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ULTRASTRUCTURAL OBSERVATIONS OF THE ARGONAUT SHELL

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Abstract

An examination of the ultrastructure of the shell of the cephalopod *Argonauta Nodosa* was carried out using scanning electron microscopy, transmission electron microscopy and polarised light microscopy. The structure of the Argonaut shell was found to consist of an inner and outer prismatic layer separated by a thin central zone which was sparsely occupied by spherulitic crystals. Fluctuations in the width and porosity of the central zone resulted in changes in the shell's opacity and gave rise to the fibrous lines visible in the structure. The central zone was the region of initial growth and was the nucleating point for the crystals which formed the prismatic layers. It was concluded that deposition of material in the Argonaut shell occurred on both the inner and outer surfaces of the shell, in contrast to the single growth surface of other molluscs. The deposition process can be explained by the periodic movement of the Argonaut's tentacles, which are responsible for the material secretion, from one surface to the other. In general it was found that the Argonaut exercises less control over the structure of its shell than is common amongst the molluscs and in particular the organic matrix of the shell does not appear to play as large a role in determining the crystal structures.

Key Words: Argonaut, paper nautilus, shell, ultrastructure, transmission electron microscopy, scanning electron microscopy, cephalopod, biomineralization, calcium carbonate, biological crystals.

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Introduction

The Nautilus and the Argonaut are the only two cephalopods to possess external shells, however, despite a superficial similarity in shape, the shells have very little in common. The shell of the Argonaut is thin and fragile with no internal partitions or chambers and, in evolutionary terms, is a relatively recent addition [Young, 1959, Wells, 1962, Morton, 1967]. It is known that only the female of the genus forms a shell using membranous extensions of its modified pair of dorsal tentacles [Adams and Adams, 1858, Allan, 1933, Miner, 1935, Allan, 1950, Wells, 1962, Morton and Yonge, 1964, Douglas, 1974, Plant, 1974, Illife, 1982, Reid, 1989]. The shell's major purpose is to carry the Argonaut's eggs until they are ready to hatch [Allan, 1933, Plant, 1974] and, for this reason, and also because it is not formed by the animal's mantle, the structure is often not considered to be a true shell [Plant, 1974]. However it has been shown [Young, 1959] that the shell plays an important role in feeding and that the animal soon dies if it loses its shell.

The pelagic Argonaut has usually been captured only when it has come inshore to release its eggs and it has been reported [McInnes, 1959, Young, 1959, Bishop, 1979] that those animals placed in aquaria usually die after being pushed from the shell by the growing mass of eggs [Allan, 1933, Allan, 1950]. Thus observation of the Argonaut is difficult in the wild and in captivity is restricted to short-lived, atypical individuals. Consequently, little information is available on the animal and almost none is available on its development. For example, loss of the shell and death may not be a necessary consequence of egg laying - Reid (1989) reported observations of female Argonauts healthy enough to outswim the observing diver after releasing their eggs in the wild; it is not clear from this description whether these animals had also lost their shells.

It has been reported [Adams and Adams, 1858, Woodward, 1880, Allan, 1950, McInnes, 1959 Wells, 1962 Morton and Yonge, 1964, Stephens, 1965, Morton, 1967, Reid, 1989] that the material used to form the Argonaut shell is secreted from glands set along the edges [Young, 1959] of the membranous webs [Miner, 1935, Allan, 1950, Wells, 1962, Morton and Yonge, 1964, Morton, 1967, Plant, 1974, Illife, 1982]. Thus the shell is not moulded to the body of the animal [Woodward, 1880] and there is no muscular

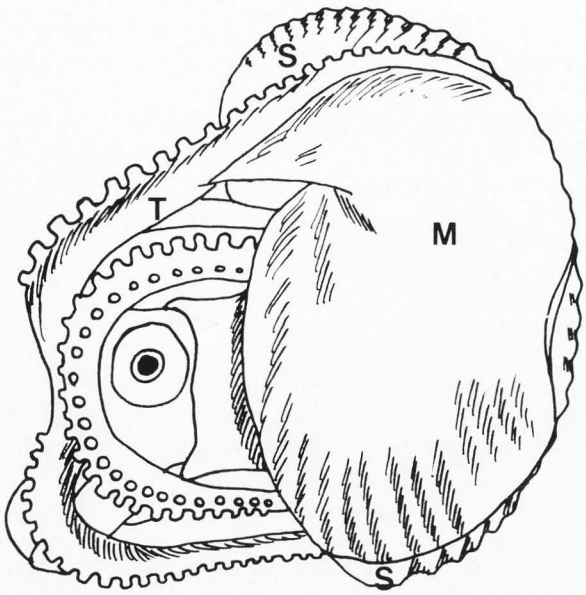


Fig. 1. A diagrammatic representation of the argonaut within its shell (S). The main part of the shell surface is covered by the membranes (M) which extend from the ends of the dorsal arm pair (T).

connection to the shell [Adams and Adams, 1858, Woodward, 1880, Allan, 1950, Reid, 1989] (Fig. 1). Although no acceptable mechanism of shell formation has been suggested in the literature, the most obvious method seems to be precipitation of the material from a supersaturated fluid, in a fashion similar to other molluscs. One explicit description of shell formation implies construction of the shell late in life of the Argonaut and without further enlargement [Miner, 1935] but this contrasts with the general belief that the shell is started within days of the animal hatching and growth continues until maturity [Adams and Adams, 1858, Allan, 1933, Allan, 1950]. Growth lines in the shell material have also been described, probably in reference to the fibrous lines which are visible within the structure [Douglas, 1974]. Thus it is convenient to refer to the tightly coiled part of the shell as the early part, and the region forming the aperture as the later part. This convention will be adopted here but, since the mechanism by which extension occurs is not clear, the terminology cannot be assumed to be absolutely correct.

A very brief description of the microstructure of the Argonaut shell has previously been given in an extensive treatise [Bøggild, 1930]. This investigation, using a polarised light microscope, showed that the shell consisted of two very fine prismatic layers, a thicker upper and a thinner under layer and, in between them, a very thin layer of a very fine and irregular grains of calcite. The crystals of the prismatic material were described as being almost perfectly aligned with the axes of the prisms themselves. Ultrastructural studies of Argonaut shell were not possible at the time the treatise was published and have not been performed since. The preparation of ultra-thin samples of fragile and brittle materials, suitable for observation with the transmission electron microscope (TEM), have been made possible by the technique of selected-area argon ion beam thinning [Phakey *et al.*, 1974, Palamara *et al.*, 1980]. This technique along with standard methods of scanning electron microscope (SEM) sample preparation, has been used in this study to examine the ultrastructure of the Argonaut shell.

Materials and Methods

Four shells belonging to the species *Argonauta Nodosa*, found on the beach near Inverloch, Victoria in the spring of 1988, were donated by Jack's Shell Museum in Inverloch for the present study. These shells were not fully intact but were sufficiently whole to be identified and were considered to be typical of the species. Areas to be studied were selected mostly from the shell wall not far from the aperture but some were also taken from the keel of the shell and the thinner region near the apex of the shell. Initial samples from these selected areas were obtained either by cutting with a diamond-edged rotary saw or by simply snapping off a piece, depending on how straight an edge was required.

All natural surfaces of the specimens and those which were polished and etched were initially examined by a light microscope (LM) using reflected light. An Olympus BHA-P transmission LM was used to observe sections of the shell between crossed polarisers. These sections were taken from a strip of material which was cut along the keel of the shell and which bisected the structure. This strip, approximately 1 cm in width, was then placed in a plastic petri dish which was filled with epoxy resin. When the resin had set, the dish and epoxy were polished away to expose the slice of shell. Cross-sectional slices, about 0.6 mm thick, were cut using a diamond saw, both along the surface of the epoxy disc and normal to it, giving cross-sections both perpendicular and parallel to the leading edge of the shell. Specimens were hand lapped and polished to a thickness of about 100 microns using graded abrasive papers. All transmitted light micrographs were taken with the samples between crossed polarisers.

