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Mantle histology, histochemistry and ultrastructure of the pearl oyster *Pinctada margaritifera* (L.)

Roula Jabbour-Zahab [1], Dominique Chagot [2] †, Françoise Blanc [1](*) and Henri Grizel [2]

[1] *Laboratoire de Zoogéographie Génétique, Université Montpellier 3, B.P. 5043, 34032 Montpellier Cedex 1, France.*
[2] *Laboratoire de Pathologie et Génétique des Invertébrés Marins, IFREMER, B.P. 133, 17390 La Tremblade, France.*
(*) to whom all correspondence should be addressed.

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Abstract

The edge and isthmus of the mantle of *Pinctada margaritifera* (Mollusc, Bivalvia) were studied using light and electron microscopy from a morphological and histochemical point of view. Six areas with several subareas were differentiated in the mantle edge. One of these areas is similar to the epithelium isthmus. Trials to elucidate the nature of the pigment responsible for the black colour of the shell and pearl indicate a melanin-like material. Possible functions of the distinctive epithelial areas in *Pinctada margaritifera* mantle are discussed according to available data on the secretion of organic matrix of the shell, protein sclerotization and deposition of crystals in some pearl-forming molluscs. Three different epithelial areas in the outer mantle edge of *Pinctada margaritifera* are probably involved in the sequential processes of deposition of calcite prisms, aragonite fibres and aragonite rhombs.

Keywords: Mantle, structure, ultrastructure, pigment, *Pinctada margaritifera*, Mollusc, Bivalvia.

Histologie, histochimie et ultrastructure du manteau de l'huître perlière, Pinctada margaritifera (*L.*).

Résumé

Le bord et l'isthme du manteau de *Pinctada margaritifera* (Mollusque, Bivalve) ont été étudiés du point de vue morphologique et histochimique par microscopies photonique et électronique. Six zones et plusieurs sous-zones ont été identifiées dans le bord du manteau. L'une d'entre elles est similaire à l'épithélium de l'isthme. Les essais de détermination de la nature du pigment responsable de la couleur noire de la coquille et de la perle, ont abouti à un type de mélanine. Le rôle des différentes zones identifiées dans l'épithélium palléal de *Pinctada margaritifera* est discuté en fonction des données acquises chez d'autres Mollusques perliers ou non sur la sécrétion de la matrice organique de la coquille, la sclérotisation des protéines et le dépôt des biocristaux. Cette analyse permet notamment d'attribuer à trois des zones décrites ici une fonction dans le dépôt séquentiel des prismes de calcite, des fibres et des rhomboèdres d'aragonite.

Mots-clés : Mantcau, structure, ultrastructure, pigment, *Pinctada margaritifera*, Mollusques, Bivalves.

INTRODUCTION

The black pearl oyster *Pinctada margaritifera* (Linné, 1758), widespread in French Polynesia, has been harvested since the nineteenth century for its nacreous matter and, more recently, cultured for the production of pearls. This species produces a striking amount of black pearls in addition to white or grey pearls.

In all molluscs, the mantle is directly responsible for shell synthesis. In Bivalve molluscs, the mantle tissue covers the visceral mass and adheres to the inner surface of the valves. The two mantle lobes are usually unattached ventrally and laterally, but are joined together dorsally along the hinge line to constitute the mantle isthmus. The central part of each lobe contains a thin connective tissue layer that covers muscle fibres, nerves and blood vessels. The connective tissue is limited by two epithelial unicellular layers (Istin and Masoni, 1973).

The mantle edge as described by Dix (1973) in *Pinctada maxima* consists of three folds: an outer, a middle and an inner fold and the pallial zone lying immediately inside these three free folds up to the line of gill attachment. The central mantle is situated between the line of gill attachment and the mantle isthmus.

The present study was carried out to describe the general structure of the mantle edge and isthmus of *P. margaritifera*. Histological and histochemical data are compared to those obtained from some other Bivalves including pearl species such as *P. martensii*, *P. maxima* and *P. radiata* and discussed according to the potential role of the mantle edge in the complex process of shell formation (Ojima, 1952; Kawakami and Yasuzumi, 1964; Nakahara and Bevelander, 1971; Dix, 1972; Dix, 1973; Saleuddin and Petit, 1983).

In *Pinctada margaritifera* and other pearl species, the shell is composed of two major crystalline forms: peripheral calcitic prisms and aragonitic rhombs. However, unlike all other pearl oysters, in *P. margaritifera* the calcitic prisms are totally black while the aragonitic rhombs are pure white nacre except the very first aragonitic layer which is slightly coloured (J. P. Cuif, pers. comm.).

Finally some assays were undertaken to determine the nature of the pigment that may be released from the mantle and be responsible for the black coloration of the shell and pearl.

MATERIAL AND METHODS

Specimens of adult *P. margaritifera* were collected by diving in Takapoto, an atoll of the Tuamotu archipelago in French Polynesia. For light microscopy, whole black mantles were dissected and fixed either in buffered formalin according to Carson *et al.* (1973) or in Bouin Hollande's fluid (Gabe, 1968).

Selected pieces of tissue as indicated in *figure* 1 were embedded in paraffin, and cut into 3 µm thick

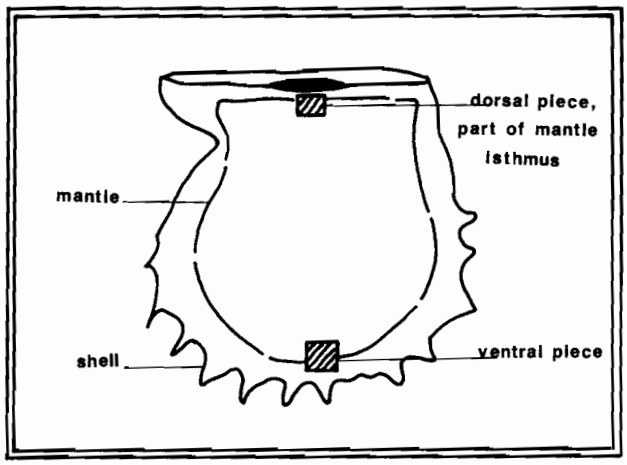


Figure 1. – Parts of *Pinctada margaritifera* used for the histological study.

sections. The sections were stained for morphological studies with either Ehrlich's hematoxylin-eosin, Goldner's trichrome (a modification of Masson's trichrome) or Giemsa's stain.

