Structure and formation of the egg capsule tendrils in the dogfish *Scyliorhinus canicula*

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SUMMARY

The tendrils of the egg capsule of the dogfish *Scyliorhinus canicula* appear to act as damped springs which become entangled with one another, attaching the capsule firmly to the seaweed *Halidrys siliquosa*. The present paper describes the structure of tendrils and their method of formation in a specialized region of the nidamental gland which we have termed the tendril-forming region (TFR). The tendrils provide a unique system for studying the assembly of a complexly ordered collagenous material.

Tendrils show primary twisting and then undergo secondary helical coiling. In cross section they have a lamellated, spiral construction. Each lamella appears to consist of a broad and a narrow lamina. Collagen fibril orientation is approximately longitudinal in the broad lamina and approximately circumferential in the narrow one. Fine, longitudinal fluid-filled canaliculi lie between the lamellae and may act as shock absorbers. Spherical granules containing a high concentration of tyrosine residues are present in large numbers in the outermost lamellae and may have rubber-like properties. The collagen is probably heavily cross-linked and gives the tendril high tensile strength.

The tendril appears to be formed from the adhesion of successive lamellae which are wrapped round a central core as the forming tendril undergoes counter-clockwise (left-handed) rotation within the TFR. The latter appears to represent a modification of the structure of the simpler capsule wall-forming region (CWFR). A wave of progressive activation of tubular glands of the TFR travelling anteriorly followed by a wave of deactivation in the opposite direction appears to be responsible for the secretion of first the posterior tendril, then the marginal rib of the egg capsule and finally the anterior tendril. Secreted material passes from glandular tubules through secretory ducts to a series of parallel transverse grooves which act as complex extrusion dies to form the lamellae of the tendril. We have gone some way towards describing how the complex three-dimensional organization of the tendril is produced by these dies. Observations suggested the following sequence of events within the extrusion dies: secreted material becomes uniaxially oriented in the secretory duct and is then passed between ciliated plates we have termed 'baffle plates'. These separate the material into an anterior flow containing vertically oriented collagen molecules and a thinner posterior flow containing approximately horizontally oriented ones. These two flows then pass through a transverse groove to become respectively the broad and the narrow lamina of a single lamella of the tendril. The lamellae become pleated within the transverse grooves probably by anisotropic shrinkage. The canaliculi appear to be formed by the partial adhesion of the pleated lamellae as they are wound onto the forming tendril by rotation within the TFR. The mechanism of rotation of the forming thread and its subsequent coiling in the posterior oviduct is discussed.

1. INTRODUCTION

How collagens self-assemble into complex structures exhibiting order at several hierarchical levels (Lakes 1993; Van Der Rest 1991) and how the remarkable and highly adaptive properties of these structures are determined raise questions of considerable philosophical interest. The answers have implications for the study of embryological development, pathology and normal physiology, and possible technological applications. Two reasons make the collagenous egg capsule of the dogfish *Scyliorhinus canicula*, and the nidamental gland which secretes it, an excellent system for studying these questions: (i) the system enables the time sequence of the whole self-ordering process to be investigated (Knight & Feng 1992; Knight *et al.* 1993); and (ii) different parts of the egg capsule (walls, tendrils, marginal ribs and anterior seal) show remarkable variations in structure and mechanical properties. The tendrils of the egg capsule of *Scyliorhinus canicula* are coiled and there is interest in the way in which coiling is produced in natural systems (Galloway 1989). There is also commercial interest in the production of coiled fibres. For these reasons, we decided to investigate the structure and method of formation of the tendrils having previously described the formation of the dogfish egg capsule wall (Knight & Feng 1992). The present paper describes the construction of the tendril from approximately concentric lamellae of collagen-containing fibrils; phe-

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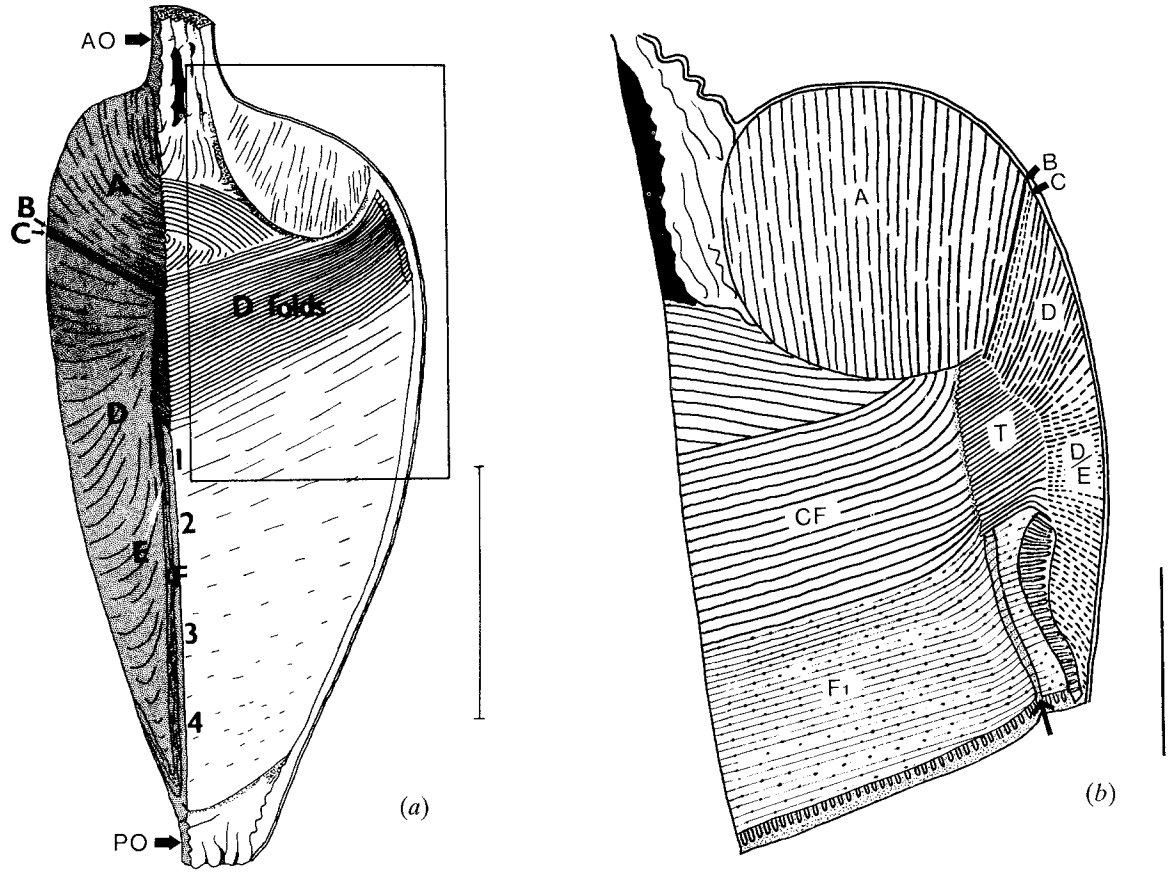


Figure 1. (*a*) Semi-diagrammatic section of the nidamental gland with the vertical longitudinal cut surface shaded showing the distribution of glands A–E. The lumen of the gland is shown to the right with the more superficial zones of glands F_1–F_4. The anterior oviduct is uppermost. The D-zone grooves lie between transverse folds which encircle the whole gland. The outline of the drawing after Metten (1939) with detail added from SEM observations. Anterior oviduct (AO) and posterior oviduct (PO) indicated. Scale bar 20 mm. (*b*) Semi-diagrammatic enlargement of the boxed area in (*a*) showing right tendril-forming region (T) viewed from the ventral surface. The gland was cut in half horizontally just ventral to the marginal groove. Capsule wall-forming region (CF), marginal canal (arrowed), and zones of gland tubules (A, B, C, D/E and F_1). Scale bar 3 mm.

nolic, probably rubber-like, polymer granules; and fine longitudinally oriented fluid-filled canals which, because of their small diameter, we have termed canaliculi. The possible biomechanical significance of these components is discussed. We describe the structure of the tendril-forming region (TFR) and go some way towards describing how the surprisingly complex three-dimensional organization of the tendril is produced as an extrusion from a specialized region of the nidamental gland. The sequence of events during the formation of the tendrils and egg capsule is discussed. The formation of the tendril may have relevance to the commercial extrusion of pleated sheets and helically crimped fibres for use as shock absorbing materials.

2. MATERIALS AND METHODS

Live, sexually mature female lesser-spotted dogfish, *Scyliorhinus canicula*, were obtained from the Solent by line fishing or from Weymouth Bay by trawling. Fish were quickly killed by beheading.

Of the 48 mature female dogfish we examined (June 1992 to May 1993), 13 were actively secreting the anterior tendrils and five the posterior tendrils while five were secreting the capsule wall. Twenty-one of the remainder had fully formed egg capsules and tendrils within the lower oviducts. Secretory activity generally appeared to be exactly synchronous in both nidamental glands.