Both polished and fractured samples of the shell cross-section, as well the natural inner and outer surfaces of the shell were used in the SEM observations. The cross-sectional specimens were prepared by embedding the samples in epoxy resin and then polishing with abrasive paper up to a grade of 4000. They were then etched with either 0.1M acetic acid for 90 seconds or 8% w/v sodium hypochlorite for several hours, depending on the sample and the type and depth of etching required. The former etchant removed the mineral component to reveal the organic matrix, while the latter removed any organic material leaving the crystals. Samples of the natural surface or of fractured material were not embedded, polished or etched but were simply removed from the shell and mounted on aluminium stubs using high conductivity silver paint. Prior to SEM examination, all samples were coated with a thin film of platinum to prevent charging of the non-conductive calcium carbonate by the electron beam. The samples were examined using a Hitachi S/570 scanning electron microscope operating in secondary electron mode with energies of 5-10 kV.

Some selected areas of the samples were examined using a JEOL 200CX transmission electron microscope operating at an accelerating voltage of 200 kV. For this, ultrathin sections were prepared by the selected-area argon-ion-beam thinning technique [Phakey *et al.*, 1974, Palamara *et al.*, 1980]. This method involved selecting an area of the polished sample (about 100 μm thick) by cementing a slotted grid onto the section. The mounted specimen was placed in the argon-ion-beam thinner where it was exposed to a beam of argon ions, incident at an angle of about 15° to the surface, until a small perforation appeared in the area of interest. One sided thinning by a single beam was used if the immediate surface was of importance, otherwise two beams acting on opposite sides of the specimen increased the thinning speed. The areas surrounding the perforation were thin enough to be electron translucent. Prior to the TEM examination, the samples were coated with a thin film of carbon to avoid sample charging.

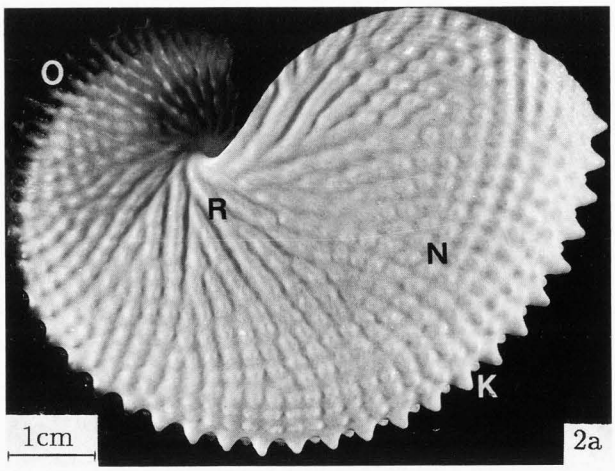
The TEM was also used to perform selected area electron diffraction (SAD) on samples. An aperture of 0.5 μm was introduced into the electron beam and the TEM adjusted to view the back focal plane of the objective lens. X-ray diffraction was carried out, using a Scintag PAD V X-ray diffractometer, on powdered samples taken from an representative area of the side wall of the shell near the aperture.

Results

Macroscopic and light microscope observations

The Argonaut shells used in this investigation were translucent, white in colour and bilaterally symmetrical plani-spirals (Fig. 2a). Numerous ribs radiated from the centre of the spiral to the keel, the furthest extension of the spiral away from its centre, and broke up into nodules along the way (Fig. 2a). The keel was defined by a double row of wide points which, like the ribs and nodules, rose in relief from the material of the shell. Near the aperture, the wide points, ribs and nodules were all well defined but on the early part of the shell they were little more than small bumps (Fig. 2a). The shell was also much thinner (about 0.1 mm) and more fragile in the early part of the shell compared to the majority of the later part of the shell which was about 0.3 mm thick. The edges of the aperture near the umbilicus were thickened into a lip of almost 1.5 mm. The inner surface, the early part of the outer surface and the edges of the aperture were glossy while the majority of the outer surface, which is mostly the area normally covered by the webs in life, possessed a dull matt finish. On the early part of the shell near the aperture, the shell was covered by a thin film of black or brown organic material (Fig. 2a).

In general the shell had the look and feel of fibre-reinforced plastic, an appearance partly due to opaque lines that radiated from the centre of the spiral to the keel through the translucent material and which appeared fibrous (Fig. 2b). Although visible to the naked eye, these 'fibres' did not show up in reflected LM pictures or in SEM pictures of the surface, indicating that they were a feature existing within the substance of the shell and not on the surface. The separation



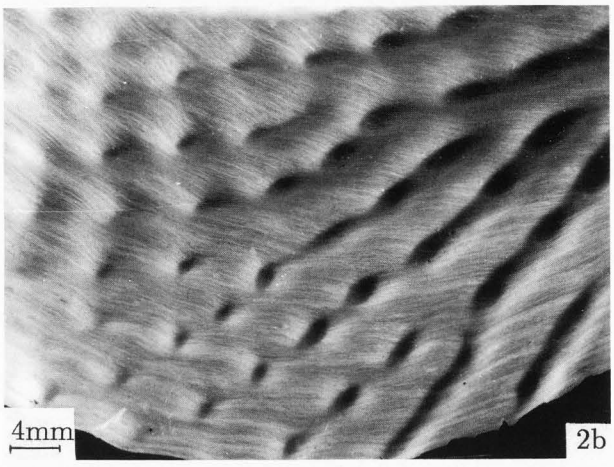
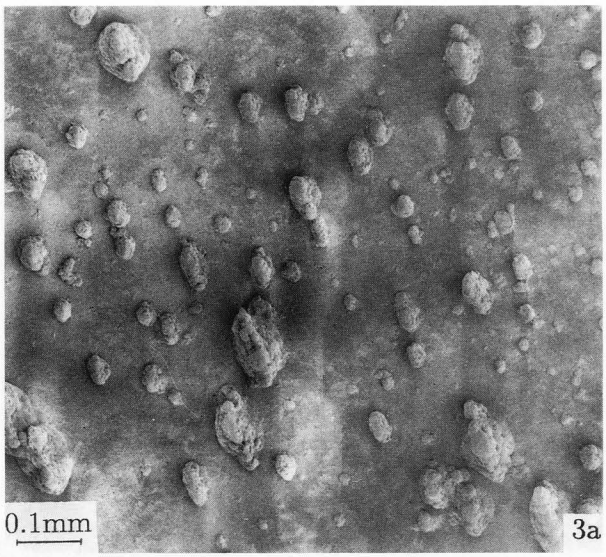


Fig. 2. (a) A photograph of the Argonaut shell showing its overall shape. The ribs (R) can be seen breaking up into nodules (N) as they approach the points of the keel (K). The dark area on the tighter part of the spiral is due to an organic film (O) over the surface. (b) A close up of the inner surface of the shell showing the fibrous appearance of the material. They are visible in both surfaces but are clearer from the inside.

between fibres was of the order of 0.2 to 0.3 mm and very irregular with a great deal of cross-linking and splitting of the fibres. Individual fibres did not appear to be continuous through the material of the shell.

The outer surface of the shell was found to be covered by innumerable small oval shaped tubercles, ranging in size from about 0.2 to 20 μm, which were elongated in a direction following the ribs on the surface (Fig. 3a). The tubercles were most numerous in the regions between the ribs and nodules but relatively scarce on top of the raised areas and not present at all on the inner surface.

