rows of bulging cells in close proximity to areas of temporary adhesion (Fig. 5). On the apical surfaces of these cellular protrusions, two populations of microvilli (short and long) were clearly observed (Fig. 6).

In areas of shell adhesion, extensive damage of the mantle surface resulted when this tissue was peeled from the shell (Fig. 7). This clearly indicated specific areas of a true mantle–shell organ existing as one non-separable entity.

At the mantle edge (Fig. 1), the periostracum was seen extruding from the periostracal groove (Fig. 8). On the tip of the inner ridge of the periostracal groove, a heavy coating of fibrous material and mucus droplets was evident (Fig. 9). This material probably coats the periostracal ribbon. In the siphonal area of the posterior edge (Fig. 1), finger-like processes, covered predominantly with microvillated cells, were seen protruding from the middle ridge of the periostracal groove (Fig. 10). Some patches of cilia were disposed along these processes (Fig. 10, insert).

At the isthmus (Fig. 1), a structure which heretofore has not been morphologically described, two distinct structures were

Fig. 5. SEM of the outer mantle epithelium showing rows of bulging cells. Middle mantle. Magnification × 500.

Fig. 6. High magnification SEM of bulging cells demonstrating two populations of microvillated cells. Cells with long microvilli, and cells with short microvilli occur in patches along these cellular bulges. Magnification × 5000.

Fig. 7. SEM of the outer mantle epithelium from a region of apparent tissue-shell adhesion. Peeling of the fixed tissue from the shell resulted in extensive cell damage. Magnification × 2000.

Fig. 8. SEM of periostracal extrusion at the mantle edge on the inner ridge of the periostracal groove. The periostracum (Pe) appears as an undulating curtain. The future growth lines of the shell outer surface are evident (arrows). Magnification × 200.

Fig. 9. SEM of the tip of the inner ridge of the periostracal groove. Extensive production of fibrous material and mucus droplets is shown. Both probably serve to coat the extruding periostracal ribbon. Magnification × 2000.

Fig. 10. SEM of the siphonal edge of the mantle. Finger-like processes are seen protruding from the middle ridge of the periostracal groove. Periostracal ribbon (Pe). Magnification × 100. *Insert:* High magnification SEM of the tip of one of the above processes. Note the presence of numerous ciliated cells. Magnification × 1000.

Fig. 11. SEM of the isthmus epithelium. A cobblestone-like epithelium, similar to that of the outer mantle epithelium, is seen. Numerous mucus granules, discharging mucocytes, and mucocyte orifices are evident. Magnification × 500.

Fig. 12. SEM of the epithelium lining the lyral gutter. Scattered ciliated cells are present. Mucocytes are also seen. Magnification × 2000.

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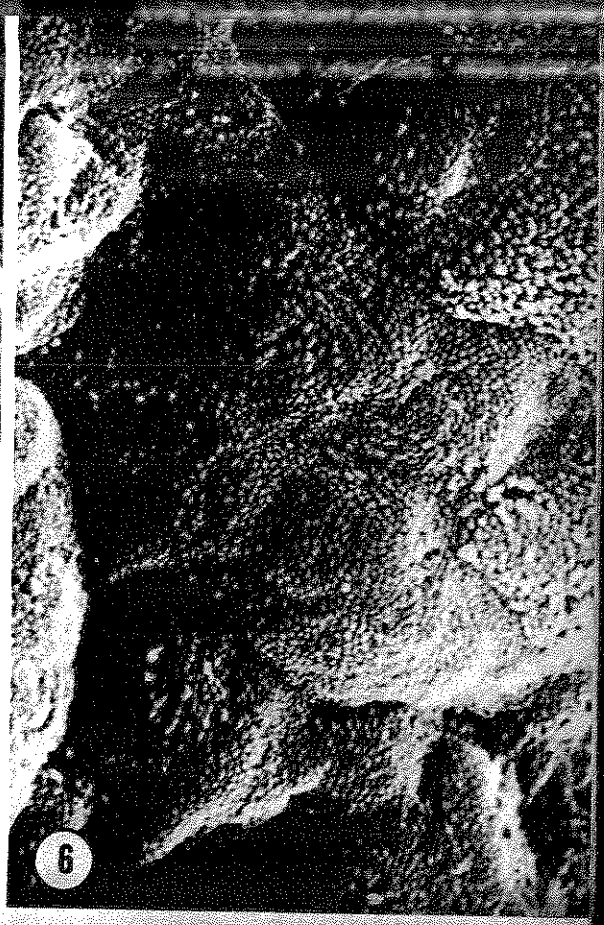

