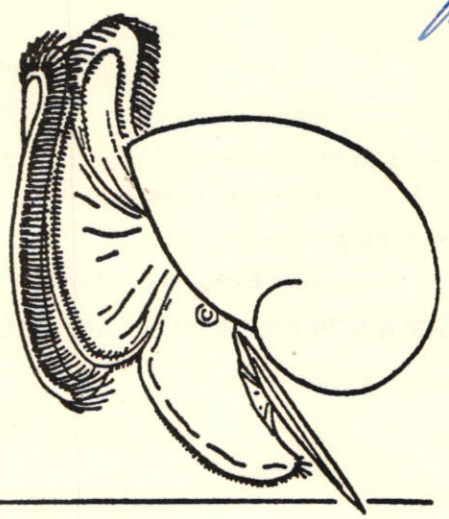
THE VELIGER



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Note: The various taxa above species are indicated by the use of different type styles as shown by the following examples, and by increasing indentation.

ORDER, Suborder, DIVISION, Subdivision, SECTION,
SUPERFAMILY, FAMILY, Subfamily, *Genus, (Subgenus)*
New Taxa

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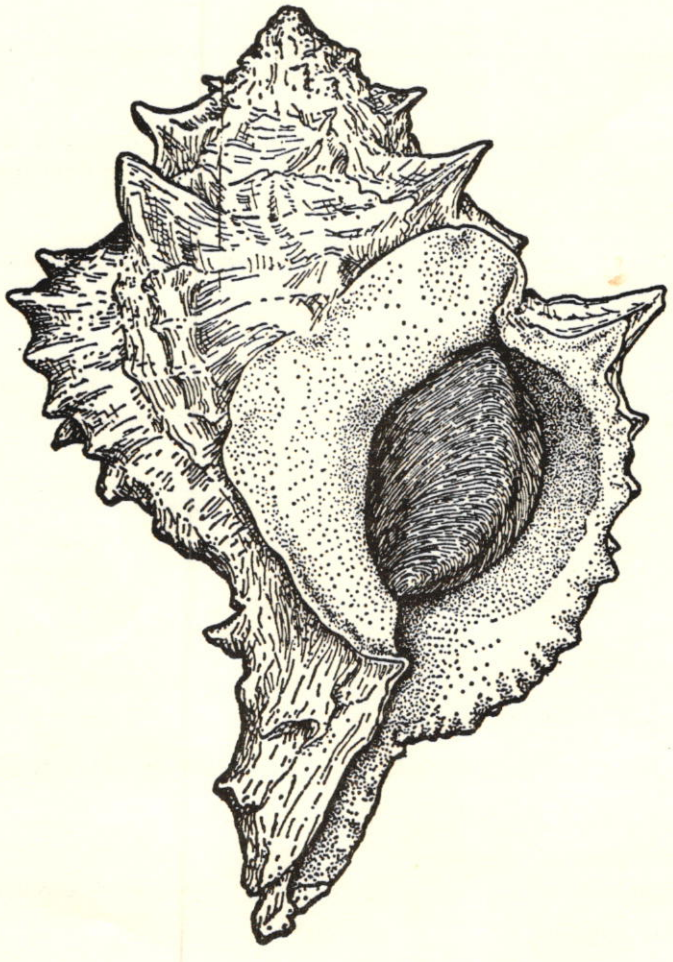

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A Comparative Study of the Structure, Development and Morphological Relationships of Chambered Cephalopod Shells

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(6 Plates; 27 Text figures)

INTRODUCTION

Among the Recent cephalopods, chambered shells are confined to the members of three families: the Nautilidae, with only a few species of *Nautilus* that represent the nearly extinct subclass Nautiloidea; in the Coleoidea (order Sepioidea) the monotypic Spirulidae and the speciose Sepiidae, the common cuttlefishes.

The shell of *Nautilus* is external, whereas the *Spirula* shell and the "cuttlebone" of the Sepiidae are internal, as are those of all coleoid cephalopods. The general aspect of these three types of chambered shell is rather different. The shells of *Nautilus* and *Spirula* show some resemblance in that they are coiled and have large chambers, in contrast to the cuttlebone which is straight and has numerous extremely narrow chambers. However, the coiling of the external shell of *Nautilus* is exogastric, whereas the coiling of the internal *Spirula* shell is endogastric. Despite its straight form, the cuttlebone presents signs of a close relationship with the coiled *Spirula* shell.

These general aspects were known in the last century. If there has never been a doubt about the buoyancy function of the gas-filled chambered shell, its actual functioning has only recently been elucidated. With the use of scanning electron microscopes, it has become possible to analyse in great detail the ultrastructure of these aragonitic shells, recent and fossil. This structural analysis provides a sound basis for the comparative study of the functional morphology of the chambered cephalopod shells, and more particularly of their siphuncular system, which is known to be responsible for buoyancy regulation.

The present study follows this line of investigation. Along with the presentation of new data on the ultrastructure of the *Spirula* shell and of the cuttlebone throughout its development, a comparative account of the structural aspects of chambered shells in general is attempted, with the aim to unravel some of the complications arising from the different interpretations presented in the literature.

MATERIAL AND METHODS

Specimens of *Sepia* were collected at Banyuls-sur-Mer, (Western Mediterranean), Port Sudan and Suakin (Red Sea); *Spirula* shells on the Canary Islands (Eastern Atlantic) and at Santa Marta (Caribbean).

Shell material was prepared for observation in the Scanning Electron Microscope ("Cambridge Instruments") by oriented breaking, washing with distilled water (no etching!), mounting on metal supports and coating with carbon and gold.

Embryonic shells were removed mechanically and washed in water. For histological investigations, specimens were either fixed in Bouin's fixative, embedded in paraffin, sectioned at a thickness of 7 μm and stained with Azan or Masson's Trichrome; or pieces of fresh material were fixed in 1% OsO_4 in sea water for 1 to 2 hours,

embedded in Epon, and sections ranging from about 0.5 to 1.5 μm were cut with glass knives on an ultramicrotome. These sections were stained with a mixture of Methylene blue and Azur blue.

CHAMBERED SHELLS IN SEPIOIDEA

Morphology and Structure of the Shell in *Sepia*

The shell of *Sepia* has been described in detail by APPELLÖF (1893). This author also discussed older literature dating back to the 18th century. The terminology we use for the different parts of the cuttlebone is largely adopted from Appellöf and translated from German (Figure 1). For some details, the terms used by DENTON & GILPIN-BROWN (1961), are given preference to those of Appellöf.

The cuttlebone consists of a dorsal shield ("Rückenschild") and the ventral chamber zone ("Wulst"). The spine ("Dorn", "Rostrum") is situated on the mid-dorsal line on the convex dorsal shield, close to its posterior end. The upper side of the dorsal shield is covered by calcareous tubercles; the posterior and the marginal portions are smooth. Appellöf called the part that surrounds the spine "Dornhülle," i. e., cover of the spine.

The ventral side of the dorsal shield bears the convex chamber complex, which thins out towards the posterior end. On the ventral surface of the chamber zone, we distinguish the siphuncular zone ("gestreifter Wulst") from the zone of the last-formed chamber ("ungestreifter Wulst"). The posterior portion of the chamber zone is embraced by the fork ("Gabel"). The fork is broad posteriorly and narrows anteriorly on each side; it ends near the siphuncular surface of the last-formed chamber (last "Wulststreifen"). A calcified rim ("verkalkte Randzone"), accompanied by an uncalcified outer rim ("unverkalkte Randzone") of the dorsal shield, surrounds the fork and the chamber zone.

The dorsal shield consists of three layers (Figure 2). These are the uppermost, dorsal layer ("Rückenplatte"), the central layer ("Mittelplatte") and the inner layer ("Innenplatte"). The central layer emerges at the rim of the dorsal shield; it is characterized by organic and mineralized lamellae. The inner layer begins somewhat inward of the mainly organic rim of the dorsal shield; it consists of two portions, an upper, coarsely columnar prismatic layer ("Pfeiler-", i. e., pillar-like crystals) next to the central layer, and a lower, spherulitic prismatic layer ("besenartige," *i. e.*, broom-like crystals). The dorsal layer covers the upper intra-marginal portion of the dorsal shield. Appellöf differentiates between two portions, the middle and anterior undulating, nodular layer ("Hökkerpartie") and the posterior area of the spine cover (cf.

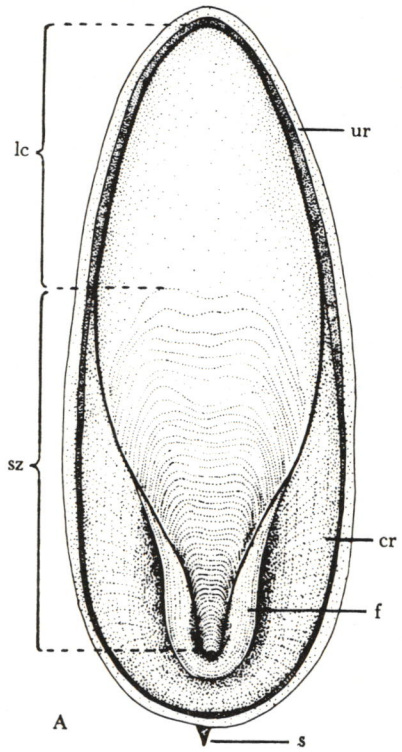
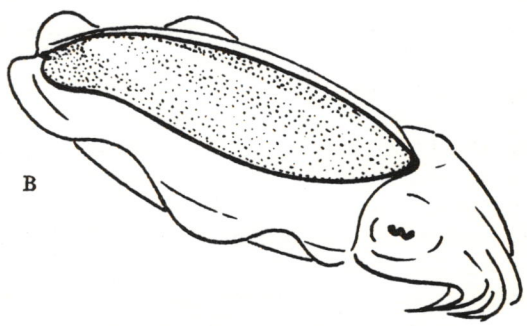


Figure 1

A:
Ventral view of a *Sepia* shell with the siphuncular zone (sz) and the last chamber (lc) surrounded by a calcified rim (cr) and an outer uncalcified rim (ur). The so-called fork (f) is restricted to the posterior part of the shell around the siphuncular zone. The spine (s), which projects from the dorsal side, is only partly seen at the posterior end of the shell.



B:
The position of the cuttle-bone in the dorsal part of the mantle is shown in a swimming animal (cf. also Figures 10, 11, 15, 17, *98* and *99*)

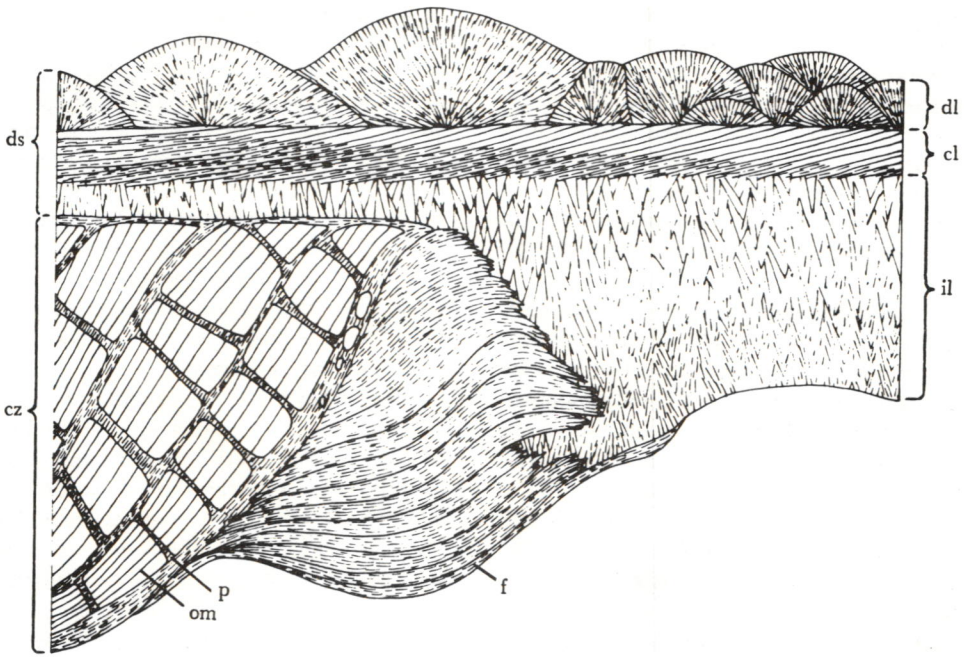


Figure 2

A cross section through the posterior part of the *Sepia* shell, showing the insertion of the chamber zone (cz) on the lower side of the dorsal shield (ds), the latter comprising 3 layers: the dorsal layer (dl), the central layer (cl), and the inner layer (il). Between the marginal part of the inner layer and the chamber zone lies the fork (f). Within the chambers, organic membranes (om) are suspended between the pillars (p).

above). In our study, the spine and its surroundings are included in the central layer because of their structural similarity. The fork consists of several separate layers ("Gabelsepta"), each of which shows a finely laminated structure.

The chambered part consists of cavities ("Höhlenschichten"), separated by septa. Each septum is made of a chamber roof (ventral portion of the septum) and a chamber floor (dorsal portion of the septum) (Figure 3). Within the chambers, vertical pillars and walls ("Pfeiler") form the supporting elements of the septa. In addition to the septa, organic membranes are suspended between the pillars ("freigespannte Membranen") (Figure 2).

After a chamber is completed by the formation of the (ventral) chamber floor, formation of a new chamber starts with the chamber roof completing the last septum (cf. Figure 3). The chamber roof consists of a prismatic layer composed of rectangular, rod-like prisms (0.4 μm wide) (Figures *28, 82*). The smallest components which

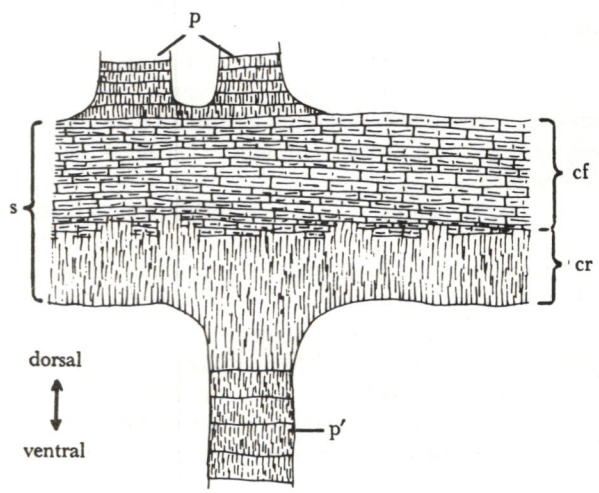


Figure 3

Cross-section through a septum of the *Sepia* shell. The septum (s) comprises the chamber floor (cf) with the base of the pillars (p) of the upper chamber and the chamber roof (cr) with the "tops" of the pillars (p') of the lower chamber

make up these prisms are angular elements with a width of 0.2 μm. At the insertion of the pillars and pillar walls, the prismatic crystal rods of the roof extend, without interruption, into the base of the forming pillar. The chamber roof is about 7 μm thick; it is continuous with the floor of the older (upper) chamber (cf. below); there are no separating structures such as organic layers.

Where they emerge from the chamber roof, the pillars are either round, columnar, or they are straight or slightly undulating wall-like structures; these walls have about the same thickness as the columnar pillars. The prismatic structure of the chamber roof disappears in the basal part of the pillar; it grades into the lamellar structure with the appearance of the first annulation. The pillar annulations (38 to 62 in the central area of the chamber zone of *Sepia orbignyana*) are continuous in many pillars of one and the same area of a chamber, but they are not so in all pillars of a chamber. This can also be noted with the organic sheets that are extended between pillars. The central zone of one chamber may have 5 to 9 such sheets, extended parallel to the septa (Figures *29, 30*). These sheets are not seen as distinct organic layers within the pillars, but probably they are continuous. Within the pillars, the organic material is incorporated in the crystal fabric. The organic sheets are often found to split into a number of thinner sheets when they approach a pillar, so that only thin organic sheets are incorporated into adjacent portions of the calcareous fabric of the pillar. The "fusion" of these thin sheets at some distance from the pillars is probably secondary; it may result from artifactual agglutination of the wet membranes during drying of the cuttlebone.

The pillar annulations are 1 μm to 8 μm wide. The crystals making up the pillar lamellae are quite irregular in shape; the most common form is a brick-like component (0.2 μm wide) with its long axis usually following the axis of the pillar (Figures *78, 79, 82*).

Close to the chamber floor, the walls show stronger bending, and pillars tend to branch (Figures *30, 31, 80, 81*). The surface of the pillars in the ventral part of the chamber is thus enlarged. Also wall-like pillars tend to divide into many branches, and columnar pillars show bilateral flattening in their different branches. Along with this crenelation and flattening, the undulating sheets thus "disintegrate". The ventral side of each chamber is therefore more permeable than the dorsal part, where the base of a pillar wall is generally continuous.

The crystal growth of the ventral pillar branches that turn into the chamber floor is continuous, without any organic or mineral layer between pillar and floor. KAELIN's (1967) statement that the wall-like pillars are not solidly fused with the chamber floor is erroneous.

The chamber floor is about 15 μm thick; it shows a lamellar structure, with lamellae that are about 0.1 μm thick (Figures *28, 82*). In its middle part, this floor layer is made of 0.2 μm wide, needle-shaped crystallites that show a common orientation within each layer. In their first-formed layers, the lamellar structure of the floor is composed of shorter rod-like elements, oriented parallel to the plane of lamellation. With its gradual transition into the roof of the next chamber, the lamellae show rod-like components with gradually changing orientation, from parallel to lamellation through vertical position, until lamellation is largely or entirely lost in the roof of the next chamber (Figure *28*).

Away from the central region of the chamber, pillars become shorter (Figures *75, 76*); in the anterior region of the chamber, they are fused into wall-like ridges or simple ridge-like structures. Towards the posterior end, close to the siphuncular zone of each chamber, the contrary is found. Here pillars become thinner and more columnar than in the central part of the chamber, and they are more closely set (Figure *31*).

The number of organic sheets expanded between pillars decreases towards the anterior margin, where the chamber height decreases. This decrease is less distinct in the lateral parts, where the chamber height decreases more abruptly (Figure 2).

In the siphuncular zone, no calcareous floor is formed. The calcareous layers of the chamber floor are continuous with the organic sheets that cover the siphuncular area (Figure *31*). In the siphuncular zone, the pillars are more numerous close to the posterior end of the chamber, where they are much shorter according to the lower chamber height. From the central area of the shell, organic sheets extend only in the foremost part of the siphuncular area. Here we find few sheets extended parallel to the roof; there are more sheets extended vertically betwen pillars (Figurs *32, 33*).