The shell in cross-section consisted of two layers of prismatic material separated by a thin central zone of disordered crystal grains (Fig. 4). When viewed between crossed polarisers, the birefringent calcite of the prismatic



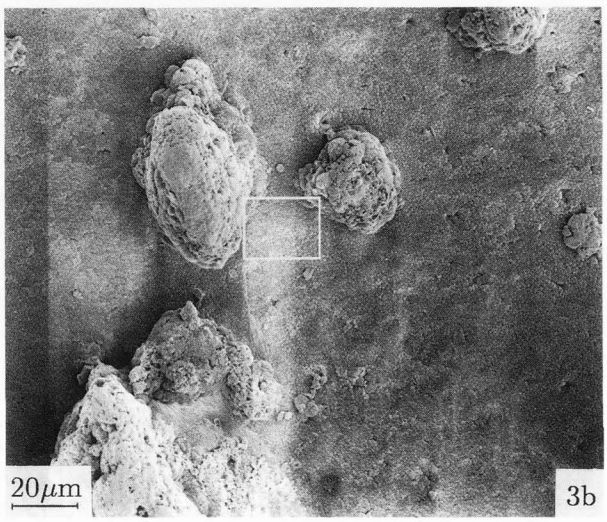
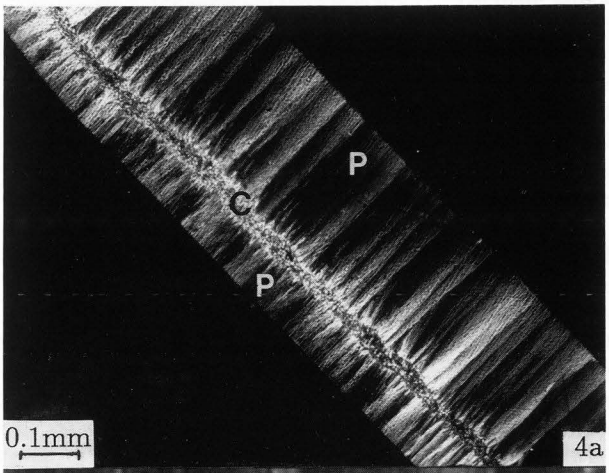
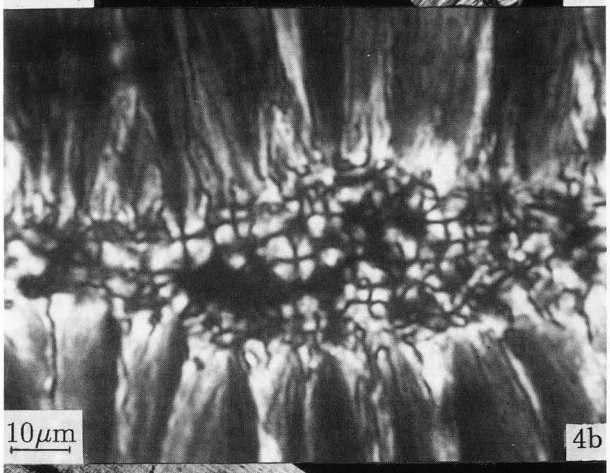


Fig. 3. Low magnification scanning electron microscope (SEM) views of the outer surface of the shell showing the many tubercles which cover it. (samples etched 7hr with NaOCl.) (a) A view showing that the tubercles are roughly aligned with each other, approximately parallel to the ribs. (b) A polygonal pattern, due to the ends of the prismatic crystals, covers the main part of the shell surface and is also visible on areas of the tubercles. (The rectangle in the centre of this picture outlines the view shown in Fig. 11a).





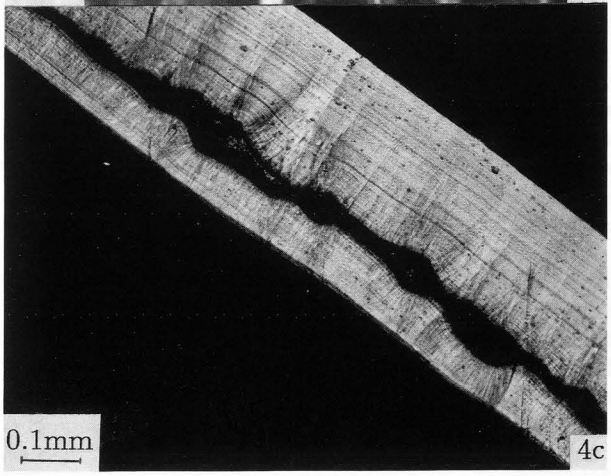
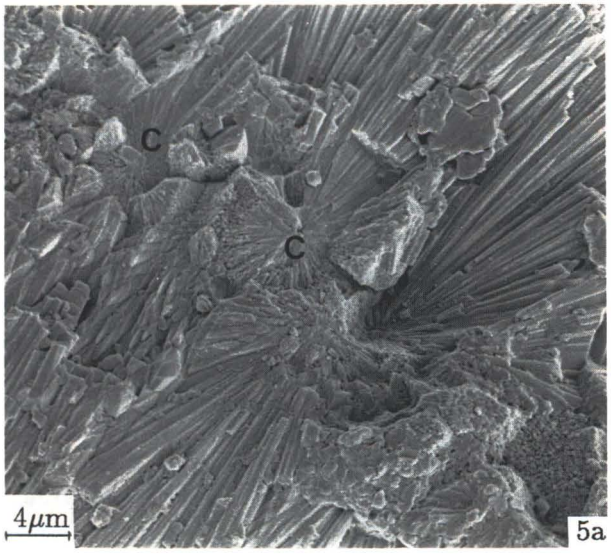
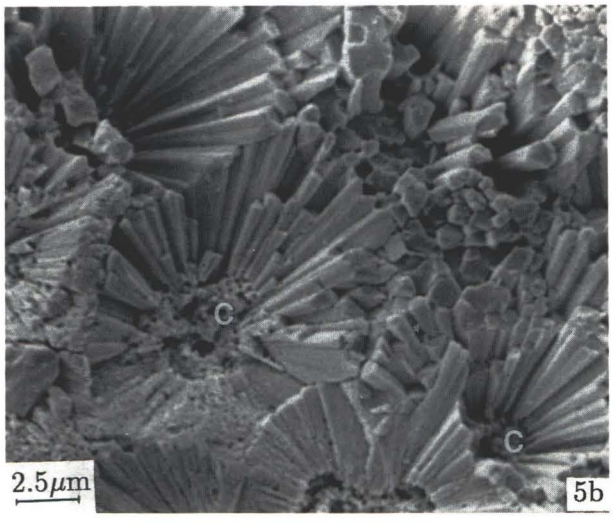
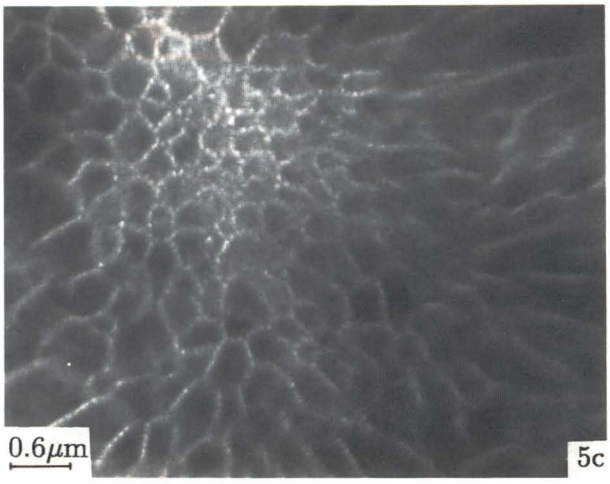


Fig. 4. (a) Light microscope (LM) picture of Argonaut shell cross-sections viewed between crossed polarisers showing the prismatic material (P) extending away from the central zone (C). A slight misalignment between the optic axes of the prisms leads to the non-uniform extinction shown here. The alignment of the crystals is not as close in the immediate vicinity of the central zone. (b) A close-up LM view of the central zone showing Maltese cross configuration on the grains in the central zone and thus the spherulitic growth pattern. (c) LM picture with crossed polarisers showing the variations in the width of the central zone in this section. The lines which run perpendicular to the prisms appear dark compared to the rest of the material due to a change in their birefringent properties. These also appeared in many of the etched SEM sections as lines of preferential etching (see Fig. 9).