Histochemical studies were made using periodic-acid-Schiff (PAS) for the detection of carbohydrates and PAS combined with alcian blue (PAS-AB) for the characterization of the different mucopolysaccharides. Trials to determine the chemical nature of the black pigment of the mantle were performed using Turnbull's blue method for detection of ferrous ions, the Prussian blue method for ferric ions, and Turnbull's blue reaction modified by Tirmain and Schmeltzer for total ionic iron. Copper and magnesium were stained with the method of Okamoto and Utamura and the magneson method respectively. Melanins were investigated using the Hueck's staining (hydrogen peroxide bleaching), potassium permanganate bleaching, the ferrous ion technique for melanins according to Lillie, and the hexamine-silver variant of the Masson-Fontana method. All staining methods were performed according to Gabe (1968) and Pearse (1980). In addition, buffered formalin fixed tissues were used to localize peroxidase activity in 5 µm thick frozen sections (Graham and Karnovsky, 1966).

For transmission electron microscopy (TEM), small mantle pieces were fixed for 12 h at 4°C in 2.5% glutaraldehyde in 0.1 M cacodylate-HCl buffer (pH 7.6, 1 000 mosm). The tissues were rinsed in buffer alone, then postfixed for 1 h in 1% osmium tetroxide in the same buffer. After a final rinse in buffer, tissues were embedded in Epon 812. Semithin sections (0.5 µm thick) were cut using glass knives and stained with 0.5% toluidine blue in 1% sodium carbonate (pH 11.0) Ultrathin sections (60 to 90 nm) were cut

with a diamond knife, automatically contrasted with aqueous uranyl acetate and lead citrate in a LKB Ultrostainer, and examined in a Jeol 1200 SX TEM.

RESULTS

Mantle edge

On the basis of specific cellular characteristic, the mantle edge has been divided into six areas with several subareas from the inner side to the outer side (*fig.* 2; *table* 1).

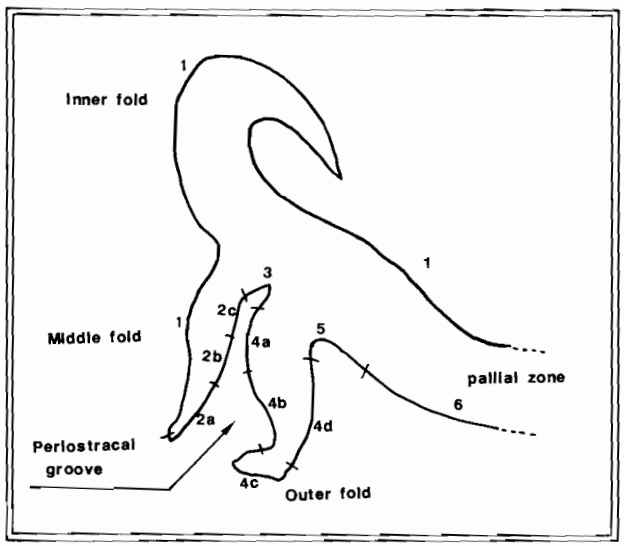


Figure 2. – Radial section through the mantle edge of *Pinctada margaritifera*.

Area (1) comprises the inner fold and the inner side of the middle fold. The epithelium lining the inner side of the free mantle edge and up to the tip of the middle fold is composed of small columnar cells that are heavily pigmented when using Bouin-Hollande's fixation. When using buffered formalin, however, these cells appear unpigmented (*fig.* 3). TEM shows that three cellular types are present: electron-lucent cells with vacuoles and microvilli, pigment containing cells with electron dense spherical granules and microvilli, electron dense cells bearing microvilli and cilia (*fig.* 15). Intercellular dilated spaces are sometimes observed near the inner fold. Neutral and acid glycoprotein-containing mucous cells (PAS and PAS-AB positive) are very abundant in the inner side epithelium of the inner fold and of the pallial zone. Many secretory cells containing acid and neutral glycoprotein granules are visible in the subepithelium of the inner side. The inner fold shows numerous radial and circular muscles. In connective tissue, the intra- and extracellular granules are electron dense.

In the middle fold, the shape of the cells is similar to that found in the inner fold. Some of the cells are pigmented. From the inner to the middle fold, the number of cells stained by the PAS and PAS-AB reactions decreases.

Area (2) is the outer side of the middle fold. Area (2*a*) (*fig.* 4) is characterized by acidophilic secretory cells and rare pigmented cells identical to those of area (1). The cells are connected by desmosomes and alternate with vacuolar cells more or less empty towards the apical cytoplasm (*fig.* 16). Some pigmented cells have microvilli and cilia while others possess brush borders. In these latter cells, the Golgi apparatus is prominent. Ciliated cells apparently

Table 1. – *Pinctada margaritifera:* Data on epithelial areas (+ presence, – absence).