In the low posterior part of the siphuncular zone, between the short, thin pillars, we find a dense growth of short, columnar crystals, plate-like crystals, and crystal aggregates (Figures *34, 35, 36*). These crystals are large in comparison with those forming the pillars, and they show well-developed crystallographic planes and faces (Figures *35, 36*).

Near the posterior end of the chamber, the growth of crystals is so dense that they form a closure between the organic floor and the calcareous roof. Further anteriorly, the crystal growth forms a porous layer together with the rearmost short pillars. More anteriorly again, at about half of the length of the siphuncular zone, crystals may form aggregations of considerable height (Figure *36*); between pillars, the first vertical organic sheets appear. Fi-

nally, in the foremost part of the siphuncular zone, the growth of distinct crystals comes to an end, and we find only columnar pillars sustaining the organic floor. This is the area where horizontal sheets are found between the pillars (cf. above). These sheets become more continuous above the rearmost part of the calcified chamber floor, and there the pillars take on a wall-like appearance.

The chamber height is approximately the same throughout the greater part of the chamber zone. Exceptions are the first chamber formed after hatching, which is often markedly lower, the zones of closely spaced chambers described by various authors (cf. BOLETZKY, 1974a), and the last chambers of the adult, senescent animal (Figure 30).

The formation of the dorsal layer begins rather late in embryonic development. In *Sepia officinalis,* it first appears in the form of an irregular crystal cover on the organic outer surface of the shell (Figure 37). In *Sepia pharaonis,* the first dorsal layer is made of nodular spherulitic structures (Figure 40). During further growth, the spherulite sectors start on the dorsal side of the organic central layer. The dorsal outer surface of the central layer is comparatively wide; the dorsal layer grows over it either by depositing directly ridge- or bump-like spherulitic structures that are surrounded by organic material (*Sepia elegans, S. pharaonis*), or with a zone of irregular fine crystal growth (*Sepia officinalis, S. orbignyana*). This may differ, however, among individuals as well as among different growth stages of an individual.

Towards the central part of the dorsal shield, the crystals are arranged in ridge-like aggregations, with a spherulitic orientation (Figure 38). Addition of crystalline material alternates with periods where organic material is added, but this periodicity seems not related to the formation of certain layers in other parts of the cuttlebone. Thus in a section or in a fraction, the dorsal layer shows varying sizes of crystal aggregates and different crystal diameters (Figure 44). Aggregates measuring 2 mm in diameter can be found on the adult cuttlebone of *Sepia officinalis.* Crystal diameters vary from 0.2 μm to 15 μm.

Near the spine or the structure corresponding to it (e. g., lamellar ridge in *Sepia elegans*), the dorsal layer is absent, as in *S. officinalis,* or it may surround the spine, as in *S. orbignyana,* or it is represented only by a very thin crystal cover of the ridge that represents the spine in *S. elegans.* In terms of its structure, the spine must be considered as part of the central layer (Figure 41), although it generally starts forming on the embryonic dorsal layers (Figure 39).

Finally, it should be mentioned that the structure of later deposits found on the posterior part of the siphuncular area consists of spherulitic-prismatic crystals and thus can be compared to the dorsal layer (cf. below).

The central layer is the earliest to appear during embryonic development, where it is represented by the protoconch and the early organic shell. In the marginal parts of the shell that are formed later, it is also mostly organic; it is composed of sheet-like smooth organic layers that have been deposited in a succession directed towards the margins. The central layer is thin near the protoconch; it gradually thickens anteriorly, and more so towards the margins. Its lateral portion is always purely organic, whereas in the ventral part calcareous material is intercalated and interlocked with the organic sheets.

Since the organic sheets that are added to the margins of the dorsal shield are not continuous with others, but are deposited on sheets formed earlier, they form a low angle with the plane of the central layer (Figures 2, 46). At 9000× magnification, the organic sheets show no other substructure than a striation with a period of 0.1 to 0.2 μm.

Towards the inner layer, very thin and long lamellar crystal rods and long needle-shaped crystals may grow along with the organic sheets. Within a short distance, purely organic layers can turn into calcified layers (Figure 42). The calcified part of the central layer is much thicker above the chambers than near the margins (Figure 2). In the central part, the inner layer shows a lamellar structure. These lamellae form a low angle with the plane of the central layer, like the purely organic lamellae closer to the rim (Figures 2, 46, 47). Lamellae are ca. 1 μm in thickness; they are continuous throughout the extent that we have been able to follow. They do not branch, but they change in thickness when turning into the purely organic zone (Figure 46). They also show some variation in their thickness close to the base of the inner layer where they end. Lamellation largely or entirely disappears where the base of the coarse prisms of the inner layer appears (Figure 47).

Each lamella of the central layer is composed of rod-like elements which usually are identically oriented within one layer (Figures 48, 51). Among different lamellae, this direction may change. Sometimes the needle-like crystallites that compose the lamellae show a feather-like arrangement (Figure 49), are gently curved (Figure 50) or branched (Figure 48). No distinct organic sheets appear between lamellae, but organic and mineral (needle-like) shell material is interlocked with one another, thus forming one complex shell deposit of organic and mineral components. The construction of the lamellae in the central layer thus is similar to the construction of the lamellae in the septa and the fork layers.

These strongly calcified parts of the central layer that are continuous in the ventral portion of the dorsal shield above the chamber zone extend, in some species of *Sepia,*

onto the outer side of the dorsal shield where they build the spine and its surroundings. In *S. elegans* there is no distinct spine, but only a ridge made of organic lamellae, which are often covered by crystal aggregates of the outer layer. Sheets similar to those of the margins of the dorsal shield alternate with deposits of the dorsal layer.

The shells of *Sepia orbignyana* and *S. pharaonis* have strongly calcified, solid spines (Figure *84*). These consist of layers very similar to those of the inner calcified portion of the central layer. From the margins towards the center of the spine, lamellae become continuously thicker and show an increasing amount of calcareous material. Thus the spine is made of cone-shaped layers that are piled up on one another. Each of these cone layers, which are thickest in their center, is continuous with a purely organic layer at the sides of the spine (Figures *41, 85*). The actual spine is a purely lamellar structure, whereas in its surroundings the organic layers corresponding to the spine layers often are covered by material of the dorsal layer so that they interdigitate with the latter (Figure 14).

The structural features of the spine of *Sepia officinalis* are intermediate between those of *S. elegans* and *S. orbignyana*. In *S. officinalis,* the change from purely organic sheets of the central layer into the lamellar calcified layers of the spine is very abrupt (Figure *41*). Thick organic sheets forming the margin of the posterior dorsal shield split into single sheets that connect the shield and the spine. Close to the spine, these layers again split into numerous free sheets, each of which is continuous with one lamella of the calcareous spine. In *S. officinalis,* the region around the spine is covered with organic sheets, which may or may not show minor growth of crystals having the structure of those that build the neighboring dorsal layer.

Certainly the layers that form the calcified spine in *Sepia* are part of the dorsal portion of the central layer; in other parts, e. g., in the marginal rim of the dorsal

Explanation of Figures *28* to *43*

Figure *28*: Fracture through septum of the chamber zone of *Sepia orbignyana*, showing a pillar rooted on the prismatic chamber floor (lower, ventral side of septum). The chamber floor is composed of the lamellar structure × 1 400

Figure *29*: Transverse section through the chamber zone of *Sepia gibba* showing narrow chambers separated by septa which are held apart by pillars. Suspended between the pillars in the chambers are organic sheets. The upper margin of the figure is ventral × 28

Figure *30*: Transverse fracture through the chamber zone of *Sepia orbignyana* showing the last formed chamber of an adult individual with decreasing chamber height. Note the organic sheets which are suspended within the chamber cavities between the pillars. The upper margin of the figure is ventral × 180

Figure *31*: Section through chambers of *Sepia orbignyana* in the siphuncular zone. Note in the lower chamber mostly round pillars close to the posterior end of this chamber. The upper chamber shows the anterior siphuncular area with short pillars. Above it the extreme posterior end of the siphuncular zone of the next chamber is visible, with irregular crystal growth and very short pillars. The siphuncular membrane is torn off and only its posterior portion is visible at the right. The upper margin of the figure is ventral × 126

Figure *32*: The siphuncular zone of a chamber of the embryonic shell of *Sepia pharaonis* during growth demonstrates the change of orientation of organic sheets from parallel to septa to vertical to septa. Also pillars become shorter and more numerous within the siphuncular zone × 190

Figure *33*: This detail of Figure *32* demonstrates the change in orientation of the organic sheets within the chamber in the siphuncular zone × 430

Figure *34*: The posterior end of the siphuncular area of a chamber of the cuttlebone of *Sepia pharaonis,* showing the organic siphuncular membrane (in the lower left), and where it is torn off the siphuncular zone of the chamber below it × 380

Figure *35*: This detail of Figure *34* shows the crystals of the posterior portion of the siphuncular zone with well-developed crystal faces × 8 000

Figure *36*: Another detail of Figure *34*, with aggregations of crystals forming pillar-like structures that lie between short pillars in the central region of the siphuncular zone × 4 000

Figure *37*: The first irregular crystal cover on the organic, outer (dorsal) shell surface in the embryo of *Sepia officinalis* ×3 700

Figure *38*: A fracture through the central portion of the dorsal shield of *Sepia orbignyana* showing the lamellar central layer (lower part of figure) and the spherulitic dorsal layer. The latter forms ridges and crests on the dorsal side of the cuttlebone × 420

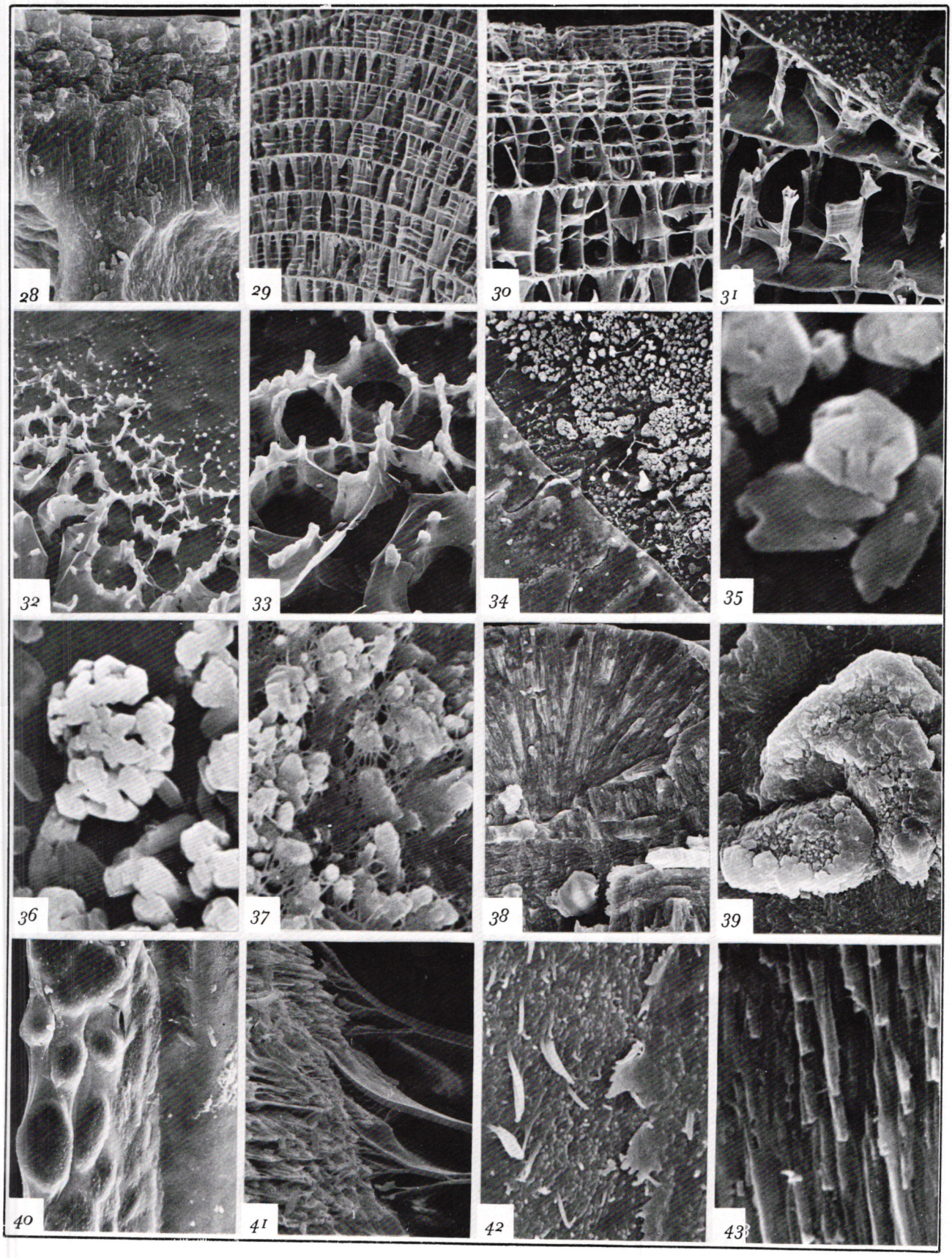
Figure *39*: Crystal growth on the posterior portion of the embryonic shell of *Sepia officinalis* at first is spherulitic, like that of the dorsal layer. Only later is it changed into the lamellar structure forming the spine at this location × 1 600

Figure *40*: The dorsal layer in the posterior rim of the dorsal shield of the shell with 6 chambers in young of *Sepia pharaonis* consists of isolated spherical structures × 370

Figure *41*: A detail of Figure *85* of the spine of *Sepia officinalis* demonstrates the rapid transition from organic sheets of the spine cover into the lamellar, calcified structure of the spine. The thick organic sheets split into thin lamellae near the calcified spine × 194

Figure *42*: The central layer of *Sepia orbignyana*, broken parallel to the growth surface, shows the rapid transition from mineralized, lamellar structure to purely organic sheets × 4 200

Figure *43*: Crystal needles are present in the mainly organic deposits covering the posterior portion of the siphuncular area of the adult shell of *Sepia pharaonis* × 3 900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shield and the area surrounding the spine, this central layer may be calcified to a much lesser extent or not at all.

On the ventral side of the shield, the inner layer comes very close to the margin; it overlaps the central layer. On the innermost organic sheets of the central layer, close to the prismatic base of the inner layer, there are more irregular needle lamellae. The needles merely show a general orientation according to a common direction; they are loosely spaced, so that there are interstices between them (Figures *49, 50*). They may also be oriented in such a way that they form whorls that unite into columnar structures (Figure *50*). In additional layers, closer to the base of the inner layer, such whorls turn into the round spherulitic nodules (5 μm wide) that form the base of the prismatic inner layer (Figure *47*).

The inner layer covers the ventral side of the dorsal shield from near the border of the uncalcified organic rim to the border of the fork layer (Figure 1). Only some of the innermost layers of the outer rim of the shield are calcified; they gradually turn into the inner spherulitic prismatic part of the inner layer, as shown above. The inner prismatic layer consists of coarse prisms that have an irregular outline (Figures *46, 47*). In a section parallel to the growth face, the prisms form an irregular network with a mesh width (diameter of prism) of about 5 μm. When viewed from the growth face, near the inner rims of the dorsal shield, the growing inner, prismatic portion of the inner layer shows well developed crystal faces (Figure *52*). The crystal needles are not arranged strictly vertical to the growth face; they may unite into columnar units of spherulite sectors (Figure *53*).

Further inside the inner rim of the dorsal shield, crystal size decreases, and the even growth of crystal heads is changed into a more nodular growth of crystal bundles; the components show less distinct crystal faces (Figure *55*). In section, one notes a spherulitic prismatic orientation of the needle crystallites, which are now much thinner. Columnar structures consisting of crystal needles inclined towards a central axis form this lower layer (Figure *53*). This lowermost (ventral) layer is only found beside the chamber layers, not above them (Figure 2). Above the chambers, there is a coarsely prismatic to spherulitic prismatic layer; its thickness decreases towards the medial part of the dorsal shield. Thus an oblong, oval field in the medial part of the dorsal shield is devoid of an inner layer. This oval field, which is broader anteriorly, is "left out" when the inner layer is formed on either side of the chamber layer (*i. e.*, not on the anterior rim of the newly formed chambers) (Figure 1). Here the calcified central layer forms the basement for the anterior portion of newly formed chambers.

The borderline between the inner layer and the fork layer is sharp and usually is marked by a furrow (Figure *55*). Sections through the contact region between the inner and the fork layer even show cavities; these are bridged by organic sheets indicating the course of growth lines (Figures 2, *54*). The region of contact between the inner and the fork layer shows signs of alternating approach and retreat of these layers during the secretion of the cuttlebone (Figure 2).

The zone of transition from the inner layer towards the central layer shows a more gradual change, especially where the medial parts of the dorsal shield grade into the strongly calcified inner part of the central layer (Figures *46, 47*). A gradual change between these layers is also found near the rim of the shield, but is restricted to the width of a few lamellae (Figure *50*).

The fork layer is broadest and thickest in the posterior part of the dorsal shield. Its anterior ends often thin out before they reach the siphuncular area of the last-formed chamber; they may also extend beyond it. There does not seem to be a direct continuation of fork layers into chamber septa. Since the fork grows in length only anteriorly, its layers are piled up on one another. In adult *Sepia,* 12 to 15 such layers with a maximal thickness of 25 μm may be present on the ventral side of the cuttlebone. In their thickest part, these layers are made up of roughly 40 lamellae (Figure *59*). These lamellae are composed of minute needle crystals (0.3 μm in width), which are uniformly oriented within a lamella (Figure *56*). Separation of thicker layers is due to the presence of more organic lamellae between the calcified ones that form the thicker layers (Figures *56, 59*).

On the margins of the fork, each layer extends to a different degree onto the chamber zone, on the one side, and onto the inner layer, on the other side (Figure 2). A more strongly mineralized layer shows a growth front consisting of flattened rod-like needles that encroach, in the form of a sheet, upon the basement (Figure *58*). There is only in some places a uniform orientation of the needles, as many lamellae grow at the same time, so that their fronts overlap one another. In mainly organic parts of the fork layer, the needle-like crystals are regularly arranged and maintained within organic sheets, where they are covered and surrounded by organic shell material (Figure *59*).