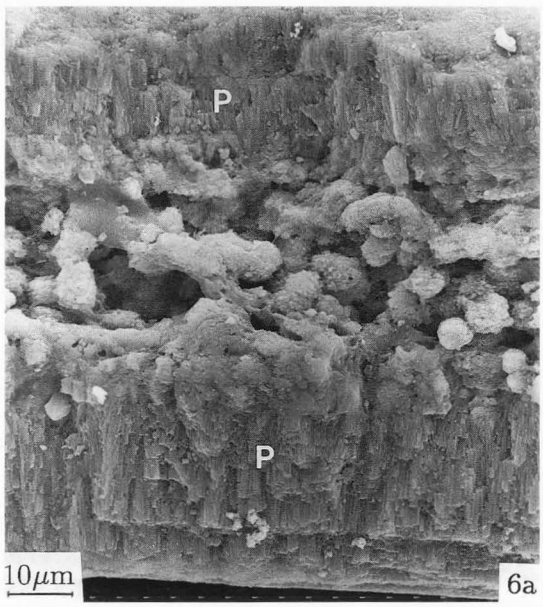
material showed only small variations in the extinction angle, indicating only a slight misalignment of the optic axes of the crystals in the prisms (Fig. 4a). Clearly defined Maltese crosses with their arms aligned with the axes of the polarisers were seen in the grains of the central zone (Fig. 4b). This meant that these grains consisted of radiating groups of crystallites, as in spherulites; and this was confirmed by SEM and TEM observations (Fig. 5). The SEM observations also showed that the centres of radiating crystals were between 5μm and 20 μm apart (Fig. 5b). The number and size of the spherulites within the central zone was variable, ranging from barely visible individuals to groups almost 5 μm across, although, as shown by the zone's porosity in most places, they did not necessarily, or even commonly, fill all the space available.

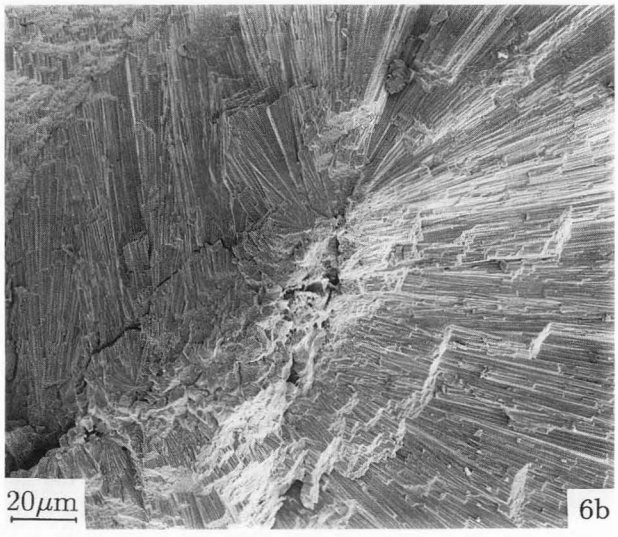
Just outside, but close to, the central zone, the uneven extinctions seen in the polarised light micrographs (Fig. 4a), indicated that the prisms were not as well aligned as they were further from the zone. It is probable that there were still some misaligned crystals included in these parts of the prismatic material which had not yet been constrained by the adjacent prisms and so had growth directions at some small angle to the perpendicular (Fig. 5a).

The aperture edges in the samples investigated were chipped or broken along most of their length, but it could be seen that the structure was very similar to the rest of the shell except that the two prismatic layers were far thinner at the immediate edge (Fig. 6a). The tapering towards the edge implied by this thinning was not obvious to the naked eye suggesting a steep reduction in width very close to the edge.

At the aperture edge near the umbilicus the central zone, which was exposed along most of the aperture, was found to be covered by the prismatic material. The central zone terminated in this region and the prismatic layers continued in a radial fashion about this terminus until the central layer was completely surrounded (Fig. 6b). The thickening that this caused at the aperture resulted in a built-up lip which followed the limits of growth to the cup of shell material in the centre of the structure which represents the oldest part of the shell (Fig. 6c). This cup was found to have the same ultrastructure as the rest of the shell but was much thinner while the lip at this point was about the same width as the bulk of the shell (about 0.3 mm). The chipped off points on

Fig. 5. Views of the spherulitic origin of the prisms from nucleation points on the central zone. (a) SEM picture of a fracture surface perpendicular to the central zone. The central zone in this section appears as a row of radiating growth centres from which the prismatic crystals extend. (b) SEM picture of a section of the prismatic layer parallel to, and adjacent to the central zone. The centres (C) of radiating growth are separated by about 5 μm to 30 μm. (sample etched 7hr with NaOCl.) (c) Transmission electron microscope (TEM) picture of a section of the prismatic layer parallel to, and adjacent to the central zone showing the cross-section of crystals radiating from the centre of a spherulite. Crystals further from the centre make more oblique angles with the plane of the section and so seem larger with elongated cross-sections.





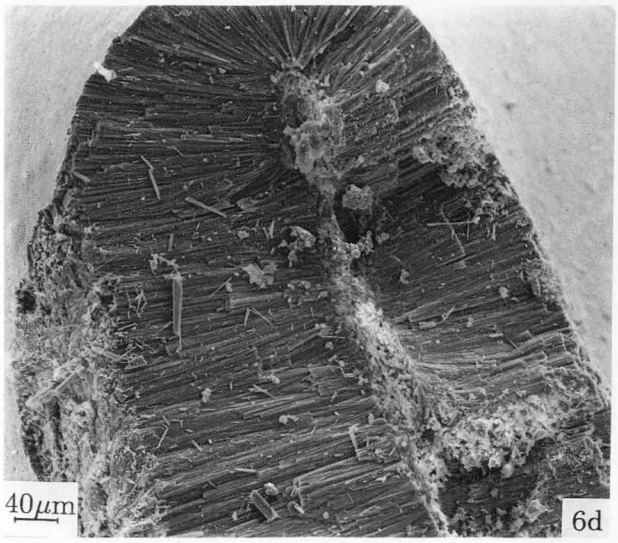
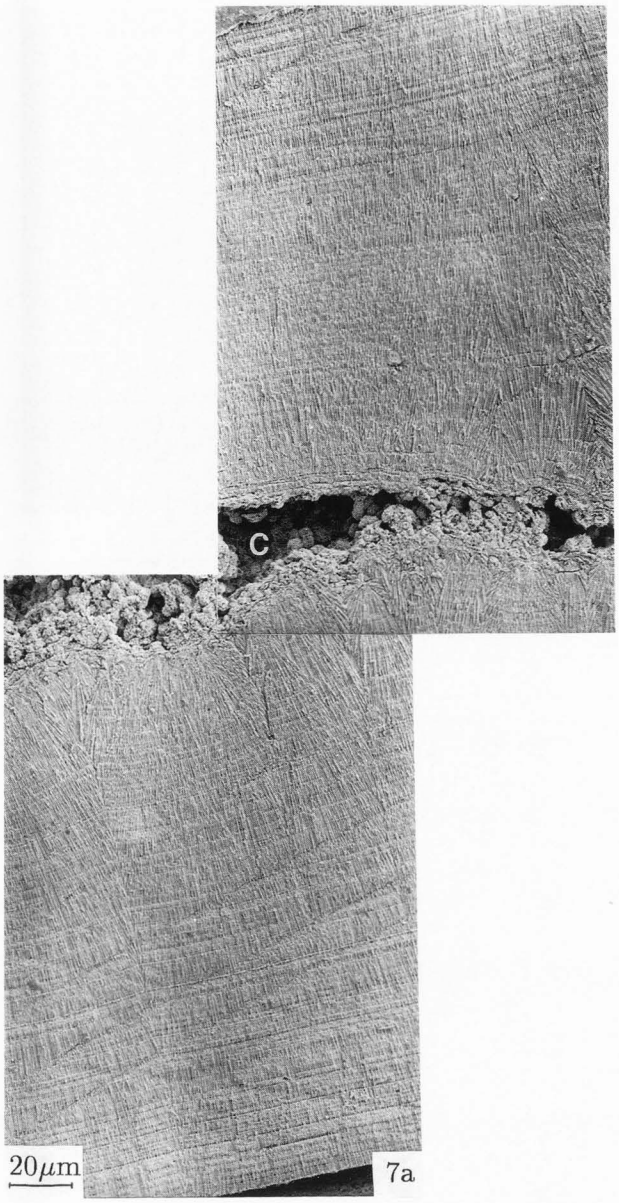


