DIFFERENTIATION OF THE BRACHIOPOD PERIOSTRACUM

by ALWYN WILLIAMS *and* SARAH MACKAY

ABSTRACT. The periostracum of living brachiopods is highly variable in microstructure, but the secretory regimes responsible for such differences give rise to either a strictly chronological succession built up on the surface of the vesicular cells at the tip of the outer mantle lobe, or a heterochronous succession secreted simultaneously from both sides of a slot between the vesicular cells and the lobate cells which constitute the junction of the outer and inner epithelium. In heterochronous successions, vesicular cells exude the basal layers of the periostracum while the lobate cells secrete superstructural features varying from the proteinous labyrinths of many terebratellaceans to the horizontally sheeted concentric ridges of *Discinisca*. Consideration of these secretory processes leads to the assumption that the regimes responsible for chronological successions are the more primitive and that those resulting in heterochronous successions are likely to have evolved repeatedly during the history of the Phylum. Despite the absence of lobate cells in *Crania*, it seems likely that they were present in the prototypic brachiopod and may well have occupied the very edge of the mantle, thereby separating skeletal-secreting outer epithelium (with vesicular cells) on the outside and ciliated inner epithelium on the inside.

THE organic cover of the brachiopod shell, the periostracum, is seldom more than a micron thick and, until the electron microscope became an aid in its study, was thought of as a simple pellicle secreted in an uncomplicated way by the inner surface of the outer epithelial lobe at the mantle edge (Williams 1956, p. 244). Within the last decade, however, it has become evident that both its structure and mode of secretion are highly variable even at familial levels within the Phylum. Yet until very recently, the interpretation of how the different components of a periostracal succession are exuded was influenced by the apparent simplicity of the inferred mode of deposition of the periostracum of the rhynchonellide *Notosaria nigricans* (Sowerby), which was the first secretory regime to be described (Williams 1968). In this species, the ultrastructure of the periostracum and the arrangement of the epithelial cells in the vicinity of its first-formed edge, suggested that periostracal successions are deposited in a strict chronological sequence from the outermost surface inwards. There were, however, obstacles in the way of accepting such a regime as a model for other brachiopod groups. In particular it was difficult to picture the secretion of the outer proteinous labyrinth of the terebratellacean periostracum, as seen in *Waltonia inconspicua* (Sowerby), in the undeniable absence of an external membrane on which such a labyrinthine aggregation of variably sized secretion droplets could be founded (Williams 1968). At that time it had not been possible to prepare sections of the terebratellacean mantle edge with the periostracum *in situ*.

The first indication that different layers of the same sector of a periostracal succession may be secreted simultaneously was found in the thecideidine brachiopod *Thecidellina baretti* (Davidson). In this species, the periostracum originates within a shallow slot in the outer mantle lobe and is demonstrably exuded by cells on either side of the first-formed strip of periostracum. Some morphological distinction was

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discernible between cells at the tip of the outer mantle lobe which secreted the internal part of the periostracum, and those responsible for exuding the external constituents; they were called the vesicular and lobate cells respectively (Williams 1973, p. 443).

This arrangement, whereby an undifferentiated inner basal layer of a periostracal succession is secreted by the vesicular cells, and an outer superstructure of varying complexity is simultaneously built up by the lobate cells has now been confirmed for all terebratellaceans investigated (Williams and MacKay 1978). Moreover, preliminary investigations of the mantle edge of *Glottidia pyramidata* (Stimpson) suggested that a distinction could be drawn between lobate and vesicular cells even when the first-formed part of the periostracum was not inserted between them (Williams 1977, p. 113). This distinction seemed to hold for *Notosaria* in which the periostracum originated superficially on the outer mantle lobe.

In these circumstances it seemed worthwhile to complete studies of the periostracum of inarticulate brachiopods, including a reinvestigation of that of *Crania anomala* (Müller) which had never been seen *in situ* (Williams and Wright 1970, p. 9); and thereby ascertain whether it is ever built up by exudation from both sides of a periostracal slot as in the thecideidines and some terebratulides. These comparative studies are not of direct palaeontological interest since, so far as is known, the periostracum does not survive fossilization in a morphologically recognizable state. They do, however, afford an opportunity to review the nature and origin of the periostracum and possibly cast some light on the organization of the mantle edge of the prototypic brachiopod.

MATERIALS AND METHODS

Living specimens of *C. anomala*, *G. pyramidata*, and *Lingula anatina* Lamarck were prepared for examination in the transmission electron microscope by a double fixation method. Fixation in 3% glutaraldehyde made up in 3% sodium chloride solution and buffered to pH 7·2 with phosphate buffer for 2 hours in the cold, was followed by a wash in phosphate buffer. Decalcification in 10% EDTA and a wash in 0·2 M sucrose solution preceded the second fixation in 2% osmium tetroxide; all these solutions were buffered to pH 7·2 with phosphate buffer. The material was then dehydrated and embedded in Taab resin. Sections were stained in aqueous lead citrate and uranyl acetate and examined under an AEI Corinth transmission electron microscope.

Specimens of *Discinisca strigata* (Broderip) fixed in alcohol and of *Discina striata* (Schumacher) and *Pelagodiscus atlanticus* (King) preserved in phenoxetol, were washed in phosphate buffer and treated according to the schedule described above from the decalcification stage onwards.

Shell surfaces were prepared for examination under the scanning electron microscope first by removing any tissue by immersion in sodium hypochlorite for some hours, followed by brief sonication in a weak detergent and then in acetone to remove any adherent particles. All natural and fracture surfaces were coated with gold/palladium for examination under a Stereoscan purchased by NERC grant GR/3/443. The technical and research assistance contributing to this study is supported by NERC grant G2/3/2555A.

THE INARTICULATE PERIOSTRACUM

Of the four Orders unequivocally assigned to the Inarticulata, only the Lingulida and the Acrotretida survive to the present day. Living specimens of both extant lingulide genera, *Lingula* Bruguière and *Glottidia* Dall, were available for study; but representatives of only one of the four Recent acrotretide genera, the cosmopolitan calcareous-shelled *Crania* Retzius, were received *in vivo*. The periostracum, however,

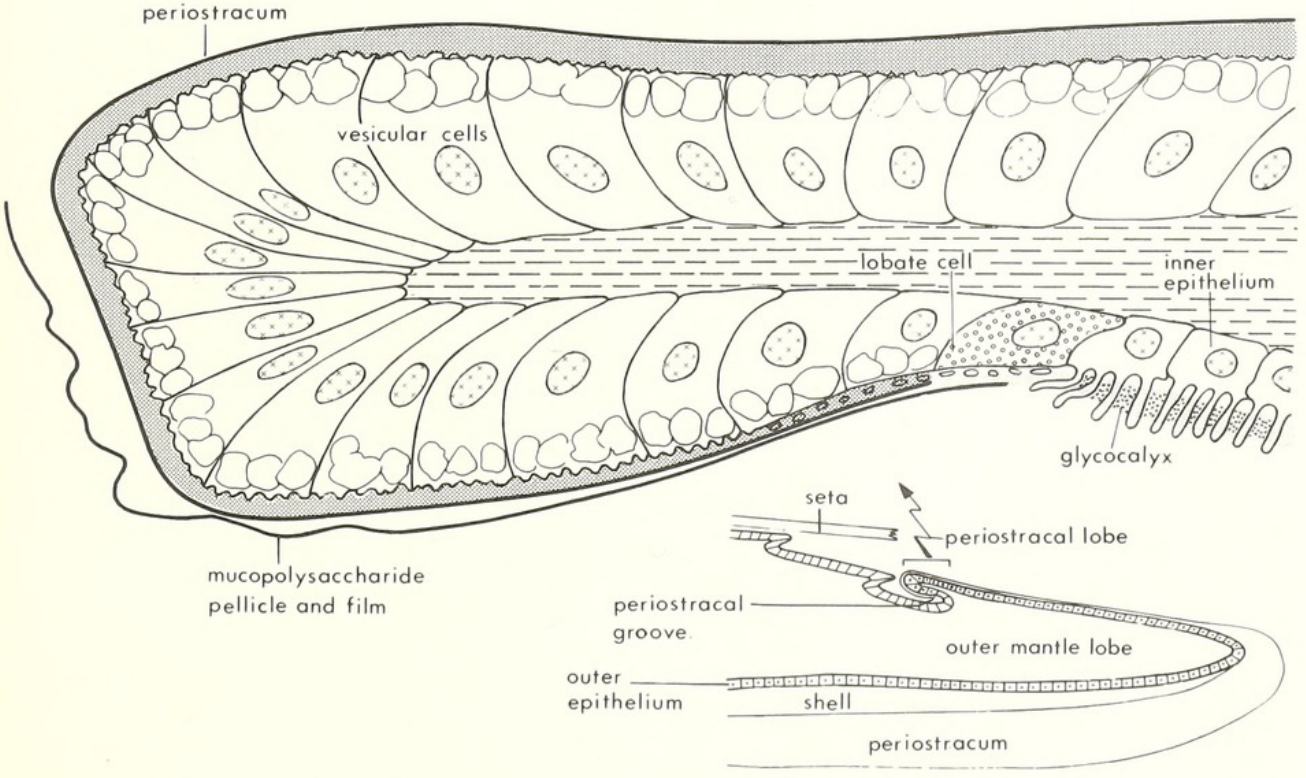
is generally tough enough to persist more or less unaltered on the shell surfaces of specimens preserved in formalin or alcohol; and, although the microstructure of the mantle edge is rendered unrecognizable by such fixatives, the configuration of the outer mantle lobe can be confidently inferred from the nature of the periostracum itself. This procedure for determining the differentiation of the outer mantle lobe has had to be adopted for the remaining acrotretide genera *Discina* Lamarck, *Discinisca* Dall, and *Pelagodiscus* Dall.