The nidamental gland is a complex structure situated between the anterior and the posterior oviduct. The nomenclature used to describe the zones of the nidamental gland which secrete both capsule wall and tendrils is that of Rusaouën *et al.* (1976), Rusaouën (1976) and Rusaouën-Innocent (1990). The tendril and capsule secreting regions both contain nine distinct transverse zones of tubular glands running from the most anterior, A, to the most posterior, F_4 (figure 1). For light microscopy, active and inactive nidamental glands were removed immediately after killing the fish. The lateral margins of the nidamental gland which secrete and form the tendrils (tendril-forming regions, TFR; see below) were separated from the region which secretes the wall of the capsule (capsule wall-forming region, CWFR) by vertical longitudinal cuts with a razor blade. TFRs were fixed as described elsewhere (Knight & Feng 1992) by immer-

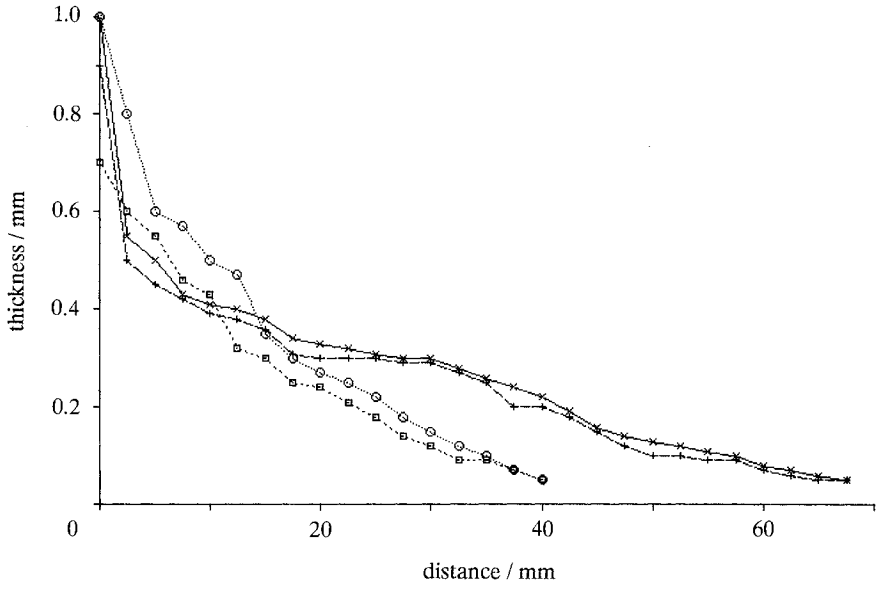


Figure 2. Graph showing how the thickness of an anterior and posterior tendrils change with distance from their origin. ×, anterior tendril maximum diameter; +, anterior tendril minimum diameter; ○, posterior tendril maximum diameter; □, posterior tendril minimum diameter.

sion in a modified Karnovsky (1965) fixative containing a final concentration of 0.45 M sodium chloride. After washing in cacodylate buffer overnight, and dehydrating in an ethanol series, tissue blocks were embedded in paraffin wax. Transverse 7–10 μm sections were cut starting at the A zone and finishing with the F_1 zone. Serial VLSs through the TFR were also prepared. Sections were stained with either toluidine blue (pH 8.5), or haematoxylin and eosin or Mallory (1901) triple stain (Gray 1973) or Millon's reagent for tyrosyl groups (Pearse 1968, 1972). Serial reconstructions were prepared by tracing tissue plans onto expanded polystyrene ceiling tiles which were then cut with a hot wire cutter.

Further details of the methods used for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have been given elsewhere (Knight & Feng 1992; Knight *et al.* 1993).

Three techniques were used to determine the pattern of activity of the tubular glands at particular stages in the secretion of the tendril: (i) plastic and paraffin sections; (ii) razor blade slices of active TFRs examined in the SEM; and (iii) whole mounts of secreted material which formed a cast of the active glands, secretory ducts, baffle plates and transverse grooves. The latter were prepared from material well fixed in Karnovsky solution by gently pulling the forming tendril from the active TFRs. These natural casts provided a rapid method and were examined wet with high magnification stereo, dark field and interference contrast microscopes and with the SEM after critical point drying. The secretion of E-zone tubules could easily be distinguished from the D zone in the casts by its light scattering (optical microscopy) and granular contents (SEM). For polarizing microscopy, cleared whole mounts of tendrils, casts of secreted material (see above) or transverse paraffin sections of nidamental glands actively engaged in the secretion of a tendril were mounted in DPX with or without Mallory staining before examination with a polarizing microscopy.

Tendrils either whole, or fractured or cut, TFRs with or without horizontal longitudinal bisection, transverse slices of the TFR in active and inactive glands and natural casts of the active TFRs (see above) were prepared for SEM.

The dimensions reported in this paper are taken from fresh tendril, fixed paraffin and plastic sections or critical point-dried specimens and were made with vernier callipers. Standard deviations are reported ($n > 10$).

The egg capsule and tendrils have a constant orientation in the nidamental gland and posterior oviduct enabling anatomical axes to be applied to them. The term 'twisting' refers to the helicoidal twisting of the lamellae of longitudinally oriented fibrils and associated canaliculi within the tendril while the term 'coiling' refers to its subsequent helical coiling.

The tensile behaviour of tendrils was subjectively assessed by stretching wet tendril between two pairs of forceps.

3. RESULTS

(*a*) *Structure of the tendril*

(i) *Gross structure and secondary coiling*

A tendril is attached to each corner of the approximately rectangular egg capsule (figure 6). The diameter of the tendril decreased with increasing distance from the capsule (figure 2). Each tendril had an elliptical cross section close to its origin from the egg capsule but became more nearly circular as it narrowed. The anterior tendrils (up to 1250 mm when gently stretched while wet) were considerably longer than the posterior ones (maximum length 470 mm). The fully formed tendril appeared to be moderately

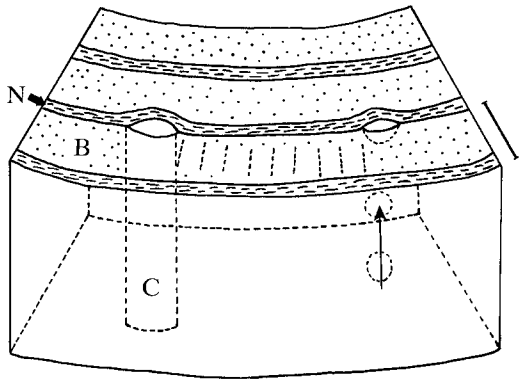


Figure 3. Diagram illustrating the lamellar construction of the tendril. The fibril orientation is longitudinal in the broad lamina (B) and circumferential in the narrow lamina (N). The slight, progressive change of orientation of the fibrils within the broad lamina is indicated (dotted lines). Canaliculus (C) and line of voids (arrow). Scale bar 6 μm.

strong when stretched, behaving like a rather slowly recoiling spring when released. The very recently formed tendril, although still in the nidamental gland just caudal to the TFR, had a very low tensile strength but this appeared to increase as it moved through the

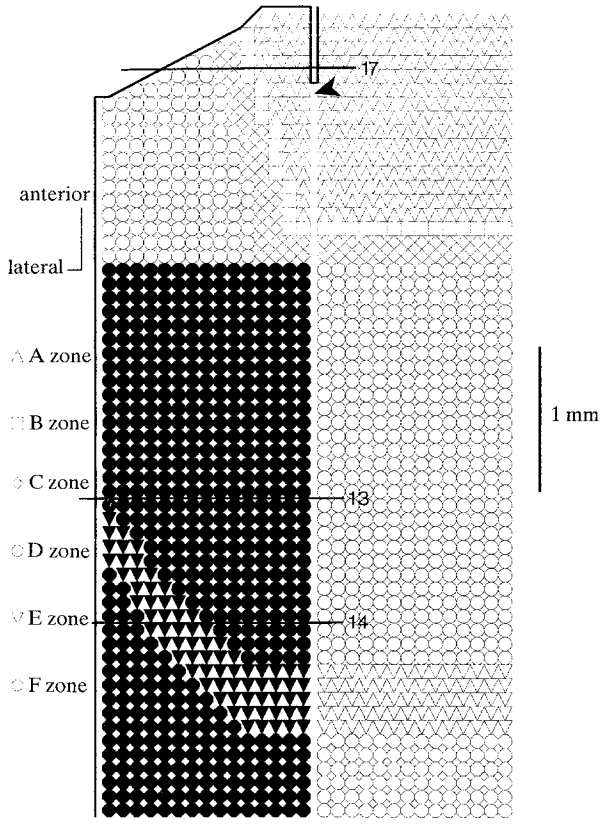


Figure 4. Somewhat simplified diagram showing the position of the openings of the different zones of tubular glands of the right TFR and part of the CWFR as reconstructed from serial paraffin sections. In this TFR, the first five rows of tubular glands opened into a short duct which joined the main lumen at the point arrowed, although the point varied from fish to fish. A horizontal row on the diagram is equivalent to a single transverse groove. Glands which were active in the sectioned gland at the time of fixation are indicated by filled symbols. The approximate plane of section of figures 17, 13 and 14 is also indicated.

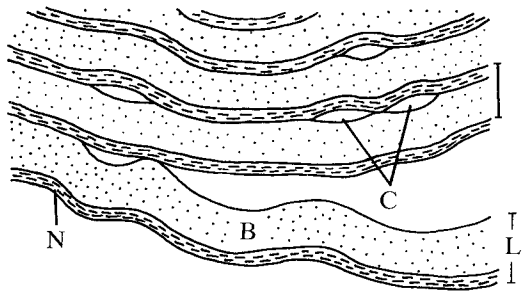


Figure 5. Diagram of a TS of part of a forming tendril to show how canaliculi (C) are thought to be formed by incomplete adhesion of a pleated lamella (L) secreted from a D transverse groove. Broad lamina (B); narrow lamina (N). Scale bar 6 μm.

nidamental gland and was retained within the posterior oviduct.

At first sight, the tendrils resembled fine, somewhat irregularly coiled springs (figure 7). On closer examination, the coiling was seen to alternate between left and right hand (figure 12) generally every one to four gyres. There appeared to be a small but probably significant preference for right-handed coiling; In twenty-two tendrils examined, 53% of the coils were right handed (paired *t*-test, 21 d.f., one-tailed probability = 0.022). The coiled regions were occasionally interspersed with short sections in which the helical folding appeared to be flattened into a planar crimp. This was particularly noticeable in the most distal extremities of the tendrils. The anterior tendrils at their origin usually bent back over the ventral part of the capsule to form short hook-like structures (figure 6) which may help to attach the egg capsule during egg laying. The posterior tendrils at their origin had a horn-like appearance, usually crossing one another in the mid plane of the capsule at an angle of approximately 90° (figure 6). The posterior margin of the capsule was concave when viewed from above.