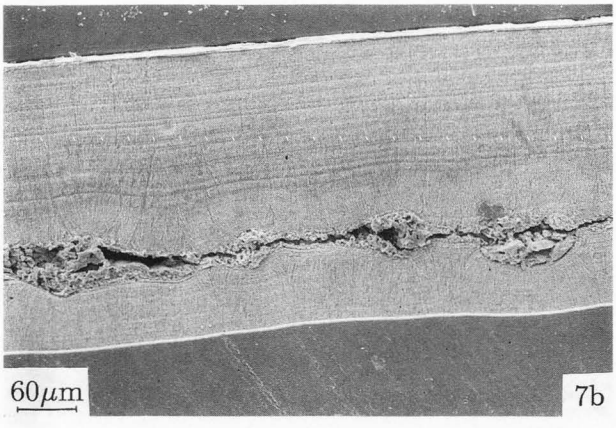
Fig. 6. (a) SEM picture of the leading edge of the shell showing a normal porous central layer, containing a number of spherulitic grains, between relatively thin prismatic layers (P). (sample etched 4hr with NaOCl.) (b) SEM picture of an area near the centre of the spiral showing the lip formed by the aperture edge. In this part of the aperture, the outer prismatic layer curves to meet the inner prismatic layer thus enclosing the central layer. (c) Reflected light photograph of the centre of the spiral of the Argonaut shell. The edge of the shell thickens at some point (arrowed) as forward extension of the material is no longer continued. The thickened lip (L) which is formed follows the limits of growth through to the original cup of shell (S) at the centre of the spiral. (d) SEM picture of a broken keel point showing the prismatic material surrounding the central zone in a pattern similar to the lip near the umbilicus. (sample etched 4hr with NaOCl.)

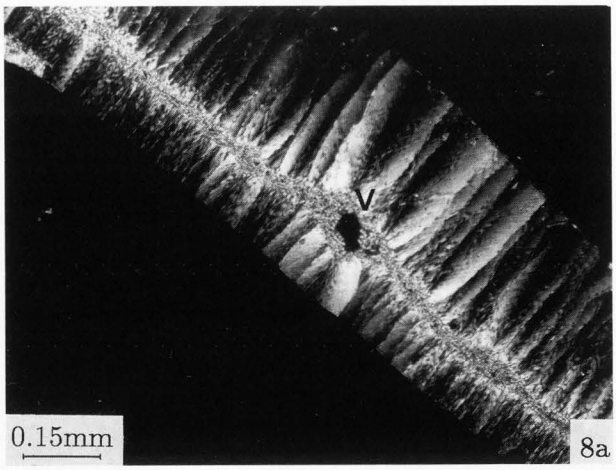
the keel of the shell also showed the central zone surrounded by prismatic material (Fig. 6d) in a pattern similar to the lip of the aperture near the umbilicus. This is because, in these points, the two prismatic layers were found to meet at an angle and eventually pinch off the central zone.

Ultrastructural observations

SEM observations showed that the central zone varied greatly in both width and porosity over relatively short distances (Fig. 7). The width ranged from roughly 5 μm to almost 50 μm over distances of about 0.2 to 0.3 mm, and porosity varied from nearly as well-packed as the prismatic material to almost empty (Fig. 7b). The major variation in porosity and width occurred perpendicular to the curvature of the shell. In radial sections, the central zone remained relatively constant in width and porosity over much greater distances. For example, the voids which occurred in the







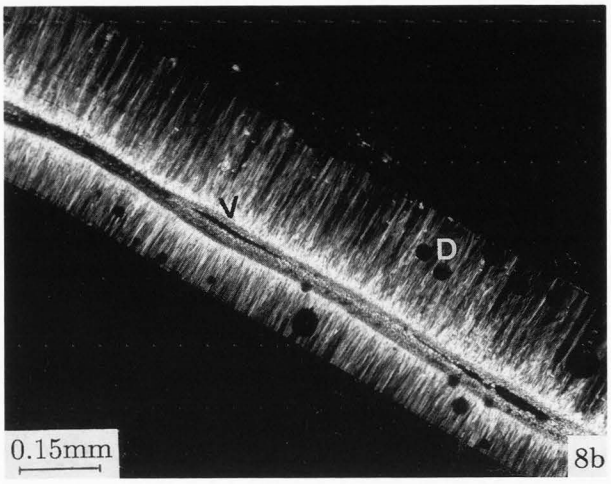


Fig. 8. (a) LM micrographs using crossed polarisers of a section cut perpendicular to the leading edge of the shell, and therefore the fibres (see Fig. 2b), showing nearly round voids (V) in the central zone. (b) LM micrographs using crossed polarisers of a section cut parallel to the leading edge, and therefore the fibres, showing elongated voids (V). The dark marks (D) are due to air bubbles in the glue used in attaching the specimen to the slide.

porous regions of the central layer were found to have elongated outlines in radial sections and round outlines in tangential sections (Fig. 8). Thus, the greatest variation in central zone width occurred perpendicular to the fibres and with spacings of a similar order of magnitude.

Fig. 7. (a) SEM montage showing a very porous region of the central zone (C). In this section, the width varies from 15 to 40 μm within a 0.25 mm length of the zone. (sample etched 30s with CH_3COOH and 3hr with NaOCl.) (b) A lower magnification SEM picture showing the scale of the width variations with respect to the cross-section of the shell. Fluctuations of up to 50 μm occur here within a space of 0.1 to 0.2 mm. (sample etched 30s with CH_3COOH and 6hr with NaOCl.)