The lingulid periostracum

The micromorphology of the mantle edge of *G. pyramidata* has been outlined in a comparison with those of other brachiopod species (Williams 1977, p. 113). Subsequent studies have confirmed that the secretory regime gives rise to the periostracum within the periostracal groove and have afforded further information about the differentiation of the outer mantle lobe (text-fig. 1).

The ciliated inner epithelial cells, which are up to 15 μm tall and are bounded by folded lateral walls, contain numerous vesicles and electron-dense droplets and exude a glycocalyx up to 1 μm thick (Pl. 94, fig. 1). The glycocalyx is usually seen as a band with electron-dense clots about 20 nm in diameter enmeshed with erect or forwardly inclined microvilli almost 2 μm long. The inner epithelial cells adjacent to the lobate cells are shorter, as are the microvilli they bear although the glycocalyx persists as a forwardly projecting sheet (Pl. 93, fig. 2).

One or two lobate cells, which are about 3 μm high and extend forward over the basal part of adjacent outer epithelial cells, have external plasmalemmas thrown into



TEXT-FIG. 1. Diagrammatic sagittal section of the edge of the valve of *Glottidia* showing the differentiation of the mantle.

irregular protruberances like prostrate microvilli (Pl. 93, fig. 2). The cells exude a mucopolysaccharide film, the external surface of which, as it emerges from beneath the glycocalyx sheet, polymerizes into a fibrillar triple-layered sheet. The fibrillar sheet is about 10 nm thick and usually persists to envelop the periostracal lobe (Pl. 93, fig. 1). This lobe is an inwardly projecting lip of the very much larger outer mantle job. Both structures are composed of vesicular cells which secrete the periostracum and then the shell after they have rotated around the mantle edge to become incorporated within the main spread of outer epithelium underlying the shell.

The vesicular cells vary in shape, tending to become attenuated to over 10 μm at the hinge of the periostracal lobe and reducing to about 4 or 5 μm in length along the inner side of the outer mantle lobe (Pl. 93, fig. 3). Vesicles may be more than one micron in diameter but are usually small and especially numerous in the vicinity of the Golgi apparati. The most striking aspect of the cell milieu, however, is the abundant rough endoplasmic reticulum which is prevalent in the folds of the lateral walls. The secretory plasmalemma is convoluted into prostrate 'microvilli' and exudation takes place beneath the loose cover afforded by the fibrillar sheet and the associated mucopolysaccharide being discharged by the lobate cells.

The outer surface of the periostracum polymerizes almost immediately after exudation into a coarsely fibrillar triple-unit membrane bounding a gradually thickening medium electron-dense, finely textured layer. When this layer attains a thickness of about 250 nm at the hinge of the periostracal lobe, internal differentiation takes place (Pl. 93, figs. 3–6). Linear arrangements of electron-dense bodies, disposed at high angles to the external surface, polymerize out of the matrix. The essential unit is an electron-dense core about 30 nm wide, composed of granules up to 10 nm in diameter, bounded, at a distance of about 9 to 10 nm on either side, by electron-dense lines 4 nm thick. As polymerization spreads inwardly the earlier appearing structures undergo further changes, with the electron-dense cores becoming less granular and their electron-dense linear boundaries losing their identity. The periostracum at this stage is triple-layered, with an outer zone consisting of electron-dense bands disposed more or less normal to the external surface; a middle zone of granular cores with identifiable lateral boundaries; and an inner zone of medium electron-dense finely textured material. In some sections the bands become broken

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EXPLANATION OF PLATE 93

Transmission electron micrographs of the mantle edge of *Glottidia pyramidata*; Recent, Florida.

Fig. 1. Transverse section showing the periostracal groove, × 3600.

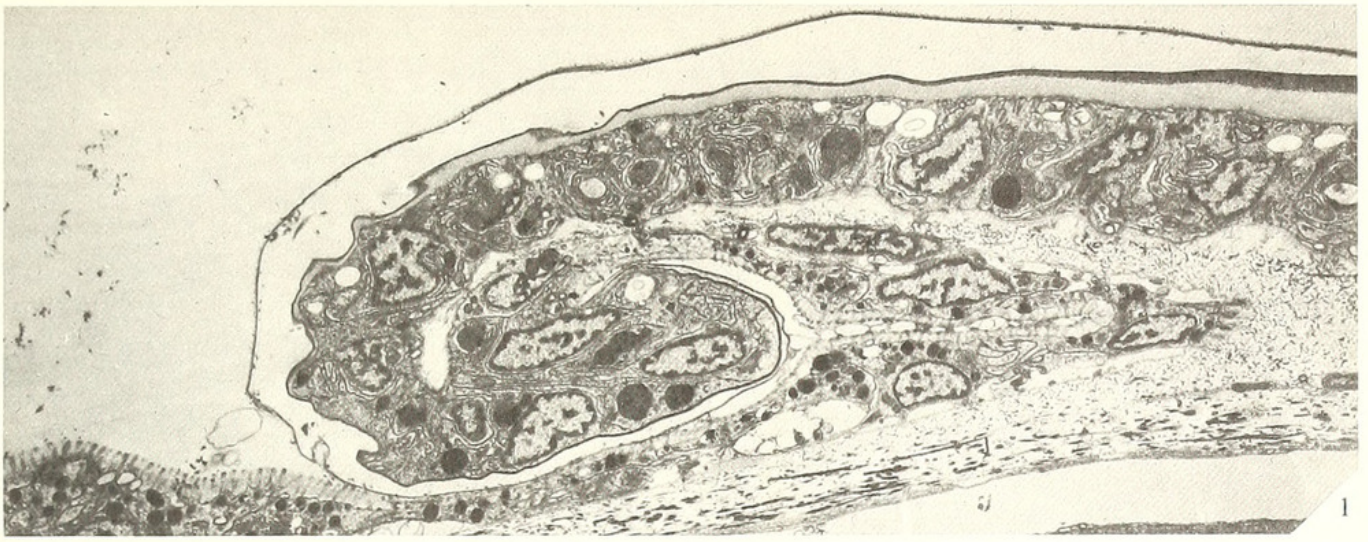
Fig. 2. Detail of the junction between microvillous inner epithelial cells (above) and the lobate cell secreting the pellicle and the mucopolysaccharide (or mucin) film, × 24 000.

Fig. 3. Detail of the immature periostracum overlying vesicular cells with prostrate microville, × 53 200.

Fig. 4. Section showing the pellicle at the top left corner, overlying maturing periostracum with electron-dense bodies and vesicular cells at the lower edge, × 35 500.

Fig. 5. Mature periostracum showing granular electron-dense bodies arranged linearly, with the fibrillar layer of the shell at the bottom, × 28 400.

Fig. 6. Detail of the mature periostracum showing electron-dense cores with linear boundaries towards top of picture and inner zone of medium electron-dense material towards the bottom, × 106 500.



