The Ligament of *Pecten*

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Summary

The ligament of *Pecten maximus* consists of two layers, the outermost situated along the extensive dorsal margin of the valves, the inner between them at the umbo. The former, composed largely of tanned proteins, is laminated and is somewhat similar to the outer layer of other ligaments. The inner layer is divided into three parts, a large central non-calcified structure and two lateral calcified regions attaching the former to the valves. The lateral parts resemble the entire inner layer of the ligament of most other lamellibranchs, which is generally uniformly calcified. The central region, consisting mainly of a tanned protein complex, is gelatinous in appearance and is characteristic of *P. maximus* and associated species. This type of structure is particularly suitable for the rapid and frequent opening of the valves, and its relatively high efficiency may be largely attributed to the non-calcified region of the inner layer.

Introduction

The shell of lamellibranchs consists of a pair of valves joined together dorsally by a horny structure termed the ligament. This structure causes the valves to open when the adductor muscles relax.

The morphology, development, and nature of the ligament of various lamellibranchs has recently been described (Trueman, 1949, 1950*a* and *b*, 1951, and 1952), and the fundamental structure of the ligament and mantle discussed (Owen, Trueman, and Yonge, 1953). Apart from a brief description of some aspects of the ligament of *Pecten* (Newell, 1937 and 1942), little attention has been given to the ligament of this genus, which is remarkable in view of the interest in its adductor muscles (e.g. Bayliss, Boyland, and Ritchie, 1930) and in the evolution of the swimming habit in the Lamellibranchia (Yonge, 1936).

The mechanical properties both of the ligament of *P. maximus* and of that of the rather similar *Chlamys opercularis* have recently been examined (Trueman, 1953). The present paper gives an account of the structure and nature of the ligament of these lamellibranchs and discusses their form in relation to their mechnical properties.

General Structure

The ligament of *P. maximus* is situated between the dorsal regions of the valves (fig. 1) and extends both in front of and behind the umbo. It may therefore be referred to as amphidetic.

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The ligament of many lamellibranchs consists of the following four layers, the periostracum, covering the outer surface of the valve and ligament, the fusion layer, and internal to these the outer and inner layers of the ligament (Owen, Trueman, and Yonge, 1953). In *P. maximus* the periostracum is very thin and apart from its attachment to the ligament laterally need not be considered.

The inner layer of the ligament of *Pecten* is a large internal structure comparable with that of other lamellibranchs. It is of a dark brown colour, is situated between the valves in the subumbonal region (fig. 1), and is joined to

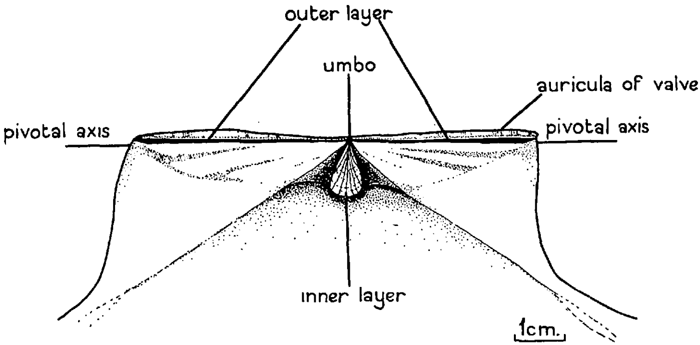

FIG. 1. Interior of the dorsal region of the right valve of *P. maximus* with the ligament cut in longitudinal section. The position of the pivotal axis is indicated (approximately natural size).

the valves in the ligament pits or resilifers. It is pyramidal in shape with a base that bulges ventrally between the valves, particularly when they are closed.

In addition to the inner layer and the periostracum, a single elongate layer (fig. 1) of a brown colour unites the dorsal margins of the valves which are extended antero-posteriorly in *Pecten* to form the auriculae. This single layer might represent either the fusion layer or the true outer layer, for convenience of reference it may be called the outer layer, but the interpretation of this layer will be discussed below.

When the elongate outer layer is viewed in transverse section, it appears approximately rectangular in shape, the width between the valves being much smaller than its thickness (fig. 2). Sections of small specimens of *Chlamys opercularis* (6 mm. overall length) were used for the examination of this layer. In transverse sections of the latter near the anterior and posterior margins of the outer layer, the periostracum may be seen attached to the dorsal edges of the ligament. There is no differentiation of the outer layer when ordinary staining techniques are used. In these specimens of *Chlamys* the more recently formed outer layer at the anterior and posterior margins stains pink in Mallory's triple stain. Sections of the outer layer of the adult *P. maximus* appear as dark structures, red when stained by Mallory's triple stain; sections of the more central region of the outer layer of the ligament of *Chlamys* are somewhat intermediate between the two conditions.