The first coil of the tendril close to its origin from the capsule wall was right handed in the anterior and left handed in the posterior tendrils in 88% of the 25 capsules examined. This may relate to the slight right-handed twisting of the egg capsule itself about its long axis and the geometry of the junction between the nidamental gland and the posterior oviduct (see below).

(ii) *Twisting*

The outer surface of the fully formed tendril, when observed with the SEM or stereomicroscope, showed fine parallel ridges indicating that it was formed as a continuous extrusion (figure 12). These ridges probably result from secretion of material from the tubules of the F_1 zone of the gland (see below). Similar ridges are seen on the outer surface of the egg capsule (Knight & Feng 1992). The ridges frequently lay parallel to the long axis in straight sections of tendril but were usually oriented at a small angle to this in coiled parts giving a twisted appearance. The handedness of this twisting was generally the same as that of the secondary coiling (figure 12). The twisted construction of the tendrils was also clearly seen in the

arrangement of the lamellae and fine canaliculi (see below and figure 8). The observation that the twisted arrangement of the tendril occurred as soon as it had emerged from the TFR (figure 9) suggested that this results from rotation of the tendril within the TFR (see below).

(iii) *Lamellar construction*

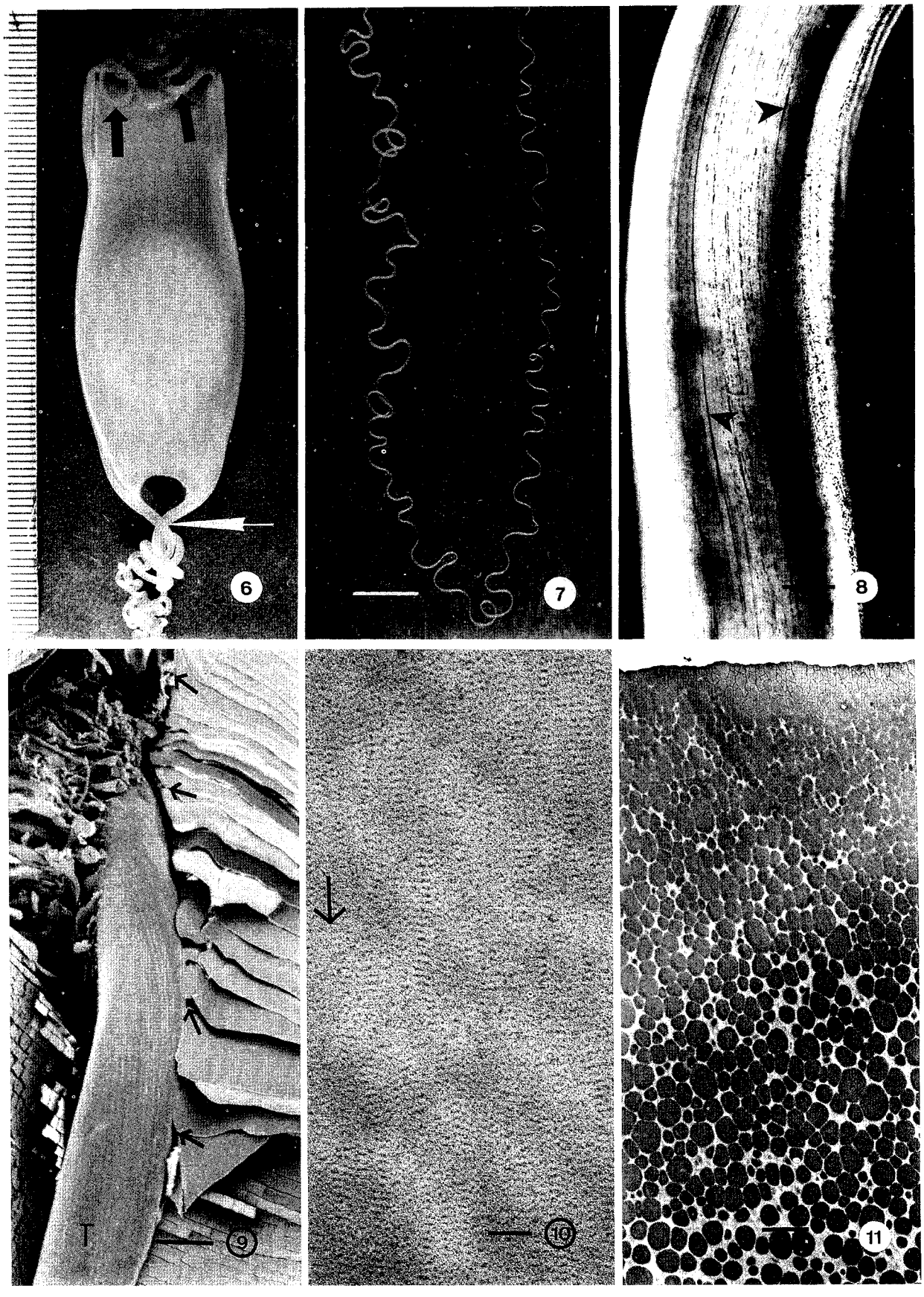
The tendril in TS superficially resembled a slice of multi-layered Swiss roll; It was constructed from lamellae which at first sight appeared to be arranged in an approximately concentric fashion but on closer inspection were seen to be arranged in a somewhat irregular spiral (figures 13 and 14). The largest diameter tendrils showed up to two complete rotations of the spiral in transverse sections. This construction suggested that a central core of rather irregularly folded lamellae with roughly circular TS was first formed within the anterior part of the TFR and wrapped in successive lamellae as it moved caudally. This method of formation was confirmed by observing whole mounts and sections of tendrils within active TFRS (see below). The lamellae had a mean thickness of 5.8 ± 0.7 μm and were seen in the polarizing microscope in TS tendrils to be constructed from alternating narrow (0.2–1.0 μm) laminae and a wide (approximately 5.5 μm) laminae (figure 13). The fibril orientation in the narrow laminae was shown to be approximately circumferential by the following observations: (i) in TS tendril, the narrow laminae showed a strong birefringence of positive sign. They gave sharp extinctions when oriented parallel or perpendicular to the axis of the analyser, thereby giving rise to a fairly distinct Maltese cross; (ii) in LS tendril the narrow laminae always appeared dark in the polarizing microscope as the stage was rotated; and (iii) in TS in the TEM the narrow laminae were seen to contain narrow, transversely banded fibrils (see below and figure 10) with a more-or-less circumferential orientation with respect to the whole tendril.

The orientation of the fibrils in the broad laminae was shown to be approximately parallel to the long axis of the tendril by the following observations: (i) in LS tendril the broad laminae showed strong birefringence, positive with respect to the fibril axis; they gave a reasonably sharp extinction when oriented parallel or perpendicular to the axis of the analyser; (ii) when TSS of tendril were rotated, the broad laminae appeared dark at all angles; and (iii) in LS tendril in the TEM the broad laminae were seen to contain well oriented fibrils running approximately parallel to the long axis of the tendril. The arrangement of fibrils within the broad and narrow laminae was confirmed by examining SEMS of material fractured longitudinally when frozen. The broad laminae in these showed parallel fibrils arranged at an angle of up to 15° to the long axis. Narrow laminae of approximately circumferentially oriented fibrils were seen between the broad laminae. Occasionally, small areas showed more obliquely oriented fibrils. The orientation of fibrils in the broad laminae was examined more closely in the polarizing microscope. When the broad laminae in LS were observed approximately parallel or perpendicular to the axis of the analyser, a narrow band of extinction was generally seen to run approximately parallel to the plane of the lamina. This band of extinction appeared to move progressively across the lamina as the specimen was rotated through a small angle. This indicates that the orientation of the fibrils changes progressively through a small angle from the outer to the inner face of a single lamina in a manner similar to that described by Bouligand *et al.* (1985) and Giraud-Guille (1988, 1989) in other collagenous systems. The mean angle of rotation from the inner to the outer face of the lamina was estimated to be $15 \pm 3°$ using the polarizing microscope. This arrangement was confirmed in SEMS of tendrils which had been fractured longitudinally while frozen. Indication of a fine crimping of the circumferentially oriented fibrils within narrow laminae was seen in paraffin TSS (figure 13).

The fibrils were seen most clearly in the broad laminae in LSS (figure 10) and in the narrow laminae in TSS in the TEM. They had a D period (34 ± 0.8 nm) closely similar to that of the fibrils of the egg capsule (35 ± 1.4 nm). The banding pattern in ultrathin sections of tendril positively stained with a combination of uranyl acetate, lead citrate and phosphotungstic acid showed a regular alternation of narrow and broad, dense transverse bands closely similar in appearance to negatively stained images of egg capsule fibrils. These similarities suggest that the tendril fibrils are similar but not identical to those of the egg capsule and are collagenous. Their collagenous nature was confirmed by observing the sharp thermal transition when short segments of tendrils were heated slowly in 0.1 M phosphate buffer at pH 7.0. This gave a mean half shortening temperature of 78°C and a shortening by about 35% of the preshrunk length, closely similar to egg capsule wall (Knight & Hunt 1974). Tendril fibrils in the TEM appeared narrower (mean diameter 71 ± 25 nm) compared with egg capsule fibrils (mean diameter 250 ± 70 nm) and showed no indication of the transverse crystalline lattice described in egg capsule fibrils by Knight and Hunt (1976). Figure 3 summarizes our observations on the lamellar unit of construction of the tendril.

