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On the periostracum of the freshwater bivalve *Lamellidens marginalis*

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Abstract. The structure and chemical composition of the periostracum of the bivalve *Lamellidens marginalis* and the change in chemical composition during growth stages were investigated. The periostracum is composed of three constituent layers, an outer part, formed of lipoproteins, the protein component containing aromatic amino acids, the middle layer showing the presence of sulphur containing protein and the innermost layer showing a general similarity in chemical nature to the outer layer. The periostracum of *Lamellidens marginalis* show transverse canals traversing its entire width. The periostracum of *Lamellidens marginalis* shows certain structural features not met with in other forms, the presence of loop-like projections of the outermost periostracal layer, downward projection from the internal layer extending into the calcareous layer and the multiple type of periostracum. Quinone tanning in the outermost layer is pronounced resulting in sclerotin formation. The middle layer of the periostracum undergoes hardening by sulphur bonding in the later growth stages. The innermost layer is untanned and shows fuchsinophily as in the outer layer.

Keywords. Periostracum; bivalve; *Lamellidens marginalis*.

1. Introduction

Previous studies on molluscan shells have indicated variation in structure and chemical composition of the periostracum in relation to environmental factors (Meenakshi *et al* 1968). For example, Jameson (1912), Haas (1935) and Beedham (1958) have found that the periostracum in the freshwater species are stronger than that of the marine species. Although such variations may be due to factors such as salinity, not much is known on this aspect owing to inadequate information on the structural as well as the chemical composition of the different layers constituting the periostracum in various freshwater and marine forms (Beedham 1965; Meenakshi *et al* 1968).

The present investigation on the structure and chemical composition of the periostracum of *Lamellidens marginalis* is undertaken with a view to throw more light on the structural peculiarities and chemical composition associated with the freshwater environment.

2. Materials and methods

L. marginalis were collected from ponds and pools in and around Madras where they occur partially buried in the soil.

There is a paucity of information on the growth size relationship of *Lamellidens marginalis*. The observations of Annandale and Prashad (1919) and Satyamoorthi (1960) on the size range of *Lamellidens* may suggest that it varies from 36 mm to about 80 mm in length. The two sizes chosen for the study are (a) 20 mm in length and 10 mm in breadth and (b) 80 mm in length and 40 mm in breadth representing a young and old stage.

The shells were fixed in 5% formalin or 10% neutral buffered formalin for a minimum period of 24 hr. They were subsequently washed and decalcified either in 5% acetic acid or in 8% ethylene diamine-tetraacetic acid (disodium salt) (Simkiss and Tyler 1957). Paraffin sections (5–8 μ) of the decalcified shell and periostracum were prepared for histological study. Sections were also prepared by double embedding in celloidin and paraffin. Stains used were Mallory's triple stain, Heidenhain's haematoxylin and Masson's trichrome stains. Frozen sections were prepared by gelatin impregnation method (Carlton 1938) and used for the application of histochemical tests.