	Area	Epithelium and cellular type	Melanin-like pigment	Secretions
Inner pallial zone and inner fold	1	Columnar epithelium Cells with microvilli and cilia Vacuolar cells with microvilli	+ + +	Mucous neutral and acid glycoproteins
Middle fold	2	Cuboidal or columnar Ciliated cells	+ –	Granular neutral glycoproteins
Periostracal groove	3	Pseudostratified epithelium Ciliated cells	–	Neutral and acid glycoproteins
Outer fold	4	Columnar epithelium Ciliated cells with microvilli Non-ciliated cells	+ –	Granular and mucous neutral and acid glycoproteins
	5	Transitional epithelium (from columnar to cuboidal)	+ –	Mucous rare neutral glycoproteins
Outer pallial zone	6	Cuboidal epithelium	–	Granular and mucous neutral and acid glycoproteins
	Isthmus	Cuboidal epithelium Vacuolar cells Cells with microvilli	–	Granular and mucous neutral and acid glycoproteins

deposit electron-dense material in the periostracal groove.

Tall glycoprotein-containing epithelial and subepithelial mucous cells (*fig.* 17), and ciliated cells are abundant in the (2*b*) area (*fig.* 5). In this area, the number of pigmented cells decreases.

The epithelium of the middle fold in the (2*c*) area (*fig.* 6), is composed of some pigmented cells. Other cells are non-ciliated, irregularly shaped. Some of them bear microvilli (*fig.* 18) with apical granules of moderate electron density containing neutral glycoproteins.

Area (3) represents the bottom of the periostracal groove (*fig.* 7). This area is covered by a pseudo-stratified epithelium. Electron microscopy shows dilated intercellular spaces into which electron dense material, thought to be periostracum, accumulates (*fig.* 19). The cells are characterized by numerous small electron-lucent vacuoles (*fig.* 20). A short area composed of ciliated cells (*fig.* 21) is seen immediately after the (2*c*) area. The periostracal material can be seen between the cilia (*fig.* 22). Based on histochemical reactions, the periostracal material is proteinaceous in nature and contains neutral and acid glycoproteins as shown by PAS and PAS-AB positivity.

Area (4) corresponds to the two sides of the outer fold. Below area (3), the epithelium (4*a*) (*fig.* 8) is columnar, with non-ciliated cells and a sparse population of pigmented cells provided with a brush border (*fig.* 23). Neutral glycoprotein-containing secretory granular cells are subepithelial.

Towards the (4*b*) area the columnar epithelial cells become less high and the intercellular spaces increase (*fig.* 9). The cells contain electron dense pigment. Some vacuolar cells have a clear cytoplasm with microvilli, others have a dense cytoplasm with microvilli and cilia (*fig.* 24). The mucous and the granular subepithelial cells show PAS positivity (acid glycoproteins).

At the outer fold tip (4*c*) area, an infolded and cuboidal epithelium, without granular secretory cells, is observed (*fig.* 10). Cuboidal cells are characterized by basal nuclei with microvilli and cilia (*fig.* 25). Mucous cell containing neutral glycoproteins are subepithelial.

In area (4*d*) (*fig.* 11), the epithelium, composed of tall columnar cells and of mucous cells with microvilli and cilia, is more infolded than that of (4*c*). Ultrastructural features of cells in this area are identical to those of area (4*c*). Epithelial or subepithelial cells contain either acid glycoproteins granular secretions or neutral mucous secretions.

Area (5) is a very small area limiting the outer free mantle fold. In this area, the cell height decreases progressively to constitute a transition zone (*fig.* 26, 27, 28) between the columnar (4*d*) and cuboidal epithelium typical of area 6. In the transition zone, the vacuolar cells are characterized by apical secretions (*fig.* 26). In this area, little of the neutral secretions are subepithelial.

Further in toward the shell, the epithelial cells flatten in area (6) (*fig.* 13). They have a basal elongated nucleus with short protruding cell processes (*fig.* 29). The epithelial cells containing acid glycoprotein secretory granules alternate with neutral glycoprotein-containing mucous epithelial cells. The two secretory types occur in the subepithelial connective tissue of the outer pallial zone.

Peroxidase activity with similar intensity was detected throughout the whole mantle edge.

Mantle isthmus

Lying inside the shell hinge line, the mantle isthmus (*fig.* 14) shows a dominant proportion of cuboidal cells with basal nuclei and polymorphic vacuoles (*fig.* 30). These cells have microvilli (*fig.* 30, 31) and sometimes few electron dense granules (*fig.* 31).

Light microscopy shows that some cells contain mucus, and that others have small or large secretory granules. Mucous cells and large secretory granule cells are positive for neutral glycoprotein reaction.

Figures 3 to 14. – Light micrographs, 3 µm radial paraffin sections of the mantle edge, and isthmus of *P. margaritifera*.

3: Area (1), epithelium (ep) of the inner fold showing pigmented cells, connective tissue (ct) and muscle fibre (mf).

4: Area (2*a*) showing acidophilic secretory cells (ac) and rare pigmented cells (pc).

5: Area (2*b*) containing tall secretory cells (sc).

6: Area (2*c*) with pigmented cells.

7: Area (3), periostracal groove bottom containing pseudo-stratified epithelium (pse) and vacuolar cells (vc). The forming periostracum (P) is clearly visible.

8: Area (4*a*), columnar epithelium with sparse pigmented cells (pc), neutral glycoprotein-containing subepithelial cells (nSc).

9: Area (4*b*), vacuolar cells (vc) with proeminent intercellular spaces (is).