(iv) *Granules and tendril surface*

Most of the tendril's surface appeared to be constructed from lamellae largely composed of numerous spherical granules. These granules had a mean diameter of 2.3 ± 0.4 μm and were amorphous in the TEM (figure 11). The granules at the very surface of the tendril were somewhat smaller in TSS, and were elongated in LSS. They appeared rather tightly packed, irregularly polygonal in outline, with small quantities of matrix between them. With increasing distance from the surface, the granules appeared progressively more spherical and with larger quantities of matrix between them. The granules often appeared to be concentrated between the broad and the narrow laminae. The outermost surface of the tendril appeared to be coated with a narrow layer of fine floccular material with the appearance of mucus. This was underlaid by a narrow layer of dense



Figures 6–11. For description see opposite.

material approximately 0.5 μm thick which appeared continuous with the matrix. The density of the matrix decreased rapidly over the first 4 μm from the surface. This appearance is compatible with the suggestion that the outer surface of tendril is infiltrated from the outside by material secreted by glands of the F_1 zone (see also Feng & Knight 1992). This was confirmed by examining the surface of the forming tendril with the SEM which showed, as the tendril passed through the F_1 zone, that a surface of flattened granules measuring 1 to 2 μm in diameter was converted to a surface with narrow longitudinal ridges covered with a fine meshwork with the appearance of mucus. Granules of similar size, staining and appearance were seen both at the surface and in lamellae deeper within the tendril but were present in relatively low numbers within the central core. The granules in some laminae fairly close to the surface of the tendril were considerably larger (7.3 ± 0.3 μm). Even the narrowest distal portions of the tendril appeared to be coated with polygonal granules. The granules gave an intense reaction with Millon's reagent indicating a high concentration of tyrosyl groups.

The structure of the outer layer of the tendril appeared closely similar to L_1 of the egg capsule. The similarity in size and staining properties of the granules of the surface lamellae of the tendril to those described in L_1 of the capsule (Feng & Knight 1992; Knight & Feng 1992) suggests that the tendril granules are also composed of strongly cross linked protein(s) containing a high concentration of tyrosine residues (see discussion below). The surface layer covering much of the tendril therefore resembles a fibrous (collagenous) composite material containing spherical granules of a phenolic polymer filler.

(v) *Canaliculi*

Numerous fine, straight, fluid-filled, non-branching canaliculi and lines of small voids were seen in SEM, plastic and paraffin sections and whole mounts of tendril (figures 8 and 15). Canaliculi ran for distances of up to 1 mm and had a lens-shaped to circular TS with mean diameters of about 10 μm. They showed a precise longitudinal or helical arrangement (figure 8) running exactly parallel to the fibril orientation in the longitudinal laminae. They generally lay between the circumferential and longitudinal laminae. They were present in large numbers between the central core and the outer granular lamellae, but were small and infrequent in the central core. The observation that these structures were also seen in completely unfixed whole mounts of tendril mounted in water confirmed that they are not artefactual in origin.

(*b*) *Structure of the tendril-forming region (TFR)*

A TFR was located anterolaterally on each side of each nidamental gland where the flattened dorsal and ventral halves (CWFRS) of the gland met (figures 16 and 18). The nidamental gland could be considered to be constructed from two identical halves, the right TFR being attached to the right margin of dorsal CWFR and the left TFR to the left margin of the ventral CWFR (figure 16). The construction of the TFR appeared broadly similar to that of the CWFR as described by Rusaouën (1976), Rusaouën *et al.* (1976) and Knight & Feng (1992). Both regions appeared to consist of more or less distinct zones of gland tubules arranged in rows. In the B, C, D and E regions each gland tubule in both TFR and CWFR was connected to a short secretory duct which opened between two baffle plates, obliquely oriented plate-like structures in the base of the transverse groove (Knight & Feng 1992) (figures 23 and 26). Each transverse groove received the secretion from a whole row of tubules (figures 21, 22 and 26). The following differences between the TFR and the CWFR were observed.

1. The TFR appeared to be built mainly from D- and E-zone tubules opening by way of approximately 42 deep transverse grooves on one side only into the longitudinal marginal channel (figures 1, 16 and 19). The other side of the channel received, by contrast, only 35 transverse grooves, a similar number to the CWFR. The TFR therefore appeared asymmetric in contrast to the dorsal and ventral halves of the CWFR which were closely similar (Knight & Feng 1992).

2. The openings of the A–F_1 zones of the TFRS started more anteriorly than the equivalent zones in the CWFR (figure 4) and appeared to be rotated through an angle of nearly 90° relative to the equivalent structures in the CWFR (figure 4). In consequence, many of the transverse grooves of the

Figure 6. Photograph of a whole egg capsule viewed from the ventral surface showing anterior tendrils forming two hook-like structures where they join the egg capsule (black arrows) and the posterior tendrils crossing at approximately 90° (white arrow). Scale in millimetres.

Figure 7. Photograph of portion of anterior tendril showing coiling. Scale bar 5 mm.

Figure 8. Polarizing photomicrograph of cleared tendril mounted whole and with long axis at 45° to the axis of the polarizer. Fine canaliculi (arrows) and rows of small voids can be seen running equidistant from each other and with a slight twist relative to the long axis of the tendril. Scale bar 50 μm.

Figure 9. SEM showing a forming tendril (T) emerging from the TFR. Fine ridges on the surface of the tendril indicate that it rotates as it is formed. Examples of transverse grooves of the TFR (arrows). Scale bar 400 μm.

Figure 10. TEM. LS of broad lamina showing moderately well-oriented, fine, banded fibrils with a closely similar D period to those of the egg capsule wall. Material positively stained with uranyl acetate, lead citrate and phosphotungstic acid. The banding pattern appears similar to that of negatively stained egg capsule wall fibrils. Longitudinal axis of the tendril (arrow). Scale bar 100 nm.

Figure 11. TEM. TS of the surface layers of the tendril showing tightly packed granules set in an amorphous matrix. Scale bar 2.5 μm.

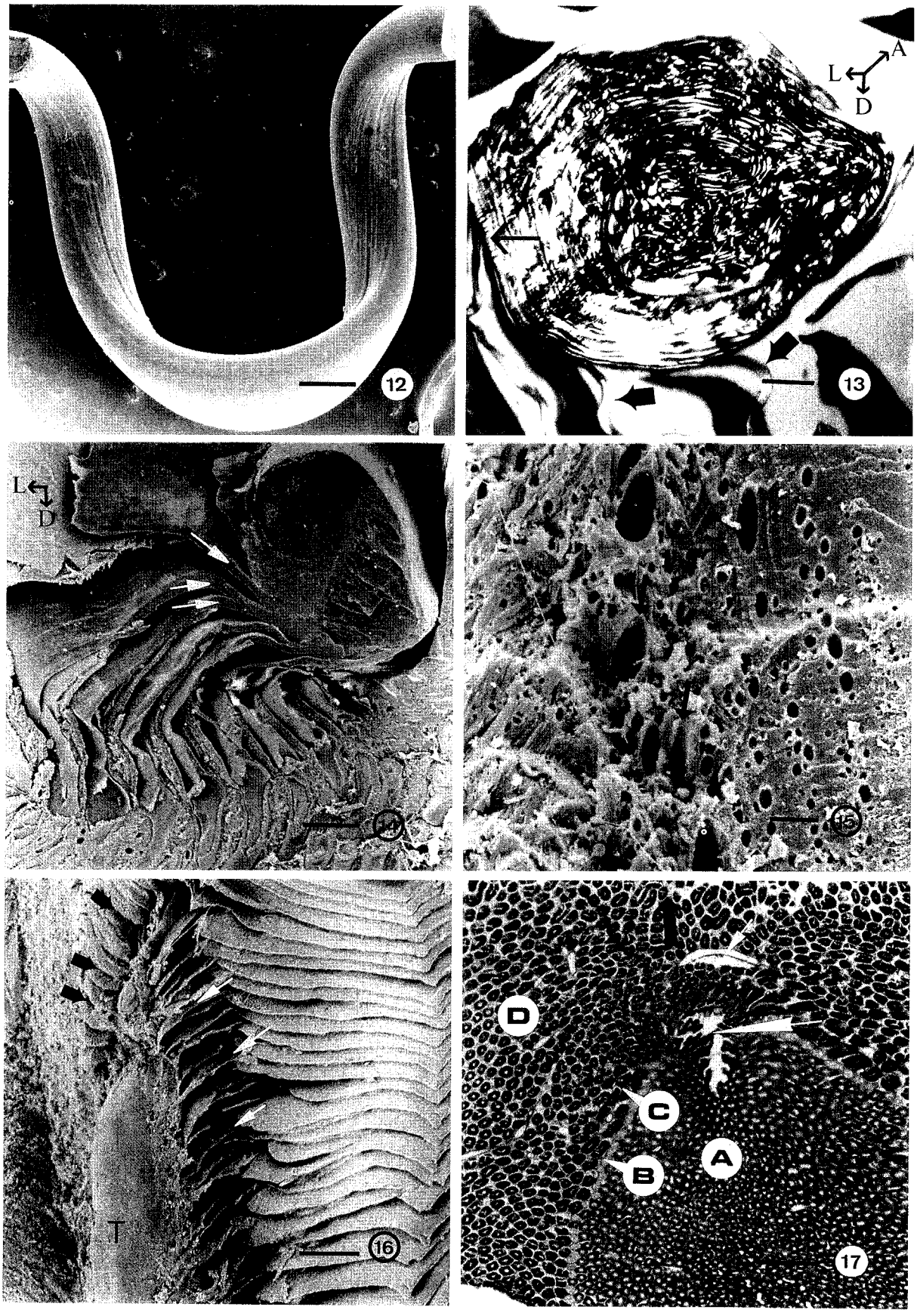


Figure 12–17. For description see opposite.