In the posterior part of the siphuncular area, adult individuals of *Sepia officinalis* and *S. orbignyana* often show tubercular deposits that consist of aragonitic needles in a spherulitic prismatic orientation, like those found in the dorsal layer. Thus the siphuncular membrane of the oldest chambers, which are refilled with liquid, becomes impermeable.

In *Sepia pharaonis,* a crescent-shaped deposit consisting of a thick layer of mainly organic sheets is secreted on a very prominent tubercular deposit in the posterior part of the siphuncular area (Figure 4). Within these

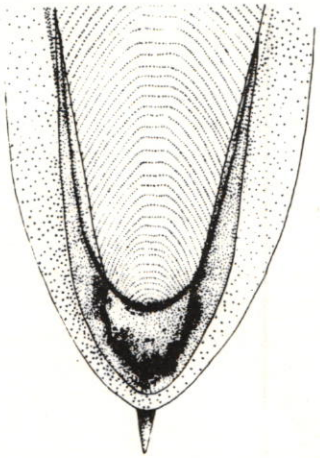


Figure 4

Ventral view of the posterior part of the cuttlebone of *Sepia pharaonis*. The rear end of the siphuncular zone (belonging to the earliest chambers) is covered by a crescent-shaped deposit that consists of mainly organic layers with calcitic and aragonitic crystals.

sheets there are mineralized spots. This is the only case where calcitic crystals exist besides aragonitic ones in the shell of *Sepia,* as has been demonstrated by X-ray diffraction. The needle-shaped crystals are distributed in distinct spots of irregular outline; they lie parallel to the plane of the organic sheets and generally parallel to one another (Figure *43*) (cf. also ADAM & REES, 1966: plt. 8).

It should finally be emphasized that the intricate system of organic sheets and threads interspersed with the calcareous structures is only partly chitinous. JEUNIAUX (1963) has analysed the shell of *Sepia officinalis* and found that 4.4% of the chambered zone is organic. Of this portion, only about ¼ is chitin. This author also indicates that the dorsal shield contains chitin (cf. also RUDALL & KENCHINGTON, 1973).

Morphology and Structure of the Shell in *Spirula*

The shell of *Spirula* was first described in detail by APPELLÖF (1893) and more recently by MUTVEI (1964a) and DAUPHIN (1976). Scanning electron microscopy was used for the study of the septal layers by MUTVEI (1970), ERBEN (1974) and DAUPHIN (*op. cit.*). Our description is therefore largely restricted to features of the *Spirula* shell that were not described or mentioned in the earlier literature.

The initial chamber of the shell is almost spherical. It is only slightly higher (0.7 mm) than wide (0.5 - 0.7 mm). Nearly the entire wall consists of a single layer, since nodular deposits of the outer layer are sparse and are entirely restricted to the anterior dorsal side of the initial chamber. The wall of this chamber has a thickness of 10 μm; it consists of a very regular prismatic layer (Figures *64, 66*). The needle-shaped crystallites composing this layer are arranged parallel to each other and vertical to the inner and outer surfaces of the chamber. In the prismatic layer one can distinguish an outer and an inner part (not including the spherulitic-prismatic structure of the dorsal layer, which is added later). The outer layer is rich in organic material, whereas the inner layer shows fewer organic deposits. The surfaces of the inner and outer layer are devoid of any organic cover.

The apertural end of the first chamber is 0.38 mm wide; it shows a strong constriction which is much more pronounced than the constrictions between later chambers. The apertural lumen is taken up entirely by the siphuncular tube; the latter is inserted on the inner walls of the constriction and extends into the lumen of the chamber (Figures *64, 66*). The end of the siphuncular tube is continuous with an organic sheet that is fixed to the opposite wall of the chamber (Figures *64, 65*). Between the prismatic outer wall of the constriction and the insertion of the siphuncular tube lies a prismatic ridge (Figures *66, 73*). The side of this ridge that slopes into the first chamber shows more organic material in its fabric than the opposite side. Crystallites are arranged vertically to the surface of the ridge. From inside the first chamber, this ridge appears as a low rim that surrounds the siphuncular tube, from which it is separated by a deep, narrow depression (Figure *64*). From the constriction (aperture of the first chamber), the siphuncular tube decreases apically in diameter until it reaches its extended tubular end which is about 0.1 mm wide.

The pear-shaped siphuncular tube is inclined towards the ventral side of the chamber. The calcified portion extends into the chamber for about 0.3 mm. It is composed of lamellar layers in the structure of the septa that are formed later on (Figures *66, 73*). This tube is generally closed by a hemispherical cap that is continuous with the organic sheet mentioned above. The longitudinal wrinkles of the sheet continue into wrinkles that cross the organic cap in a radial orientation (Figures 5, *65*).

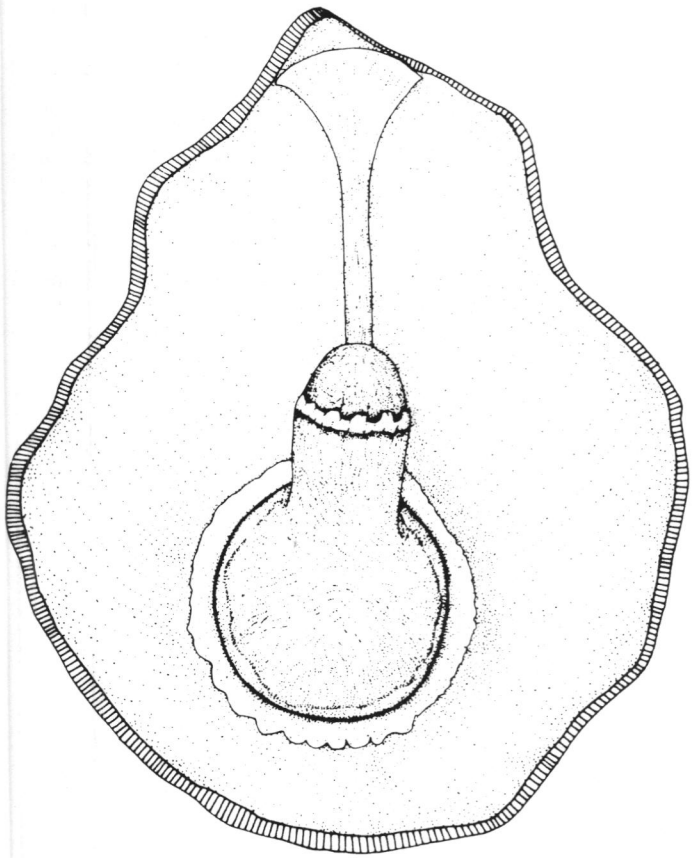


Figure 5

The first chamber of the *Spirula* shell opened up, exposing the earliest part of the siphuncular tube with its wrinkled surface, and its attachment to the inner wall via the organic sheet that is roughly spatulate. The site of attachment presumably marks the protoconch area

The organic cap may continue into the mineralized portion of the siphuncular tube without any break in its outline. But it may be altered in its structure when it has collapsed and the resulting shape has been fixed by the addition of organic material (Figure *87*). More commonly, however, the diameter of the cap is slightly smaller than the diameter of the uppermost part of the calcareous tube, so that the transition from one structure to the other is marked by an edge (Figures *64, 66*). The wrinkles mentioned before cross this edge and continue in the outer organic cover of the calcareous siphuncular tube.

After the primary siphuncular tube of the first chamber (which is devoid of a septum) is completed, the normal growth of the shell begins with the formation of septa and the siphuncular tube. In contrast to later sections, however, in the second section of the siphuncular tube the pillar zone is extremely short (Figures *66, 68*).

The outer wall of the *Spirula* shell consists of 3 layers, except for the first chamber (Figure *67*). The outermost, dorsal layer may or may not be continuous on the dorsal side; it has a sculpture of nodular structures. On the ventral side, the dorsal layer is continuous; it consists of prismatic to spherulitic needle-like crystallites. The main portion of the shell wall is represented by the central layer (Figure *67*). In polished sections viewed under the light microscope, it appears to consist of a prismatic structure with many parallel organic lamellae that cross the prisms, parallel to the inner and outer surfaces of the shell (cf. MUTVEI, 1964a: plts. 17, 18, fig. 2). In fractures, this layer shows a construction of small granular to brick-like crystallites that are not arranged in vertical needles; they present a slightly irregular arrangement in lamellae that lie parallel to the growth face (Figure *72*). Single crystallites are about 0.4 μm long and 0.2 μm high; they are always enveloped by organic shell material. The construction of the central layer very much resembles

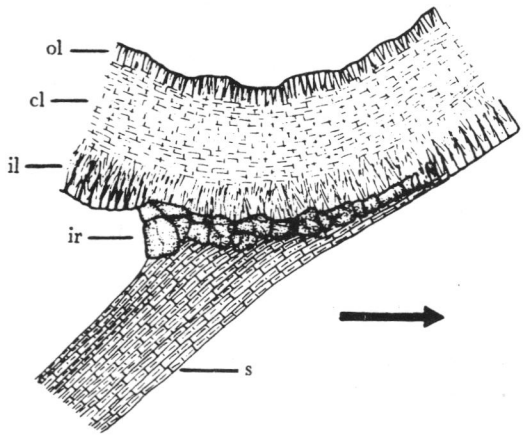


Figure 6

A longitudinal section through the outer wall of the *Spirula* shell, at the insertion of a septum (s) on the coarsely prismatic inner rim (ir). The outer shell wall consists of the outer, irregularly prismatic layer (ol), the central, lamellar layer (cl), and the inner, prismatic layer (il). The arrow points towards the aperture

that of the columnar siphuncular pillars (cf. below). The central layer forms more than half of the outer wall. It grades into an inner prismatic layer of somewhat variable thickness (Figure *67*). This change is characterized by the arrangement of the crystallites in vertical needles of continuously increasing width towards the inner surface of the chamber. In tangential sections, these needles show up as a polygonal network (cf. MUTVEI, 1964a: plt. 18, fig. 2).

Where the septa are inserted on the outer wall of the shell, the inner layer shows a particularly coarse prismatic structure (Figure 6). It forms an inner rim that reinforces the constriction of the chamber aperture (Figure *88*).

The septum consists of a lamellar needle layer. At the insertion on the outer wall, the inner side differs in its structure from the outer side. The inner side (facing the newly closed chamber) is marked by an abrupt ending of the lamellar needle layers (Figures 6; *88*). They are inserted on the prismatic ring mentioned above, which is similar to the additional constriction formed at the aperture of the first chamber (Figures *66, 73*).

On the apertural side of the septum, lamellae extend further anteriorly, on the inner surface of the newly formed chamber ("living chamber") (Figure 6). The growth surfaces of the last-formed septal layers show a gradation from fine crystallites on the septum to coarse crystallites towards the inner prismatic layer of the wall that follows anteriorly. The outermost layer of the septum is lamellar, but the orientation and the composition of the needles are not as regular as in the layers next to it. The lamellae of the septum, from the first formed one to those lying under the last formed lamellae, consist of needle crystals that have a width of 0.2 μm and show the same orientation within a lamella. The thickness of each lamella is given by the width of the single layer of needle crystallites and their organic cover. Although the orientation of crystals may change from one lamella to another, one sometimes finds series of lamellae in which the crystals

Explanation of Figures *44* to *59*

Figure *44*: A section through the dorsal layer of the cuttlebone of *Sepia orbignyana* showing the irregular spherulitic structure × 335

Figure *45*: A detail of Figure *74* showing the first mineral deposits on the dorsal side of the embryonic shell cap of *Sepia officinalis* that raise the contrast of radial sculpture and transversal growth lines × 140

Figure *46*: Section through the cuttlebone of *Sepia orbignyana* shows the transition from the lamellar structure of the calcified portion of the central layer to the columnar base of the prismatic inner layer × 1 800

Figure *47*: Detail of Figure *46* shows the rapid transition from the central layer (lower part of figure) to the inner layer × 4 600

Figure *48*: Calcified central layer of *Sepia officinalis*, broken nearly parallel to lamellation, demonstrates the composition by rod-like crystallites × 4 700

Figure *49*: A feather-like arrangement is present in the central layer of the *Sepia orbignyana* shell, broken parallel to the growth surface, near the outer side of the shield and close to the transition into the inner layer. The needles are loosely spaced × 4 000

Figure *50*: Crystallites of the central layer in almost the same position as in Figure *49*, forming whorls that unite in columnar structures and form the base of the coarse prisms of the inner layer × 4 000

Figure *51*: The central layer of *Sepia officinalis*, broken nearly parallel to the lamellation, demonstrates its composition of small rod-like crystallites composed of small basal units of 0.1 to 0.2 μm in size × 5 000

Figure *52*: Growth surface of the lower portion of the inner layer in the shell of *Sepia orbignyana*, near the inner rim of the dorsal shield, with well-developed faces × 4 500

Figure *53*: The inner layer of the cuttlebone of *Sepia orbignyana* showing below the dorsal coarse, prismatic portion and above the ventral finer, spherulitic-prismatic portion × 930

Figure *54*: A section through the fork layer (right) and the inner layer (left) of the shell of *Sepia orbignyana* shows the sharp border between both layers and cavities separating them, bridged only by organic sheets × 270

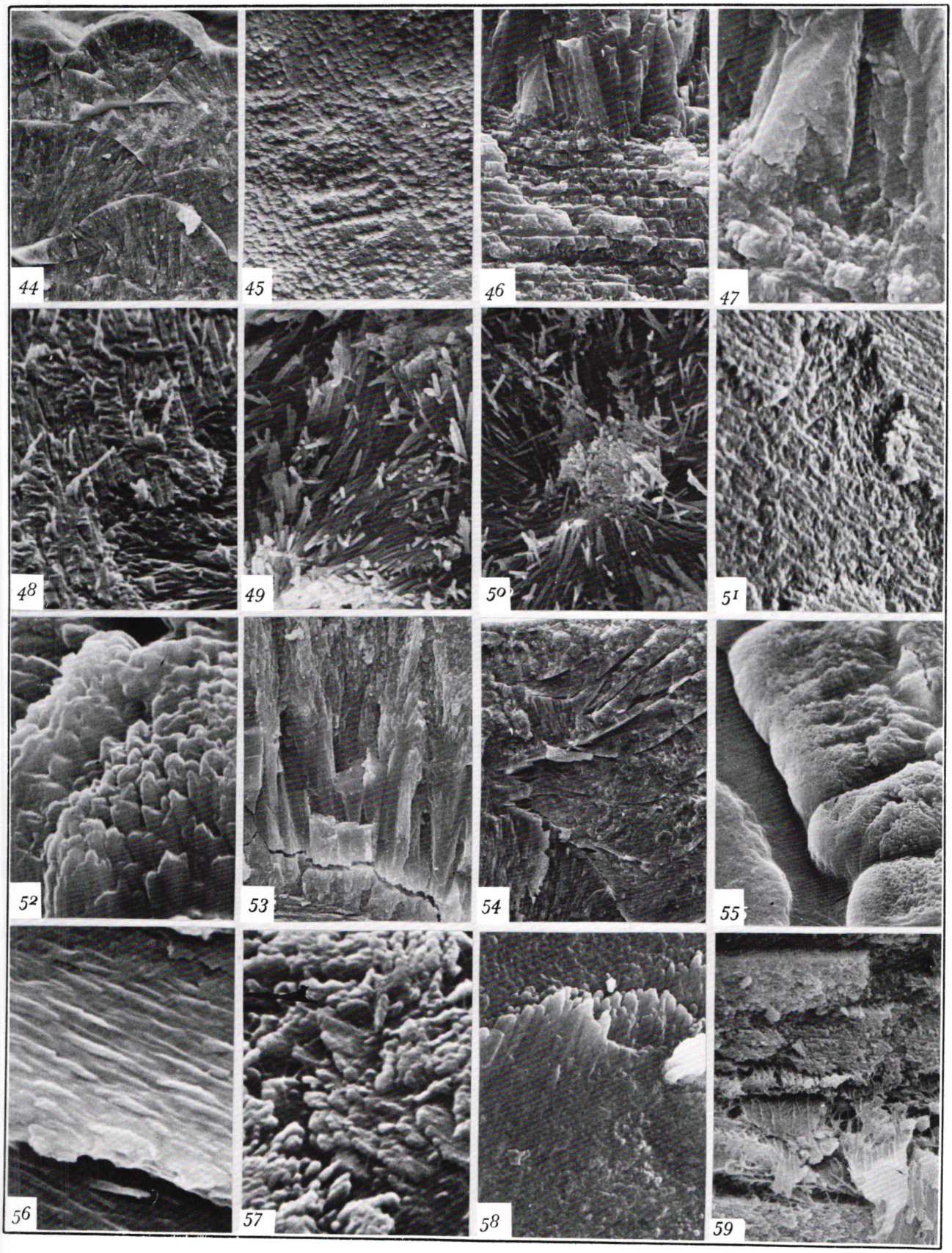
Figure *55*: The boundary between the inner layer (right) and the fork layer (left) in the shell of *Sepia orbignyana* is formed by a deep groove on the growth surface × 880

Figure *56*: The fork layer of *Sepia gibba* is composed of needle-like crystallites surrounded by and intergrown with organic material. Needles of each individual layer show the same orientation, but this orientation may differ among layers × 4 500

Figure *57*: Detail of Figure *84* shows the orientation of the first crystal growth near the spine of *Sepia pharaonis*, in an animal ready to hatch. Crystal needles have the same orientation within each aggregate, but are not yet oriented as regularly as within the lamellar structure of the spine itself × 7 800

Figure *58*: The growth surface on the central portion of the fork of *Sepia orbignyana* shows flattened, rod-like needles encroaching upon its surface × 3 800

Figure *59*: The fork layer of *Sepia officinalis* in a fracture shows the fine lamellation formed by 0.2 μm thick layers composed of crystal needles and organic sheets. Some thicker organic sheets extend over the fracture and are hanging down from it × 2 300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are all oriented identically. They are always well separated by organic material (Figure *62*).

On the apertural surface of the septum, the lamellar crystal growth is more dendritic (Figure *60*). Single lamellae tend to split into 2 lamellae by dendritic branching of crystal needles, with the thickness of needles remaining similar (0.2 μm) to that observed in deeper layers. Towards the periphery of the septum (apertural side), needles and rods or prisms are oriented along 2 to several axes (Figure *61*). The crystallites then become much wider and the crystalline faces become clearly visible. Further away from the septum, the size of crystals may remain unchanged, but lamellation completely disappears and is replaced by a vertical arrangement of needles. The orientation of growth increments on the crystal heads in 2 or several directions gradually disappears until a random pattern is established in the normal inner prismatic layer.