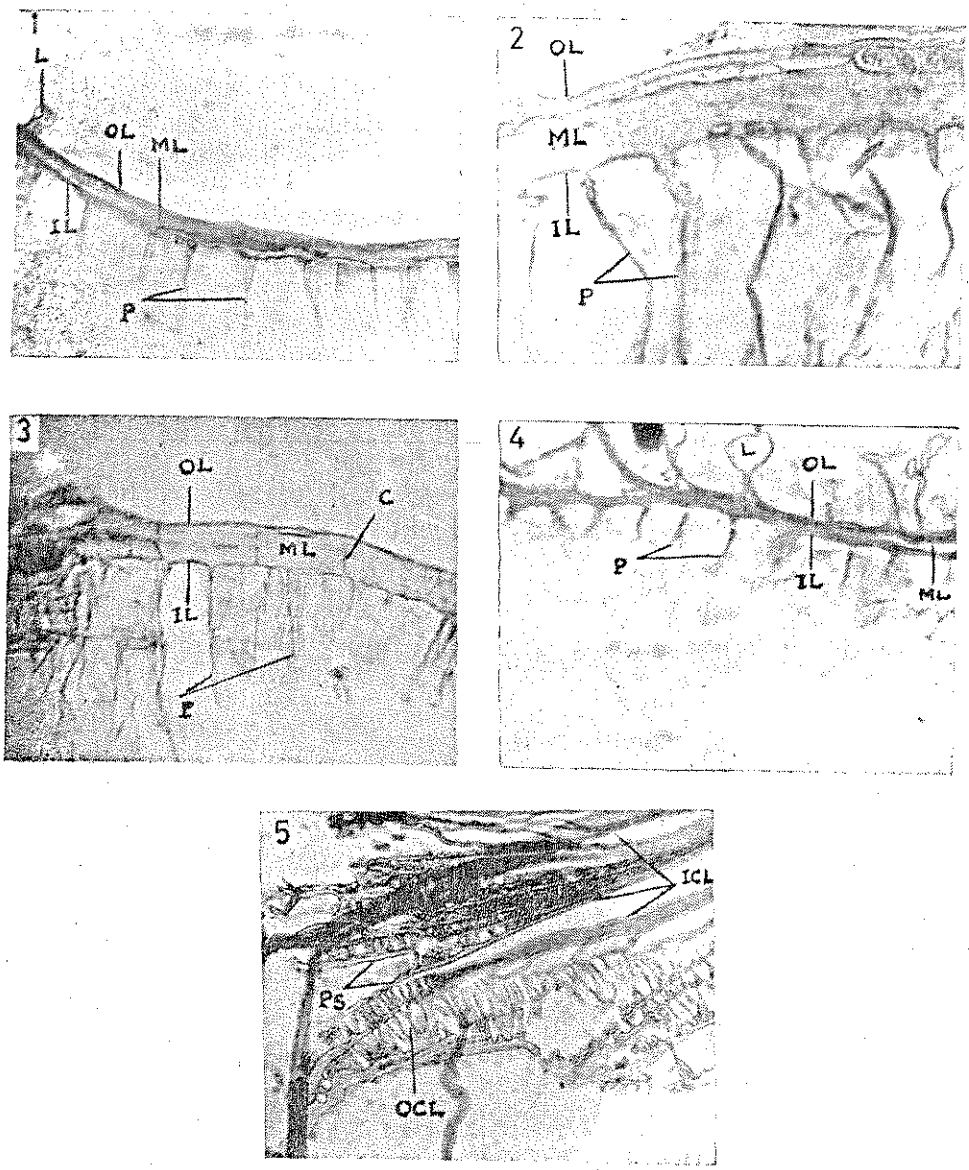
For the detection of chitin, the chitosan test (Campbell 1929), formic acid test (Rudall 1955), PAS test (Runham 1961) the modified chitosan method (Clark and Smith 1936) and Schultz test were applied (Lillie 1954). The product resulting from the chitosan test was analysed for sugar constituents by circular paper chromatography and unidimensional ascending chromatography (Giri and Nigam 1954).

3. Results and discussion

The unstained sections of the periostracum in an early growth stage (figure 1) show some differences in structural characters. The outermost layer which is yellowish in unstained preparations is thrown into " ∩ " shaped loops along the outer margin of the periostracum. Another unusual feature is that the innermost thin layer is produced into downwardly projecting processes penetrating the underlying calcareous layers. In a later growth stage, the periostracum presents similar features but the middle layer has become considerably wider (table 2 and figure 2).

In stained preparations, the thin outermost layer takes up a red colour with Mallory's stain and deep blue in Heidenhain's haematoxylin. The parts of the outermost layer forming the loops also stain red in Mallory. The middle region also takes up a light red in Mallory. The innermost layer is fuchsinophil, the downward projections from this layer are aniline blue staining (table 1 and figure 1). Similar reactions were obtained on the later growth stage but the outer fuchsinophil layer has lost its reactivity to stains but the innermost layer still retained its fuchsinophily. The downward projections also remain blue with Mallory.

The histochemical reactions are summarized in table 1. The thin outer layer in the early growth phases yield a positive reaction to Millon's Hg/nitrite and xanthaproteic tests and in a later stage loses its reactivity to Millon's and xantho-



Figures 1–5. (1) Transverse section through the periostracum at an early growth stage, stained in Mallory's triple stain. (2) Transverse section through the periostracum at a late growth stage stained in Mallory's triple stain. (3) Transverse section through the fully hardened periostracum pretreated with sodium hypochlorite, stained in Mallory's triple stain. (4) Transverse section through the fully hardened periostracum treated with alkaline sodium sulphide. Stained in Mallory's triple stain. (5) Transverse section through the shell (decalcified) showing the repeated formation of periostracal layers (× 1000). (OL—Outer layer ; ML—Middle layer ; IL—Inner layer ; P—Projections ; L—Loop ; C—Canal ; OCL—Outer calcareous layer ; ICL—Inner calcareous layer ; PS—Periostracum).

Table 1. Results of staining reactions and histochemical tests obtained with the periostracum of *Lamellidens marginalis* at different growth stages.