TFR received secretion from more than one zone of secretory tubules (figure 4). The larger number of transverse grooves in the TFR appeared also to be a consequence of this rotation.

3. The transverse grooves of the TFR were considerably deeper than the equivalent structures in the CWFR.

4. The first few rows of tubules in the TFR opened into a short duct which ran posteriorly before opening into the lateral margin of the main lumen of the nidamental gland (marginal canal, figure 4). The position at which this duct joined the main lumen varied from individual to individual. In some fish the duct opened just anterior to the hindmost row of the A tubule openings of the CWFR (Knight & Feng 1992) whereas in others the duct did not join the marginal channel until the middle of the D region (figure 16).

Observations on actively secreting glands showed that both posterior and anterior tendrils were secreted mainly by the side of the channel with 42 grooves (figures 16, 18, 19 and 21). This side appeared to be invariably located on the dorsal side of the right marginal channel and ventral side of the left one in each nidamental gland.

(*c*) *Formation of the tendril within the nidamental gland*

Our understanding of the processes involved in the formation of the tendril has been built up from observations on TFRs fixed in the act of forming tendrils and on the structure of the fully formed tendrils. The latter act as a record of the processes leading to tendril formation. Our observations on the relative numbers of fish observed in the act of secreting the tendrils and capsule wall suggest that it takes longer to secrete the tendrils than the capsule wall and that the anterior tendrils take more time than the shorter posterior tendrils.

The SEM demonstrated that the surface of newly formed tendrils often exhibited left-handed helical twisting as soon as they emerged from the TFR (figure 9). One instance of right-handed twisting was observed in this situation. Twisting was confirmed by examining similar material with the polarizing microscope. These observations strongly suggested that the forming tendril undergoes a counter-clockwise (left-handed) rotation as it is formed within the TFR. This was confirmed by observing transverse sections of TFRs fixed in the act of secreting a tendril. In these, the concentric spiral arrangement of the tendril appeared to arise as successive lamellae were applied from the transverse grooves of the TFR to the forming tendril (figures 9 and 19). Although the narrowest regions of the tendril were already folded into a planar crimp with a wavelength of about 0.5 mm within the TFR and marginal canal, secondary coiling in thicker tendril did not generally occur until the newly formed portion had moved down the marginal channel into the posterior oviduct (figure 20). These observations suggested that physical constraint of the wide tendril within the marginal canal prevented coiling until the less confined space of the posterior oviduct was reached.

SEM, transverse sections and natural casts indicated that when the nidamental gland became active, the 42 transverse grooves of the TFR first secreted the posterior tendril. The TFR then secreted the marginal rib of the capsule in co-operation with the 35 transverse grooves on the opposite side of the marginal channel. Finally the TFR secreted the anterior tendril. Only the first 12 or so of the 35 grooves on the opposite side of the marginal groove to the TFR appeared to contribute material to the forming tendrils (figure 21).

When the distribution of active tubular glands in the TFR is studied, all gland tubules and transverse grooves did not appear to be continuously active. Activation of the glands appeared to be an all-or-nothing process, the lumen of the tubular glands appearing full of secretion or entirely without secretion without intermediate states. Coherent fields of activity in the tubular glands appeared to expand and then contract during the formation of the tendrils.

Figure 12. SEM of a portion of tendril showing a parallel change of handedness of secondary coiling and primary twisting. Scale bar 500 μm.

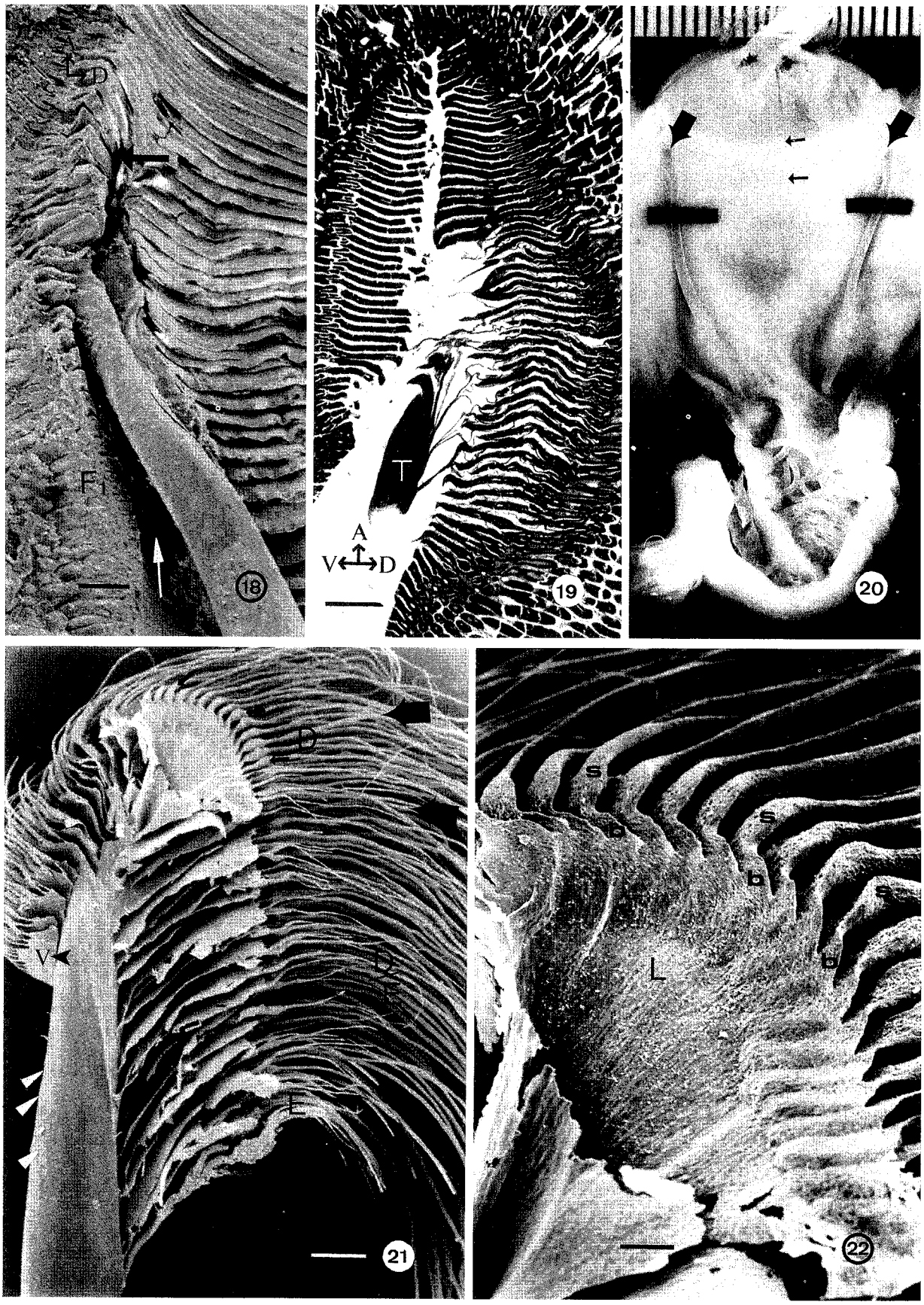
Figure 13. Transverse paraffin section of an active TFR viewed from posterior surface showing construction of forming tendril from concentric lamellae laid down in a spiral. Pleated lamellae (broad arrows) emerging from the transverse grooves appear to be wound onto the forming tendril as it rotates in the marginal groove. The circumferentially oriented fibrils in the narrow laminae appear finely crimped in places (narrow arrow). Axis of the analyser (A), dorsal (D), lateral (L). Scale bar 50 μm.

Figure 14. SEM showing a nearly transverse section of the posterior part of an active, right TFR with forming tendril viewed from the anterior surface. The method of assembly from lamellae emerging from the transverse grooves (arrowed) can be clearly seen. Dorsal (D), lateral (L). Scale bar 200 μm.

Figure 15. SEM of a slightly oblique transverse section of tendril showing canaliculi (arrowed) and small voids which lie between the lamellae. The core of the tendril was to the left of the micrograph. Scale bar 15 μm.

Figure 16. SEM of an active TFR on the right-hand side of a nidamental gland. The tendril (T) is formed within the marginal channel from the coalescence of lamellae (white arrows) which arise from the D/E transverse grooves of the TFR. Little material appears to be contributed from the transverse grooves on the ventral side of the marginal channel (black arrows). Scale bar 400 μm.

Figure 17. Paraffin transverse section of the anterior part of an inactive TFR cut at the level indicated on figure 4. The separate zones of gland tubules (A, B, C and D) open into a duct (long arrow) which is separated from the marginal channel at this level of section. Vein (short arrow); lateral direction (black arrow). Scale bar 500 μm.



Figures 18–22. For description see opposite.

Our observations are compatible with the suggestion that the increasing diameter of the posterior tendril results from a progressive wave of activation of the TFR starting in the posterior part of the D/E region and spreading anteriorly along the length of the TFR. Only the hindmost four transverse grooves of the TFR contained secretion during the formation of the narrowest region of the tendril. Activity appeared to spread posteriorly to an addition 30 transverse grooves (figure 4) for the secretion of a much thicker portion of tendril. The entire length of the TFR appeared active during the secretion of the widest (proximal) portion of the posterior tendril. A similar progressive wave of deactivation of tubular glands starting at the most anterior end of the TFR and moving in a posterior direction accounted for the decrease in thickness of the anterior tendril. The presence of granules on the surface layer, and banded collagen fibrils in the core, of the distal extremity of tendrils confirmed that both E and D tubules remained active while these parts were being secreted.