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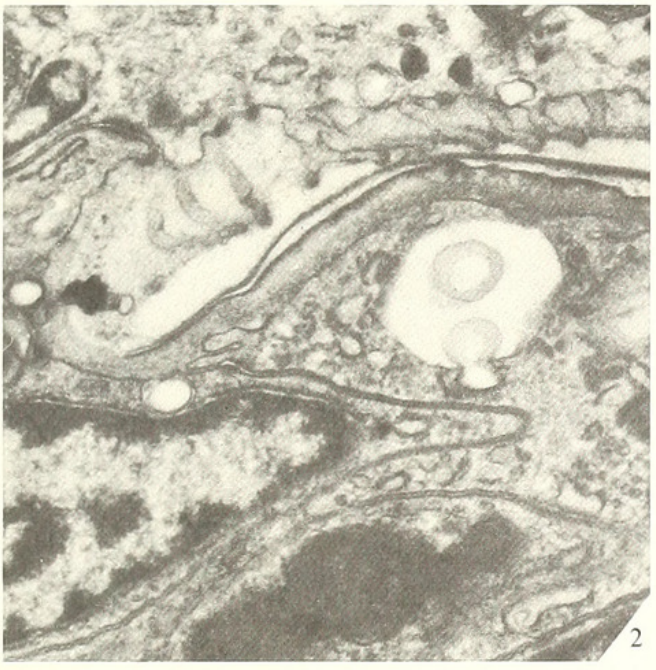

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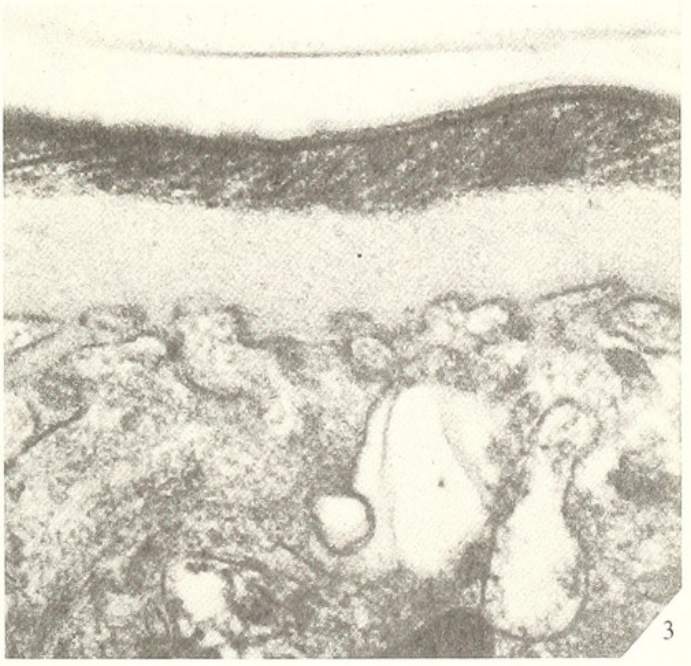

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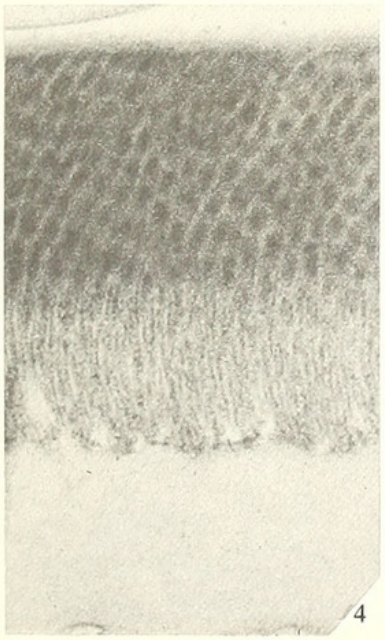

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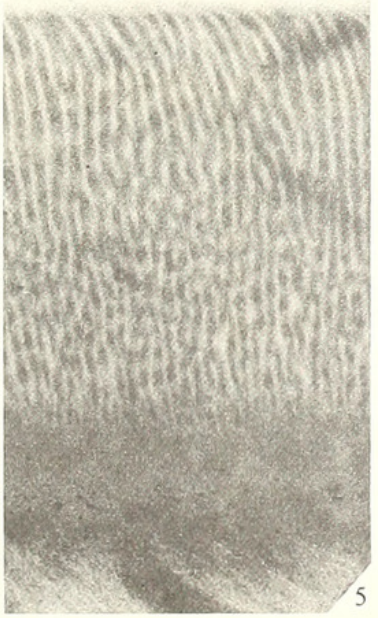

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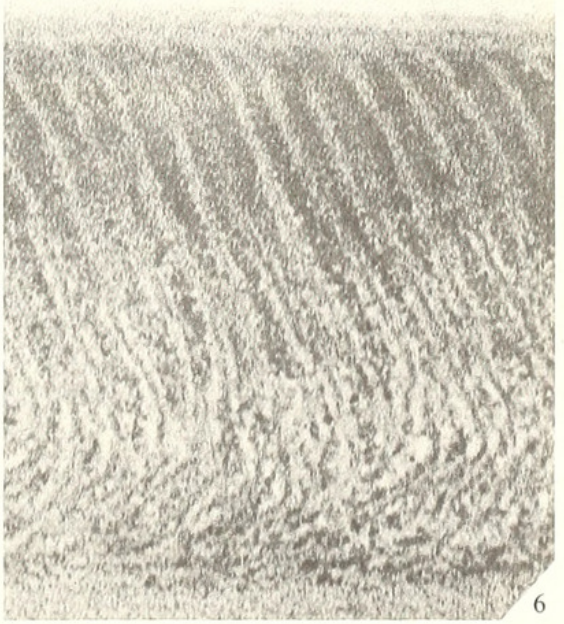

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WILLIAMS and MACKAY, brachiopod periostracum

into discrete elliptical or circular units, indicating that they are really hexagonally stacked rods.

Each zone gradually thickens until they attain a total thickness of about 5 or 6 μm about 100 μm externally to the hinge of the periostracal lobe. Thereafter the thickness remains constant although polymerization continues so that the fully developed periostracum consists of an outer layer, 3 to 4 μm thick, of hexagonally stacked electron-dense rods separated by electron-light partitions, and a medium electron-dense layer about 2 μm thick sharing an irregularly interdigitating boundary with an underlying electron-light, somewhat fibrillar layer, which may be as much as 10 μm thick at the hinge of the outer mantle lobe. We have identified this layer as the outermost organic constituent of the shell proper. It is permeated, as are the inner apatitic as well as the organic layers of the shell, by canals about 60 nm wide. Identical canals are especially conspicuous in *Lingula anatina* where they form a variably spaced system accommodating strands of outer epithelium (Pl. 95, figs. 3, 4). The canals terminate immediately below the layer underlying the hexagonal packed rods.

The periostracal succession (Pl. 94, fig. 5) and the microstructure of the mantle edge of *Lingula* differ only in detail from those of *Glottidia*. The inner epithelium is remarkable for the extraordinary length of the fibrils which emanate from the erect microvilli as anastomosing meshes up to a micron long, and are usually in contact with the sheet bounding the mucopolysaccharide layer exuded by one or two lobate cells (Pl. 94, figs. 1, 2). The vesicular cells, which are rich in glycogen, have regularly developed prostrate microvilli attached by desmosomes to the developing periostracum (Pl. 94, figs. 3, 4).

Although no histochemical tests have been carried out during the present investigations, the studies of Jope (*in* Williams *et al.* 1965, p. 161) leave no doubt that the lingulid periostracum is composed of a glycine-poor protein with some traces of chitin and hydroxyproline. Hunt and Oates (1978, p. 447) have commented on the tendency of such proteins to be ultrastructurally arranged in helicoidal layering like that identified by them in the periostracum of the gastropod *Buccinum undatum* Linnaeus. Why the fine structure of the lingulid periostracum is so different has yet to be explained.

EXPLANATION OF PLATE 94

Transmission electron micrographs of the mantle edge of *Lingula anatina*; Recent, Hawaii.

Fig. 1. Transverse section of the periostracal groove, × 3600.

Fig. 2. Detail of the junction between inner epithelium at the top left corner with fibrillar meshes around microvilli and lobate cells towards right, showing the origin of the pellicle and mucopolysaccharide film, × 35 500.

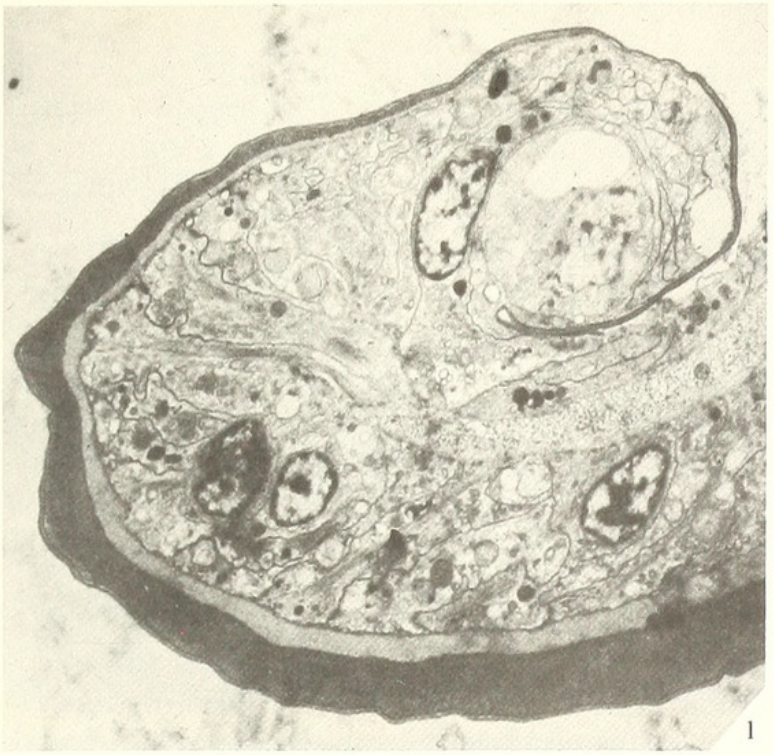
Fig. 3. Detail of the immature periostracum overlying vesicular cells with prostrate microvilli, × 88 750.