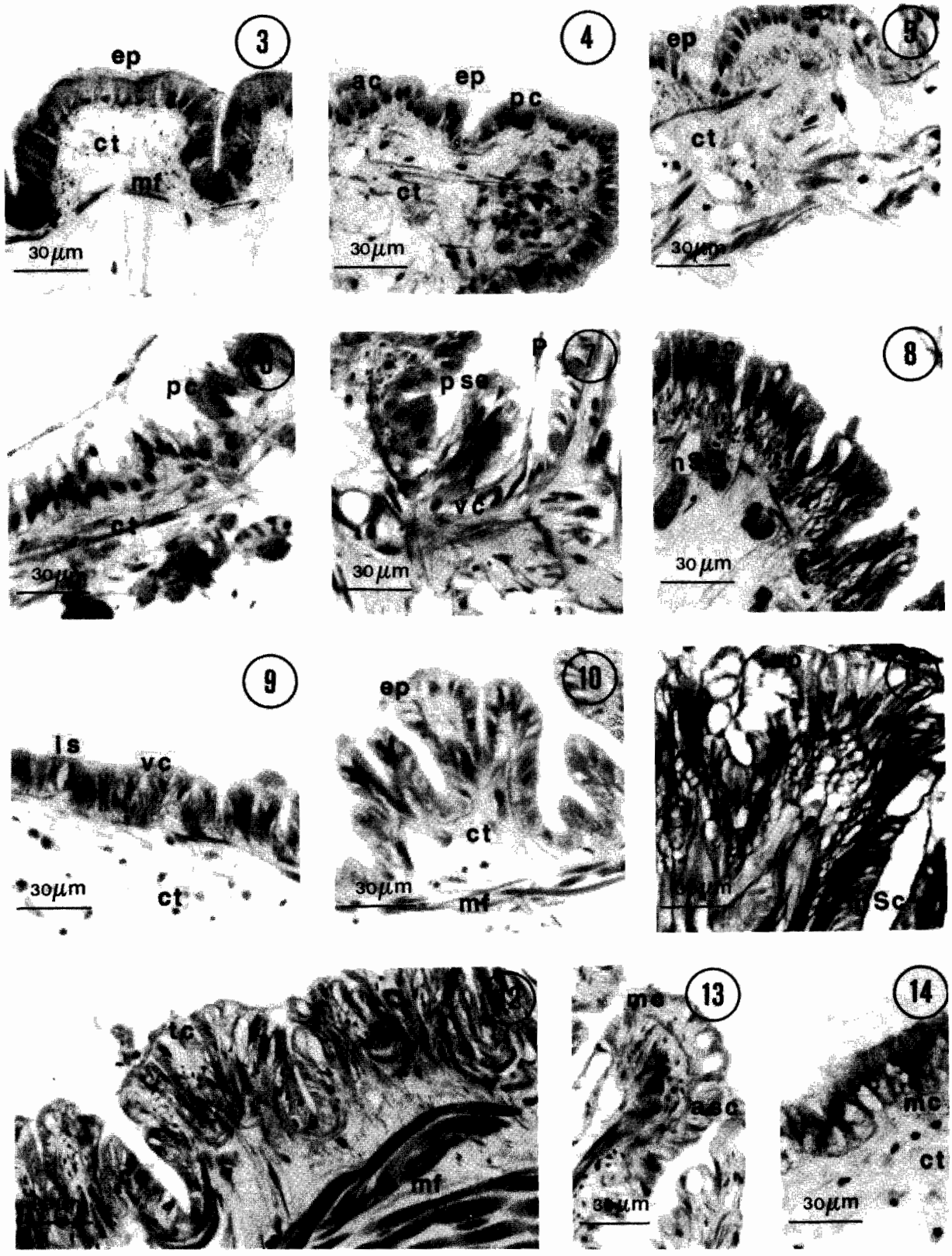
10: Area (4*c*), infolded cubic epithelium.

11: Area (4*d*), columnar epithelial cells and mucous subepithelial cells (mSc).

12: Area (5) showing two cellular types: cubic cells (cc) and taller cells (tc).

13: Flattened cells in area (6) with mucous cells (mc) and acid glycoprotein containing secretory cells (asc).

14: Mantle isthmus showing vacuolar cells (vc) and mucous cells (mc).



Figures 3 to 14

Figures 15 to 22. – Electronmicrographs.

15: Two main cellular types in area (1) (inner fold), pigmented cell (pc) containing spherical electron dense granules (dg) and bearing microvilli (mv); electron dense cells (dc) bearing microvilli and cilia (ci); nucleus (N); mitochondria (mi).

16: Arca (2 *a*) acidophilic secretory cell (asc) containing granules (ag), vacuolar cell (vc); desmosome (d); mucous gland (mu).

17: Area (2 *b*) Ciliated (ci) and mucous (mu) cells.

18: Area (2 *c*) Microvilli-bearing cells containing apical granules (ag).

19: Area (3) Pseudo-stratified epithelium into which intercellular spaces (is) are dilated and countain periostracum (P).

20: Same area than figure 19 showing cells with numerous electron lucent vacuoles (lv).

21: Ciliated (ci) cells lying immediately after 2c area.

22: Periostracum (P) accumulating between cilia (ci).

Some small granule secretory cells appear to contain acid mucopolysaccharides while others are devoid of glycoproteic secretion (PAS negative).

Pigment histochemistry

The histochemical reactions performed to determine the nature of the black pigment in the mantle edge are presented in *table* 2. The reactions were negative for ferrous iron, ferric iron, ionic and non-ionic iron, copper, and magnesium. The hexamine silver staining variant for melanin and the Hueck staining were also negative. In contrast, the ferrous iron technique for melanin (Lillie's reaction, 1957) and the bleaching by the permanganate method showed positive reactions.

DISCUSSION

According to Wilbur and Saleuddin (1983) the formation of shell can be described in terms of two major phases: 1) cellular processes of ion transport, protein synthesis and secretion and 2) a series of physicochemical processes in which crystals of $CaCO_3$ are nucleated, oriented, and grow in intimate association with a secreted organic matrix. Shell formation is a complex process involving three steps: the secretion of the organic matrix of the shell, protein tanning, and deposition of the crystals.