The layers of the septum are continuous with the siphuncular tube. The structure of the lamellar needle layer of the outer wall of the tube, which we find already in its blind end (Figure *66*), continues through the chamber. Only below the septal neck of the following septum, the lamellar layer splits into 4 parts (Figure 7). The outermost layer is continuous with organic sheets. In newly formed, closed chambers, these organic sheets form a layer that closes the entrance to the space in which the pillars lie (Figure *69*). These pillars are rooted on the inner side of the siphuncular tube, the foremost (apertural) ones being set on the concave surface of the septum where it turns into the septal neck. Their growth ends with the formation of the posterior part of a new siphuncular tube (Figures *68, 70*).

Organic sheets extended between the pillars are found only in the apertural part of the pillar zone (Figure 7).

From the innermost lamellar layer of the last formed siphuncular tube, pillars always grow up without any

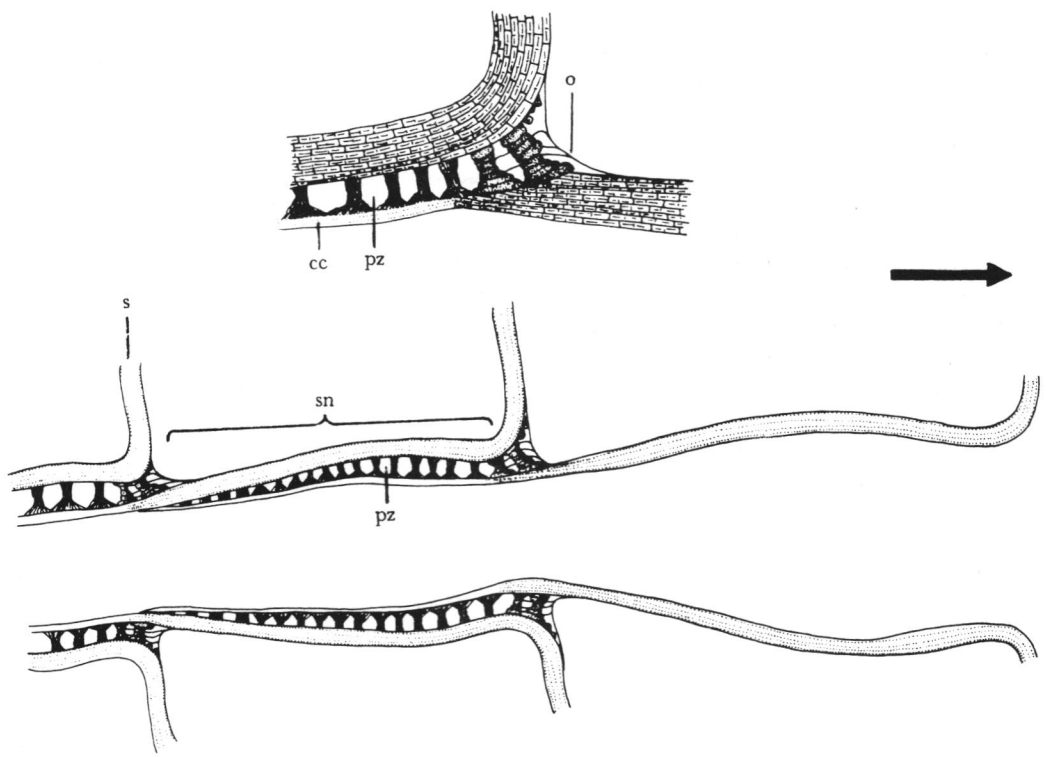


Figure 7

A horizontal section through the siphuncular tube of *Spirula*. The layers of the septum (s) continue into the siphuncular tube. At the entrance to the septal neck of the next older septum, the lamellar layers split into organic sheets (o) that shut up the pillar zone (pz), organic sheets and pillars of the entrance, pillars of the middle pillar zone, and finally into the organic and crystal cover (cc) of the pillar zone. The arrow points towards the aperture.

separation by organic sheets. The shape of the pillars varies, however, according to their respective position along the siphuncular tube.

In the anterior zone, they form solid structures of biconical appearance with terrace-like annulations; they are broadly rooted on the bend of the septal neck and on the apertural part of the siphuncular tube (Figure *70*). In front of complete pillars, small mounds and half-grown pillars with the typical annulations are found. The foremost complete pillars show only 10 to 15 annulations or growth zones, each of which is the continuation of one lamella of the anterior part of the tube. In this part, the lamellae may measure up to 15 μm in thickness. The free space between pillars is about equal to the diameter of these. On the inner siphuncular tube (the "floor"), the ends of the pillars fuse to form a solid, non-porous tube (in the anterior-most part of the pillar zone only!) (Figure *71*).

Further inward, pillars have a more columnar shape and smooth surface, on which 50 to 60 lamellar layers can be counted (Figure *86*). These are continuous with lamellae of the central part of the anterior siphuncular tube. Spaces between pillars are much wider than in the anterior pillar zone; they correspond to twice the diameter of a pillar. The lamellar structure of the base of columnar pillars is similar to the central layer of the outer shell wall. It consists of short needle- or brick-shaped crystals that are arranged vertically to the axis of the pillar (Figure *63*). In contrast to the pillars of the anterior pillar zone, the pillar ends do not form a solid cover of the inner siphuncular tube. Instead, the radiating crystals and crystal aggregations form an interlocking system with many small spaces (Figure *71*). In these, numerous thin organic sheets are transversally suspended. This porous layer of interlocking needle aggregations is covered by a smooth, thick organic sheet. The fourth (inner) section of the lamellar layer of the anterior tube is continuous with this uncalcified layer; it forms the innermost part of the shell that is in direct contact with the living tissue of the siphuncle. The transition from the calcified lamellae of the anterior tube to the organic inner layer is quite abrupt (Figure 7).

Further inward, towards the end of the inner tube, pillars are very short and drum-shaped; the interlocking crystal aggregations are growing into the spaces between the pillars.

A section through a double-walled siphuncular tube thus shows 4 layers: an outer lamellar layer, the pillars, the interlocking needle aggregations, and the inner organic layer. The anterior tube of the last septum shows a single wall that consists of the lamellar layer only. The 3 inner layers are added when the next septum is formed (Figure 7). It should be noted here that the inner organic layer is destroyed by acids, whereas the organic components of the layers with lamellar structure are resistant.

The formation of a new septum and siphuncular tube section can be figured in the following way. First of all, the animal has to begin the outer wall of the new chamber (Denton & Gilpin-Brown, 1971). Then an annular prismatic ridge is formed at the border between the old and the new chamber, so that the constriction between them is reinforced. Meanwhile the tissue of the siphuncle must have grown to a sufficient density to be stretched over the length of one chamber. When the living tissue withdraws from the old chamber, it produces organic sheets that cover the wall left behind. When all the tissue has withdrawn, these organic sheets entirely cover the walls of the liquid-filled space. Now calcification begins, from the septal rim all through the septal neck into the siphuncular tube of the older chamber. The organic covers of the septum and the anterior siphuncular tube are integrated into the first lamellar layers, so that they can no longer be traced. As we have said above, free organic sheets will cover only the outer walls and the apertural side of the (old) septum and close the aperture between the anterior and posterior part of the siphuncular tube when the tissue withdraws from the "living chamber." Apparently the first lamellar layers deposited on the rear flanks of the prismatic ridge are secreted at the same time as the lamellar layers in the anterior part of the tube, the thick layers of the anterior pillar zone (with intercalated organic sheets), and the thin lamellae at the base of the pillars in the middle zone. The medial parts of the septum and the anterior tube form along with the upper portions of the anterior pillars that are fused into a calcareous ring, and with the upper portions of the medial pillars. The growth of the last septum layers is connected with the formation of the irregularly prismatic, porous layer and the first layers of the organic, soluble inner sheet. The septum is now completed, whereas in the siphuncular tube calcareous and organic lamellae continue to form at lower rates of growth until a new chamber is added. With the completion of the septum, the newly closed chamber can be used as a buoyancy element.

Development of the Shell Complex in *Sepia*

The early development of the shell complex in *Sepia* embryos has briefly been described by Appellöf (1893), and observations made by earlier authors are discussed there. A handicap of all these descriptions was the lack of a comprehensive staging system. The relation between morphogenetic processes and differentiation in different

parts of the embryo does not clearly show up, therefore. It was a great advance when NAEF (1923, 1928) introduced a good system of embryonic stages (stages I to XX). On his figures one can follow a great part of the complex morphogenetic processes by which an originally thin cap of embryonic tissue (the typical blastodisc of the telolecithal cephalopod egg) takes the form of a compact organism. In the center of this embryonic cap, the future shell epithelium can be made out at very early stages. It is surrounded by an annular thickening, which is the rudiment of the muscular mantle.

In all decapod cephalopods (cuttlefish and squids) so far studied, the subsequent process of the closure of the shell sac rudiment is essentially identical. First an annular ridge forms at the periphery of the mantle epithelium. This ridge then becomes a fold which grows centripetally over the mantle epithelium. The central opening of this mantle fold becomes slightly cornered, with one anterior (dorsal) and 2 lateral angles, before the shell sac is closed. The "scars" of the anterior and lateral angles will later differentiate into the so-called organ of Hoyle, which acts as a hatching gland.

As soon as the shell sac is closed, at stage XI of Naef, formation of the embryonic shell begins. The histological aspects and the general differentiation of the early mantle epithelium and of the closed shell sac have recently been described by SPIESS (1972). His description ends with stage XVI when histological differentiation is still in progress. Some complementary remarks must therefore be made for the later embryonic stages, and in some respects also for the earlier stages described by this author. The following brief description is based entirely on our own observations.

At the earliest stage of shell sac formation (stage VIII of Naef), the flat shell epithelium is characterized by roughly columnar cells, which are much higher in the anterior and middle parts of the epithelium as compared to the posterior part. The nuclei lie closer to the cell base, so that the apical parts of the cells together form a "plasma border"; the latter contains numerous vacuoles. In the middle and anterior parts of the epithelium, the nuclei of the closely packed cells lie at different levels, so that in these parts of the epithelium 2 or 3 layers of cells would seem to exist. However, there is no horizontal stratification of the cells. This primary shell epithelium is clearly separated from the underlying mesoderm.

The further development of the annular ridge, which already takes the shape of a fold by stage VIII, leads to the formation of the so-called "secondary shell-epithelium," which will be the "ceiling" of the shell sac. From histological sections, it appears that this lower (inner) cover of the closing fold is stretched during the closure of the shell sac. Mitotic figures observed close to the opening show, however, that cell division continues also in this inner part of the fold. This means that the secondary shell epithelium forms *in situ* from ectodermic cells given off by the parts derived from the early annular ridge. This secondary shell epithelium remains thin, with its flattened nuclei widely separated. Its thickness gradually increases towards the periphery of the shell sac, *i. e.*, in the zone of transition to the primary epithelium.

By stage IX, the greater part of the primary shell epithelium is covered by the closing fold (the latter is filled with mesodermic cells that form a rather loose "mesenchyme"). Complete closure of the shell sac occurs between stages X and XI. The closing pore lies in the posterior part of the sac, about $\frac{1}{3}$ of its length from the posterior end.

The closure of the shell sac is coordinated with the general contraction of the embryo cap. The organ rudiments which had been lying in the single plane of the thin cap are now "assembled" in a three-dimensional arrangement, which largely corresponds to the definitive organisation of the animal. During these stages of assembly, the primary eye vesicles and the statocysts close, the stomodaeum is invaginated, and the funnel and mantle margins rise as distinct folds. The mantle complex thus takes its cup shape. The shell sac, which occupies the dorsal part of the mantle complex, remains comparatively flat, but its posterior and lateral parts are slightly bent to the ventral side.

The initial shell or protoconch, which is secreted immediately after closure of the shell sac, is roughly spoon-shaped, with its deepest part near the posterior end. It is a simple organic membrane, without any growth lines, about 0.9 mm long and 0.7 mm wide. During the following stages, organic material is added to the margins of this protoconch. The outline of the shell becomes ovoid. Along with the deposition of more organic shell material, the posterior slope of the embryonic shell becomes steeper, until an angle of about 90° with the plane of the anterior dorsal shield is attained. Around stage XII, the posterior edge bends and grows outwards into a brim. This differentiation of the shell sac is related to the differentiation of the mantle muscle (on the ventral side), as the latter is inserted on the lower face of this brim (Figure 15).

Up to stage XI, the shell sac shows no signs of any local excrescence or folding of either one of the epithelia. Around stage XII, however, both the primary and secondary epithelium present such phenomena.

In the posterior part of the shell sac, the secondary epithelium forms a lateral diverticulum on either side, in contact with the base of the fin rudiment. This process has been mentioned briefly by NAEF (1922). The lateral

pockets thus formed (Figure 10) correspond to those described in the sepiolid *Rossia macrosoma* (BOLETZKY & BOLETZKY, 1973), where the uncalcified shell is extremely reduced. Thus, independently of the presence or absence of a shell in the area of the fin base, the fin cartilage forms on the outer wall of these pockets.

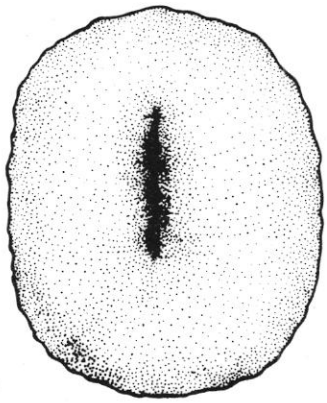


Figure 8

The early embryonic shell (protoconch) of *Sepia officinalis* often shows a ventral ridge or fold that is formed by the longitudinal fold of the primary shell epithelium shown in Figure 95

The primary epithelium forms a groove that can become a very distinct deep and narrow slit, in which a ridge-like fold of the embryonic shell is anchored (Figure 8). It is not yet clear whether this acute form of epithelial depression does not occur in all embryos, or whether it is a short-lasting phenomenon that may occur sometime between stages XII and XIII; in fact, only part of the embryos preserved at these stages present a deep groove. Except for a very small infolding of the siphuncular epithelium, in which a tiny lobe of the first septum was anchored, this sort of groove has not been found after stage XIII.

From stage XII to stage XIV, the mantle and the shell sac grow considerably, and the primary shell epithelium becomes thinner. If large parts of it have appeared 2- to 3-layered at stage XII, most of it is clearly monolayered epithelium by stage XIV. It is conceivable that this rapid stretching is partly prepared by the formation of a groove that would represent a "surface reservoir." The apparent irregularity of this process is not so surprising if one considers the range of variation in the other

Explanation of Figures *60* to *73*

(Figures *60* to *73* are taken from *Spirula* shells)

Figure *60*: View onto the apertural surface of the septum demonstrates lamellar crystal growth with somewhat dendritic components × 8 800

Figure *61*: Septum broken nearly parallel to the growth surface shows the lamellar layers composed of needles arranged parallel to each other within each layer, but not so in neighboring layers × 1 500

Figure *62*: View onto the peripheral portion of the apertural side of the septum shows continuously coarser crystals transitional between the lamellar structure and the prismatic structure × 3 900

Figure *63*: Detail of Figure *86* showing the short brick-like crystals that are arranged vertical to the axis of the pillars which they compose × 9 500

Figure *64*: The opened initial chamber of the shell showing the blind end of the siphuncular tube that continues as an organic sheet fixed to the opposite wall of the chamber × 108

Figure *65*: The end of the siphuncular tube with its organic cap that continues as an organic sheet × 1000

Figure *66*: The end of the siphuncular tube comprises the organic cap and the calcified portion with lamellar structure (detail in Figure *73*). The tube is fixed to the outer prismatic shell wall in the constriction between the first and second chamber by a prismatic ridge (central right side). The inner tube of the siphuncle is filled with a secondary organic deposit × 320

Figure *67*: Section through the outer shell wall with the outer prismatic layer on the lower side of the picture and the coarse inner prismatic layer at the upper side. The lamellar central layer forms the bulk of the shell × 550

Figure *68*: The entrance into the pillar zone of the second portion of the siphuncular tube extending into the blind initial section of the siphuncular tube already shows the typical apertural pillars, as at later stages. The length of this first pillar zone is much smaller than in later sections, however × 350

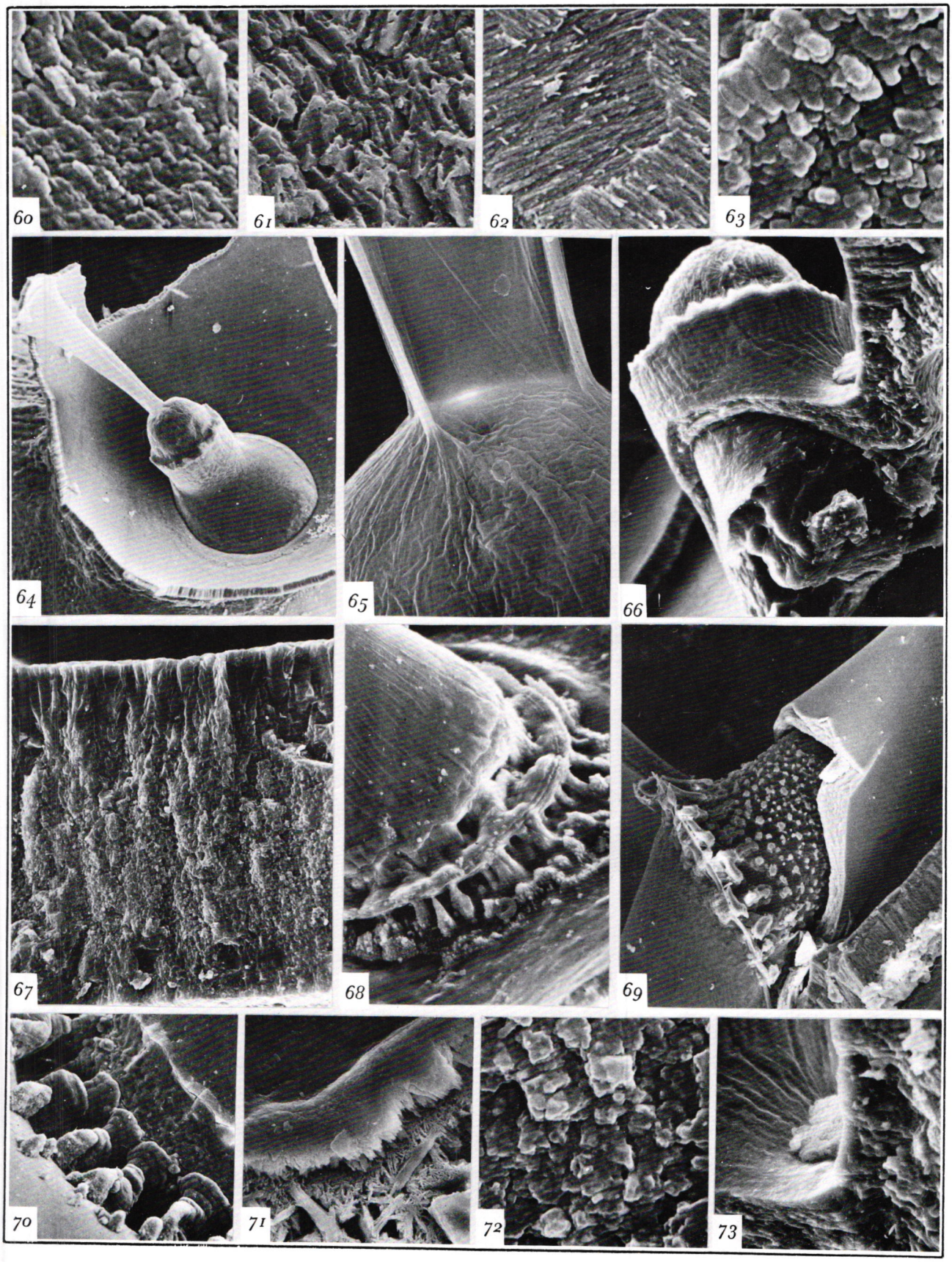
Figure *69*: A septum with its septal neck is broken to show the continuity of the siphuncular tube extending into it. At the entrance into the pillar zone, organic sheets are seen that had separated the liquid held in the pillar zone from that present in the chamber × 130

Figure *70*: The entrance to the pillar zone is formed by biconical solid pillars with 10 to 15 annulations; note free space between them. Their end on the inner siphuncular tube is fused to form a solid layer (see Figure *71*). × 175

Figure *71*: The inner side of the inner siphuncular tube below the entrance to the pillar zone shows the solidly fused pillar ends against the (apically) following porous crystal cover of the pillar zone × 1 500

Figure *72*: Detail of Figure *67* showing the central portion of the outer shell wall that is composed of small brick-like basal units in a lamellar structure × 4 500

Figure *73*: Detail of Figure *66* demonstrates the lamellar structure of the end of the siphuncular tube (left). The latter is attached to the prismatic outer wall (right) by a coarsely prismatic ridge × 660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morphogenetic processes going on in the embryo. All through these stages of later organogenesis, one will rarely find 2 embryos that are exactly identical in their morphological features.