In such transverse sections as those of *Chlamys* the outer layer is only faintly birefringent. This is in contrast to the marked anisotropic properties of this layer in certain other lamellibranchs, e.g. *Tellina tenuis* (Trueman, 1949), where the optical properties appear related to the tensile stress to which the outer layer is subjected. The outer layer of the ligament is laminated, the laminae appearing in transverse section to be parallel to its ventral surface

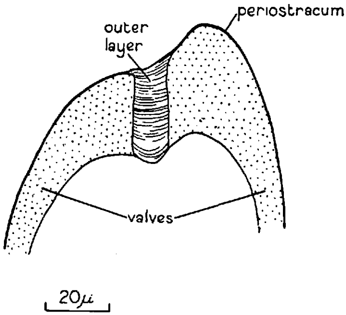

FIG. 2. Transverse section of the outer layer of the ligament of *C. opercularis* showing the characteristically laminated appearance. In this figure and in the one following the probable position of the valves is indicated by the stippled area.

(fig. 2). Each group of lamellae extends to the outer surface at the anterior or posterior margin of the layer, where they are thicker than those more centrally (fig. 4, B). Growth of the outer layer of the ligament of *Pecten* takes place both by elongation and by the simultaneous thickening of the more central outer layer, which occurs in *Pecten* in the absence of an extensive inner layer. As the shell grows the more dorsal part of the outer layer frequently becomes worn away and is replaced by more ventral secretion.

Transverse sections of the outer layer of the ligament of small specimens of *C. opercularis* (1·5 mm. overall length) were examined with particular care regarding its differentiation into fusion and outer layers. It is considered that if there was such differentiation it should be apparent in sections of an early post-larval stage. The fusion and outer layers of the ligament of many lamellibranchs, e.g. *Tellina* or *Mytilus*, may be easily differentiated in sections stained by Mallory's triple stain, but when sections of the small specimens of *C. opercularis* were stained in this manner only the laminae could be seen. Thus the region termed the outer layer appears to consist of a single kind of layer.

The inner layer of the ligament may be divided into three parts: a large non-calcareous central region, dark brown in colour and of rubber-like consistency, and two smaller lateral calcified regions attaching the former to the valves. The shape and relationship of these parts to each other and the outer layer are best understood by reference to fig. 3. The inner layer of the ligament of many lamellibranchs, e.g. *Mytilus*, *Ostrea*, or *Tellina*, is calcified. The central part of that of *Pecten* or *Chlamys* is not calcified although it shows structures similar to the inner layer of the other genera. The two regions of the inner layer may be easily differentiated by use of Mallory's triple stain, which colours the central part red and the lateral parts blue.

In transverse and longitudinal sections the inner layer presents a laminated appearance, the lamellae being nearly parallel to the ventral growth surface of

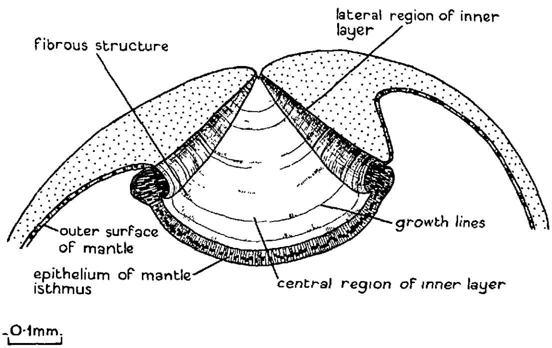

FIG. 3. Transverse section through the centre of the inner layer of *C. opercularis* showing the structure of the central and lateral regions as described in the text. The outer layer has become worn away and the inner layer is slightly fractured dorsally.

this layer (fig. 3). These lamellae probably represent growth stages and correspond to the structures previously described as the growth lines of the inner layer of the ligament of other lamellibranchs, e.g. in *Tellina tenuis* (Trueman, 1949, figs. 3 and 4). Examination of hand sections of the central region of the ligament of *P. maximus* clearly demonstrates the presence of fibres whose main axis lies normal to the ventral growth surface of the ligament and to the growth lines (fig. 3). The growth lines may be traced into the lateral calcified regions, where they are particularly prominent, indicating corresponding growth stages in all parts of the inner layer. In transverse hand sections of the lateral regions a well-marked fibrous structure may be observed, which appears similar to that of the central region. The structure of the lateral regions is very much more noticeable than that of the central, the latter being relatively amorphous.