We tried to find a relationship between the mineralogical sequence occurring in shell formation and the cellular differentiation of the pallial epithelium, especially that of the outer fold. The presence of pigment in some epithelial areas may also indicate their possible role in black calcitic prism deposition.

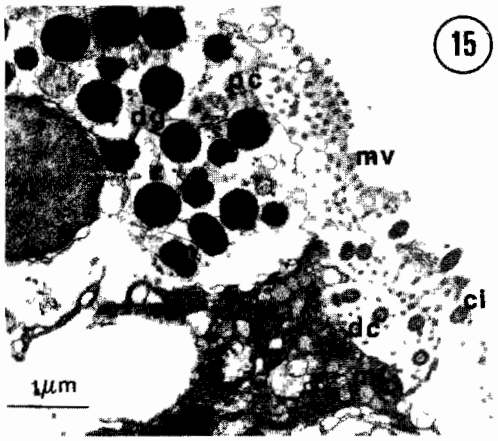
In *Pinctada margaritifera*, the epithelium in area (1) is characterized by acid and neutral glycoprotein-filled mucous cells, and numerous pigmented cells with microvilli and cilia or with microvilli only. One type of these secretory cells is similar to those described in *Anodonta cygnea*. These cells are supposed to play a role in controlling the osmotic pressure of the hemolymph (Machado *et al.*, 1988). In *Pinctada maxima*, in this area, acid and neutral glycoproteic secretions are granular (Dix, 1972) instead of mucous as in *P. margaritifera*.

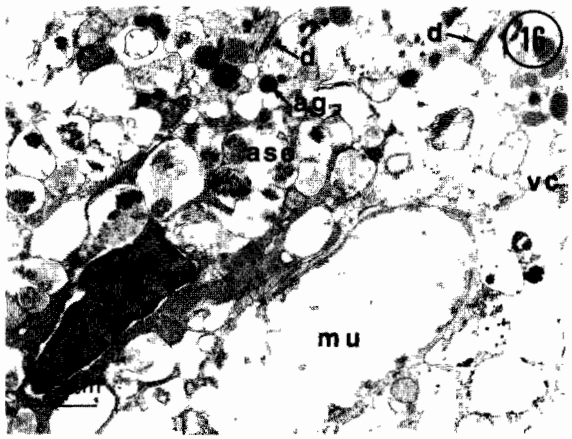
Our test on the chemical nature of the pigment found in areas (1), (2) up to (6) and absent in isthmus, have led to inconsistent results with respect to the presence of melanins: two assays were found positive (Lillie, $KMnO_4$ bleaching) and two were found negative (Hueck, Masson-Fontana). It must be mentioned that the first two reactions had been tested in *P. maxima* (Dix, 1973), another pearl-forming species, and produced the same positive result, leading Dix to assume that the pigment was indeed melanin. In *P. margaritifera*, the whole set of reactions regarding melanin is not positive. Considering, according to Pearse (1985), Lillie's reaction as the most specific for melanins, we consider the black pigment in *P. margaritifera* to be melanin-like.

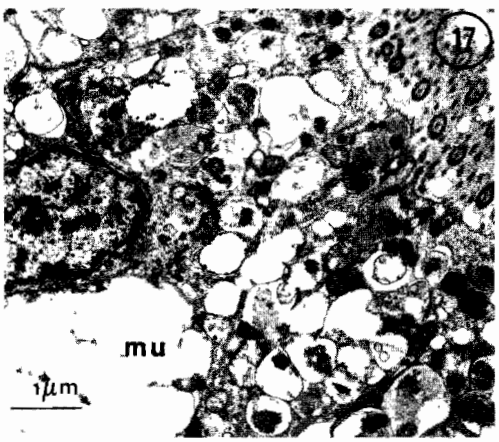
Some metals originating from basaltic substratum, such as iron (Fe) or magnesium (Mg) have been found in lagoon waters (Serra, 1989). These metals, if selectively concentrated in *Pinctada* mantle, could be involved in the mantle coloration. Nevertheless, reactions for iron and magnesium were also negative.

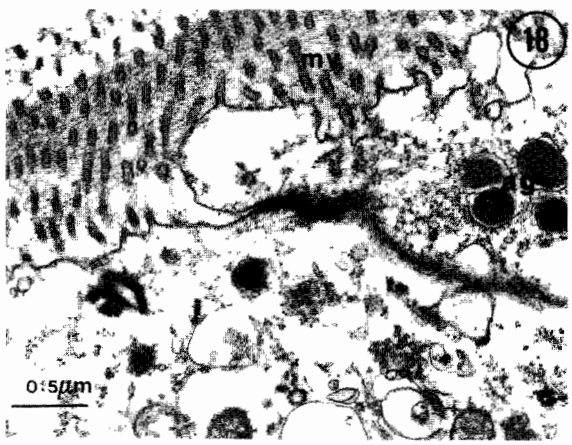
Area (2) is characterized by ciliated cells and rare pigmented cells. Epithelial and subepithelial secretory cells contain acid and neutral glycoproteins like in *Pinctada maxima* (Dix, 1972). According to Petit (1978), Petit *et al.* (1980), Petit (1981) and Richardson *et al.* (1981), the epithelial cells lining the periostracal groove in *Amblema* and *Cerastoderma edule* maintain and guide the initial periostracal material. These cells have microvilli in both species.