The fast growth of the shell sac is reflected by the increments that are broadest in the anterior part of the shell; they measure between 0.03 and 0.08 mm each. In addition to these growth lines, there are several other morphological features that characterize the purely organic shell between stages XIII and XIV. In the posterior part (protoconch area), a longitudinal groove or a pair of grooves can be made out (Figure 9). The corresponding

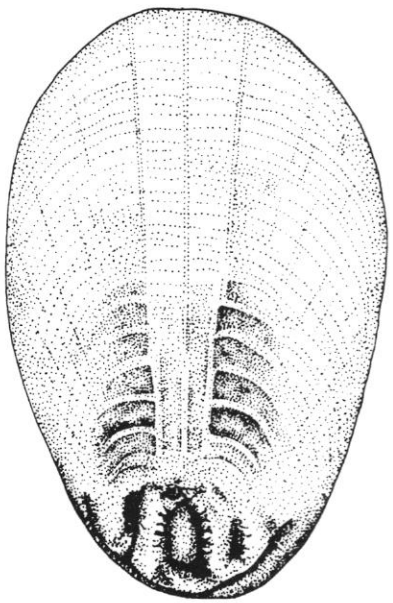


Figure 9

A later stage of shell formation in *Sepia officinalis*, before the onset of calcification. The protoconch area is marked by a depression that is surrounded by radial wrinkles. Along the growth lines, pairs of grooves appear, combined with radial ridges

form of the shell epithelium has apparently resulted from the folding and stretching processes mentioned above. The depressions in the shell are sometimes surrounded by radial wrinkles; occasionally there are also concentric wrinkles (Figure 9). On newly added portions of the organic shell, one finds radially arranged sculptural ridges. These become increasingly distinct with the growth of the organic dorsal shield. More laterally, one finds grooves that are generally forming pairs arranged along the growth lines. Seven to 11 such pairs of crescent-shaped depressions have been found on different embryonic shells just before the onset of calcification (Figure 74).

In the organic shell of embryonic *Sepia pharaonis*, these morphological features were not found. There is only a central depression or a pair of depressions in the protoconch, and at later stages the shell shows only growth lines.

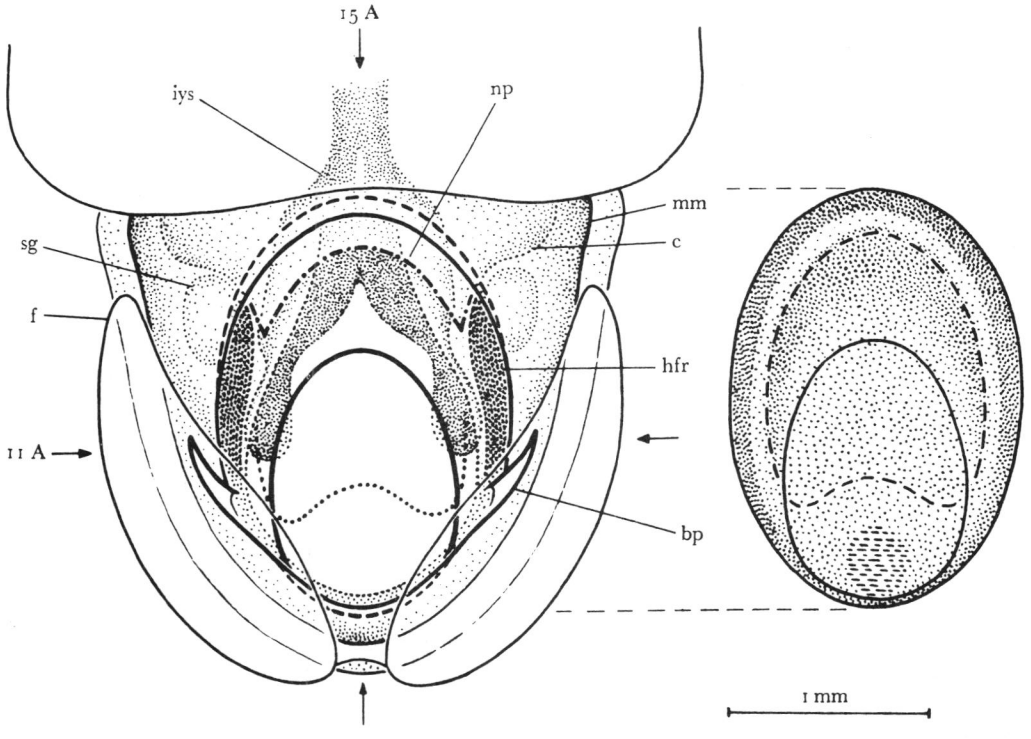
During the phase of mainly marginal shell growth described above, the marginal zone in the anterior and lateral parts of the primary epithelium is characterized by particularly high cells. This rim of columnar cells remains a typical feature of all the later stages of shell formation (Figures 11, 18).

Calcification of the embryonic shell begins between stages XIV and XV, when the shell has a length of about 2.3 mm. The first aragonitic layer is formed on the inner, ventral side of the shell, which it covers entirely except for a narrow marginal rim; the sculpture of the organic shell is thus fixed in its form. The radial ridges on the dorsal shell surface become distinct (Figure 45).

In its first phase, calcification is restricted to the ventral side of the dorsal shield, where a continuous layer of minute aragonite crystallites that form needle lamellae is deposited. This is the basement for the first pillars that are formed around stage XV.

In histological sections, the embryonic shell is usually distorted and often partly broken, and the closed shell sac balloons during fixation. It is often difficult therefore to relate shell structures to morphological and cytological features of the shell sac epithelium. However, the site of pillar formation is generally marked by a depression in the epithelium, so that it is easy to reconstruct the original position of the shell if it is well preserved. The question is whether these depressions reflect an actual cytological differentiation into "pillar forming cells," or whether they are insignificant or even artificial, the depressions being merely imprints of the pillars (preserved during the early part of fixation, before the shell sac expanded). Our observations suggest that these depressions are not significant for the mode of pillar formation. For these observations, it is crucial to have encountered optimal conditions of fixation, so that the shell structures are well preserved.

If the last-formed section of a chamber has, at least partly, been covered by an organic membrane when the animal was fixed, the "liquid" contents of the cavity next to the primary epithelium may be preserved in their original state. One then finds a stratification, which can only be made out by the refractive lines that mark the interfaces of the unstained layers (Figure 12). Thus there appears to be a stepwise secretion of the medium in which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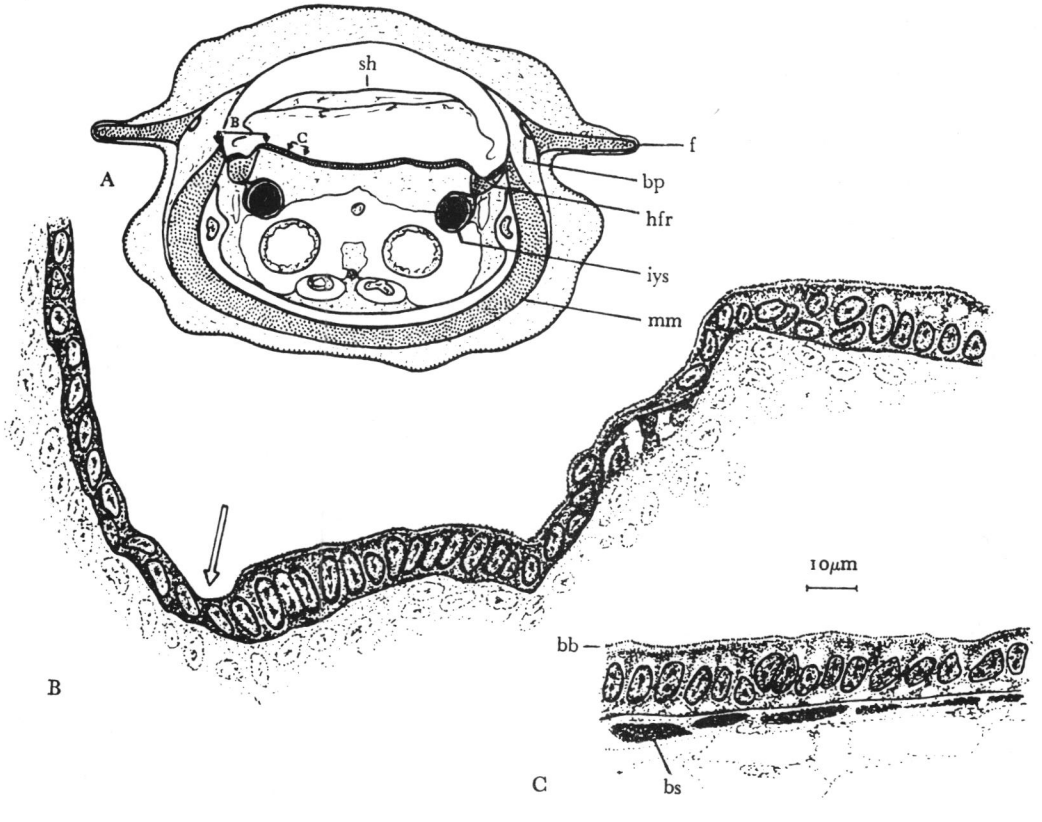
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Figure 10

An embryo of *Sepia officinalis* at stage XVI of Naef. The pallio-visceral complex is reconstructed from cross-sections. Of the visceral mass, only the inner yolk sac (iys) is represented as the lowermost organ visible in this dorsal view of the body. The uppermost parts are the fins (f) with their basal pockets (bp) that are a differentiation of the secondary shell sac epithelium. In the shell sac, the embryonic shell is represented with its first complete chamber (broad oval line, at left), and the posterior part of the outline of the second chamber in formation (dotted line, at left). The broken line with dots marks the depth of the nuchal pouch (np). The insertion of the head and funnel retractors on the marginal part of the primary shell epithelium is marked by dark stippling (hfr). The mantle muscle (mm), the funnel pouch or collar (c) and the stellate ganglia (sg) are also indicated. The pairs of arrows indicate the plane of the section presented in Figure 11A and in Figure 15A. At right, the shell is represented with its first chamber and the outline of the second chamber in formation (broken line). The adhering primary epithelium is represented by the stippling, darker parts marking high columnar cells, lighter parts lower cells. The oval dashed field in the posterior part indicates the typical siphuncular epithelium

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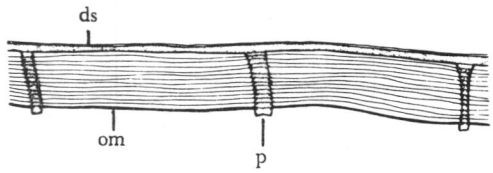

Figure 12

A schematic presentation interpreting the aspect of chamber contents as shown in Figures 96 and 97. When the animal was fixed, the shell epithelium had secreted what appears to be an organic membrane (om) and one or two gelatinous layers that have not been preserved except where crystallisation of pillar layers (p) had taken place. Between the dorsal shield (ds), in this particular situation (or an organic membrane or a septum, in general), and the organic membrane (om), the presumably gelatinous contents of the chamber are preserved and exhibit a periodical striation that is particularly distinct in the vicinity of the pillars (cf. Figures 96 and 97) where it appears to match the sequence of "nodular" thickenings.

the pillar lamellae crystallize, layer after layer. The fact that the unmineralized part of these layers is preserved suggests that they are gelatinous for some time after they have been secreted by the epithelium. These observations support the hypothesis put forward by BANDEL (1977a), according to which nacreous and lamellar layers form via a gelatinous phase.

On the solid, mineralized basement covering the ventral side of the embryonic shell, the first round pillars are set. Throughout their growth, only the uppermost portion shows mineral apposition, whereas further towards the base, they do not grow in thickness. These pillars are randomly distributed on the chamber ceiling, only the foremost ones forming walls that are radially arranged (Figure 75). Pillars are most densely set in the presumptive siphuncular area; they remain columnar in this posterior part of the chamber, whereas more anteriorly situated ones change in form during their growth. The latter measure about 10 μm in diameter; they have transversal bands, and their growth face has a central groove (Figure 77).

In the first chamber of *Sepia orbignyana* and *S. elegans*, the pillars are similar to those of *S. officinalis*. In *S. pharaonis*, however, the pillar base is often branched. The middle part of these pillars is again columnar (Figure 78). Between these growing pillars, the chamber roof is usually covered by a thin organic sheet; such sheets may also be extended vertically between pillars (Figure 79), so that the first chamber already has a labyrinth-like partition.

When the pillars have grown to a length of about 15 to 30 μm, an additional organic sheet is formed; it is extended horizontally between the pillars. When viewed from the ventral side of the shell, such a sheet may cover most of the chamber, except the marginal parts (Figure 76). In the first chamber, 4 to 7 organic sheets may be formed in the central part, in *Sepia officinalis* as well as in *S. pharaonis* (Figure 80).

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Figure 11

Cross section from the embryo shown in Figure 10 (camera lucida drawings). A: an entire section of the posterior part of the body (cf. arrows in Figure 10). The vertical expansion of the shell sac with the shell is an artifact. The arrow lines B and C mark the parts represented in the detail views (other abbreviations as in Figure 10). B: marginal part of the shell sac. The arrow indicates the limit between the secondary (left) and the primary shell epithelium (right). Note the similar height, but different aspect of cells in the marginal zone forming the organic rim and the middle zone (C and right part of B) where chambers are formed. C: the chamber forming epithelium shows a particularly distinct brush border (bb) with very long microvilli. Under this epithelium lie extensive blood spaces (bs)

Towards their apices (in the direction of growth, *i. e.*, towards the ventral end), the pillars change from their initial columnar form to a more wall-like structure. Near the chamber floor, these flattened pillars branch and form crenelations which may come close to those of neighbouring pillars. Thus the chamber floor shows a characteristic pattern of meandering lines (Figure *80*).

The siphuncular zone of the first chamber is a small oval field (Figure *76*). It differs from the anterior part of the chamber only by a denser distribution of pillars. The siphuncular area of every following chamber is a crescent-shaped band that shows the characteristic structure known of the cuttlebone (Figure *83*).

The tendency of the pillars to form wall-like structures increases throughout the growth of the shell, but even in adult specimens, one always finds some columnar pillars within the actual chambers; in the siphuncular area, this remains the typical pillar form in *Sepia officinalis, S. orbignyana* and *S. elegans*. In *S. pharaonis,* wall-like and columnar pillars may alternate in one and the same meandering row of pillars.

The height of the embryonic chambers measures from 0.5 to 0.8 mm; this corresponds to the chamber height of juvenile individuals. The first chamber formed after hatching is often markedly lower, however.

The first calcification of the dorsal shell surface appears only after a few chambers are formed, *i. e.*, around stage XVIII. Aragonite crystallites of about 0.5 μm appear in a random pattern on the surface of the dorsal shield (Figure *37*). Prominent sculptural elements, such as radial ridges, wrinkles and growth lines, are covered first. Thus they become very distinct for some time (Figure *45*). Later on they will be covered by newly-added aragonitic layers.

The crystallites of this initial dorsal cover do not show any particular orientation. They grow along with the formation of interspersed organic fibers (Figure *37*). Except for the margin of the dorsal shield, the entire dorsal surface is rapidly covered by a continuous layer of aragonite crystals; the spherulitic arrangement typical of this dorsal layer is soon established.

In *Sepia officinalis,* the posterior region of the embryonic dorsal shield is first covered by the same initial layer as the anterior part. The earliest traces of a spine appear only towards the end of embryonic life. The first crystallites that build the rudimentary spine are aragonite aggregations very similar to those forming the embryonic dorsal layer (Figure *39*). The spine forms behind the protoconch grooves that can still be made out through the mineral cover.