Fig. 4. Detail of the microvilli with desmosomes and other features of the vesicular cells underlying mature periostracum, × 53 250.

Fig. 5. Detail of the mature periostracum, × 36 000.

Transmission electron micrograph of the mantle edge of *Crania anomala*; Recent, Firth of Clyde.

Fig. 6. Transverse section showing the transition from microvillous inner epithelial cells (above) to periostracal-secreting vesicular cells, × 15 000.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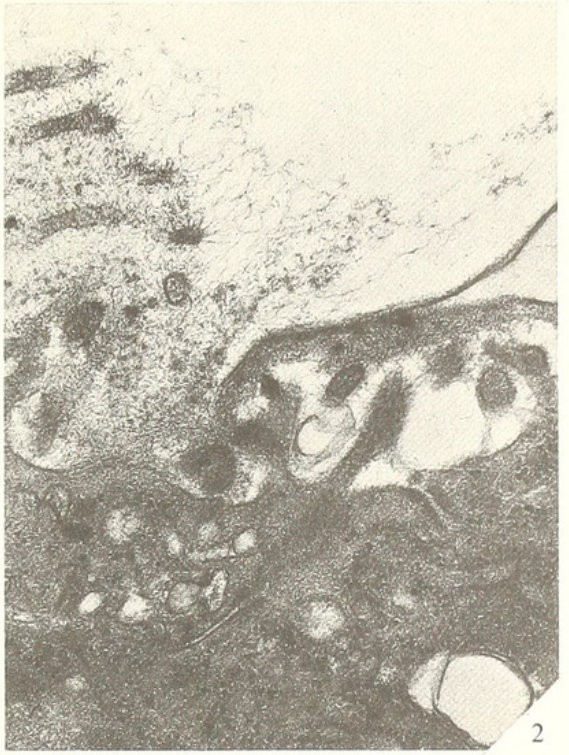
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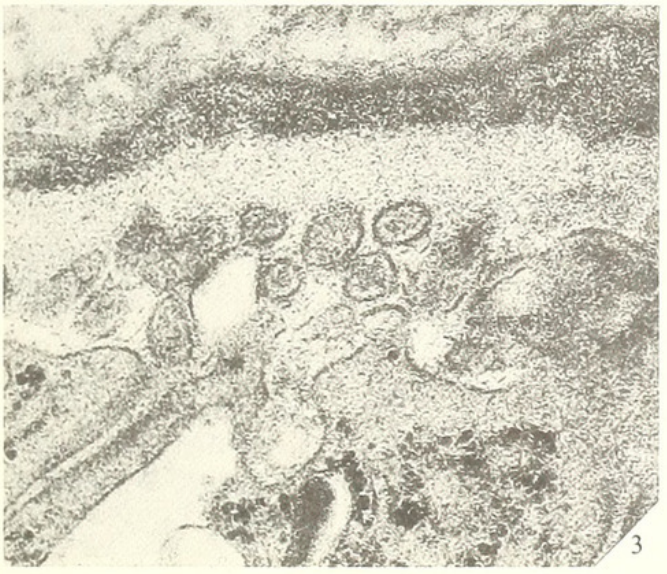
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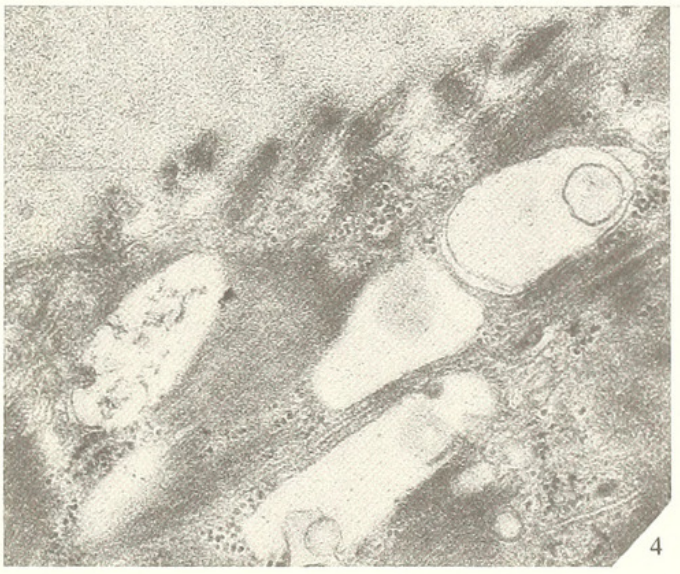
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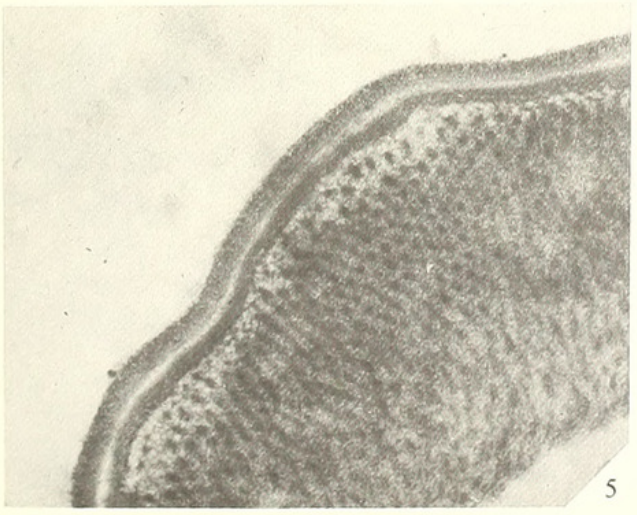
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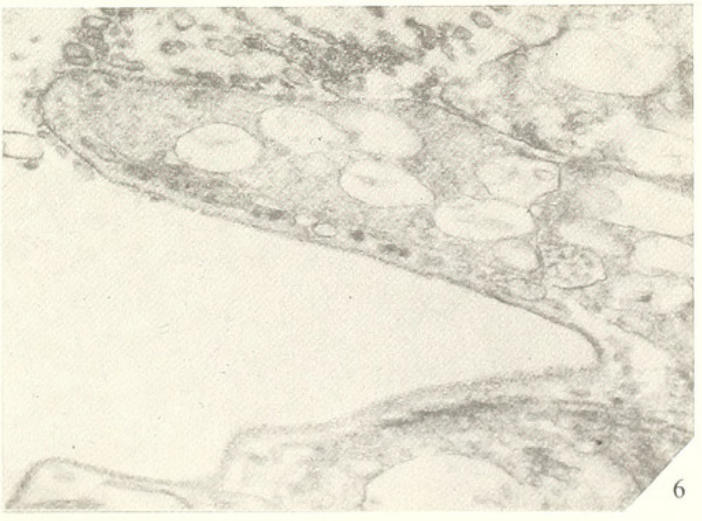
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WILLIAMS and MACKAY, brachiopod periostracum

The lingulid periostracal succession is evidently isotopic in that different parts of the same layer were secreted at different times during shell growth. It is also strictly chronological so that, in any section, the external fibrillar triple-unit membrane is the first-formed part of the succession followed by the zone of hexagonal stacked rods, and so on.

The craniid periostracum

The fully developed periostracum of *C. anomala* is a layer of mucopolysaccharide permeated by fibrils and attaining a thickness of 5 μm or more beneath the crests of impersistent concentric external folds (Pl. 95, fig. 2). The outer bounding surface is an undifferentiated electron-dense layer about 12 nm thick, supporting closely packed, bulbous-tipped, fibrillar rods about 27 nm high (Williams and Wright 1970, p. 10). When this succession was described previously, no sections could be prepared showing the newly formed edge of the periostracum *in situ* on the outer mantle lobe. During recent investigations sections have been cut which reveal the origin of the periostracum (Pl. 94, fig. 6; Pl. 95, fig. 1). They show that the outer membrane arises on the surface of vesicular cells beneath forward projecting microvilli of adjacent inner epithelium. These cells conform closely to their designated types: the former are highly vesicular with prostrate cylindroid extensions of the secretory plasmalemma; the latter bear erect microvilli up to 2·7 μm long. The absence of lobate cells is noteworthy. Consequently the extraneous matter seen adhering to the outer surface of the periostracum is more likely to represent residual glycocalyx derived from contact with inner epithelial microvilli than a mucopolysaccharide film as identified by Williams and Wright (1970, p. 19).