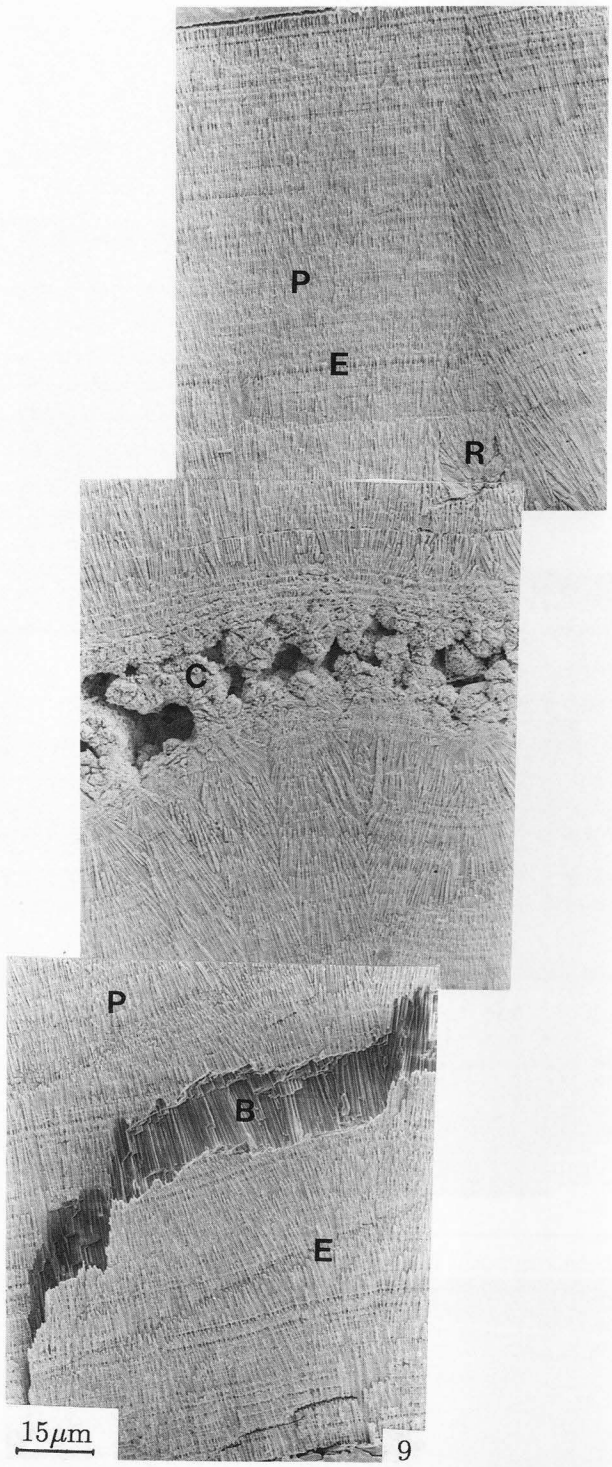


Fig. 9. SEM montage of the etched cross-section of the shell showing the two prismatic layers (P) of nearly equal thickness and the central zone (C) varying in width between about 20 μm to 30 μm. The shell is about 0.3 mm thick. In the central zone, many voids and a number of crystalline grains which have not continued to grow into prisms can be seen. Lines of preferential etching (E) are seen passing perpendicular to the prismatic material (see Fig. 4c) without disrupting the continuity of the prisms. These lines appear closer together near the central zone. A break (B) in the shell structure appears at the bottom of the picture and there is also a restart point (R) visible just above the central zone. (sample etched 30s with CH_3COOH and 3hr with NaOCl.)

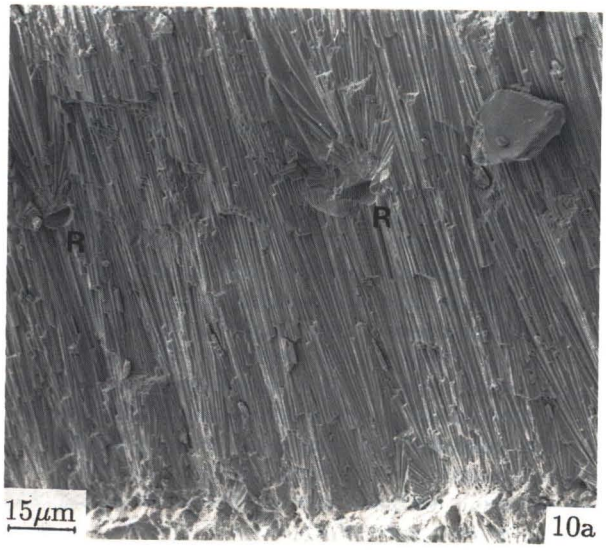
The two prismatic layers showed nearly identical ultrastructures although their relative widths varied (Fig. 7b); the inner layer being generally thinner than the outer. In the tightly coiled early part of the shell, where the material was fairly thin, the inner layer was as little as half the width of the outer (Fig. 4,7b) while near the aperture, where the shell opened out more, the two layers were almost the same width.

Scanning electron microscopy of cross-sectional samples showed lines of preferential etching perpendicular to the prisms (Fig. 7,9). These lines were also visible as darker strips in the unetched thin sections viewed in polarised light (Fig. 4c); which suggests an alteration to the normal crystal growth within the lines. These lines were not identified in TEM sections nor were they obvious in the SEM micrographs of the fracture surface. The relative number of lines on either side of the central zone was difficult to determine because, while some lines were quite distinct and definite, others were barely distinguishable and also because the lines, particularly those less well defined, did not have sharp boundaries. The lines were less distinct in the inner prismatic layer than in the outer where they were broader and better defined (Fig. 4c,7b).

Within the prismatic material, there were a number of points where prisms were interrupted (Fig. 10) and a short section of material appeared with a structure similar to the central zone. At these points, there occurred radiating growth of crystals rapidly constrained to a single direction. These crystals extended only in the direction away from the central zone, since the previously formed prisms underlying the interruption prevented any growth toward the zone.

The prismatic material consisted of prismatic crystals which had polygonal cross-sections, with no consistency between crystals as to actual size, which varied between 0.3 to 3.0 μm, or shape (Fig. 11).The crystals were separated by an organic intercrystalline matrix of very regular width (about 0.015 μm) (Fig. 11c). Space-filling considerations seemed solely responsible for the polygonal outline of the prismatic crystals.

Both SEM pictures of the shell surface and TEM pictures of sections cut perpendicular to the prismatic layers showed a large number of intrusions of the matrix within individual crystals (Fig. 11). The intrusions showed no apparent difference from the organic intercrystalline matrix and had the same regular width (Fig. 11c). Some of these intrusions were continuous with the intercrystalline matrix while others seemed to be completely isolated within crystals, although this may be an effect caused by the two dimensionality of the section. The pattern formed by the organic matrix showed some similarity to the patterns usually found in thin films due to surface tension; for example, it was usual that the angle between two interfaces was 120° and that only three lines met at any one point. This pattern also covered parts of the tubercles on the outer surface of the shell (Fig. 3b).



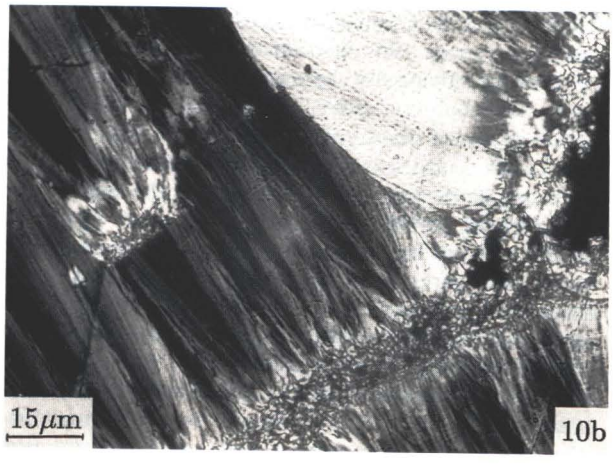
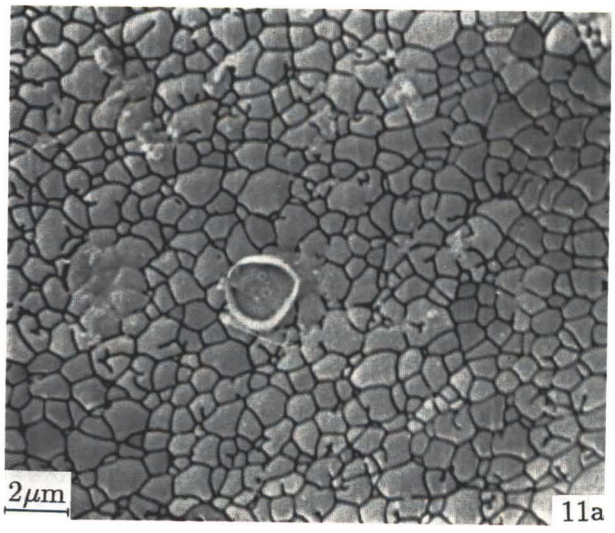
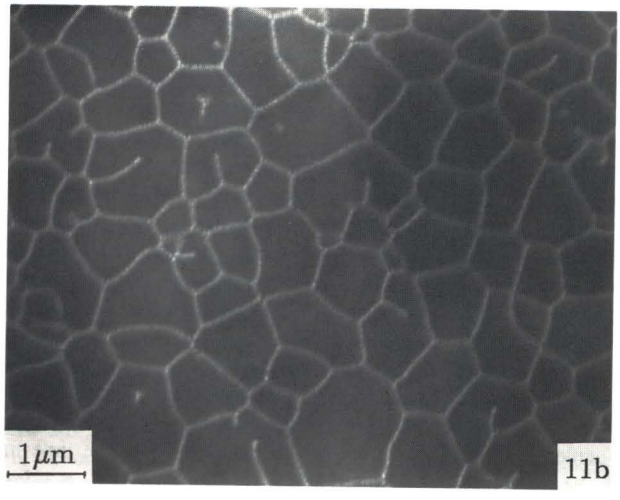
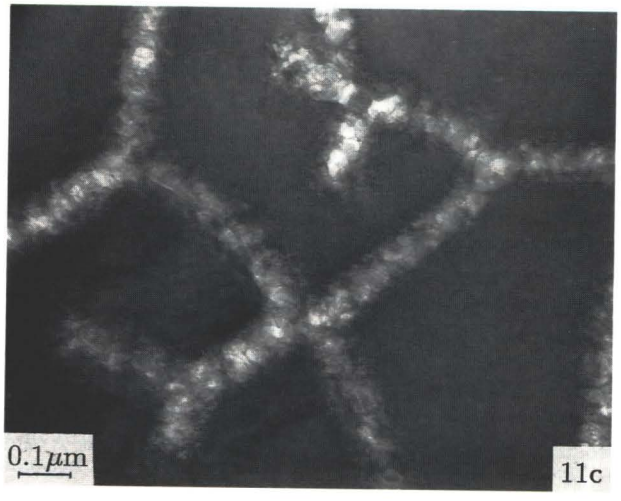