(*d*) *Formation of the lamellae of the tendril*

Each tendril lamella was seen to be formed within a D/E transverse groove in the TFR (figure 19). Polarizing microscopy indicated that the orientation of secreted material in the D zone became progressively defined as it flowed through the secretory duct; the material appeared irregularly oriented at the base of the secretory duct but by the apex it was strongly and coherently birefringent with a positive sign with respect to the long axis of the duct (figure 25). Polarizing microscopy also suggested that the baffle plates (Knight & Feng 1992) were able to define the orientation of the material passed to them from the transverse groove, the material emerging from the apex of the baffle plates as two laminae in which the final molecular orientations of the tendril laminae were already defined; the longitudinal fibrils of the tendril arising from the thicker anterior lamina and the circumferential fibrils from the thin posterior lamina. In this respect the function of the baffle plates of the TFR appeared to be similar but not identical to that of the equivalent structures in the CWFR: the baffle plates of the CWFR appear to define the five orientations of orthogonal arrangement of the egg capsule wall (Knight & Feng 1992) whereas the baffle plates of the TFR define the two orientations of the tendril laminae. A comparison of the way the secretory ducts opened into the space between the baffle plates in the TFR (figures 4 and 26) and the CWFR helped to account for this difference. In the TFR, the secretory ducts opened much closer to the anterior than the posterior surface of the transverse groove whereas in the CWFR the opening appeared exactly equidistant between anterior and posterior surfaces. Polarizing microscopy and TEM of actively secreting transverse grooves, together with observations on the orientation of fine, roughly parallel ridges on the surface of the secreted material in casts, suggested the following sequence of events in the extrusion die of the TFR (figure 26): as the molecules flow through the gland tubule and secretory duct they become oriented parallel to the long axis of the duct (approximately vertical) by shear orientation (flow birefringence) possibly assisted by nematic liquid crystallization (Knight and Feng 1992). From the apex of the secretory duct most of the secreted material is passed directly to the anterior surface of the transverse groove without change in orientation to give rise to the broad anterior lamina. A smaller flow of material originating from the secretory duct passes posteriorly across the baffle plate and is subject to shear orientation produced by ciliary action so that the molecules now lie approximately horizontally. This flow leaves the free edge of the baffle plate and passes vertically next to the posterior face of the transverse groove to become the narrow lamina of circumferentially oriented fibrils in the final tendril. A very thin layer of molecules in immediate contact with the posterior surface of the transverse groove may undergo a further shear

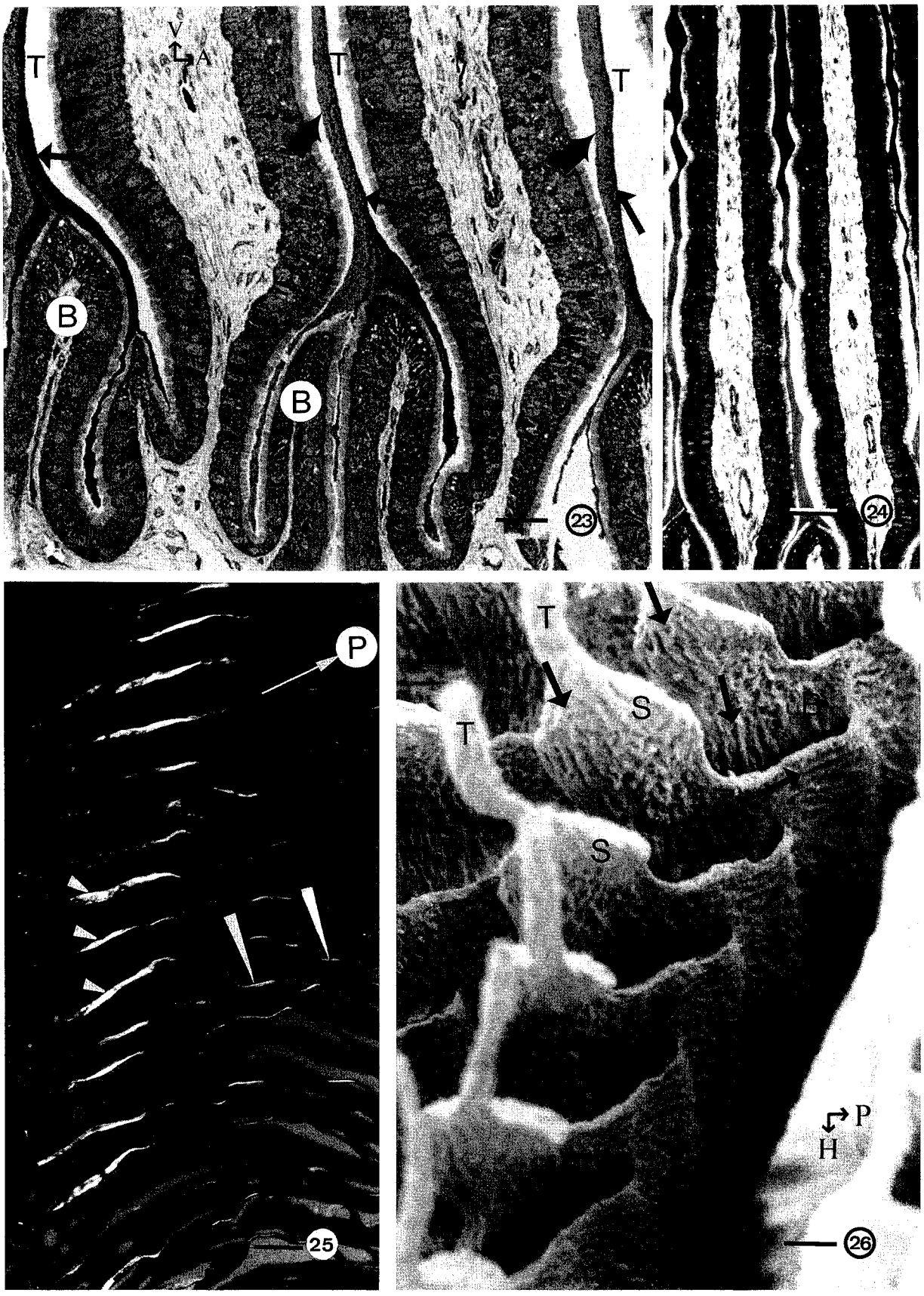
Figure 18. SEM of a tendril forming in an active right TFR. The separate duct described in figure 17 joins the main lumen at the point indicated (black arrow) and continues as the marginal channel (white arrow) which receives openings of the F_1-zone glands. Anterior (A), dorsal (D). Scale bar 300 μm.

Figure 19. Paraffin HLS of an active, right TFR forming the proximal part of an anterior tendril (T). Only the posterior 30 or so transverse grooves on the dorsal side of the marginal channel are active at this stage. No secretion is seen in the anterior TFR grooves or the grooves on the ventral side Anterior (A); dorsal (D); ventral (V). Scale bar 500 μm.

Figure 20. Whole nidamental gland and anterior part of the posterior oviduct opened with a ventral mid line incision. The forming tendrils emerge from the TFRs (broad arrows) and remain uncoiled until they reach the posterior oviduct. The tendril appears to hank randomly in the posterior oviduct. First and last D-zone transverse grooves (small arrows) of the capsule wall-forming region. Scale in millimetres.

Figure 21. SEM of a forming, right anterior tendril and natural cast of a right TFR viewed from the ventral surface. The cast reveals that most of the tendril is derived from the secretion of about 28 of the dorsal transverse grooves in the D and D/E region. The last four to five lamellae (E) receive secretion only from E-zone tubules (see also figure 4). About 12 transverse grooves on the ventral side (V and arrow head) of the marginal channel contribute some secretion. The cast reveals the three-dimensional arrangement of the lumen of glandular tubules (large black arrows) and secretory ducts (small black arrows). The slight transverse ridges on the tendril probably represent secretion from the F_1 zone. Scale bar 400 μm.

Figure 22. Close up detail of an area shown in figure 21 showing secretory ducts (s), space (b) between the baffle plates and walls of the transverse grooves, and lamella within the transverse grooves (L). Scale bar 100 μm.



Figures 23–26. For description see opposite.

reorientation as the material flows through the transverse groove. These vertically oriented molecules would join the similarly oriented fibrils of the broad lamina from the next transverse groove when adjacent lamellae adhere to form the tendril.

The ability of the baffle plates to define the separate laminae of the lamellae was clearly demonstrated by the following observation (figure 23). When examining the region where the D and E zones meet, separate flows appeared to have been fixed as they emerged from the apex of the baffle plate, Anterior flows containing a large number of granules probably originated from E-zone tubules while the posterior flows containing many fewer granules probably arose from those of the D zone. These separate flows appeared to show little mixing as the material flowed apically through the transverse groove to become the laminae of the forming tendril.

SEM and sectioned material indicated that the secreted lamellae within the transverse grooves appeared rather regularly pleated (wavelength approximately 150 μm), particular in the mid to posterior D-zone grooves (figure 24). The axis of the pleat ran parallel to the long axis of the nidamental gland and hence parallel to the molecular orientation in the anterior lamina as revealed by polarizing microscopy. Observations on transversely sectioned, forming tendrils in active TFRs suggested that the tendril canaliculi described above arise from the failure of the pleated lamellae from the transverse groove to adhere completely to each other as the forming tendril slowly rotates and moves posteriorly (figure 5). Thus the longitudinal orientation of the canaliculi within the formed tendril appeared to be determined by the axis of pleating which in turn appeared to be determined by the molecular orientation within the anterior lamina.