The lateral parts of the inner layer of *Pecten* are similar to the complete inner layer of other lamellibranchs. This suggests that they represent the more typical part of the layer, the central region being a modification characteristic of this type. The inner layers of *Chlamys*, *Amussium*, and *Hinnites*

(Yonge, 1951) are similar to that of *Pecten*, so that the modified inner region of the ligament appears characteristic of the Pectinidae.

The pivotal axis of the ligament lies immediately below the outer layer (fig. 1), but as the shell grows the axis tends to move ventrally. A small part of the inner layer thus becomes situated above the pivotal axis and subjected to tensile stress when the valves are closed, so causing slight splitting of the most dorsal region of this layer (fig. 3). The inner layer of the ligament of *Ostrea* and *Mytilus* (Trueman, 1951 and 1950*b*) has been shown to split similarly when subjected to tensile stress. As the fracturing of the inner layer occurs in the ligament of *Pecten*, so the dorsal part of the outer layer also splits. The outer layer may thus become completely worn away from above part of the inner layer (fig. 3). This phenomenon may be observed in large specimens of *P. maximus* and in these the replacement of the older parts of the outer layer by a more ventral secretion may easily be seen in longitudinal section. Above the outer layer of such specimens a region of previous attachment of the outer layer may be observed on the valve, showing parallel striations probably equivalent to the laminae of the outer layer now worn away. This occurs to a very much greater extent in *Hinnites multirugosus* (Yonge, 1951), apparently in response toc onstraint between rock surfaces when the upper valve is raised.

The ventral movement of the pivotal axis also occurs in *Ostrea* and appears to be characteristic of those lamellibranchs with internally situated ligaments, Dall (1895) observed that the longer the line covered by a functional ligament the more rigidly will the opening and closing of the valves be controlled. There are two methods of obtaining a long straight pivotal axis near the border of a rounded body: either the axis must pass inwards, or the outline of the body must be modified. The auriculae and the relatively long outer layer of the ligament of *Pecten* probably reduce the amount of ventral migration of the axis as compared with other lamellibranchs with internal ligaments.

The Mantle Epithelium

The relation of the mantle to the various layers of the ligament, discussed recently by Owen, Trueman, and Yonge (1953) may be briefly noted. The periostracum covers the valves and ligament, although it is frequently worn away at the umbones. It is secreted from the periostracal groove, which is continuous around the mantle edge between the middle and outer lobes of the mantle. The fusion layer is secreted by the outer mantle lobe immediately below the periostracum in the dorsal midline. It is so called because it represents material secreted by the region of the outer mantle lobes which coalesce in very early post-larval life. This region is considerably elongated in many lamellibranchs and together with the periostracum forms a type of secondary ligament, which may extend both anteriorly and posteriorly to the outer and inner layers of the ligament.

The true outer layer of the ligament is generally secreted by the epithelium adjacent to the boundary of the mantle isthmus in the midline. The term 'mantle isthmus' is applied to the epithelium of the outer surface of the mantle, bounded peripherally by the outer mantle lobe, where this epithelium forms a connecting neck of tissue between the two lateral lobes of the mantle. The general outer surface of the mantle secretes the inner calcareous layer of the valves except at the mantle isthmus, where it secretes the inner layer of the ligament (fig. 4). The inner layers of the valves and ligament are thus closely related to each other.

The mantle of *Pecten* shows the characteristic periostracal groove and outer lobe of the mantle. Examination of the tissues of whole specimens shows a

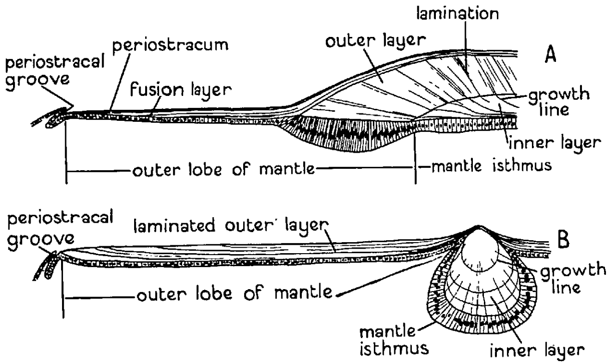

FIG. 4. Diagram of longitudinal sections through either anterior or posterior ends of lamellibranchs. A, basic type of ligament with periostracum, fusion, outer and inner layers. B, the ligament of *Pecten*. The epithelium of the mantle is shown below the ligaments. Not to scale.