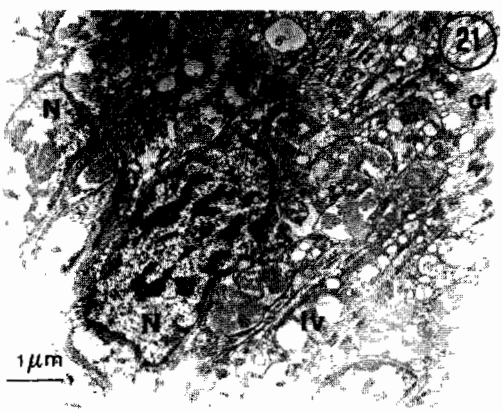
In *P. margaritifera*, like in all Bivalves, the periostracum arises from a groove located between the outer fold and the middle fold. Nascent periostracum can be seen in intercellular spaces at the pseudo-stratified epithelium level (area 3). These ciliated cells may be involved in guiding the initial periostracum towards the exit of the groove. The two epithelial layers (2 *c*) and (4 *a*), composed of cells with microvilli and which secrete neutral glycoproteins may play an important part in periostracum maturation, as suggested by the PAS-positive reaction of the periostracum. The newly formed periostracum in *P. maxima* is also found PAS positive (Dix, 1972).

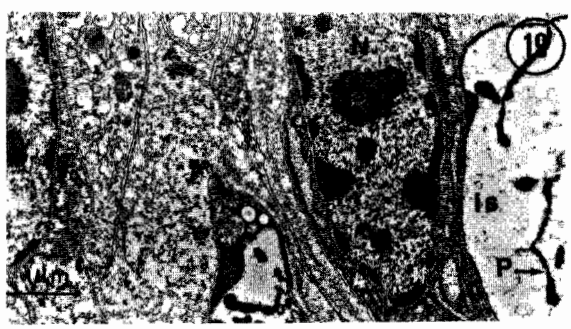


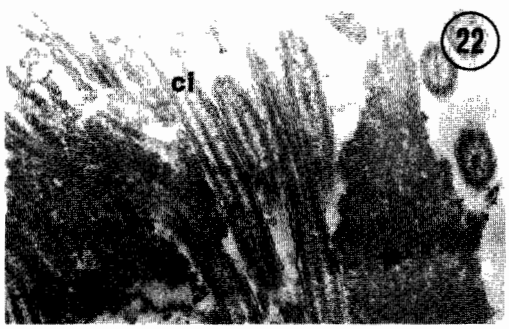


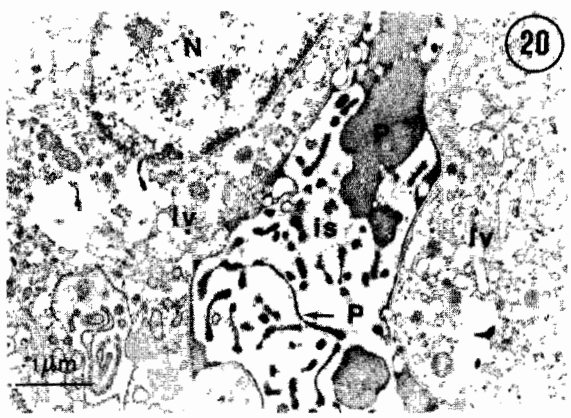












Figures 15 to 22

Figures 23 to 29. – Electronmicrographs (contd.).

23: Non-ciliated columnar cell bearing microvilli in area (4*a*).

24: (4*b*) area dense cytoplasm cell with microvilli and cilia.

25: (4*c*) area, cuboidal cells with basal nuclei, microvilli and cilia.

26: Area (5): cells containing vacuoles (va) and apical granules (ag).

27 and 28: Area (5): cells becoming less high compared to *figure* 26.

29: Area (6) pallial zone. Flat cells with short cell processes (cp).

Kawakami and Yasuzumi (1964) mentioned that in *P. martensii* the periostracum is formed by a special modification of the internal surface of the outer fold.

In *Amblema*, (Petit, 1978) the periostracal material originates from intercalated cells located in the inner side of the periostracal groove. These cells are devoid of microvilli and have a protruding tongue. The epithelial layers along the periostracal groove secrete a glucidic coating on the two sides of the periostracum with the help of two cellular types similar to those we describe for *P. margaritifera:* cuboidal cells with short microvilli in area (2*c*) and epithelial cells with high microvilli in area (4*a*) (Petit, 1978; Saleuddin and Petit, 1983).

In *Macrocallista maculata* (Bevelander and Nakahara, 1967), the periostracum is first elaborated in a limited cellular area at the bottom of the periostracal groove by cells bearing long microvilli, a situation different from *Astarte* (Saleuddin, 1974) in which the periostracum origin can be found in the large epithelial cells of the outer fold devoid of microvilli but containing channels. Their cytoplasm has electron dense inclusions, which are membrane-bound vesicles supposed to be periostracum precursors.

In *Cerastoderma edule* (Richardson *et al.*, 1981), periostracal groove cells play a role in periostracum production: these are basal cells of the outer fold and cells called accessory basal cells. A type of the latter, located between the first basal cell and the first epithelial cell of the outer fold, is supposed to push the periostracum towards the outside of the groove and to protect it from tearing when the mantle retracts during shell closure.

The secretion of periostracum is accompanied by sclerotization of the proteins, a process presumed to be similar to quinone tanning, by polymerization of phenolic compounds resulting from oxidative degeneration of tyrosine. Thus, melanins, a group of polymeric molecules formed from tyrosine (Verhecken, 1989) have been described in several species of Molluscs (Fox, 1983), and particulary in *P. maxima* (Dix, 1973) in the middle and inner fold epithelia of the mantle.

Enzymes of the mono- and polyphenol oxidase classes are copper proteins. The tyrosinase of the wall of the ink sac in the octopus and squid is especially rich in copper. However, in our study copper was not detected in any mantle tissue cells indicating that, if present, copper concentration is below the sensitivity of the used method (Pearse, 1985). The strong peroxidase activity might indicate the involvement of such an enzyme in the tanning system (Waite, 1983).