Explanation of Figures *74* to *85*

Figure *74*: The embryonic shell of *Sepia officinalis* before calcification shows a central depression in the area of the protoconch. Following the initial shell cap radial sculpture elements and paired grooves situated in the growth lines have been formed when the shell was still free of mineral deposits. First mineral deposits (detail in Figure *45*) fix these morphological features × 28

Figure *75*: Ventral side of embryonic shell of *Sepia officinalis* during formation of the first chamber shows the random pattern of the pillar insertion, with exception of the radially arranged foremost ones that form walls × 42

Figure *76*: The central portions of the embryonic shell of *Sepia officinalis,* towards the end of formation of the second chamber, is covered by a continuous organic sheet of the last intracameral lamella. The siphuncular zone of the first chamber shows up as a small oval field × 30

Figure *77*: Pillars in the central portion of the first chamber in *Sepia officinalis* show a growth face with a central groove. They project over an organic sheet suspended between them and are connected by vertical organic sheets × 730

Figure *78*: The pillars of the first chamber of *Sepia pharaonis* show a branching base turning into a columnar shape. The annulations of the pillar are clearly visible × 1 200

Figure *79*: Pillars of the first chamber of *Sepia pharaonis* connected by a vertical organic sheet × 1 300

Figure *80*: The round and the wall-like pillars of the first chamber of *Sepia officinalis* are crenelated near their ends in the septum. Organic sheets form 4 floors. The first septum has not yet been secreted × 130

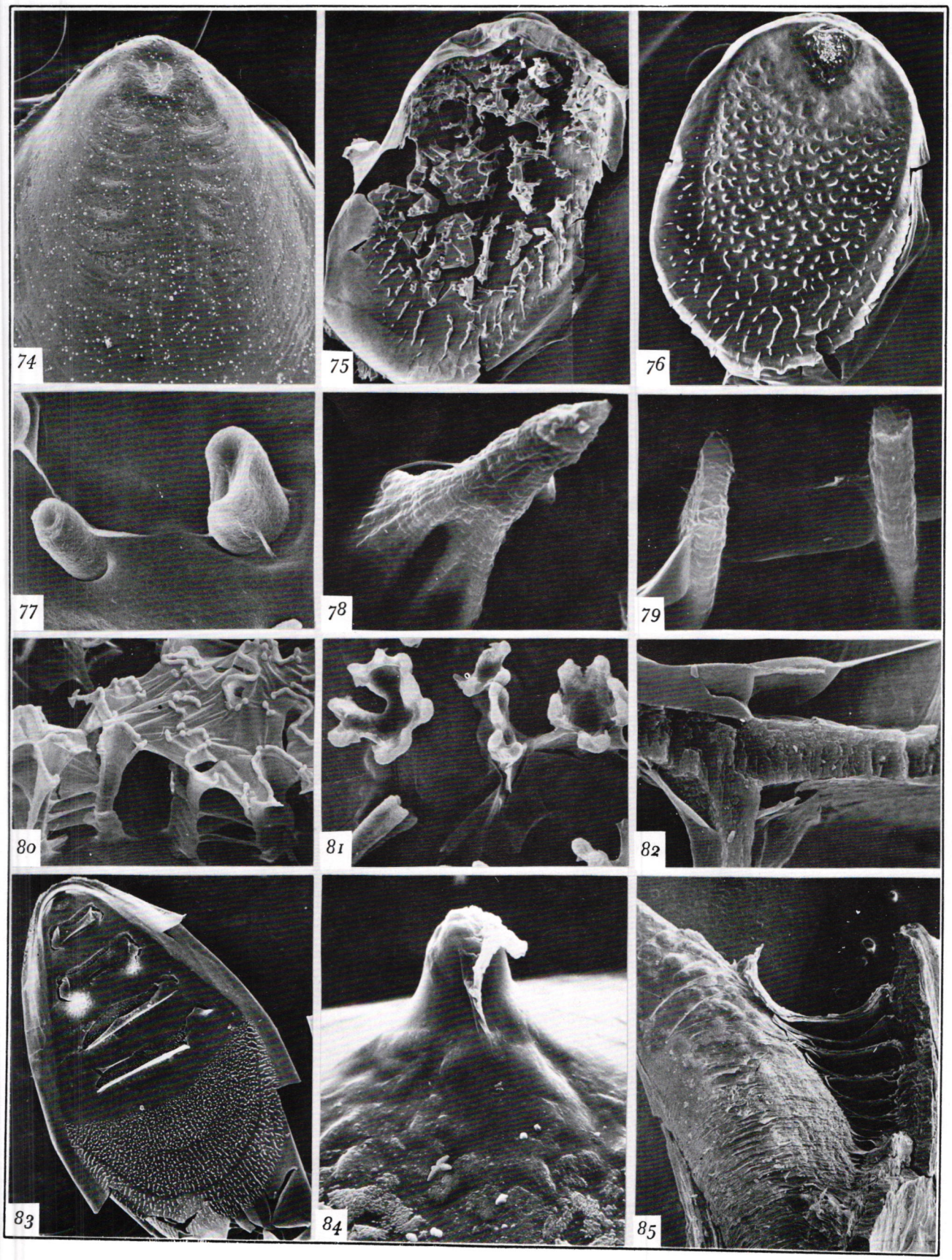
Figure *81*: Round pillars at the first chamber of *Sepia officinalis* branch before they turn into the chamber floor × 370

Figure *82*: Fracture through a septum of the chamber zone of *Sepia gibba* showing the lamellar chamber floor and the prismatic chamber roof with a pillar rooted on it. Organic membranes do not cover the roof or floor surface, but are expanded through the chamber room × 1 250

Figure *83*: The embryonic shell of *Sepia pharaonis* during formation of the 6th chamber, after which the embryo would normally hatch. In the siphuncular zones of the chambers the organic covers are partly rolled up, exposing the posterior end of the chamber (details in Figures *34, 35, 36*) × 12.5

Figure *84*: The spine of the hatching *Sepia pharaonis* is partly covered by organic material. Detail of base in Figure *57* × 620

Figure *85*: The spine of *Sepia officinalis* is composed of lamellar structure which at the spine margins shows a rapid transition into purely organic sheets; these continue across the spine cover into the uncalcified portion of the central layer. For detail see Figure *41* × 19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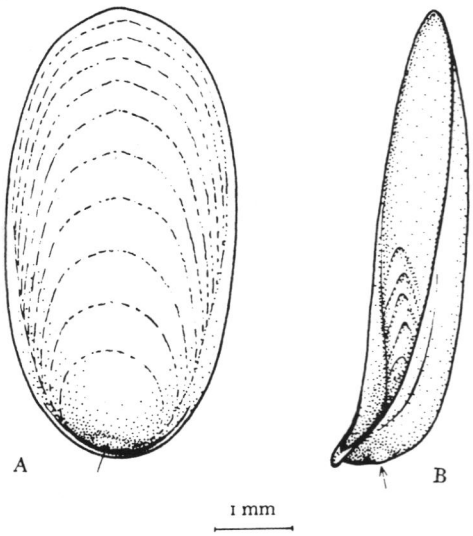


Figure 13

The cuttlebone of a newly hatched *Sepia orbignyana*. The rudiment of the spine (arrow) is barely visible on the rounded posterior part. A: dorsal view; B: ventro-lateral view

It is interesting that the spine is not yet formed in the newly hatched animals of *Sepia orbignyana,* a species with a very prominent spine in the adult stage. In the youngest animals, one only finds an inconspicuous thickening at the site where the spine will later form (Figure 13).

In *Sepia pharaonis,* the embryonic dorsal layer begins to develop in the form of a series of dispersed crystallisation centers, where aragonite crystals aggregate into complete spherulites. These may fuse in the central part of the dorsal shield, whereas near the margins they remain isolated (Figures 14, *40*). These spherulites consist of needle-like crystallites that radiate from the center of the nodules, which are 12 - 50 μm wide. They are embedded in the organic sheets of the outer and marginal shell layers of the embryonic dorsal shield.

In the region of the spine, the shell of embryonic *Sepia pharaonis* also differs from that of *S. officinalis.* Towards hatching, *S. pharaonis* has completed 6 shell chambers (Figure *83*); this is less than in hatching *S. officinalis,* but the spine is already a large and solid structure (Figure *84*), whereas in newly hatched *S. officinalis* it just begins to form. In the spine region of *S. pharaonis,* the crystal needles show the same orientation within an aggregate; *i. e.,* there is neither a spherulitic nor a random orientation (Figure *57*). These crystallite aggregations tend to

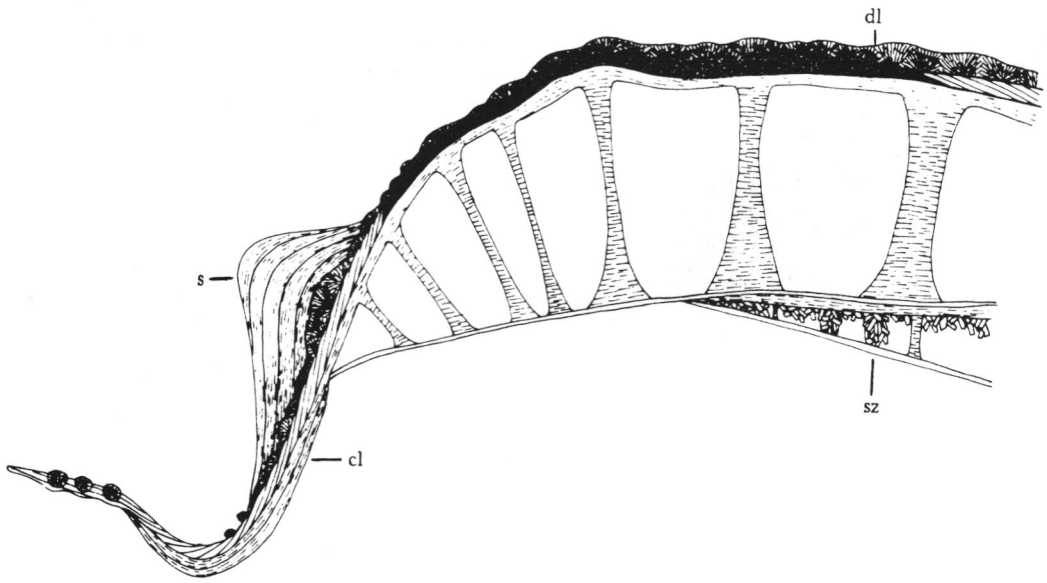


Figure 14

Longitudinal section through the cuttlebone of an embryo of *Sepia pharaonis* (with 5 completed chambers), showing the spine (s) with lamellar structure, the prismatic dorsal layer (dl) forming spherulitic aggregates near the posterior rim of the shell, and the central layer (cl) that is purely organic in the region of the early embryonic shell and then becomes partly mineralized. In the siphuncular zone (sz) of the second chamber, the space between the pillars is partly filled with aragonitic crystals that show an inorganic type of crystal growth.

flatten out, with the needle axes following the plane of the organic sheets next to them (Figure 57). Thus their structure is intermediary between the patterns observed in the dorsal and central layers, respectively. The needle-like crystals of these early spine structures measure 0.2 μm in width, like those of the central layer. The spine of newly hatched *S. pharaonis* is often covered by organic material (Figure 84); it strongly resembles the spine of the adult shell, which is similar to that of adult *S. orbignyana*.

During the later embryonic stages, the different parts of the primary shell sac epithelium reflect the increasing complexity of the shell structures they form. APPELLÖF (1893) described and figured several typical forms of shell-secreting cells of the adult cuttlefish. DENTON & GILPIN-BROWN (1961) described the micro-anatomy of the siphuncular wall of the shell sac. Finally some ultrastructural aspects of the primary epithelium in the anterior chamber zone were described by KAWAGUTI & ODA (1963).

SPIESS (1972) tried to relate the histological differentiations he observed at stage XVI directly to the different types of cells described by APPELLÖFF (1893). With the completion of the first chamber before stage XVI is attained, the embryonic cuttlebone has indeed acquired the

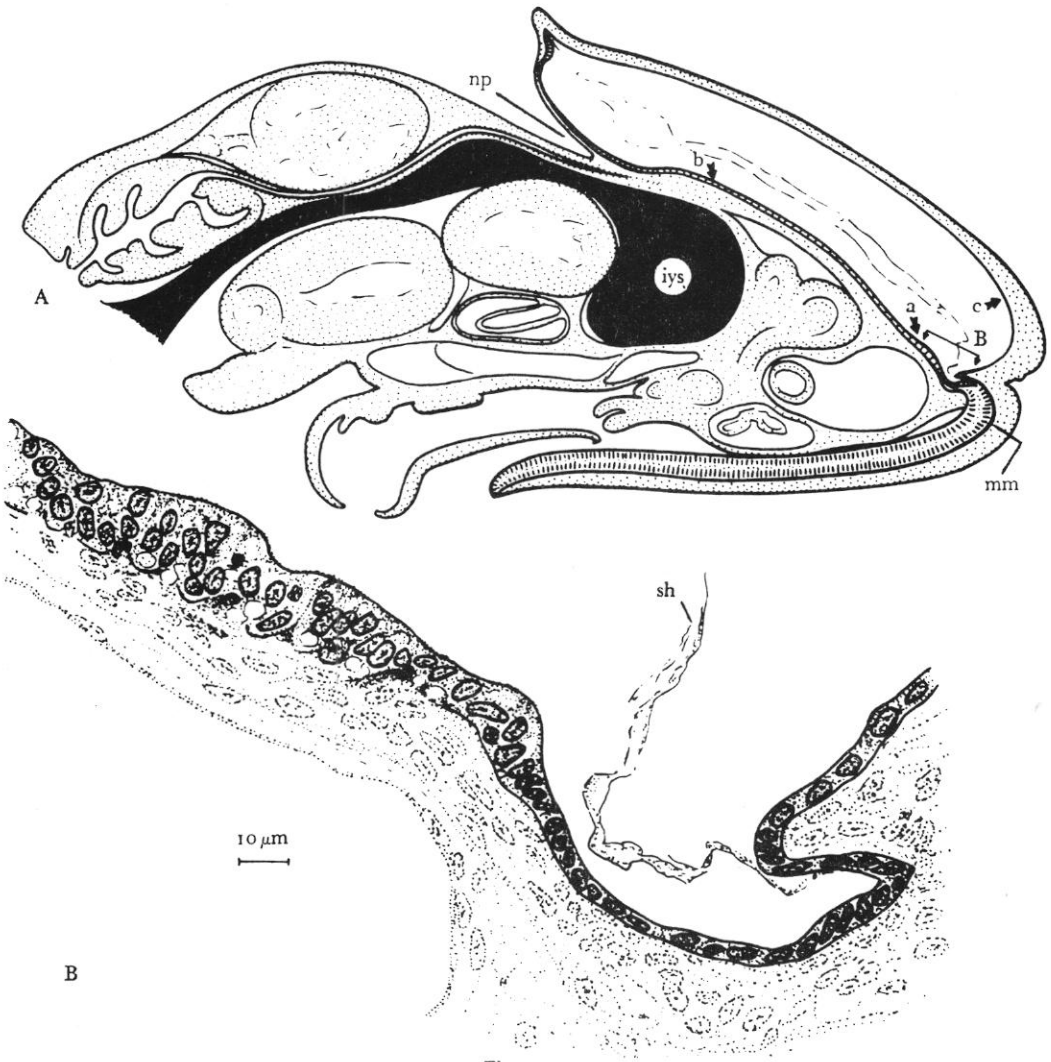


Figure 15

Medial longitudinal section of an embryo of *Sepia officinalis*, at stage XVI of Naef (cf. Figure 10, arrows). A: Semi-schematic presentation of the entire embryo in a medial section. The arrow line B indicates the area enlarged in B. The thick arrows a, b, and c mark parts corresponding to those represented in Figure 16, from a later stage (abbreviations as in Figure 10). B: Histological aspect of the typical siphuncular epithelium with the early basal infoldings (left part) and of the epithelium of the posterior brim of the shell (sh), the upper right part belonging to the secondary epithelium

main elements of the future buoyancy apparatus. However, several parts of the adult cuttlebone, such as the spine, the dorsal layer and the fork, are still lacking. Furthermore, a comparison with later embryonic stages shows that the histological differentiation in general is still in its early phase at stage XVI. Thus, *e. g.*, the siphuncular tissue (type D of the 5 cell types listed by SPIESS, 1972) only begins to take on its typical structure with the basal infoldings described by DENTON & GILPIN-BROWN (1961) (Figures 15B, 16A). Also the chamber-forming epithelium (type C of Spiess) will attain its adult structure after stage XVI (Figures 16B, 18B).

We do not agree, therefore, with SPIESS (1972) who states, without presenting any figures of later stages, that "the following stages up to hatching do not present any crucial change in the tissue of the shell complex," and that there is only "an insignificant reduction of the height of cells in the primary epithelium." The contrary is true, as one may see by comparing Figures 11, 15 and 16. What is crucial in these changes is that they lead to structures that are very similar to the histological aspect of the adult tissue. From the figures presented by APPELLÖF (1893) and by KAWAGUTI & ODA (1963) it is clear that the cells forming the calcareous material of the chamber zone are high, columnar cells. The assumption of SPIESS (*op. cit.*: 197) that high cells always form uncalcified structures, whereas calcified structures are always built by cubiform cells, is untenable.