Fig. 10. Two views of interrupted prismatic growth which has restarted in a spherulitic form similar to that seen in the central zone. It can be seen that the growth is quickly constrained to a single direction once more. (a) SEM picture of a fracture surface. The two restart (R) points shown here both appear to have hollow regions below them that may have contained foreign matter which caused the interruption to normal growth. (b) LM picture of a section between crossed polarisers. The misalignment of crystals found in the prismatic material close to the central zone also appears at the restart point.

Fig. 11. (a) Scanning electron micrograph of the surface of the shell showing polygonal pattern formed by the ends of the prismatic crystals. (sample etched 7hr with NaOCl.). (b) TEM view showing organic inclusions both wholly within the crystals and as extensions of the surrounding organic matrix. (c) A high magnification TEM picture showing the similarity between the matrix inclusions inside the crystals and the matrix surrounding the crystals. Both are very regular in width (about 0.015 μm).







Both X-ray diffraction (XRD) patterns and electron diffraction patterns of the Argonaut shell showed that it consisted of almost pure calcite with undetectable amounts of any other crystalline phase present.

Discussion

In most molluscan shells, crystal deposition is known to occur only on the inside surface of the shell. Consequently, the shell cross-section shows a series of layers from the organic crystal-nucleating periostracum present on the outer surface of the shell to the last deposited material adjacent to the mantle. The observations presented in this study show that, in the Argonaut, the central zone is the first part of the shell formed and this acts as the nucleation site for the rest of the shell. The crystallites at the edges of the central zone initially show a spherulitic growth pattern and then extend into prisms due to the restriction in growth direction created by the presence of neighbouring spherulites. Since only those crystallites which are oriented perpendicular to the central zone have room for unrestricted growth, there is very good alignment between the resultant prismatic crystals.

The central zone itself is formed of calcite spherulites most of which grow no larger than about 5 μm in diameter. Only those spherulites which are at the fringe of the central zone continue growth and operate as nucleation points for the prisms. This radically different construction is almost certainly due to material deposition by the webs rather than the mantle. This explanation assumes that the mechanism of shell secretion follows the normal molluscan pattern with a supersaturated fluid filling the space between the growing shell and the secreting surface of the animal. The crystalline and organic parts of the shell then precipitate from this fluid to form the new material.

The presence of two essentially identical prismatic layers around the central zone implies that both were formed by the same mechanism. This means that one web must rest on the inside of the shell for a significant proportion of the time in order to deposit the inner layer. If the Argonaut resides within the shell as material is being deposited, then a web must rest between the animal and the shell while forming the inner layer. Thus, the most probable procedure of shell deposition would involve alternation of the webs between inner and outer surfaces. This is in agreement with reports [Young, 1959, Stephens, 1965] that the web is retracted within the shell for irregular, albeit unspecified, lengths of time. It is, therefore, possible that the inside surface of the shell is being laid down during these periods.

This method of shell formation would result in a periodic secretion of the shell material, which would cause discontinuities in the prismatic crystals. Minor deterioration of the exposed surface and slight compositional changes would also occur whenever the surface is left exposed by the web and growth is interrupted. These factors would explain the lines of preferential etching shown by the SEM observations. It is also possible that the lines are due to an increase in the amount of organic material within the crystals in these regions. For instance the lines may be part of a diffuse extension of the organic matrix which exists between the prismatic crystals. However, it seems more likely that preferential etching is a consequence of an increase in the number of imperfections inevitably created in the crystal lattice by periodic exposure of the growth surface to the environment. The higher potential energies within the lattice caused by these imperfections would increase the rate at which the etchants interact with the crystalline material.

The alternation of the webs from one surface to the other should create an equal number of lines on either side of the central zone; however this could not be unambiguously established. Extreme variations should and do exist in the width and spacing of the lines, dependent on the length of time for which the web was absent from the surface and the disruption caused by its departure. Differences in the environments to which the inner and outer prismatic layers are exposed would also affect the lines and this is confirmed by the observation that less distinct lines occur in the more protected inner prismatic layer.

Given the shell structure as revealed here, questions are raised by the suggestion that the Argonaut shell is enlarged as the animal matures [Stephens, 1965, Douglas, 1974]. In most molluscs, specific areas of the mantle can be associated with the specific layers in the shell which they deposit. Since the webs move, this association cannot be made in the Argonaut implying that there are no specialised areas of the webs designed to deposit certain areas of the shell. The development of the Argonaut shell has not been reported in the literature and it is not clear from the observations here how any extension could come about as the animal grows. For forward growth of the shell, material must be deposited at the aperture but in the adult, the webs do not generally cover this part of the shell. The conclusion is, that in juveniles, the webs do extend to cover the leading edge of the shell so that deposition can take place. The appearance of the edge shown here, with the central zone exposed, suggests that this may in fact be the case. This is supported by the fact that, near the umbilicus, the central zone is surrounded by the prismatic material because, in this area, forward growth of the aperture is no longer necessary or desirable.

The alternative to continual growth of the shell is that it is formed as a full size unit either around the animal or so that the animal can enter it after formation is complete. This method is implied by Miner (1935) who describes the shell formation as starting with the webs held together and secreting a gelatinous substance which moulds to the webs and hardens in the water to create the shell. Two halves of the shell are formed and joined along the keel before the female Argonaut enters and lays the eggs. If no growth occurs in the shell at all then there are several possibilities; each of which has problems associated with it. For example, successive moulting and growth of new shells is unlikely given the observation [Young, 1959] that the animal cannot leave the shell for any length of time without dying. Moreover, the formation of a new shell would almost certainly take a considerable length of time and require much of the animal's physical resources. On the other hand, if the Argonaut does not form a shell until after reaching maturity when further growth of the animal has ceased, then juveniles without shells would exist; also all shells found would be roughly the same size, i.e. the size of an adult animal; neither of these is true. The growth mechanism described by

Miner (1935) may be valid for the cup of shell formed by the newly hatched animal, although a discontinuity would be expected if the shell was formed in two sections as suggested. Since no sharp mismatch in the structure was observed in this study and the fibres were found to be continuous as they crossed the keel around the full curvature of the shell, the continuous growth mechanism seems most likely.