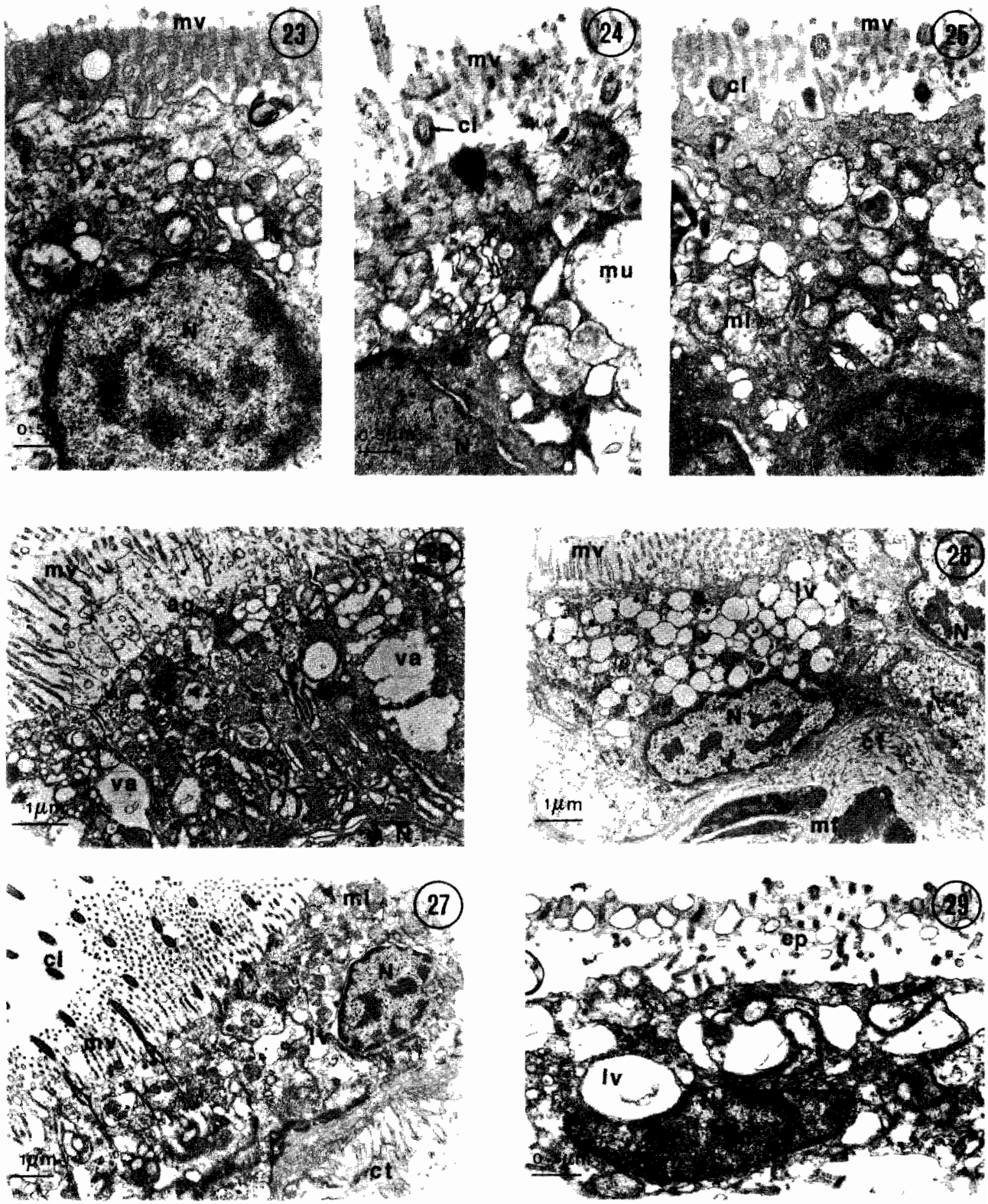
In *P. margaritifera*, brown pigmented cells are sparsely distributed in (4*a*) and (4*b*) areas of the outer fold an in (2*a*) and (2*c*) areas of the middle fold. The pigment in these areas persists partly when using buffered formalin fixation. However, the pigment of others areas is dissolved in the same buffered fixation. Despite this differential behaviour, histochemical reactions used indicated the whole pigment to be of melanin nature.

In *Cerastoderma edule* (Richardson *et al.*, 1981), the ion pump transporting calcium from mantle towards the pallial cavity, is assumed to be present in the cells of the outer fold which are provided with brush border and characterized by numerous mitochondria and large endoplasmic reticulum. These cells are similar to those described in *P. margaritifera* area (4). According to Istin and Masoni (1973), in Bivalves, the number of mitochondria underlying the epithelial border of the outer fold indicates a metabolic activity for such cells. This activity is not linked with calcium movements but may be linked with matrix component synthesis.

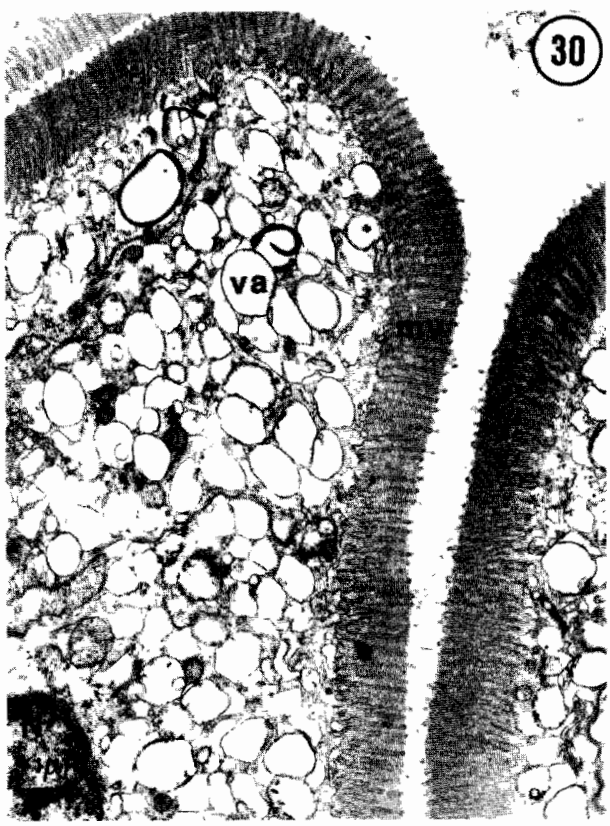
In *Pinctada margaritifera* the columnar epithelium in area (4*d*) is characterized by ciliated cells with microvilli. Numerous acid and neutral glycoprotein-filled granular and mucous cells are epithelial and subepithelial.