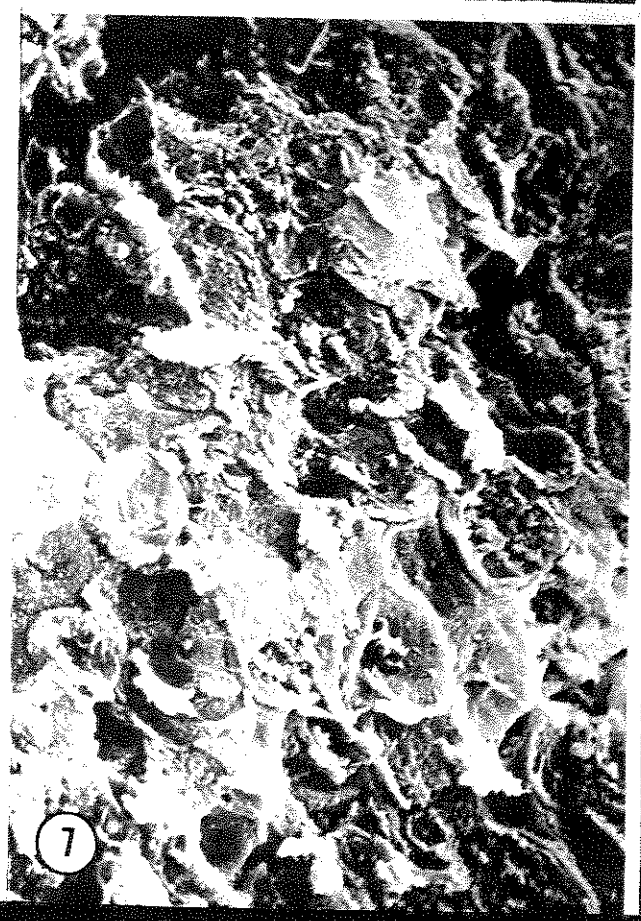

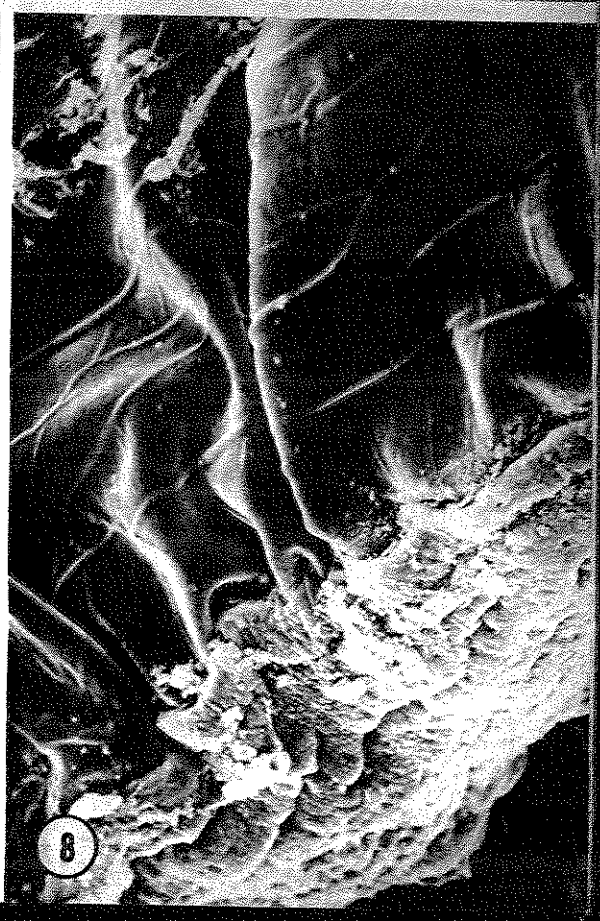

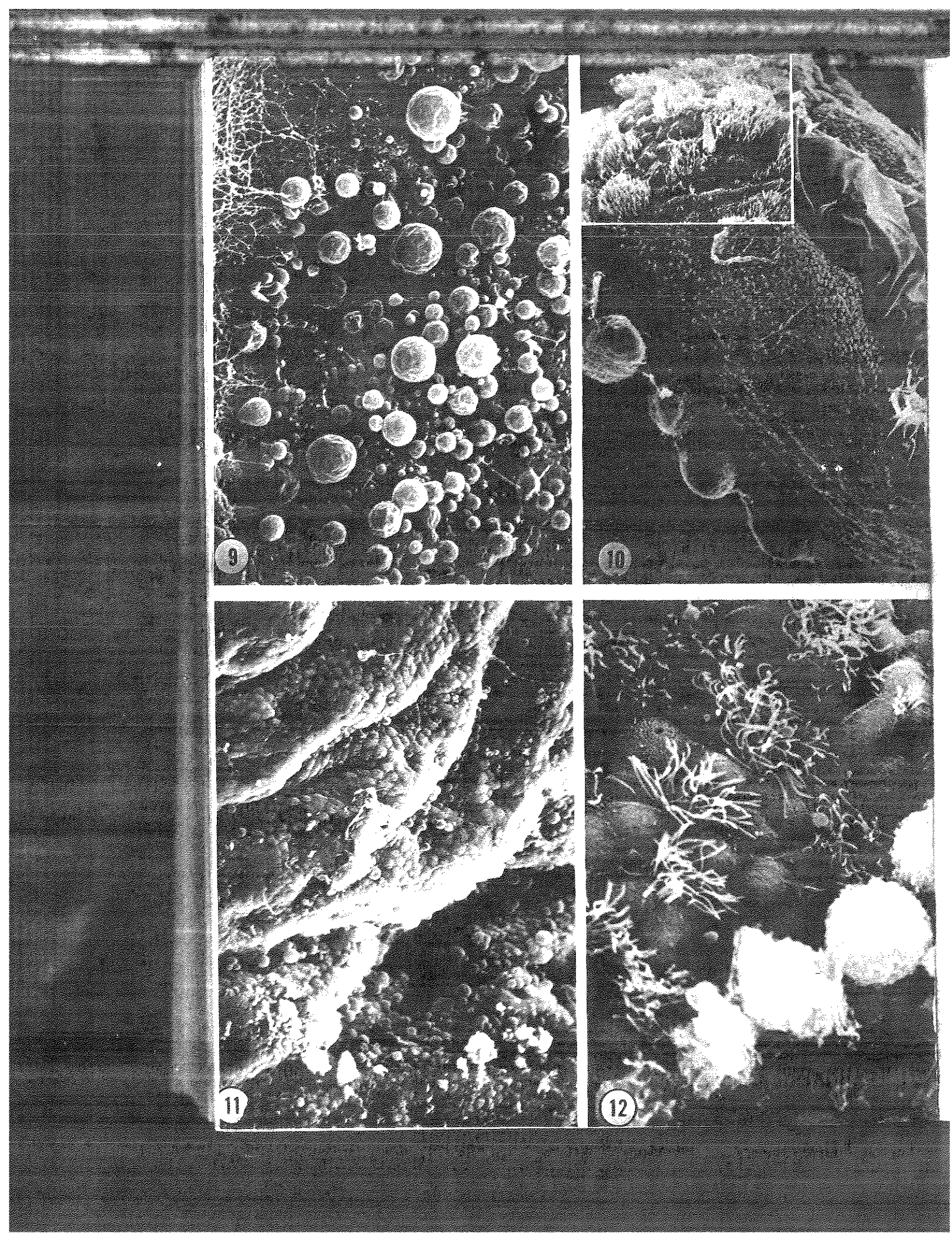
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identified. A common lyre was found all along the hinge ligament. This is connected to the body by a triangular common lamina which is related anteriorly to the pseudocardinal teeth and posteriorly to the lateral teeth. The outer aspect of the lyre, as well as the common lamina, were basically identical in surface morphology to the outer epithelium of the middle mantle (see above). However, both sides of the common lamina demonstrated a marked increase in the number of mucocyte orifices (Fig. 11). This gives this area a 'mattress-like' appearance. Inside the lyral gutter (Fig. 1), scattered ciliated cells were seen (Fig. 12). At the edge of the isthmus, the periostracum is produced (extruded) similar to that seen at the distal mantle edge. However, no finger-like processes were seen here. Additionally, only one groove, the periostracal groove, was found in this region. In this area, the periostracum is connected to the inner calcified ligament.