In the D zone of the TFR, the secreted material within the secretory ducts, between the baffle plates and in the basal parts of the transverse groove, probably consisting mainly of collagen, appeared to be in a lamellar or smectic A liquid crystalline phase closely similar to that described Knight *et al.* (1993) for the CWFR. As it flowed through the transverse grooves, the material appeared to undergo a rapid transition approximately 600 μm from the apex of the baffle plates to give rise to a different phase. The latter apparently consisted of individual molecules or small protofibrils with a defined longitudinal orientation but no obvious transverse banding pattern or defined lateral order. As in the CWFR, formation of the final, banded fibrils appeared to take place within about 100 μm from the apex of the transverse groove as the newly formed tendril moved posteriorly from the TFR.

4. DISCUSSION

(*a*) *Structure and function of the tendril*

The tendrils of the dogfish *Scyliorhinus canicula* have a unique structure. There is, however, some structural resemblance between the tendril and the capsule wall. Both appear to be constructed from similar components: transversely banded collagen fibrils and amorphous granules. Both are constructed from lamellae built up from laminae. However, there are marked differences in the way in which the lamellae are assembled in the two structures. The tendril has a spiral concentric construction apparently derived from rotation of the tendril within the TFR whereas the lamellae of the egg capsule form as a simple laminated extrusion (Knight & Feng 1992) which does not appear to rotate in the CWFR. The tendril exhibits a marked primary twisting and secondary coiling whereas the egg capsule of the dogfish, unlike that of the shark *Heterodontus* spp., described by Smith (1942),

Figure 23. HLS of a plastic section showing the base of three transverse grooves (T) in an active left TFR. Baffle plates (B). The lamina (narrow arrow) containing a high concentration of spherical granules secreted by E-gland tubules arises from the space between a baffle plate and the anterior surface of the wall of the groove. The lamina containing a much lower concentration of granules (broad arrow) and consisting of material secreted by D-zone tubules arises from the space between the baffle plates and the posterior surface of the transverse groove. Note the apparent lack of mixing of these flows as the material flows through the transverse grooves. Anterior (A); ventral (V). Scale bar 30 μm.

Figure 24. As figure 24 showing a fairly regular pleating of the secreted lamellae in the transverse grooves in the middle of the D region of the TFR. This pleating is thought to be involved in the formation of the fine canaliculi of the formed tendril (see figure 5). Scale bar 60 μm.

Figure 25. Polarizing micrograph of a paraffin HLS through an active, right TFR showing the D/E-zone grooves and associated structures. Note the progressive development of birefringence with a positive sign as the secreted material flows through the secretory ducts (short arrow heads). Note also the pleating of the lamellae within the transverse grooves (long arrow heads). Axis of the polarizer (P). Dorsal is to the left of the micrograph and anterior to the top. Scale bar 100 μm.

Figure 26. SEM of a natural cast from the D region of an active right TFR showing the three-dimensional arrangement of the gland tubule lumen (T), secretory ducts (S) and spaces left by the baffle plates (B). Note that each secretory duct in the TFR joins the space between two baffle plates close to the anterior surface of a transverse groove and not midway between the anterior and posterior surfaces as it does in the CWFR (see text). Note also fine more or less regularly oriented ridges on the surface of the secreted material which are thought to give an indication of the molecular orientations in the secreted material (see arrows). These orientations suggest that the baffle plates serve to produce two laminae, an anterior lamina which is produced by material flowing directly from the secretory duct without reorientation and a posterior lamina which becomes re-oriented as it flows across the anterior parts of the baffle plates. Posterior (P), horizontal (H). Scale bar 30 μm.

is only slightly twisted. In the tendril, the fibrils are arranged at approximately right angles to one another in adjacent laminae in contrast to the orthogonal arrangement with an angle of approximately 45° between fibrils in adjacent laminae in the capsule wall (Wourms & Sheldon 1971; Knight & Hunt 1976; Gathercole *et al.* 1993). There is also a marked resemblance between the structure of the tendril and that of another shock absorbing tissue, mammalian intervertebral disc as described by Cassidy *et al.* (1989). Both are lamellated structures exhibiting crimping or pleating. The similarity to the structure of elasmobranch fin rays is noted below.

The tendrils are used to attach the egg capsule closely and firmly to the alga *Halidrys siliquosa* in the sublitoral zone or to pipework or similar firm upright supports in aquaria (J. C. Thomason; personal communication). The egg-laying behaviour has been observed on several occasions in *Scyliorhinus canicula* in captivity (Dodd 1983). The fish selects a firm support to which to attach the eggs when the posterior tendrils have just started to protrude from the cloaca. The fish then swims rapidly and repeatedly round the support in a tight circle propelled only by the tail. Eventually the posterior tendrils extending from the cloaca snag on each other or on the support. The tendrils tighten and further swimming pulls the egg out of the oviduct. The anterior tendrils of the first egg of the pair become entwined with the posterior tendrils of the second. The fish continues to swim round the support until both egg capsules are firmly anchored.

The repeated change in handedness of the secondary coiling of the tendrils means that when the tendrils are laid across one another they snag firmly and repeatedly, making it very difficult to disentangle them. Repeated tangling in the dense mass of tendrils produced by communal egg laying makes separation of the capsule from the support practically impossible without cutting the tendrils. The efficiency of this remarkable anchoring mechanism can be demonstrated by replicating the movements made by a laying fish using an egg capsule with tendrils taken from the posterior oviduct. This results in rapid and firm fixation from a simple behaviour pattern. This suggests possible commercial uses for man-made fibrils with counter-coiling helical crimp.

The dogfish's reproductive strategy involves the production of a small number of very large, well protected eggs. The success of this strategy would appear to depend on the possession of a tough egg capsule which acts as a fluid-filled shock absorber (Knight & Feng 1992) and tendrils which anchor the capsule firmly, preventing removal by predators and wave action. The tendril's mechanical properties, which include marked tensile strength, toughness and elasticity, probably confer considerable survival value. The structure we have described helps to account for these properties in the following ways.

(*b*) *Mechanical properties*

(i) *Tensile strength*

The predominantly longitudinal orientation of the collagen fibrils together with their slight fanning about the longitudinal axis resembles the arrangement of collagen in tendon, a material with considerable tensile strength (Vincent 1982). The extreme insolubility of the collagen (D. P. Knight & D. Feng, unpublished results) and high thermal transition temperature suggest that the collagen in the tendril has extensive intermolecular covalent cross-links. These may be introduced by an oxidative phenolic mechanism (Feng & Knight 1992). The tensile strength is therefore at least partly attributable to the arrangement of the collagen fibrils and their intermolecular cross-linking.

(ii) *Elasticity*

Observations on the tyrosine-rich granules secreted from the E-zone cells suggest that they may represent an elastic component. They are completely amorphous in the TEM as are the rubber-like materials resilin and abductin. Calculations on amino acid analysis data presented by Knight & Hunt (1974) indicate that they contain a moderately high proportion of hydrophobic residues (31%), a high percentage (44%) of helix breakers (Vincent 1982) and a very high tyrosine content (20%) compatible with the suggestion that they are rubber-like proteins. Observations on strips of egg capsule extended by approximately 20% of their initial length suggest that these granules are reversibly deformable, tightly cross-linked to the collagen fibrils and have a higher modulus of elasticity than the surrounding fibrils (Knight & Feng 1993). For these reasons we suggest that the tyrosine-rich granules function as an elastic component. Their presence in large numbers in the longitudinally oriented collagen fibrils of the broad laminae and their concentration at the interface between the broad and narrow laminae place them in an ideal location for storing strain energy when the tendril is stretched or bent.

(iii) *Shock absorption*

The longitudinally oriented fine canaliculi appear well constructed and localized to act as fluid-filled shock absorbers. Their narrow radius would give them a very high resistance to the flow of fluid forced along them as the tendril shortened or bent, enabling them to dissipate strain energy as frictional heat. A similar suggestion has been made by Fox (1980) for the function of fluid-filled canals in tooth enamel, wood and other tough biological materials. The circumferential collagen fibrils which are adjacent to the canaliculi may prevent dilation and splitting of the canaliculi when tendrils are deformed. They may, however, be involved solely in the formation of the canaliculi (see below).

(*c*) *Origin of the twisting and coiling of the tendrils*

Three hypotheses help to account for the origin of the primary twisting and secondary coiling of the tendrils.

1. The newly extruded tendril can be regarded as a straight rod with an internal helical or helicoidal

structure. Reduction in the water content gives rise to stresses which result in coiling.

2. Secondary coiling results from stresses resulting from the inhomogeneous nature of the fibril.

3. The handedness of the coiling is partly determined by chance as the still plastic, recently secreted, tendril is forced out of the nidamental gland and irregularly hanks in the confined space of the oviduct.

The first hypothesis is suggested on the basis of the similarity between the structure and behaviour of the tendril and the elasmobranch fin ray collagen. The latter also has a concentric arrangement of laminae apparently constructed from longitudinally oriented collagen fibrils (Fitton Jackson 1968). Fin ray collagen undergoes a remarkably regular, right-handed coiling as it dries (McGavin 1976) which is accompanied by a twisting or tilting of the laminae of collagen molecules (Hukins *et al.* 1976) probably representing a smectic A to smectic C liquid crystal transition (Hukins & Woodhead-Galloway 1977, 1978). This suggests that the collagen fibrils of the tendrils may undergo primary twisting and secondary coiling as a result of a smectic A to smectic C transition as the water content is reduced. The observations that the fibrils were tilted within the broad laminae of the tendril and that there was a statistical preference for right handedness in the coiling support this hypothesis. Thus a smectic A to smectic C transition in the tendril may represent a final lyotropic liquid crystal transition in the series we have previously described (Knight *et al.* 1993). We speculate that the crimping of mammalian collagen fibrils (Gathercole & Keller 1991) may also result from a smectic A to smectic C transition. This suggestion is supported by micrographs of crimped fibrils published by Dlugosz *et al.* (1978) which show an oblique rather than exactly transverse banding pattern indicating a smectic C configuration.