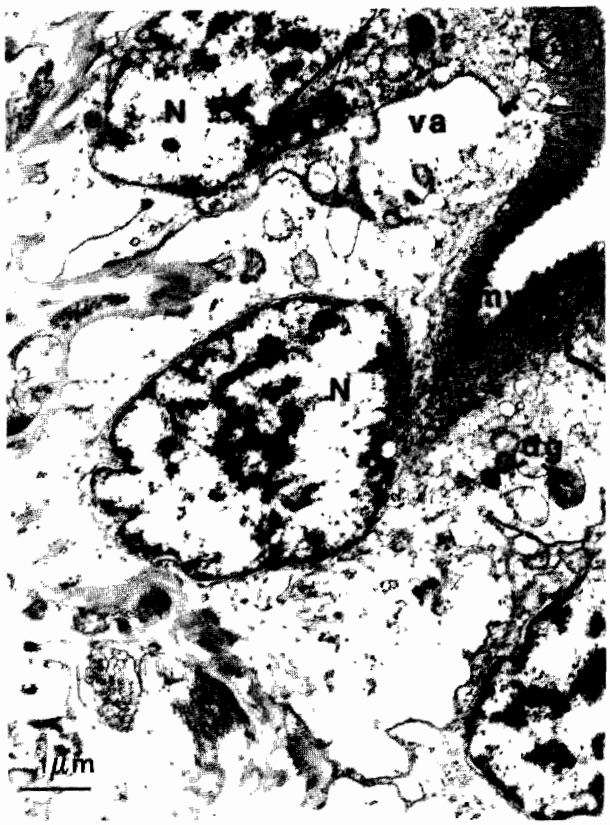
According to Nakahara and Bevelander (1971), in *P. radiata*, the prismatic layer of the shell is derived exclusively from the tall columnar cells lining the outer surface of the outer mantle fold. These cells are identical to those described in *P. margaritifera* area (4*d*). According to Petit (1978), in *Amblema* the middle layer of the periostracum is involved in the prismatic layer of the shell. Unlike in *Amblema*, mantle epithelial cells located in the outer fold are assumed to contribute to this prismatic layer in *P. radiata* and in *P. margaritifera* as well. Furthermore, similar cells are described in the periostracal or prismatic pearl sac in *P. maxima* (Dix, 1973) and *P. martensii* (Machii, 1959; Aoki, 1966).

In area (6), flat epithelial cells are found to contain extremely abundant acidic and neutral glycoproteins secretions.



Figures 23 to 29





Figures 30 and 31

Figure 30. – Mantle isthmus cells with polymorph vacuoles (va) and brush border (mv).

Figure 31. – Same area *figure* 30 showing few electron dense granules (dg).

Table 2. – Pigment determination by using histochemical staining reactions.

Reaction	Result
Turnbull's blue (Fe^{2+})	–
Prussian blue (Fe^{3+})	–
Turnbull's blue (modified by Tirmain and Schmeltzer) (whole ionic Fe)	–
Okamoto and Utamura (Cu)	–
Magneson (Mg)	–
Hueck (peroxide bleaching)	–
$KMnO_4$ bleaching	+
Lillie's (melanins)	+
Masson-Fontana ($AgNO_3$)	–

Area (5) may be considered as a transition zone between (4*d*) and (6) epithelia. However, unlike areas (4*d*) and (6), area (5) is found very poor in neutral as well as in acidic secretions.

Mantle isthmus cells are more similar to area (6) cells than other areas of mantle edge: cuboidal epithelium, no pigmented cells and rich in acidic and neutral glycoproteins secretions.

As Samata *et al.* (1989) have pointed out, Ca-binding glycoproteins have been found very acidic in gorgonian spicules and in several Bivalve shells.

Thus, in *P. margaritifera*, areas (4*d*) and (6) can be related to intense activity in crystalline deposition due to special richness in acidic glycoprotein secretions. Conversely, area (5), found poor in neutral as well as in acidic secretions, may be involved in a different process related to aragonitic fibrous variety described by (Caseiro and Gauthier, in press). Furthermore, the morphological change noticed between (4*d*) and (6) areas allows us to correlate (4*d*), (5) and (6) areas to the sequential processes of deposition of calcitic prisms, aragonitic fibres and aragonitic rhombs. Thus, epithelial and subepithelial cells in area (6) may be involved in the nacreous layer of the shell in *Pinctada margaritifera*, unlike in *Amblema* (Petit, 1978; Saleuddin and Petit, 1983).

Assuming the potential role of areas (5) and (6) in the deposition of aragonitic fibres and rhombs, these areas are supposed to be convenient as graft in pearl

culture. Furthermore, similar cells to those of (5) and (6) areas in *P. margaritifera* are found in the nacreous pearl sac of *P. maxima* (Dix, 1973).

Moreover the description of the healthy tissues can be used to examine possible histological and histochemical alterations of the mantle inducing dysfunctions in crystal or proteinaceous matrix production. Such dysfunctions leading to proteinaceous hyperproduction and decrease in crystal production are sometimes observed in this species.

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