The semi-diagrammatic representation of the primary epithelium at stage XVI (Figure 10) shows the peripheral zone of the high cells that form the organic rim of the dorsal shield (cf. Figure 11B). Next to this peripheral

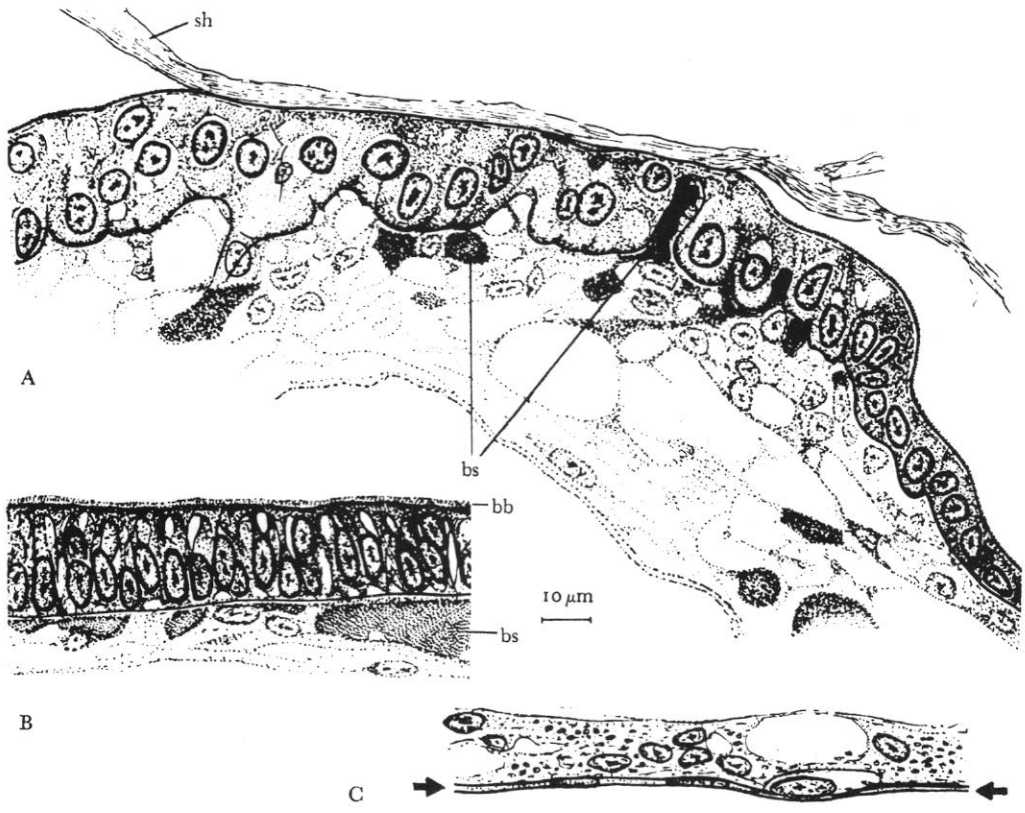


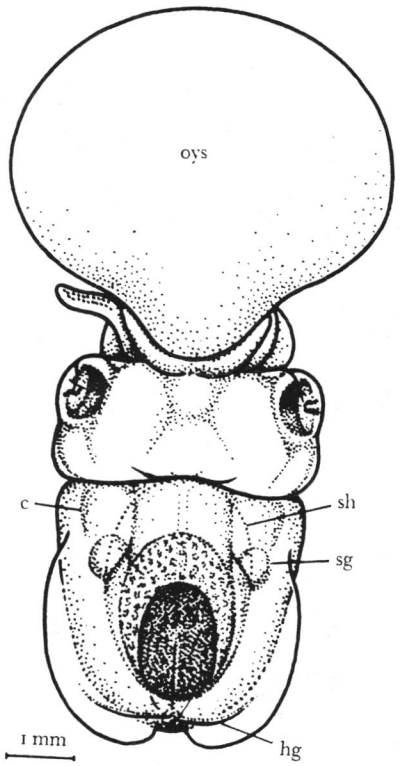
Figure 16

Sagittal sections from an embryo of *Sepia officinalis*, at stage XVII-XVIII of Naef (for their location cf. Figure 15). Note that these camera lucida drawings are at the same magnification as Figure 15B. A: the typical siphuncular epithelium, with the siphuncular wall of the shell (sh) adhering to it. B: the epithelium that forms the chambers, with high columnar cells and large vacuoles and a broad brush border (bb). The large blood spaces (bs) do not penetrate the epithelium, as they do in the basal infoldings shown in A. C: the extremely flat secondary epithelium (arrows!)

Figure 17

Embryo of *Sepia officinalis*, at stage XVII of Naef (dorsal view, drawn from living specimen). Note the large outer yolk sac (oys) which will be absorbed by the time of hatching when the animal will have doubled its size (cf. Figure *98*). The shell (sh) has two complete chambers, and the third chamber is being formed. The anterior limit of the insertion of the head and funnel retractors can be made out by the position of the stellate ganglia (sg) (cf. Figure 10), which lie behind the collar (c). On the posterior mantle and fin surface lies the anchor-shaped hatching gland (hg)

(adjacent column →)



zone lies a band of very low cells that secrete the mineralized (ventral) marginal zone of the dorsal shield. The actual chamber-forming epithelium, however, is again composed of columnar cells, which are highest in the medio-lateral and anterior parts (Figure 10). The typical siphuncular tissue is rather limited and occupies an oval field close to the posterior end of the shell. This highly vascularized zone is surrounded by an area of apparently the same type of cells that do not form, however, the deeply folded epithelium that is so typical of the central part of the siphuncular zone (cf. Figures 16A and 18C).

The growth of the shell complex during the later em-

Explanation of Figures *86* to *94*

Figure *86*: Central pillar zone of *Spirula* broken open to show the septal neck (base of figure) with its lamellar structure. The pillars (detail Figure *63*) are covered with the porous layer consisting of irregular crystals that line the inner space of the siphuncular tube. The lamellar structure of the pillar shows gradation into the irregular structure of the inner cover of the pillar zone × 1 700

Figure *87*: The collapsed and then solidified end of the siphuncular tube of *Spirula* with its organic ribbon that extends to the opposite wall of the initial chamber × 350

Figure *88*: At the insertion to the outer wall (right) the septum of *Spirula* sits on a coarsely prismatic ridge. The septum is composed of well differentiated lamellar layers, which end abruptly at the prismatic ridge × 620

Figure *89*: The end of the siphuncular tube of *Nautilus pompilius* showing the first pillar zone on the nacreous inner wall of the initial shell cap. This pillar zone borders on the chalky layer at the margins of the siphuncular tube. The apical side of the first septum is seen in the lower part of the figure × 95

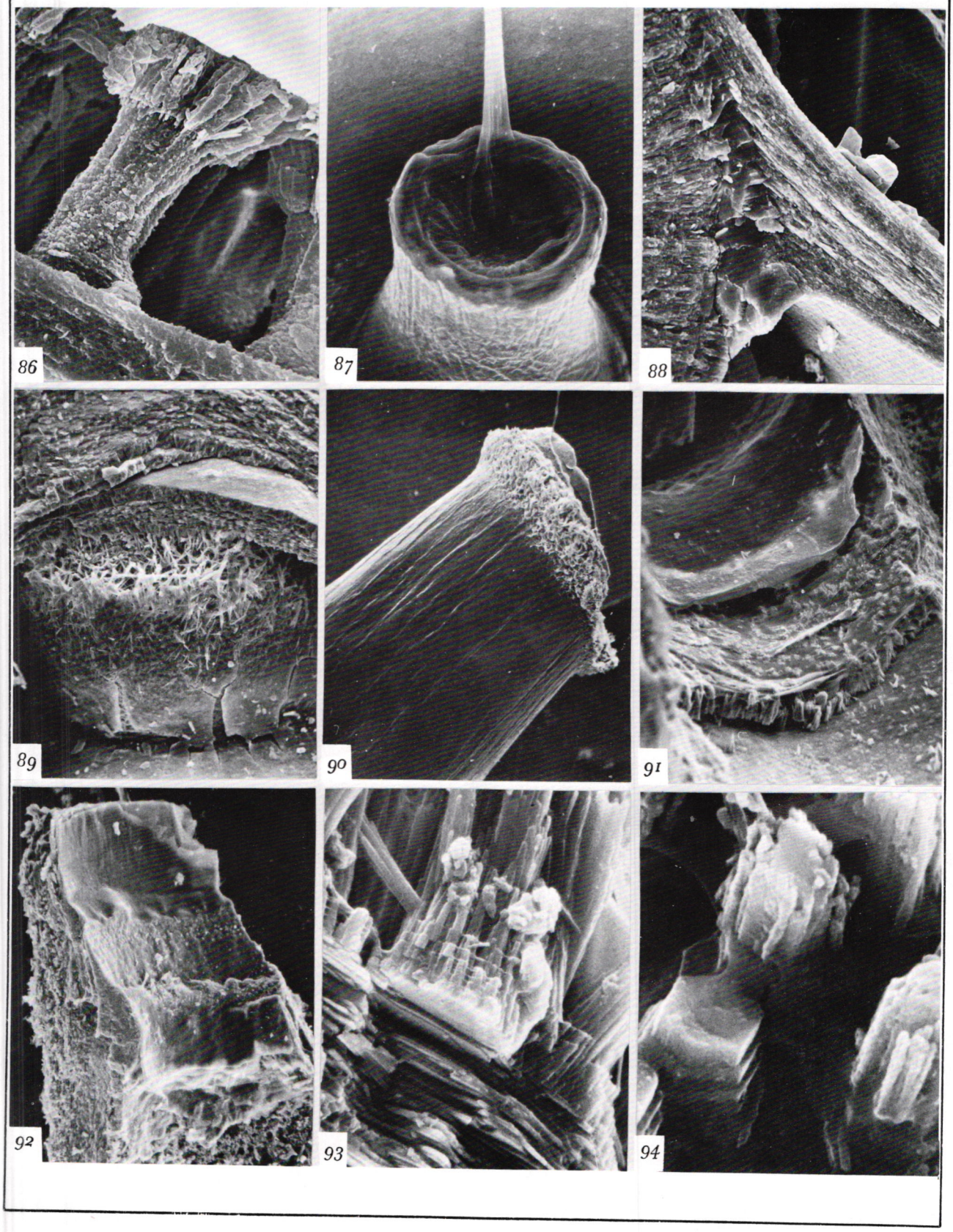
Figure *90*: The horny siphuncular tube of *Nautilus pompilius* ends in the spongy interfusion of the chalky layer and the organic sheets of the tube. This porous zone is in contact with the pillar zone (Figure *91*) within the septal neck. A chalky layer is not developed in this particular section of the siphuncular tube × 34

Figure *91*: The inner side of the septal neck of *Nautilus pompilius* opened to demonstrate the inner non-porous ridge (upper portion of figure) covered by the organic sheet that continues into the horny tube (here torn off). The spongy apical end of the horny part of the siphuncular tube rests on the pillar layer seen in the central portion of the figure × 112

Figure *92*: A fracture showing the end of the septal neck of the siphuncular tube of *Nautilus pompilius*. The horny tube and the cover of the chalky layer are seen in the upper part of the figure. In the center is the non-porous ridge forming the attachment of the next section of the siphuncular tube. The tube is torn off near the base of the figure to show the end of the pillar zone × 82

Figure *93*: Crystals of the frontal pillar zone of the siphuncular tube of *Nautilus* show the transition from the lamellar nacreous layers into the prismatic pillars × 1 500

Figure *94*: The inner pillar zone of the siphuncular tube of *Nautilus* shows the transition from stacks of nacre platelets to pillars of prismatic structure × 2 950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bryonic stages is most intensive in the anterior part of the mantle (cf. Figures 10 and 17), so that the insertion of the large head and funnel retractors continually approaches its definitive extent in the posterior part of the shell, on either side of the siphuncular zone (cf. TOMPSETT, 1939, for the anatomy of the muscular and other systems of the adult cuttlefish).

The secondary shell epithelium that forms the dorsal layer of the dorsal shield from stage XVIII onward remains very flat (Figure 16C). At its periphery, it becomes gradually thicker before it turns into the marginal part of the primary epithelium. In this outermost marginal area, the cuttlebone is firmly attached to the shell epithelium, which in turn is fixed via a delicate cartilaginous band to the dorso-lateral margin of the muscular mantle (Figures 10, 11B). The lateral pockets at the base of the fins (Figure 10) are gradually separated from the secondary epithelium.

When the animals hatch, their general aspect is very similar to that of the adult animal. However, the body proportions still differ markedly from those of the adult. This is also true for the proportions of the shell; its width to length ratio is about 1:2 at hatching, against something like 3:8 at the adult age. Also the relative length of the last septum, which in young animals is shorter than the siphuncular zone, will increase during later development (MANGOLD, 1966). Only under artificial starving conditions will the last chamber always be shorter than the siphuncular zone; except for extreme starvation leading to constant positive buoyancy of the animal, these abnormal proportions of the chamber zone do not influence the buoyancy mechanism of the animal (BOLETZKY, 1974a).

We are still far from a detailed knowledge of the function at the cellular level of the different parts of the epithelium that surrounds the cuttlebone, builds its complex structures and forms the physical and physiological link between the other living tissues and the shell. In order to elucidate all the biological processes going on in the shell complex, a very detailed histochemical and ultrastructural study of the shell sac epithelium will have to be carried out on material that must be obtained under various well-defined experimental conditions.

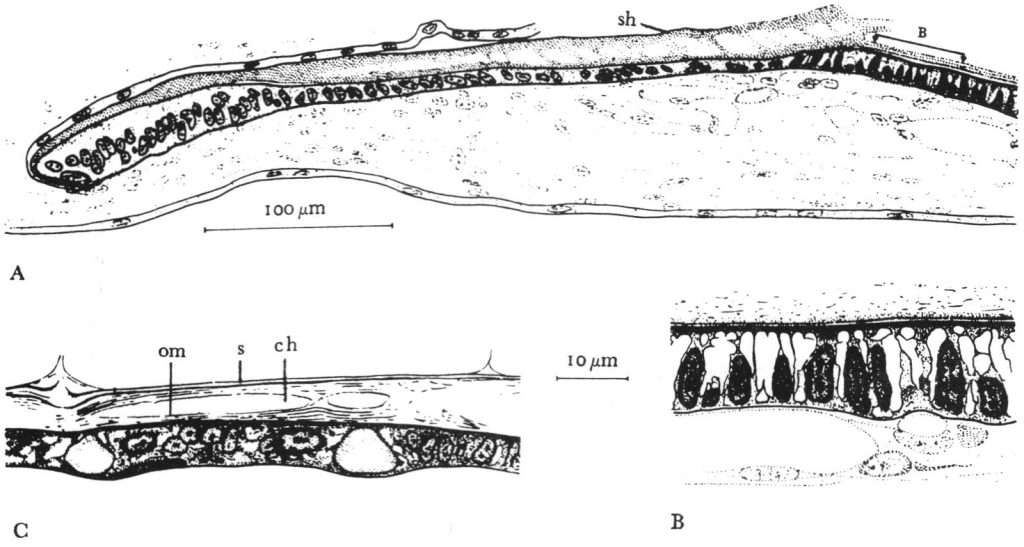


Figure 18

Sections from a juvenile *Sepia officinalis*, a few weeks after hatching. A: Cross section close to the anterior end of the cuttlebone. The left part of the shell (sh) is the organic rim (uniformly stippled). The middle part next to this shows artificial cavities due to the dissolution of calcareous shell material during fixation. At right is the peripheral part of the chamber zone, enlarged in B (cf. Figure 16B). C: Cross section from the lateral part of the siphuncular zone in the same specimen. In contrast to the central part of the siphuncular zone, the epithelium is rather flat and shows no basal infoldings. It adheres to the organic membrane (om) forming the bottom of the chamber (ch) in the siphuncular zone. The small chamber height indicated by the next upper septum (s) shows that this section passes through the rearmost part of the chamber.

Development of the Shell Complex in *Spirula*

The embryonic development of *Spirula* is still unknown. It is also uncertain whether the smallest animals caught with plankton nets are newly hatched young. However, NAEF (1923, 1928) estimated from the size of mature ovarian eggs as indicated by CHUN (1910), that the newly hatched animals might have a total length of about 4 mm and that their shell would then have not more than 2 or 3 completed chambers.

In the Sepioidea so far studied, the dorsal mantle length of newly hatched animals corresponds roughly to the length of the mature ovarian egg from which they have developed. In *Spirula*, mature ovarian eggs measure 1.7 mm according to CHUN (1910). The smallest specimens we have been able to study, thanks to the kindness of Prof. Dr. E. J. Denton (cf. DENTON & GILPIN-BROWN, 1971), had a dorsal mantle length of about 2.7 mm, and their shell already had 3 complete (closed) chambers. The smallest specimens observed by CLARKE (1970) had a mantle length of only about 2 mm; probably they had not more than 2 closed chambers. If the newly hatched animals are markedly smaller, with a mantle-length of about 1.7 mm, it seems likely that they have only the first chamber closed, which measures 0.7 mm. Since in the known specimens the closed part of the shell never takes up more than half of the mantle length, one can practically exclude the possibility that the mantle complex that is markedly shorter than 2 mm holds a shell with 2 closed chambers. As only the posterior part of the digestive gland ("liver") occupies the open chamber ("living chamber"), the anterior part has to find its place in front of the shell, inside the mantle. In the smallest specimens we have seen, the posterior part of the head with the statocysts is also retracted into the mantle cavity, as in the adult.

It seems reasonable, therefore, to assume that *Spirula* hatches with the first chamber of the shell completed (and probably containing some gas to give the animal neutral buoyancy) — possibly with the second chamber completed, if the mantle-length of the newly hatched animal is not less than 2 mm (cf. Figure 19B).

JOUBIN (1910) and NAEF (1928) have clearly shown that the hypothesis of HUXLEY & PELSENEER (1895) postulating a partly external formation of the early shell in *Spirula* is untenable. Certainly the embryonic shell of *Spirula* is formed inside a closed shell sac that is more cup-shaped, however, than it is in other decapods. The early hypothetical stages figured in JOUBIN (*op. cit.*: figs. 15, 16) are not actually convincing, since the shell rudiment there appears as a narrow "pen" in the dorsal anterior part of the mantle, similar to the rudimentary shell of sepiolids. The following stage, however (stage III of JOUBIN, *op. cit.*: fig. 17) is likely to come close to reality. We have redrawn it in our Figure 19A. NAEF (*op. cit.*) suggested that in the early embryonic shell complex of *Spirula*, there should be some rudimentary formation representing the proostracum, which would disappear during later embryonic development. At any rate, the initial chamber does not show any trace of an early proostracum rudiment (which might simply be represented by the primary insertion of the dorsal part of the mantle rudiment on the margin of the shell sac).

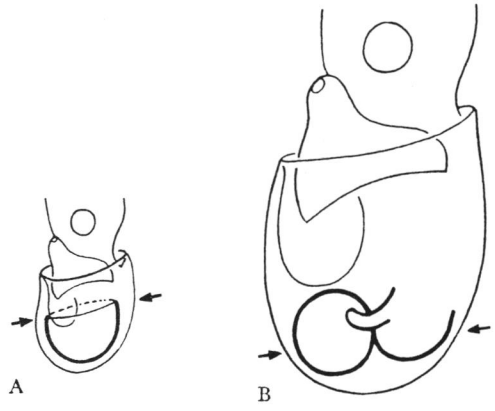


Figure 19

Hypothetical developmental stages of *Spirula*, after JOUBIN (1910, figs. 17 and 18). A: stage III of Joubin, with formation of the first chamber. At this stage, the embryo would have a large outer yolk sac. B: stage IV of Joubin, with formation of the second chamber. Arrows indicate the plane of insertion of the mantle muscle on the shell complex

What is important is that the insertion of the mantle muscle apparently "moves" from its primary location on the edge of the shell sac (where it probably lies at early organogenetic stages) to the outer surface of the shell sac, so that the first chamber can take up its position inside the muscular mantle. This displacement of the muscle insertion probably starts on the ventral side, but it also attains the dorsal side of the shell sac, the foremost part of which will then always lie under the muscle insertion. A comparison of Figures 19A and 19B may help to understand this process. Figure 19B (stage IV of JOUBIN, 1910) would represent a stage near hatching, according to our estimation of size and shell development.