The second hypothesis is suggested on the grounds that asymmetrical co-extrusion of two polymers with different shrinkages has been suggested as a commercial means of producing helically crimped fibres (for example, the following patents: Japan Exlan Co. Ltd. JP6682290 (661214); Veb Chemiefaserwerk Schwarza Fr 6689350 (661229); Du Pont NL 6818468 (681220); see References). This could be achieved by rotation of the forming tendril within the bilaterally asymmetric TFR. This would result in the deposition of tyrosine-rich granules in a helicoidal pattern on the surface of the rotating tendril. Differential shrinkage between the collagen fibrils and this outer coating would be likely to result in secondary coiling. Differential shrinkage may also account for the observation that strips of unfixed egg capsule curled inwards on drying. The rotation of the tendril within the TFR is suggested by our observations on the structure of the tendril and method of formation. It may result from the liquid crystal transition suggested above or from the action of the ciliated epithelium which covers all parts of the TFR and marginal groove. An alternative suggestion is that the rotation within the TFR results from transmission down the length of newly formed tendril, of torsional forces resulting from the secondary coiling within the posterior oviduct. This seems unlikely as the newly formed tendril appeared to have very little tensile strength.

The observation that the tendril in the posterior oviduct is irregularly hanked suggests that collisions between the tendril and already hanked portions or the walls of the posterior oviduct could result in changes in handedness of the coiling. Similarly, the three-dimensional arrangement of the junction between the nidamental gland and the posterior oviduct may confine the handedness of the proximal coil, generally right handed in the anterior and in left handed in the posterior tendrils (see above).

(*d*) *Sequence of events in the secretion of tendrils and egg capsule*

The following account extends the suggestions we have already made for the cycle of events involved in the formation of tendrils and egg capsule (Knight & Feng 1992) (figure 27).

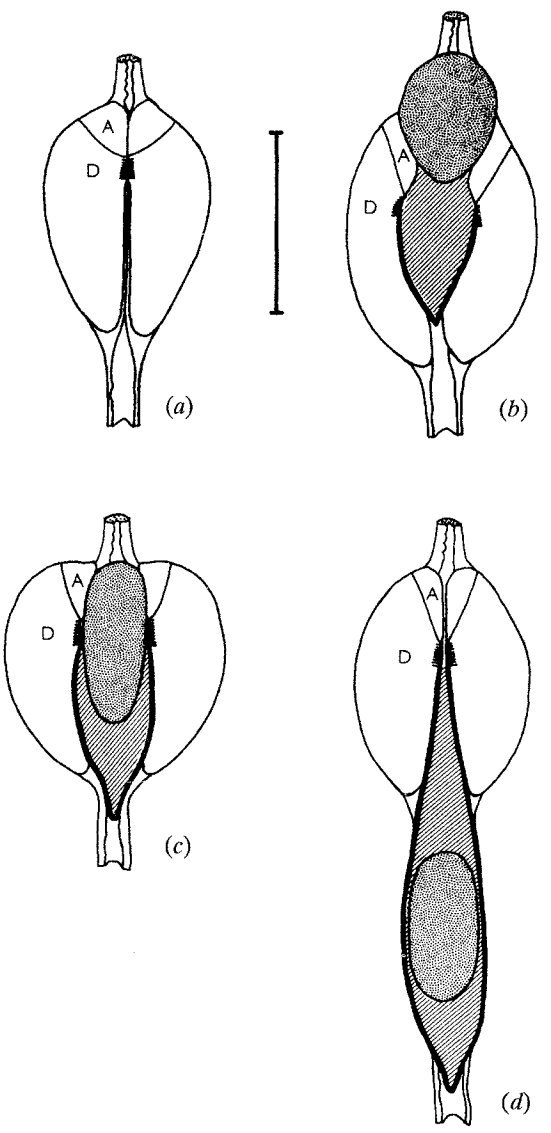


Figure 27. Semi-diagrammatic drawing of dorso-ventrally sectioned actively secreting nidamental glands showing the stages involved in the formation of the egg capsule (see text). Scale bar 20 mm.

First, the gland tubules which contribute their secretion to the posterior grooves of the TFR are activated to secrete the distal extremity of the posterior tendril. Activation then spreads progressively in an anterior direction along the TFR to secrete the widening posterior tendril. The lengthening tendril is continuously moved down the marginal channel by cilia. After the whole length of the TFR has been activated to secrete the proximal end of the posterior tendril, activation spreads across to the D and E zone of the CWFR starting from the lateral margins inward. This produces the curving posterior horns and posterior margin of the capsule wall. The latter forms as a plug which occludes the lumen of the nidamental gland. Activation then spreads to the whole of the CWFR. The anterior oviduct is occluded at this stage by elastic collapse or muscular contraction of its walls. The secretion of jelly by the A-zone tubules into the lumen between these two occlusions commences and acts as the primary driving force for extrusion of the forming capsule wall. The margins of the capsule are secreted by all the gland tubules of the TFR together with those which feed into the 35 transverse grooves on the opposite side of the marginal channel. The ovum is transported along the anterior oviduct and arrives at the junction between this structure and the nidamental gland shortly after the posterior margin of the egg capsule has been formed (figure 27*b*). The ovum is, for a while, prevented from entering the nidamental gland by the narrowness of the opening at this point. When the anterior opening of the gland has been sufficiently dilated by the secretion of jelly from the A zone, it will allow the ovum to enter the gland (figure 27*c*) and this occurs when approximately one third of the capsule wall has been completed. Secretion of the jelly from the A zone must be stopped for a while to allow the ovum to move in a posterior direction relative to the D-zone grooves (figure 27*c*) to allow it to enter the capsule. This is probably achieved simply by the pressure of the ovum on the openings of the A-zone tubules as the ovum moves posteriorly through the lumen. Secretion of jelly from the A zone will continue as soon as the ovum moves further down the lumen. After the ovum has entered the forming capsule, the number of active A-zone tubules in the CWFR is progressively reduced from the anterior end resulting in a progressive dorsoventral narrowing of the capsule. After the A zone has been deactivated, inactivation of the B and C zones of the CWFR allows the two capsule walls to stick together (figure 27*d*) to initiate the formation of the anterior marginal seal (eclosion slit). Progressive deactivation of the D folds from anterior to posterior accounts for the progressive thinning of the anterior margin. When secretion of the anterior margin is complete the tubular glands of the CWFR are deactivated but the TFR continues to secrete the proximal part of the anterior tendril. A progressive deactivation of the TFR starting from the anterior end then reduces the diameter of the secreted tendril.

It is interesting to note that two branches of the sympathetic nerve (Young 1933) enter each nidamental gland very close to each TFR suggesting that sympathetic activity may initiate the activation of the TFRs, the first event of the secretory cycle.

(*e*) *Formation of the tendril within the TFR*

Both TFR and CWFR represent local modification of the same remarkably advanced extrusion die design. These local modifications together with some difference in the biochemical composition of the extruded materials result in the extrusion of structures with markedly different mechanical properties: the coiled, damped spring of the tendril and tough plywood construction of the capsule wall. The dogfish egg capsule and associated tendrils can be considered as a smart structure whose biophysical properties vary adaptively with thickness and from region to region. Three aspects of the method of formation of the tendril in the TFR are worthy of further comment: (i) determination of the orientation of collagen fibrils within the laminae; (ii) formation of the canaliculi; and (iii) co-ordination and control of secretion of the tubular glands.

(i) *Determination of the orientation of collagen fibrils within the laminae*

The TFR appears to represent an advanced extrusion die for the formation of laminae in which the orientation of collagen fibrils is well defined. The secretory ducts, baffle plates and transverse grooves appear to define orientations in a similar, but not identical way to that described by Knight & Feng (1992) for the CWFR. As in the CWFR, it is likely that shearing orientation and liquid crystallization are involved.

(ii) *Formation of the canaliculi*

Another aspect of the advanced nature of the TFR is its ability to form precisely oriented fine canaliculi. This appears to result from the incomplete adhesion of longitudinally pleated lamellae. The orientation of this pleating appears in turn to be defined by the orientation of the collagen molecules within the broader, anterior lamina. It is possible that pleating results from anisotropic shrinkage as studied in a theoretical model by Roddeman *et al.* (1987). A similar suggestion has been made to account for the origin of crimping in other collagens (Dale & Baer 1974). The shrinkage in the lamellae of the forming tendril may result from the progressive removal of water as the secreted material flows between the ion transporting epithelia of the transverse grooves (Knight & Feng 1992). Alternatively, as in the case of twisting and coiling, the pleating may result from a liquid crystal transition consequent on the removal of water. Pleating of tendril lamellae would appear to represent an extended version of the planar crimp found in a variety of collagens as reviewed by Gathercole & Keller (1991). Thus the TFR appears to be specialized for the extrusion of helical shock absorbers whose ability to dissipate energy may result in part from the presence of numerous fine fluid-filled canaliculi. The structure of the TFR may therefore have implications for the design of extrusion dies for

the production of pleated sheet materials and coiled shock absorbing fibrils, the subject of a provisional patent application based on this work.

(iii) *Co-ordination and control of secretion of the tubular glands*

Finally, the observation that the secretion of glandular tubules of the TFR and CWFR appeared to be switched on and off in a highly controlled and co-ordinated fashion suggests that this material may represent a good model for the study of the control of collagen secretion.

Our analysis of the method of formation of the tendril goes some way to explaining how the remarkably complex structure of the tendrilis assembled at different hierarchical levels.

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