Deposition and extension of the shell with the webs covering the edge may produce the ribs and nodules on the surface. These structures are useful to lend rigidity to the rather thin and flexible material of the shell and also provide a grip for the webs when they are laid upon the shell. However, the webs themselves are too flexible to have any pattern to their surfaces that might imprint onto the shell as it is deposited. Minor variations in the position of the webs, as they overlap the edge during extension of the shell, could result in a slight change in the curvature of the material. This would average out with curvatures toward both the inside and the outside of the shell, so that the general shape of the shell would remain spiral but, on a smaller scale, ripples would appear on the surface.

Once growth of the shell has commenced, it is sufficient that the webs alternate between the outside and the inside of the shell, to ensure continuous growth. However, this mechanism is unable to initiate the first nucleation of crystals in the central zone. The fluid from which the shell precipitates would require a confined space in which to reach supersaturation, and a substrate on which the material can precipitate. The most convenient substrate is the other web; so that the most likely possibility is that the first formed material is created between the two webs. If the webs are not in full contact, the space between them would contain a quantity of the supersaturated fluid; and crystal nucleation within this space would result in the observed spherulites. The nuclei which form in contact with the surface of the webs then become the spherulites on the fringe of the central zone and extend into prisms. Exhaustion of available ions between the webs would restrict the growth of the spherulites inside the central zone so that they do not necessarily grow to fill the available space.

It is not known whether a difference exists in the nature or composition of the organic material within the central zone and that in the prismatic layers. The above description does not require any alteration of the organic components to provide the observed structures since the indications are that the matrix has no obvious influence on the formation of the crystalline components of the shell. Extension of the fringe spherulites into prisms is simply due to the concentration gradient in the component ions set up by the webs, and so the only function of the organic material is to maintain crystal separation and coherence. The prismatic structure would naturally occur since deposition of the material only takes place on the surface of the crystal closest to the web; which will necessarily become the growing face of the crystal. In other words, when the supersaturated fluid is secreted into a space, for example between the webs, spherulitic growth occurs, while prismatic growth results when there is unilateral secretion on the surface of a previously established crystal. This is in contrast to other molluscan shells in which almost all matters related to the crystal deposition are believed to be influenced by the matrix.

It is unlikely that the apparently empty places observed in the central zone originally contained organic material which was removed by the sample preparation because specimens of the fracture surface, which underwent no treatment, also showed similar spaces. This is relevant to the restart points observed in the prismatic layers where an interruption to the prismatic growth occurred and spherulitic growth of the crystallites took place. Since no organic nucleation site is present in the central zone, it is presumably not required for spherulitic growth. Hence, these restart points can be explained as places in which the webs, for some reason, could not continue growth of the underlying prisms and thus new spherulites were created. This could be due, for example, to an impurity landing on the shell while the growing surface was exposed so that the webs could not come into full contact with the underlying prisms. This is supported by the fact that all restart points observed were in the outer prismatic layer which is exposed to the external environment and therefore is more likely to have come into contact with foreign matter.

The nature of the fibres, which give the shell an appearance similar to fibre-glass reinforced plastic, has not been explained previously in the literature. Although the fibres were readily visible within the material with either the naked eye or with a reflected LM, they were not visible in cross-sections of the shell with transmitted LM, SEM or TEM. The well aligned calcite crystals of the prismatic layers are mostly transparent under normal conditions. However, any change in the porosity or thickness of the central zone, with its disordered crystal grains, would cause an alteration in the zone's opacity. This would be easily visible as a change in the translucency of the shell but not readily apparent in a sectioned sample. Hence the observations reported here indicate that the variations in the opacity, i.e. the thickness and porosity, of the central zone cause the fibrous appearance of the shell.

The pattern of polygons which appears in transverse sections of the prismatic layers is reminiscent of minimum surface energy forms seen, for instance, in soap bubbles. The equiangular intersections between polygons, with angles of approximately 120° between line segments and only three lines meeting at a point, imply some sort of surface tension effect in the organic matrix. The matrix was not directly visible in most cases since it was removed during the preparation of most TEM samples, however it was apparent from the spaces that it left in the material. These observations suggest that the organic matrix which surrounds the crystals is secreted first and assumes a low energy configuration. Later deposition of crystal then fills the pre-formed matrix shapes and results in the observed polygonal structure. This does not contradict the earlier suggestion that the organic material is not responsible for the configuration of the crystals which make up the prismatic layers, because there is still no requirement for organic nucleation of the prisms.

The non-crystalline intrusions seen within the cross-section of the crystals do not differ from the organic matrix surrounding the crystals. Therefore, they are almost certainly parts of the matrix trapped inside the growing crystals. The constant width of the non-crystalline material

may be governed by the structure or size of the proteins forming the organic matrix. The origin of the intrusions is uncertain: they may result from a broken organic film which gets incorporated in the growing crystals. Alternately they may just be folds in the matrix caught up in the growing crystal, although this explanation requires that the matrix is not under tension while the polygonal appearance of the crystals implies that it is under tension. The appearance of some intrusions entirely within a single crystal without any apparent connection to the matrix may be due to the two-dimensional nature of the images.

The matt appearance of the outer surface of the shell is almost certainly due to the small tubercles covering the surface. The areas of the outer surface which do not possess tubercles, such as the centre of the spiral and the immediate edge of the aperture, are as glossy as the inside. These are the areas not usually covered by the webs when they are extended over the outside of the shell. The formation of tubercles suggests that some minor secretion of material is taking place in localised areas covered by the webs even though the shell is fully grown and shell growth has ceased.

The observations presented in this study suggest a possible shell formation process quite different from the method used by other molluscs and which is tacitly assumed in the literature to be applicable to the Argonaut. The description given here does not account for all aspects of the shell formation nor all observations, however it does provide a viable construction method. There are still many questions concerning the process of shell formation in Argonauts which cannot be answered by simple observation of the ultrastructure. These include the exact method of extension of the shell during maturation, or even whether the shell is extended.

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Discussion with Reviewers

K. F. Hirsch: Can you exclude the organic matter from having an influence in the nucleation of the crystalline matter and its shape?

A. C. Smillie: Is it certain that the organic material plays no part at all in the initial nucleation of the spherulitic granules?

Authors: Unlike the crystals found in other, more developed shells, those found in the argonaut shell have habits which are common for calcium carbonate which is formed inorganically. However, given the large role played by the organic matrix in the structure and composition of other molluscan shells, it is unlikely that the influence of the organic material can be entirely ruled out, despite the great differences in the method of shell formation. The point is not that the matrix has no influence but only that it need not be invoked in order to explain the ultrastructure of the argonaut shell. It is possible that the organic matter plays a role in the initial nucleation of the granules but the shape of the prisms in particular, seems to be determined primarily by space constraints during growth.

K. F. Hirsch: Might there also be difference in the nature of the organic material within the central zone and that in the prismatic shell layer?

Authors: This is possible, but the techniques used in this investigation are not suitable for detecting differences in the organic matter from different parts of the shell.

A. C. Smillie: Could the organic material surrounding the prisms (and in some cases intruding into them) have come to be where it is as a result of the growth of adjacent crystals squeezing the organic material into an ever-diminishing space rather than that it assumed a low energy configuration from the outset?

Authors: The very uniform thickness of the organic material surrounding the prisms suggests that some structure existed prior to the growth of the crystals and the final low-energy configuration seems most likely. If the material had been squeezed into its final position by the growing crystals then it would be expected that the films would be uneven in thickness around the prisms.