The periostracum of *Crania* clearly constitutes a simple chronological succession which is unaffected by differential polymerization, except for the appearance of anastomosing fibrils within the maturing mucopolysaccharide layer.

The discinid periostracum

The first-formed part of the periostracum of *Discinisca strigata* has not been seen *in situ*, but the fully developed succession is so extraordinary (Pl. 95, fig. 5) that its relationship to the outer mantle lobe can be reasonably inferred.

EXPLANATION OF PLATE 95

Transmission electron micrographs of the mantle edge of *Crania anomala*; Recent, Firth of Clyde.

Fig. 1. Detail of the origin of the periostracum secreted by vesicular cells, × 60 000.
Fig. 2. Section showing folded periostracum overlying vesicular cells (bottom right), × 68 700.

Transmission electron micrograph of the shell of *Lingula anatina*; Recent, Hawaii.

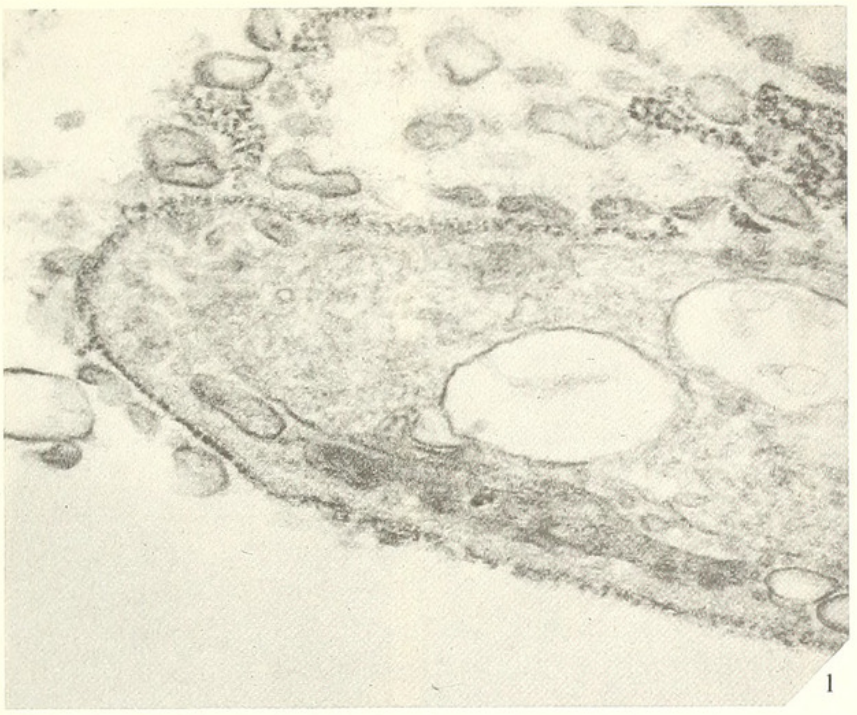
Fig. 3. Transverse section showing the base of an internal canal traversing the shell, × 35 500.

Scanning electron micrograph of the shell of *Lingula anatina*; Recent, Hawaii.

Fig. 4. Internal surface showing variably spaced openings of canals which permeate the shell, × 2000.

Transmission electron micrograph of the periostracum of *Discinisca strigata*; Recent, Peru.

Fig. 5. Transverse section showing decalcified fibrillar layer of the shell permeated by canals, overlain by the medium electron-dense basal layer of the periostracum and the overlying sheeted ridges, × 13 750.



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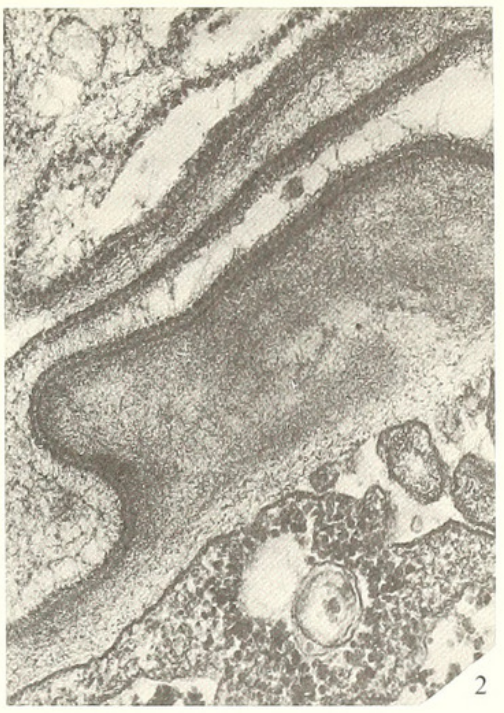

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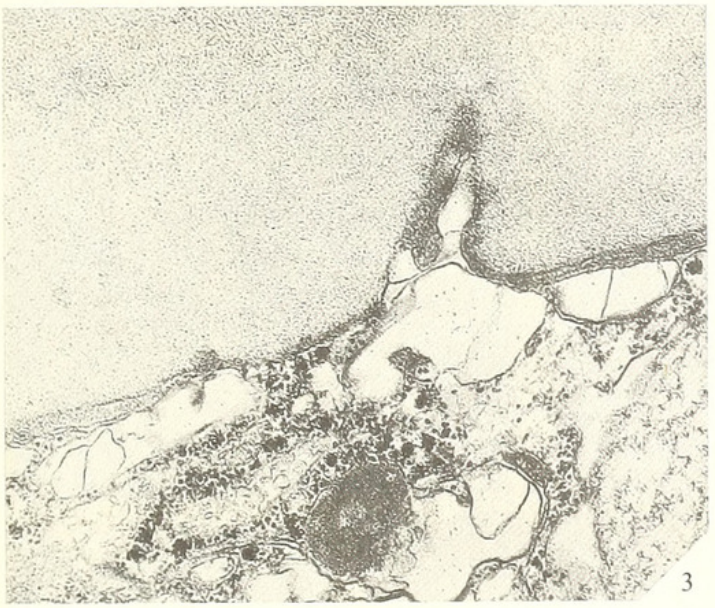

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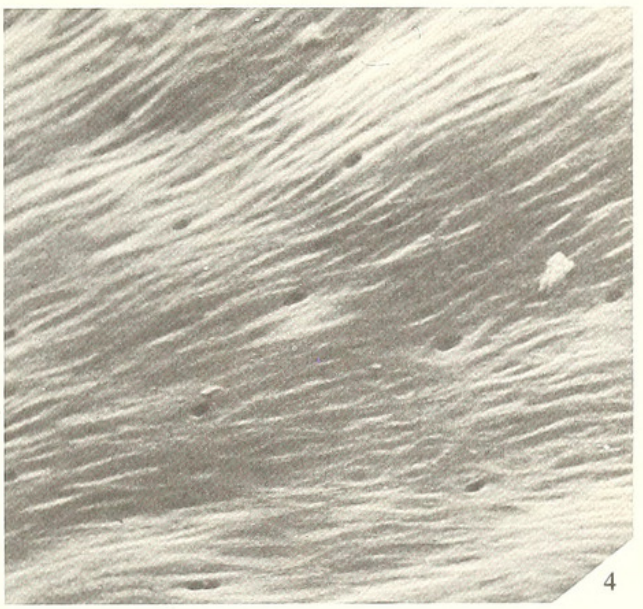

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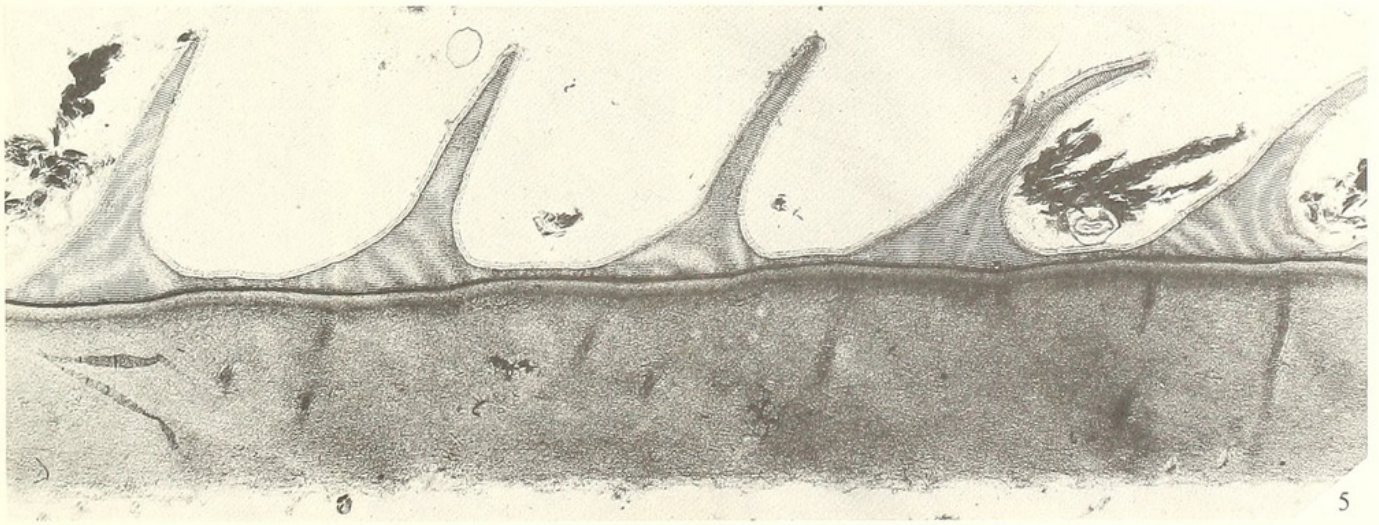