The attachment of the body mass to the valves is complex. Some specific muscles, the suspensory muscles, perform this function. However, the anterior and posterior adductor muscles, which function in the closure of the valves, also participate in the fixation of the soft tissues to the shell. All of these muscles transpierce the mantle, to which they are fused, before inserting into a specific mineral arrangement at the level of the shell muscle scars (Petit, 1977b).

Discussion

To our knowledge, this paper represents the first regionalized morphologic description, using scanning electron microscopy, of the molluscan bivalve mantle, the shell-forming organ of these animals (Wilbur, 1964, 1972). Additionally, this study represents an initial attempt to describe the regionalized mantle structure in coordination with its elaborated products (shell, periostracum, etc.). From this study we have shown that extreme care must be exercised during tissue preparation in order to precisely maintain the various described mantle–shell relationships. The methods we have utilized in this communication for preserving these relationships have demonstrated significant differences in the surface morphology of the outer mantle epithelium in specific regions of the shell. Thus, in certain areas such as the adductor muscle attachments, the mantle is strongly attached to the shell via the muscular fibers which transpierce it. In other regions, such as the pallial muscle attachment, the mantle is simply permanently adhered to the shell surface. However, along the bulk of the mantle surface, there is no permanent shell attachment; rather, some apparent temporary adhesions occur, apparently in pulsatile-like waves, as demonstrated by the rows of alternating, bulging cells (Fig. 5).

The described permanent attachments appear to be related to the insertions of the adductor muscles. The events of shell growth induce a migratory movement of the adductor scars, thus necessitating a permanent attachment. On the other hand, the pallial muscle is continuously moving distally, concurrent with mantle growth, and therefore requires only a simple surface adhesion.

In the regions where no attachments or only transient adhesions are seen, a space exists between the outer mantle epithelium and the nacreous layer of the shell. This probably allows for the formation of fluid-filled (extrapallial fluid) compartments in which the initial mineral of the nacreous layer is precipitated (Petit, 1977b). These compartments, which are numerous (seven to nine), appear to demonstrate a constant pulsatile activity resulting in the transient epithelial adhesions and the subsequent movement of fluid.

Of the above features on the mantle surface, only adhesions appear to be related to nacreous deposition.

Adhesions seem to be temporary (transient) and located at various areas of the mantle. These observations, coupled with quantitative analyses on the temporal moment to moment changes in the ionic composition of the extrapallial fluid compartment (Petit, 1977b), suggest a wave-like movement of the mantle flap which in turn leaks and/or secretes the shell components. Reinforcing this hypothesis is the constant observation of long microvilli on the tips of protruding (bulging) cells in close proximity to the nacre. These may function in the secretion and/or reabsorption of both the organic and inorganic components of the shell. The presence of long microvilli on the cells of the outer mantle epithelium in close proximity to the mineralized shell has been observed by other investigators (Bevelander and Naka-

hara, 1969; Nakahara and Bevelander, 1967) These have been reported to play a role in secretion (Bevelander and Nakahara, 1969) and particulate ingestion (Nakahara and Bevelander, 1967).