Stains and tests	References	Early growth stage A (20 mm length 10 mm breadth)			Late growth stage B (80 mm length 40 mm breadth)		
		Outer layer	Middle layer	Inner layer	Outer layer	Middle layer	Inner layer
Mallory's triple stain	Mallory (1938)	Deep red	Light red	Red	Unstained	Unstained	Red
Masson's trichrome stain	Pantin (1948)	Deep red	Light red	Red	Unstained	Unstained	Red
Heidenhain's haematoxylin	Lillie (1954)	Deep blue	Light blue	Blue	Unstained	Unstained	Blue
Millon's test	Bensely and Gersh (1933)	+	–	+	–	–	+
Hg/nitrite test	Baker (1956)	+	–	+	–	–	+
Xanthoproteic test	Pearse (1961)	+	–	+	–	–	+
Sudan black B	Lison (1936)	+	–	+	–	–	+
Sudan black B in acetone at 60° C	Berenbaum (1958)	+	–	+	–	–	+
Ferric chloride test	Lison (1936)	+	–	–	0	0	0
Diaphanol test	Kennaugh (1957)	0	0	0	+ Loses amber colour	–	–
Argentaffin test	Lison (1936)	+	–	+	–	–	+
Sodium hypochlorite test	Brown (1950)	0	0	0	+ Loses amber colour	–	–
Nitroprusside test	Pearse (1961)	–	+	–	–	–	–
Blue tetrazolium test	Barnett and Seligman (1954)	–	+	–	–	+	–
Lead Acetate test	Pearse (1961)	–	+	–	–	+	–
Sodium sulphide test	Brown (1950)	–	+ Swells	–	–	+ Swells	–
Chitosan test	Campbell (1929)	–	–	–	0	0	0
Modified chitosan	Clark and Smith (1936)	+	+	–	0	0	0
Schultze test	Lillie (1954)	+	+	–	0	0	0
Mineral acids	Brown (1950)	0	0	0	Resists	Resists	Slowly dissolved

Key : + Positive reaction : – Negative reaction : 0 Not carried out.

Table 2. Thickness (μ) of the layers of the periostracum of *Lamellidens marginalis* at different growth stages.

Growth stages	External layer	Middle layer	Internal layer	Total thickness
Early growth stage	4	8–10	2–3	14–17
Late growth stage	10–12	35–45	2–3	37–45

proteic tests. When this layer is subjected to treatment with diaphanol or sodium hypochlorite the colour is lost and the layer was rendered stainable (figure 3).

In previous work, this test has been applied to tanned arthropod cuticles to reverse the effect of tanning and restore the tanned cuticle protein to its original condition (Kennaugh 1957). The histochemical reactions of the outermost peristracal layer, its assumption of amber colouration in the later growth stages and refractility to stains as well as resumption of staining properties after treatment with detanning agents, provide evidence for tanning in this region. In freshwater bivalves periostracum shows a higher degree of tanning than that of the allied marine types (Haas 1935; Beedham 1958).

The middle layer remains light red in the earlier growth stages and is colourless and refractory in later stages of growth. It increases markedly in width during the growth of the periostracum. It is negative to tests for proteins containing aromatic amino acids but positive to nitroprusside and sulphur (table 1). Thus sulphur involved in the hardening of this region is inferred from a positive effect with thioglycollate and alkaline sodium sulphide reagents (figure 4) but not to sodium hypochlorite and diaphanol.

The innermost third layer reacting positively to Millon's Hg, nitrite and xanthoproteic tests is fuschsinophil throughout the growth stages. It is essentially untanned or only partially tanned as in the periostracum of *Meretrix casta* and *Mytilus viridis* (Sowmini 1970). The nature and significance of the downwardly projecting processes arising from this layer are not known. They are aniline blue staining with Mallory's stain and differ chemically in not containing a protein precursor of tanning suggesting that they may provide organic continuity between the outer layers of the periostracum and inner region leading to the mantle wall.

The occurrence and distribution of chitin is identical to those reported for *Meretrix casta* (Sowmini 1970). All the layers of the periostracum except the innermost fuchsinophil layer and the projections associated with it contain chitin which reacts typically to the modified chitosan test of Clark and Smith (1936) by turning violet on addition of iodine sulphuric acid to the KOH treated test material (table 2). With chitosan test of Campbell (1929) however, the layers in question quickly dissolve. Chromatographic analysis indicated that the chitin-like component in this bivalve contains glucose, galactose and rhamnose.

In some instances, the periostracum in the regions inside the pallial line is formed repeatedly one below the other, each layer alternating with a calcareous layer (figure 5). Sections of the decalcified shell show that the sub-periostracal layers arise, close together but at more distal points they appear to branch and give rise to new periostracal layers forming a ramifying system in the calareous part of the shell. Such periostracal layers resemble in structure, staining and histochemical reactions, to the normal periostracum, the essential difference is that tanning is much reduced. Similarly, –S–S–bonding in the middle layer is also less pronounced. In general the sub periostracal layers correspond to the condition of the normal periostracum in the early growth stages of the shell.

Structural features such as the " ∩ " shaped loops formed at the outermost periostracal layer and the downward projections arising from the innermost layer appear to be unique to this type. The more pronounced tanning seen in *Lamellidens* is in accordance with what has been reported by the earlier workers who correlated such a feature with the freshwater habitat of these animals. The multilayered periostracum occurring in some regions of the shell referred to above in the species corresponds to the condition reported in *Anodonta cygnaea*. The significance of such a multiple type is not clear although the observations of Beedham (1965) suggest that new periostracal layers may arise under the old one due to damage to the periostracum and the subsequent regeneration. A multiple type of a periostracum may be an adaptive device to the adverse conditions of the environment.

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