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WILLIAMS and MACKAY, brachiopod periostracum

The foundation of the periostracum is a finely textured, medium electron-dense layer about 85 nm thick. It is underlain by a thick, finely fibrillar layer which has been regarded as an integral part of the shell because it is permeated by regularly branching canals, up to 60 nm in diameter, terminating at the interface between the two layers (Pl. 96, fig. 1). The outer boundary is an electron-dense layer between 30 and 40 nm thick, which supports a series of impersistent, concentric ridges disposed at intervals of about 2 μm.

In cross-section the ridges are seen as triangular structures, with a base normally about 1·5 μm across rising for as much as 3 μm to a posteriorly curving, rounded apex less than 0·2 μm wide. Each ridge is composed of up to 100 or more electron-dense sheets about 6 nm thick, which are disposed at regular intervals of 12 nm almost parallel with the basal layers of the periostracum so that those at the apex are a fraction of the area of those at the base, where two or three sheets are frequently continuous from one ridge base to the next (Pl. 96, fig. 1). The ends of the sheets normally coalesce with one another to form a continuous surface for each ridge which is additionally coated with parallel sheets, usually two but exceptionally up to six in number, at intervals of 18 nm. These bounding layers occasionally break down and the ridge then assumes a frayed appearance.

EXPLANATION OF PLATE 96

Transmission electron micrographs of the periostracum of *Discinisca strigata*; Recent, Peru.

Fig. 1. Transverse section of a periostracal ridge showing the succession of sheets parallel to the basal layer and the frayed edges, ×41 250.

Fig. 2. Transverse section of a periostracal ridge showing tension cracks delineating vertical pillars, ×82 500.

Transmission electron micrograph of the periostracum of *Discina striata*; Recent, Ghana.

Fig. 3. Transverse section showing ridges composed of electron-dense sheets overlying basal layers and decalcified fibrillar shell with a canal in the bottom right-hand corner, ×15 000.

Fig. 4. Transverse section showing the disposition of concentric ridges, ×3600.

Transmission electron micrograph of the periostracum of *Thecidellina barretti*; Recent, West Indies.

Fig. 5. Transverse section showing the periostracum underlain by vesicular cells, ×82 500.

Transmission electron micrograph of the periostracum of *Pelagodiscus atlanticus*; Recent, Mid-Pacific.

Fig. 6. Transverse section of the periostracum overlying decalcified fibrillar shell, ×15 000.

Fig. 7. Transverse section showing the horizontally stacked sheets of the outer layer of the periostracum, ×90 000.

Transmission electron micrograph of the periostracum of *Notosaria nigricans*; Recent, New Zealand.

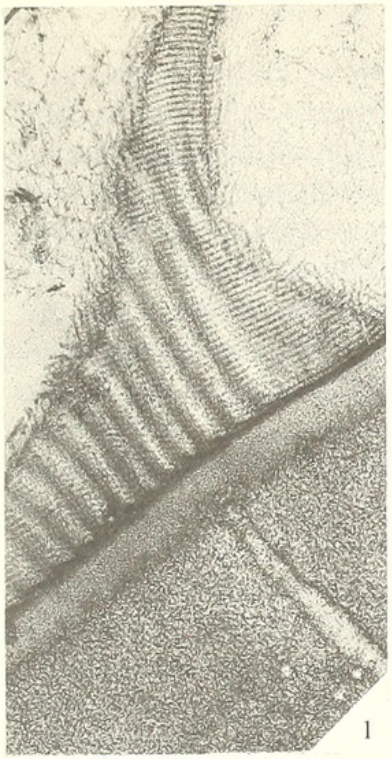
Fig. 8. Transverse section showing outer triple-unit membrane with fibrils at top, the main mucopolysaccharide layer and inner bounding membrane below, ×137 500.

Transmission electron micrograph of the periostracum of *Waltonia inconspicua*; Recent, New Zealand.

Fig. 9. Transverse section showing basal layer with labyrinthine superstructure, ×36 000.

Transmission electron micrograph of the periostracum of *Gwynia capsula*; Recent, Anglesey.

Fig. 10. Transverse section showing basal layer supporting folded superstructure, with vesicles beneath a loop of a mucopolysaccharide film above, ×60 000.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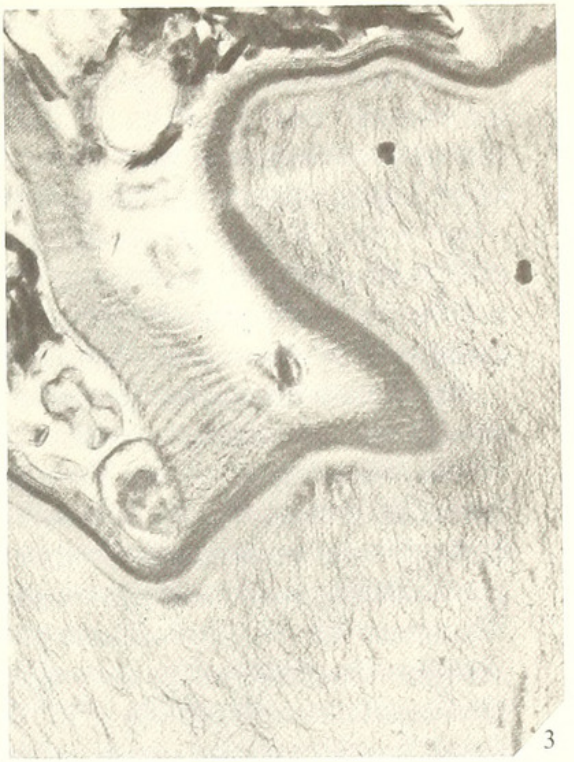
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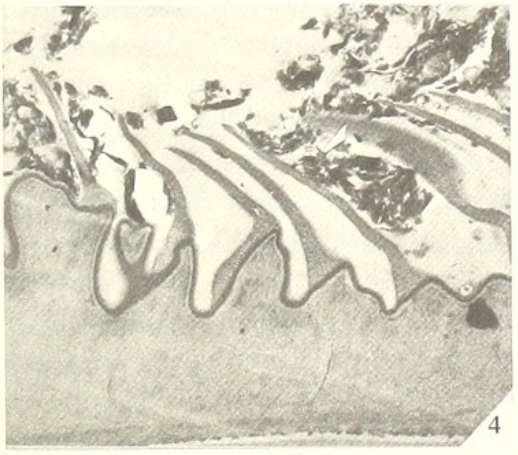
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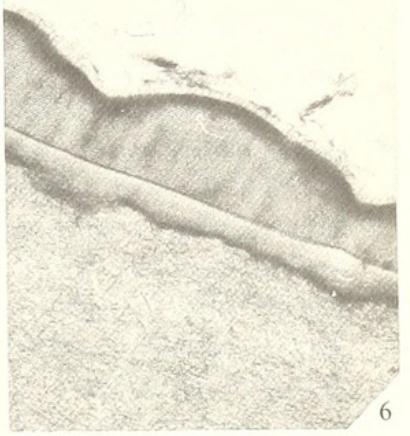
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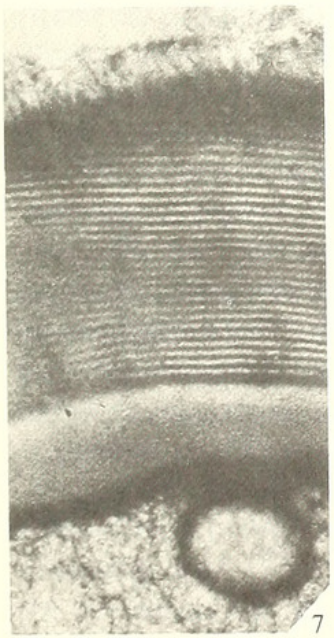
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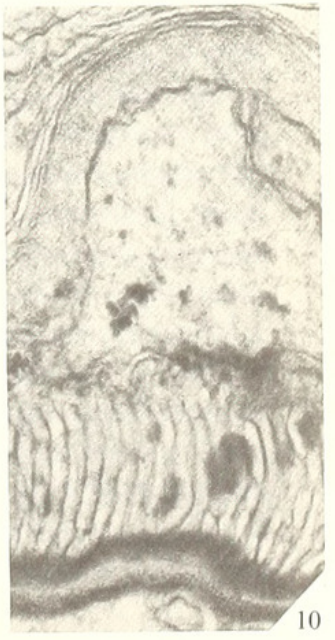
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WILLIAMS and MACKAY, brachiopod periostracum

The periodicity of both the internal and bounding sheets is noteworthy. It is maintained partly by the fibrillar coats of the sheets which enmesh with one another. However, the sheets must also be composed of regularly arranged units aligned in the same plane. Thus when ridges shrink they develop regular tension cracks up to 25 nm wide, which lie normal to the periostracal base and delineate vertical pillars usually about 30 nm wide, composed of equal lengths of internal sheets stacked one above another (Pl. 96, fig. 2). They exhibit a superficial likeness to the protein ribbons described by Hunt and Oates (1978, pl. 2) but are clearly quite different in structure.