considerable depression in the dorsal region of the animal corresponding to the position of the inner layer of the ligament and representing the mantle isthmus (fig. 5). Between the outer lobe of the mantle and the central depression two thin ridges of epithelial tissue extend anteriorly and posteriorly along the dorsal midline (fig. 5). The epithelium of these ridges, although rather thicker than that adjacent secreting the valves, shows no localized regions of hyperdevelopment such as have been observed near the mantle isthmus in certain other lamellibranchs, e.g. *Tellina* (Trueman, 1949, fig. 8, *Ep. 1B*). The epithelium of the mantle isthmus is, however, considerably enlarged (fig. 3) as compared with the epithelium of the outer surface of the mantle secreting the inner layer of the valve.

THE NATURE OF THE LIGAMENT

Some observations on the nature of the ligament of *Anodonta* have been made by Trueman (1950*a*) and on that of *P. maximus* by Roycroft (1951). The outer layer of the ligament of *Pecten* is not particularly suitable material for investigation since only small portions are available, the central part of the inner layer being much easier to study. The term 'conchiolin' is frequently used to indicate the non-calcareous part of the molluscan shell. Consideration of whether or not there is any substance present in the organic matrix of the shell to which this term may specifically refer, is beyond the scope of this paper. 'Conchiolin' may be conveniently used to refer to the non-calcareous parts of the shell.

In appearance the central part of the inner layer is somewhat gelatinous, resembling a solidified 10 per cent. gelatin solution that has been tanned by being placed in a 1 per cent. benzoquinone solution for about 48 hours. The outer layer appears to be much more fibrous, and to be generally similar to

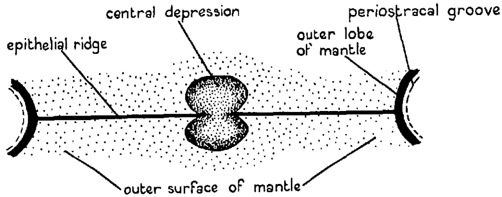

FIG. 5. Diagram of the dorsal region of the mantle of *Pecten* viewed dorsally.

that of the ligaments of other lamellibranchs. The median part of the inner layer of the ligament of *Pecten* is markedly different from its counterpart in other bivalves, largely because of the lack of calcification. The calcium carbonate content of the inner layer of the ligament of certain lamellibranchs has been approximately determined by the ignition of parts of the ligament. The residual ash was assumed to be calcium oxide and the calcium carbonate content of the inner layer estimated therefrom (Roycroft, 1951). The central part of the inner layer of the ligament of *P. maximus* contains only 0·6 per cent. by weight of calcium carbonate in comparison with that of the inner layers of *Lutraria lutraria* or *Anodonta cygnea* which amounts to 22 per cent. and 40 per cent. by weight respectively.

The lateral parts of the inner layer appear both to be calcified and to have undergone some quinone-tanning, a condition rather similar to that of the inner layer found in many lamellibranchs, e.g. *Ostrea edulis*. The lateral parts might be described as being somewhat intermediate between the central region and the calcified shell.

Tests on the ligament of *Pecten* showed that it was extremely resistant to chemical action. It was soluble in concentrated alkali, however, and the chitosan test for chitin was negative. Positive results in tests for proteins, e.g. ninhydrin, biuret, indicated that they compose a large part of the ligament. The dark brown coloration of the outer layer and the central part of the inner layer suggest that the proteins had been affected by quinone-tanning.

Roycroft (1951) has investigated the type of protein linkage present in the ligament, by use of the methods described by Brown (1950). He placed sections of the ligament in a graded series of swelling agents, and found that the outer layer and the central part of the inner layer were soluble only in sodium hypochlorite solution and not in the other swelling agents. Solution only in sodium hypochlorite solution suggests the presence of aromatic tanned proteins (Brown, 1950).

The argentaffin reagent gave a positive reaction with both layers of the ligament; this may be considered due to the presence of polyphenols. Investigation of the precursors of the tanning agent in the tissues concerned with the secretion of the ligament, e.g. the ferric chloride test for orthodiphenols was carried out with inconclusive results. The presence of some lipoid material in the central region of the inner layer was also faintly indicated by the appearance of frozen sections stained by sudan black.