The large accumulation of mucus droplets in certain areas of the mantle (isthmus, facing the teeth) may be associated with several possible functions. These include the lubrication and cushioning of the fragile isthmus tissue which is situated between the large rough teeth. Additionally, this proteinaceous material may be involved in the organization of the crystalline components of the massive teeth. In other areas, such as the edge facing the periostracal extrusion, the increased accumulation of proteinaceous mucus may also be related to the mineralization phenomenon.

The periostracum appears to be the primary regulator for the growth and calcification of the shell (Petit, 1977b). This organ is a complex heterogeneous ribbon (Petit, 1977a, b) secreted as a pellicle and coated on both sides within its course in the periostracal groove. Structural and functional differences are seen in the periostracum from the isthmus and the periostracum from the edge. The former is involved in ligament formation and the latter in edge growth.

The finger-like processes identified at the siphonal edges may be related to light or shadow sensitivity. When the siphons, the only protruding part of the resting animal, were shadowed, an immediate closure of the valves resulted (Petit, 1977b). By SEM, these ridges were seen to consist of both microvillated and ciliated cells. The presence of photosensitive elements in this region has been well documented (Charles, 1966).

In conclusion, the molluscan mantle appears to be more than just a flap of tissue functioning solely in the elaboration of the shell with which it is often intimately associated. Other possible functions for this organ include: mucus secretion; support via muscular attachments and insertions; circulation via its pulsatile movements; and sensory perception. Such physiologic variables are reflected in the regionalized morphologies of the mantle. As further studies on this tissue are developed, additional functions may become apparent.

Acknowledgements

This research was supported in part by grants from the Ministere des Affaires Etrangeres, Paris, France, and from Baylor College of Dentistry, Dallas, Texas. The authors also gratefully acknowledge Jane Coleman for her secretarial assistance in the preparation of this manuscript.

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References

ANDERSON, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *Trans. N. Y. Acad. Sci., Sec. II*, **13**, 130–134.

BEVELANDER, G. and NAKAHARA, H. 1969. An electron microscope study of the formation of the nacreous layer in the shells of certain bivalves. *Calc. Tiss. Res.*, **3**, 84–92.

CHARLES, G. H. 1966. Sense organs (less cephalopods). In *Physiology of Mollusca* (eds. K. M. Wilbur and C. M. Yonge), Vol. II, pp. 455–521. Academic Press, New York.

KAWAGUTI, S. and IKEMOTO, N. 1962. Electron microscopy of the mantle of a bivalve, *Fabulina nitidula*. *Biol. J. Okayama Univ.*, **8**, 21–31.

NAKAHARA, H. and BEVELANDER, G. 1967. Ingestion of particulate material by the outer surface cells of the mollusc mantle. *J. Morph.*, **122**, 139–146.

PETIT, H. 1977a. A scanning electron microscopic (SEM) study of the mantle–shell relationship in the fresh-water mussel *Amblema plicata perplicata* Conrad. *Anat. Rec.*, **187**, 680–681.

PETIT, H. 1977b. The mantle–shell in the fresh-water mussel *Amblema plicata perplicata*. Doctoral dissertation, submitted to the faculty of Baylor University.

SALEUDDIN, A. S. M. 1970. Electron microscopic study of the mantle of normal and regenerating *Helix*. *Can. J. Zool.*, **48**, 409–416.

SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, **26**, 31–43.

TIMMERMANS, L. P. M. 1969. Studies on shell formation in molluscs. *Netherlands J. Zool.*, **19**, 417–523.

WILBUR, K. M. 1964. Shell formation and regeneration. In *Physiology of molluscs* (eds. K. M. Wilbur and C. M. Yonge), Vol. I, pp. 243–282. Academic Press, New York.

WILBUR, K. M. 1972. Shell formation in mollusks. In *Chemical Zoology* (eds. M. Florkin and B. T. Scheer), Vol. VII, pp. 103–145. Academic Press, New York.