Not surprisingly, the periostraca of the other extant discinid species, *Pelagodiscus atlanticus* and *Discina striata*, are like that of *Discinisca*.

The foundation of the *Pelagodiscus* periostracum is a basal layer about 200 nm thick (Pl. 96, figs. 6, 7). It is median electron-dense in appearance except at its interface with an underlying fibrillar layer, where it becomes strongly electron-dense for about 20 nm, and with the outer layer which forms an electron-light band in section. The inner fibrillar layer contains traces of canals about 100 nm in diameter and also vesicles up to 200 nm across which are sporadically distributed immediately beneath the basal layer (Pl. 96, fig. 7). The outer layer consists of electron-dense sheets, each about 5 nm thick and disposed more or less parallel with the basal layer at intervals of 5 or 6 nm. This superstructure is composed of up to thirty sheets intersected by a gently undulating outer surface, beyond which the sheets terminate as loose unconnected extensions to form a fringe about 50 nm high.

The periostracum of the allegedly more distantly related *Discina* is almost identical with that of *Discinisca*, differing only in dimensions and the attitude of the sheeted concentric ridges which form the outer layer (Pl. 96, figs. 3, 4). An inner fibrillar layer is again identified as part of the shell because it is permeated by canals up to 800 nm in diameter (Pl. 96, fig. 3). The basal layer of the periostracum, which is also increasingly electron-dense externally, is about 120 nm thick. Judging from some of the less distorted sections, the concentric ridges are finer than those of *Discinisca* and stand more or less erect in the natural state, being up to 7 μm tall with a base only 1 μm across. The ridges, however, are proximally connected with one another by up to 5 or 6 sheets and show the same tendency to fraying laterally or cracking vertically into banded pillars.

The ultrastructure of the mantle lobe responsible for the secretion of such unusual periostraca is presently unknown. However, in view of the structural regularity of the sheets it seems unlikely that those at the apex are secreted before those at the base. Moreover, the sheets coating each ridge are more likely to have polymerized out of a mucopolysaccharide exuded as a uniform layer over the ridges as they are completed. If this is the mode of secretion, the lobate cells are extensively developed in *Discinisca* and are separated from vesicular cells by a periostracal slot. This would allow for the secretion of the basal layers of the periostracum by vesicular cells and the penecontemporaneous deposition of ridges by lobate cells as superstructures on such newly formed layers. According to this regime, ridges are isotopic features each secreted in chronological sequence from base to crest.

THE ARTICULATE PERIOSTRACUM

A correlation of the main types of periostraca characteristic of articulate brachiopods has recently been proposed (Williams and McKay 1978, p. 207), but warrants a brief review in the light of the variation now established for the inarticulates.

Despite the wide range of its constituents, the periostracum of the rhynchonellide *Notosaria nigricans* (Sowerby) is the product of the simplest secretory regime. The periostracal succession is strictly chronological and consists of: an outer bounding triple-unit membrane with coiled fibrils disposed in rhombic arrays; a main mucopolysaccharide layer with scattered membrane-lined vesicles, mucin inclusions, and vertically arranged fibrils polymerizing after secretion of the layer; and an inner bounding membrane with distal fibrillar surface (Pl. 96, fig. 8). Four or five lobate cells intervene between the inner epithelium and the vesicular cells, but no periostracal slot has been seen in this species nor the related rhynchonellide *Hemithyris psittacea* (Gmelin) which has the same type of periostracum.

The terebratulacean periostracum, on the other hand, is potentially more complicated in origin because, although it is a monolayer of a uniformly electron-dense mucoprotein in *Terebratulina retusa* Linnaeus, a similar layer covering the shell of *Liothyrella uva* (Broderip) arises in a shallow slot bounded by vesicular and lobate cells, and bears sporadically distributed secretion droplets and vesicles on its outer surface. These constituents are exuded by the lobate cells. They represent the beginnings of a superstructure secreted simultaneously with the basal layer of the periostracum. The entire succession is, therefore, heterochronous.

The thecideidine periostracum is also very simple in structure, being only 100 nm or so thick and consisting of a triple-unit membrane which separates a thicker inner polysaccharide layer and an outer fibrillar electron-dense layer (Pl. 96, fig. 5). The periostracum, however, invariably originates in a deep periostracal slot between the proximal vesicular cells and about six lobate cells. The latter contribute to the formation of the outer layer, which thus represents a superstructure so that the periostracal succession is heterochronous.

The most complex periostraca yet investigated belong to various terebratellacean species. The commoner type was first seen in sections of the terebratellid *Waltonia inconspicua* (Sowerby). It is now known to be characteristic of other genera belonging to the Terebratellidae as well as the Dallinidae, Kraussinidae, Laqueidae, and possibly the Platidiidae. It originates within a well-developed periostracal slot and consists of: a uniformly electron-dense basal layer secreted by the vesicular cells; and clusters of secretion droplets and large vesicles, exuded by the lobate cells, and amalgamated into a labyrinthine superstructure (Pl. 96, fig. 9). An infill between the droplets and within collapsed vesicles, which is also secreted by the lobate cells, quickly polymerizes into regular, closely packed hexagonal arrays of rods about 15 nm thick. The rods may be helicoidal in structure and are joined to one another by a fibrillar web.

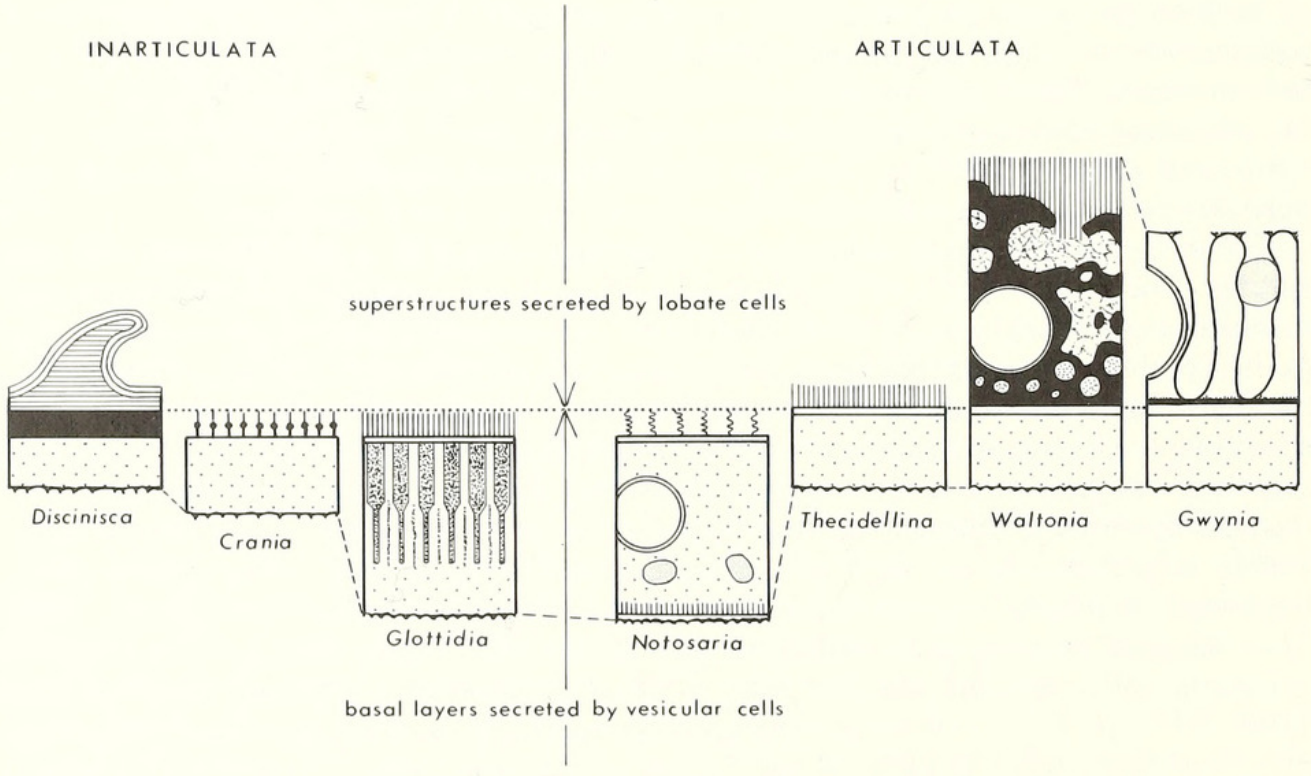
Comparison with the fine structure of the scleroprotein of *Buccinum* (Hunt and Oates 1978, p. 438) is striking, and together with the biochemical identification of glycine-rich proteins in bulk samples of the terebratellacean periostracum (Jope *in* Williams *et al.* 1965, p. H161) may indicate that both sheeted and helicoidal proteins are consecutively secreted by lobate cells.