The main constituent of the ligament may thus be shown to be a tanned protein complex, fibrous in the outer layer and more gelatinous in the inner. The presence of the non-calcified region of the inner layer of the ligament of *Pecten* and *Chlamys* is unusual in comparison with other lamellibranchs, although a similar condition has been recorded in the ligament of *Nucula* (Trueman, 1952).

Discussion

The structure and composition of the ligament of *P. maximus* and associated species has been outlined above. Whereas the ligamental structure of many lamellibranchs consists of the periostracum, fusion, outer and inner layers (fig. 4, A), in that of *Pecten* there is the periostracum and an inner layer but only one of the other two layers (fig. 4, B). From its location below the periostracum this layer could be termed the fusion layer but from its relationship to the inner layer it could be called the outer layer. Both the fusion and outer layers are secreted by the outer mantle lobe, the latter near the mantle isthmus by a band of rather enlarged epithelial cells in many lamellibranchs. No such area of epithelium has been observed in *Pecten*. The fusion layer is secreted from the more distal parts of the outer mantle lobe, which are fused together in very early post-larval life (Owen, Trueman, and Yonge, 1953). In many lamellibranchs, e.g. *Tellina* (Trueman, 1949), the fusion layer is relatively more extensive and thinner than the outer layer. The fusion layer is commonly laminated, the laminae generally being at their maximum thickness centrally, near the anterior and posterior margins of the outer layer (fig. 4, A).

Sections of the outer layer of the ligament of most lamellibranchs also show laminations, indicating stages in the secretion of the layer, corresponding to the growth lines of the inner layer (fig. 4, A).

In *Pecten* the outer layer is laminated, the laminae being at their maximum thickness peripherally (fig. 4, B). The fusion and outer layers, in contrast to the inner layer, are somewhat similar in structure and nature, and their main structural differences possibly result from their different rates of secretion.

If the so-called outer layer is actually the fusion layer, the true outer layer is probably entirely absent from *Pecten*. Alternatively the fusion layer is not present and the epithelium secreting the outer layer is much more widespread than in other lamellibranchs, e.g. Tellina (Trueman, 1949, fig. 8), extending right along the epithelial ridges in the dorsal midline of the mantle (fig. 5). From a functional aspect this layer is directly comparable with the outer layer of other lamellibranchs. It is convenient to refer to it as the outer layer of the ligament of *Pecten* without prejudicing the possibility of this layer being the fusion layer. A detailed study of the mantle of *Pecten* in early post-larval life is required to elucidate this further. The possibility that the central part of the inner layer represents a much modified outer layer should not be entirely overlooked. A similar view was held by Newell (1937). It may be suggested that in such circumstances the mantle isthmus would be divided along the mid-line into two parts each secreting the lateral regions of the inner layer. The central part of the inner layer is, however, markedly different in appearance and structure from the outer layer of the ligament of other lamellibranchs and it appears improbable that this view is correct.

Apart from the structure of the outer layer discussed above, the ligament of *P. maximus* shows two important differences from that of other lamellibranchs; first the elongated outer layer and secondly the unusual non-calcified region of the inner layer. The significance of these may be related to their mechanical properties.

The outer layer of all ligaments is normally subjected to tensile stress when the valves are closed (the inner layer simultaneously to compression) and the removal of small parts considerably reduces the opening moment of the ligament. Experiments have shown that removal of such parts of the outer layer of *Pecten* does not always have a very marked effect (Trueman, 1953). Removal of all of the outer layer either from in front of or from behind the umbo causes the tearing of the inner layer and leads to the misalignment of the valves. Thus the elongate outer layer of *Pecten* is not primarily concerned with the opening strength of the ligament, but may be considered to behave as a fairly rigid hinge structure, related to the prevention of the rotation of the valves one about the other.

The ligaments of *Pecten* and *Chlamys* are particularly efficient for the rapid opening and closing of the valves, having a lower work-loss than those of other lamellibranchs (Trueman, 1953). This may be attributed to the non-calcified inner layer of the ligament. The calcified inner layer of *Lutraria lutraria*, for example, has a modulus of elasticity in compression approximately seven times the similar but non-calcified inner layer of *P. maximus*. Calcification increases the resistance of the inner layer of the ligament to compression, but it has been further observed that this is associated with an increased internal resistance.

The central non-calcified region is thus important in respect of the relatively high efficiency of the ligament. Such a structure is particularly well adapted to the rapid and frequent opening of the valves found in *P. maximus* and *C. opercularis* in association with the swimming habit.

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