The only other type of terebratellacean periostracum so far identified is that covering the shell of the neotenously derived megathyrid *Gwynia*. The periostracum again originates in a deep periostracal slot. The basal layer secreted by the vesicular cells is uniformly textured but divisible into two zones according to variation in electron density (Pl. 96, fig. 10). The layer supports a superstructure secreted by the lobate cells, which is essentially a vertically folded monolayer sporadically interrupted by vesicles. Whether this heterochronous succession is a novelty restricted to *Gwynia* or whether it is characteristic of other megathyrids remains to be seen.

CONCLUSIONS

The Inarticulata and Articulata have existed as two independent Classes of brachiopods since Precambrian times, yet the differentiation of the mantle edge, and indeed the origin and nature of the periostracum, show how long-lived organic systems can be.

The intramarginal position of the boundary between ciliated and non-ciliated mantle epithelium, and the folding of the latter sheet into an outer mantle lobe underlying the shell edge, are characteristic of all living species examined by us. With one exception (*Crania*), the outer mantle lobe is differentiated into a subperipheral narrow zone of lobate cells responsible for the secretion of an impersistent mucin film and a variable number of vesicular cells at the hinge of the lobe which exude persistent periostracum. The absence of lobate cells from the mantle edge of *Crania* is regarded as an evolutionary novelty rather than an indication of a less differentiated

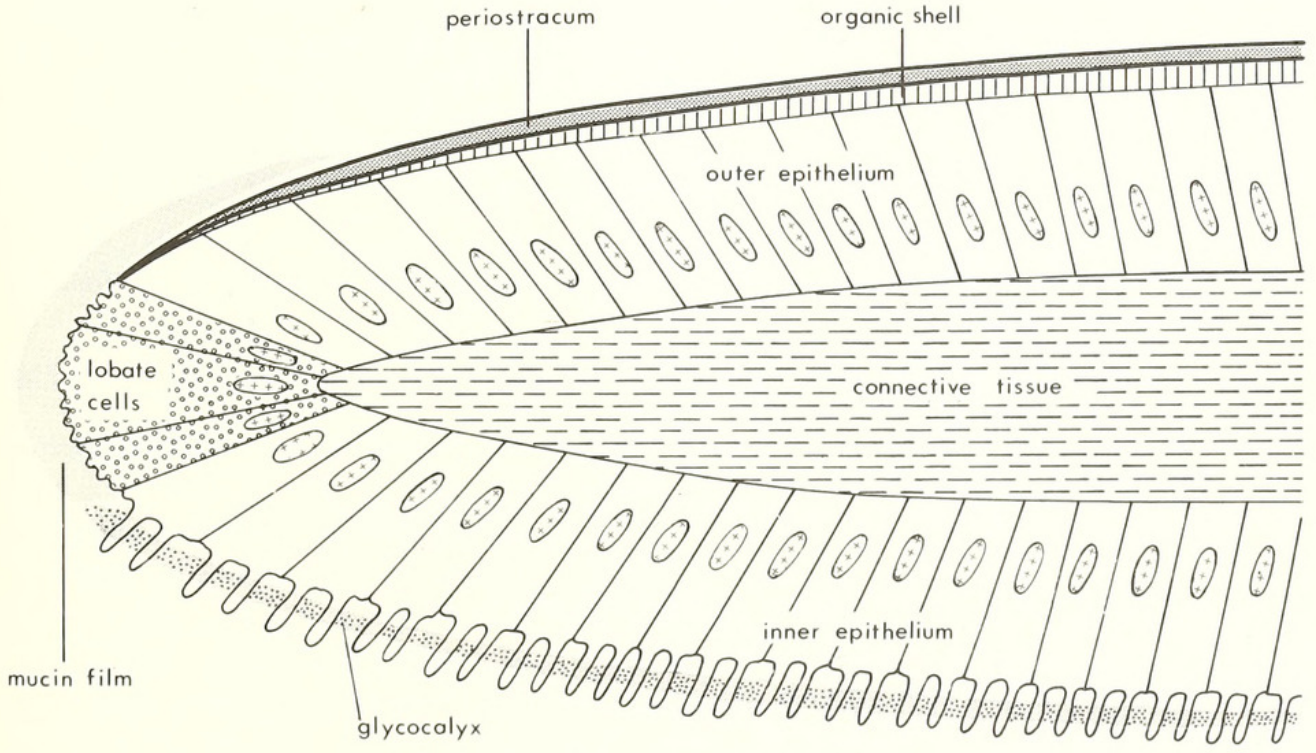


TEXT-FIG. 2. A correlation of characteristic types of periostraca among inarticulate and articulate brachiopods.

prototypic mantle. The calcareous-shelled craniaceans are post-Cambrian derivatives from a chitinophosphatic inarticulate stock; they additionally lost their setae and evidently underwent a number of paedomorphic changes. On balance we consider that the prototypic mantle (text-fig. 3) was also differentiated into inner epithelium, lobate cells, and outer epithelium (including vesicular cells).

The periostracum is highly variable in structure but the secretory regimes responsible for its development invariably follow one of two sequences in all species examined to date. In both Classes of brachiopods a periostracal succession may be chronological when it is deposited superficially by vesicular cells, or heterochronous when it originates within a slot bounded by vesicular and lobate cells, which respectively secrete a basal layer(s) and a variable superstructure (text-fig. 2). It seems more likely that the more primitive succession is the chronological one, with the further assumption that assembly of the prototypic periostracum on the secretory surfaces of the vesicular cells would have been facilitated by the protection afforded by a covering film of hydrophilic mucin exuded by the lobate cells. If this were so, heterochronous successions evolved independently and probably repeatedly in unrelated species of both Classes as the secretion of the periostracum began to take place within a slot developing between vesicular and lobate cells.

There are basic differences in the composition of the periostracum of chitinophosphatic inarticulates and that of calcareous-shelled brachiopods (including *Crania*). In particular, the periostraca of the calcareous-shelled brachiopods yield more glycine and less alanine on hydrolysis than those of the chitinophosphatic species, which additionally contain significant quantities of hydroxyproline commonly indicative of collagens and glucosamines derived from chitin (Jope 1967,



TEXT-FIG. 3. Diagrammatic reconstruction of a sagittal section of the mantle edge of a prototypic brachiopod.

p. 596). These differences, however, do not seem to be reflected in any fundamental contrast in the ultrastructural styles of the periostraca. Sheeted and/or fibrillar proteins are prevalent in both Classes; and the only structures which appear to have helical conformations are the rod-like extensions to the outer bounding membranes of *Notosaria* and the hexagonally stacked rods polymerizing out of the infill in the terebratellacean periostracum.

In retrospect, investigations to date confirm the model of the mantle edge of the prototypic brachiopod recently proposed by one of us (Williams 1977, p. 11). This assumes that the lobate cells originally occupied the hinge of a symmetrically folded mantle and secreted mucin which was inwardly confluent with the glycocalyx of the ciliated and microvillous inner epithelium (text-fig. 3). Externally the mucin formed a cover to the first-formed periostracum as it polymerized into a fibrillar triple-unit layer, beneath which a chronological succession of sheeted or fibrillar protein accumulated with continuing secretion by outer epithelium. The displacement of the lobate cells to an intramarginal position is envisaged as occurring with the development of an inflexible mineral exoskeleton. This would have tended to protrude as a protective ledge overlying the hinge of the mantle, and selection pressure would have favoured a permanent inward migration of the epithelial junctions by development of the outer mantle lobe.

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