With the further growth of the coiled shell, the displacement of the muscle insertion on the lateral and ventral parts of the shell complex continues, and early in

juvenile life the part of the shell sac that contains the first chambers becomes entirely detached from the mantle. The "movement" of the growing shell in relation to the mantle can best be compared to the movement of the innermost part of a spring in a clock-work that is being wound up.

The faster addition of shell material on the dorsal side of the shell as compared to the ventral side, which generates the spiral growth, seems to begin right after the formation of the protoconch; the posterior attachment of the "prosiphon" presumably lies in the protoconch area, and this attachment in fact lies on the ventral side rather than opposite the aperture (Figures 20, *64*).

As we stated earlier, in the first chamber of the *Spirula* shell, the entire surface of the siphuncular tube is covered by a wrinkled organic sheet, from the aperture of the chamber to the line along which the "prosiphon" is attached to the wall (Figure 5). This organic sheet is the original embryonic siphuncular tube of the first chamber. Its formation and the subsequent modifications can be imagined to take the following course.

Before the siphuncle forms, the aperture of the first chamber is further constricted by a ring-shaped ridge that is secreted on the inner surface of the apertural constriction (Figure 20A). The formation of the second chamber has begun. As DENTON & GILPIN-BROWN (1971) have shown that only a small part of the new chamber wall is formed when the third chamber is closed, one can assume a similar situation for the closure of the first chamber.

Then the epithelium that has formed the prismatic wall of the first chamber separates from this wall, except for a line of attachment in the presumed protoconch area. It then secretes the organic sheet forming the first embryonic siphuncular tube while it is slowly retracted, the first chamber becoming filled with liquid (Figure 20B).

The next step is the formation of a calcareous tube that is wider near the apertural side than at its posterior end. The tissue of the siphuncle by then is well differentiated. The elastic organic tube, from which the tissue is now being removed, is apparently twisted near its (apertural) base before the calcareous tube is formed. The latter fixes the radial wrinkles resulting from this twisting movement, the significance of which is obscure (it reminds one of the hypothesis proposed by KERR, 1931, according to which the endogastric coiling of the *Spirula* shell had been brought about by the rotation of an originally exogastric coil, in some remote ancestor. This hypothesis appears rather imaginative).

The organic cap that closes the siphuncular tube is not sustained so that it may collapse (Figure *87*). It either forms an edge on the end of the calcareous tube, or it retains its rounded cap-shape (Figures 20C, *64*). This is fixed before liquid is extracted from the first chamber, which then becomes part of the functional buoyancy apparatus.

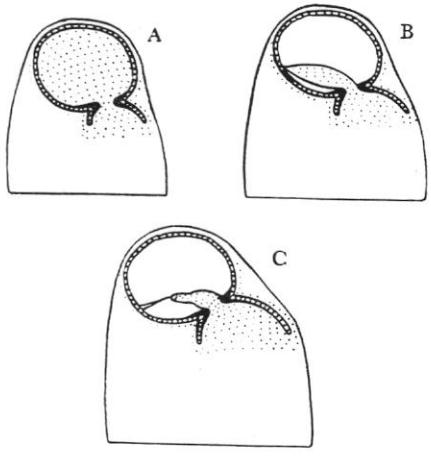


Figure 20

A schematic presentation of the processes by which the tissue withdraws from the first chamber of the embryonic *Spirula* shell.
A: the aperture of the first chamber is further constricted by a ring-shaped ridge. B: the epithelium that has formed the prismatic wall of the first chamber separates from the wall, except for the linear attachment in the (presumed) protoconch area. C: the calcareous tube forms after the siphuncular tissue has withdrawn from the elastic organic tube

Development of the Shell Structures in *Sepia* and *Spirula*

The mineral composition of the shell of *Sepia* and *Spirula* is essentially the same. In both forms, aragonitic crystallites compose 3 types of layers:

1. the irregular structure made of aragonite crystals and crystal aggregations;
2. the regularly constructed prismatic layer;
3. the more complex lamellar layer.

Type 3 forms the major part of the shell wall in *Spirula*, the whole septum and the anterior part of the siphuncular

tube. The pillars of the posterior part of the siphuncular tube of *Spirula* also show the lamellar structure, like those supporting the chamber septum of *Sepia*. In the latter, the calcified parts of the central layer including the spine, of the fork layer, and the septa of the chamber zone have a lamellar structure.

Type 2 is found in the inner layer of *Sepia* and, as a more regular type consisting of coarse needle crystals, in the inner shell wall of *Spirula*. Still coarser crystals are found in the constriction that forms in the inner shell wall of *Spirula,* before insertion of a new septum takes place. Irregular, coarse crystals in spherulitic prismatic orientation (type 1 - 2) compose the outer secondary layer of the *Spirula* shell, the dorsal layer of the cuttlebone of *Sepia,* and the crystalline covers that appear late in the ontogenesis of *Sepia* in the posterior part of the siphuncular zone. The last-formed sheets of the inner layer of *Sepia* show a structure of spherulitic-sector and are composed of thin crystal needles. In fractures made parallel to the sector axis, they have a feather-like appearance.

Type 1 forms the cover of the siphuncular pillars of *Spirula,* and the crystalline filling of the posterior-most part of each chamber in the siphuncular zone of *Sepia.*

The simplest form of aragonitic shell structure is represented by the crystal aggregations and the single crystals that form a loosely interlocking porous layer. The crystals of the early dorsal layer in *Sepia officinalis* and of the siphuncular deposits in *Sepia* and *Spirula* have this structure. They are the product of an undisturbed crystal growth from liquids or mucus rich in calcium carbonate, where the shell secreting epithelium does not influence crystallisation. Such crystals are known from many aragonitic mollusc shells where crystals grow very rapidly; for example, during formation of a first layer of shell septa in the apertural whorls of gastropods (BANDEL, 1975), or closure of other cavities in gastropod and bivalve shells (BANDEL & HEMLEBEN, 1975), and also within cavities closed off from the living tissue after the animal has withdrawn from them and formed a septum. Without any contact with the living tissue, remaining liquid or mucus rich in calcium-carbonate may then form crystals of the same shape and size as crystals that occur in mucus or body liquid still in contact with the secreting epithelium. The crystals and crystal aggregations of the porous layers formed in *Sepia* and *Spirula* can, therefore, be considered as largely uncontrolled formation of aragonite with the inorganic type of growth (BANDEL & HEMLEBEN, *op. cit.*; BANDEL, 1977a). They are not to be considered as biocrystals in the strict sense of the term (*i. e.*, structures of higher organisation where crystals are not "allowed" to form in their typical crystallographic shape, with well-developed crystal faces).

Such an indirect pathway of crystallisation without contact with the shell-secreting epithelium must be taken by the aggregates and crystals that lie between the pillars of the posterior pillar zone in *Sepia* and in *Spirula.* In the latter there are drum-shaped, short pillars; in *Sepia* they are thinner, more columnar. In both forms, these pillars show the lamellar type of construction, which is of much higher organisation than the inorganic type represented by the crystals and crystal aggregations that lie between the pillars. In this siphuncular zone, the completed pillars are covered by organic sheets; these apparently are permeable to liquid loaded with calcium carbonate that is secreted by the siphuncular epithelium.

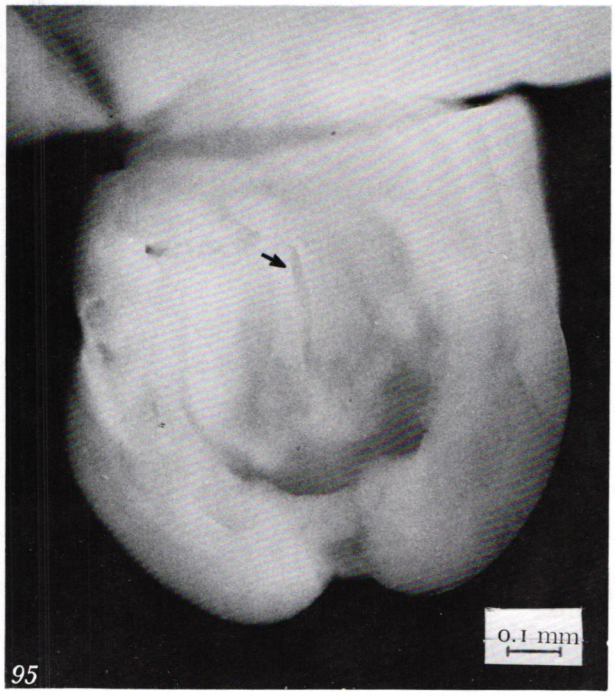
Crystals and crystal aggregations of inorganic fabric may also grade into the 2 types of higher structure present in the shells of *Sepia* and *Spirula.* In *Spirula,* the pillars of the siphuncular zone (posterior part of the tube) that are made of lamellae show a gradation into the irregular porous layer, via a transitional zone. The small, rod-like crystals of the pillar lamellae grow in thickness and become continuous across lamellae. Thus large crys-

Explanation of Figures 95 to 99

Figure 95: Dorsal view of the mantle of a *Sepia officinalis* embryo at stage XIII - XIV (fixed specimen). The dorsal part of the shell sac and the thin organic shell (cf. Figure 8) are removed to expose the primary epithelium. The arrow points to the longitudinal groove

Figures 96 and 97: Cross sections of the embryonic shell of *Sepia officinalis* at stage XVI of Naef (interference phase contrast photograph of histological section of material fixed in Bouin's solution). These sections are of the anterior part of the shell where the second chamber which is being formed lies under the dorsal shield (ds). The cavity of the chamber (ch) is filled with the future chamber liquid that appears still to be in a gelatinous state, exhibiting periodical striation that matches the annulations of the pillars (p). (Note: the closed first chamber, not shown by these sections, does not present this striation between the pillars, probably because the chamber contents are already liquefied)

Figures 98 and 99: Newly hatched *Sepia officinalis*; anaesthetized live specimen. The skin of the mantle has been removed to show the shell complex, with the gas spaces of the cuttlebone showing up with a dark outline in transmitted light (Figure 98; co = collar, sg = stellate ganglion) and as bright reflecting surfaces in incident light (Figure 99)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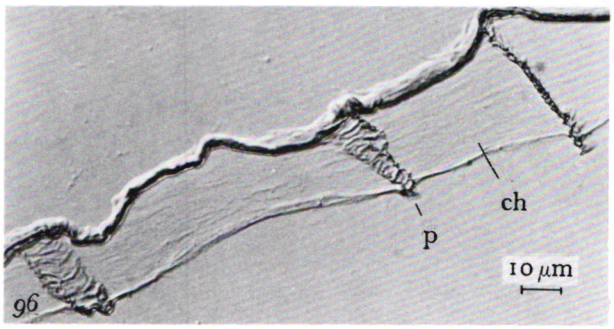
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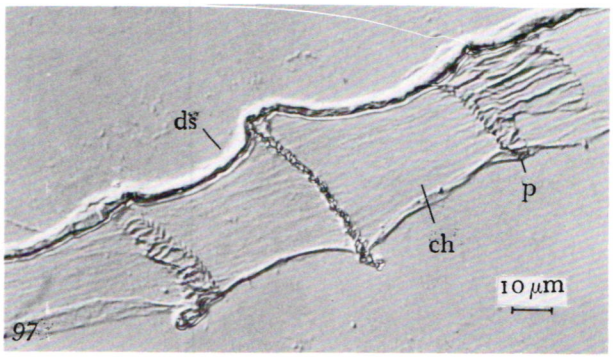
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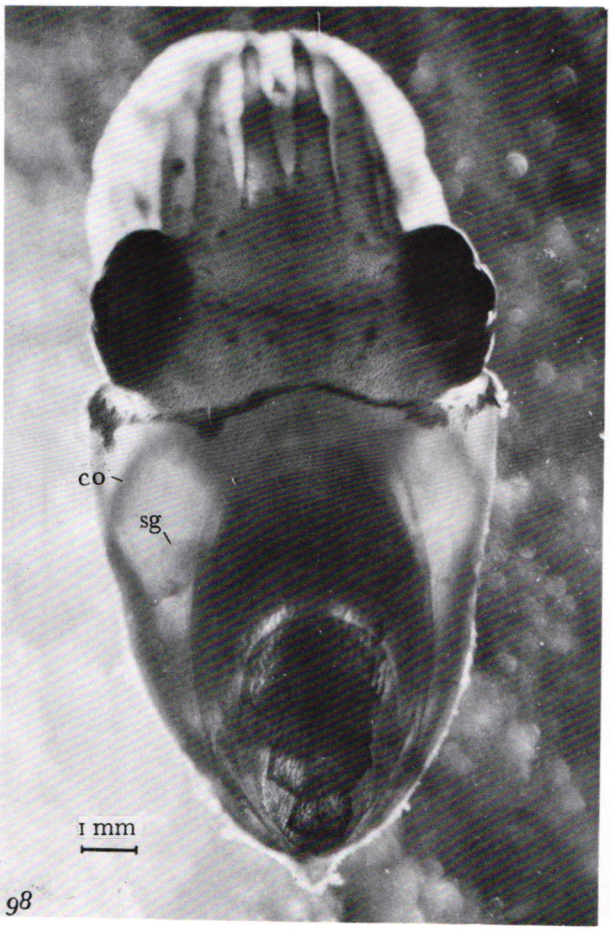
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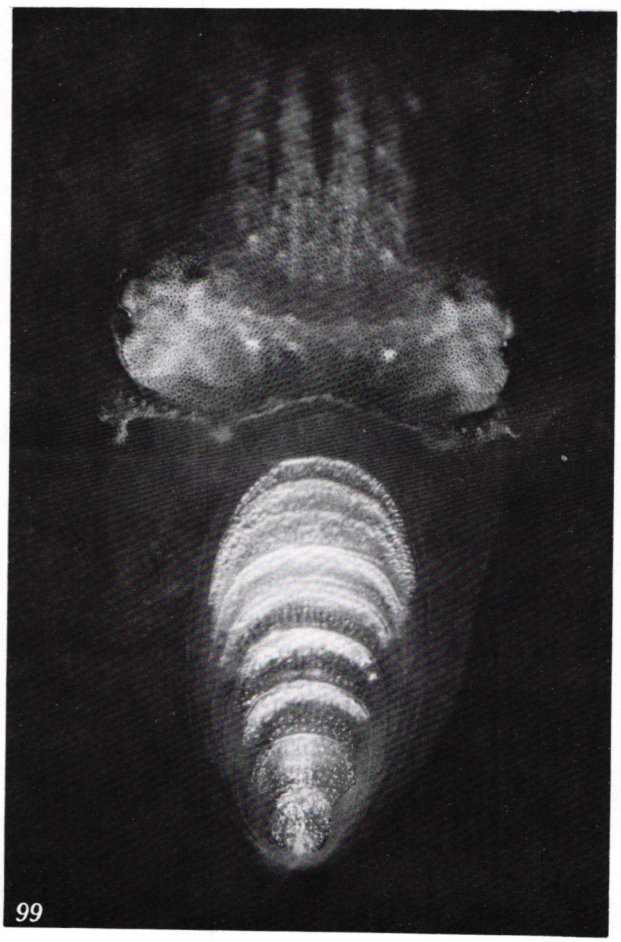
99

tallites form in which lamellation is still visible at some distance until it disappears entirely. The crystals then show well-developed faces. The irregular layer following the highly regular pillar layer consists of crystals and crystal aggregates that are interwoven with organic fibers and sheets. As there is no alternation of crystalline material and organic sheets, the organic material must have polymerized in the interstices left between the crystals. It therefore seems likely that the organic shell material of these layers differs in composition and fabric from the organic sheets that are suspended between pillars, reflecting the different degree of complexity in their mode of formation.

The layer of irregular crystals and crystal aggregates resembles very much the first layers produced in the shell apex of certain marine gastropods, when a cavity is closed off by a septum (BANDEL, 1975: plt. 2, fig. 5). A continuous transition from lamellar layers (nacreous structure in this particular case) to crystal aggregates of inorganic fabric was found in the hollow spines of 2 archaeogastropod species (BANDEL, 1977a). In these cavities, pillar- or pyramid-shaped stacks of nacreous plates grade into crystal aggregations with a radiating structure. Similar transitions exist in the *Nautilus* shell (MUTVEI, 1972; cf. our Figures *93, 94*).

The first irregular crystal cover, by which the formation of the dorsal layer in *Sepia officinalis* begins, also resembles the cover of crystal deposits in the shell repair of gastropods (BANDEL & HEMLEBEN, 1975). As in some gastropod septa, this layer grows into a prismatic or spherulitic prismatic structure with needle-like crystals of variable thickness that are oriented vertically to the growth face (cf. BANDEL, 1975: plt. 5, fig. 5; BANDEL & HEMLEBEN, *op. cit.*: figs. 2, 4, 11).

The dorsal layer of the *Sepia* and *Spirula* shell is made of crystal needles of different sizes; they either form a coarse prismatic layer, or spherulitic-prismatic ridges and bumps. The latter often show a radial arrangement of the needle crystals around the central base, in a spherulitic manner. The dorsal layer grows by mere enlargement of the needle crystals that show well-developed crystal faces. This kind of growth does not require much interaction with the shell-secreting epithelium. The latter creates a chemical micro-environment that favours the formation of aragonite and the final production of an organic cover to end the crystal growth.

The inner prismatic layer of the *Spirula* shell and the inner layer of the cuttlebone of *Sepia* show a spherulitic prismatic structure with a more regular arrangement of crystals than in the dorsal layer. But the influence of the epithelium on the mineral structure is still limited; crystals are large and show well-developed crystal faces. In the lower part of the inner layer of *Sepia,* the width of crystals is smaller, and the spherulitic sectors they compose are more apparent than in the upper part where crystallites are broader. The needle crystals that grow on the inner surface in round bump-like structures are inclined towards a common central axis, thus forming columnar units. The single needles are crystals with well-developed heads at the growth face.

In contrast to the structures so far discussed, with crystals that may attain considerable size and that show crystal faces, the lamellar layer is composed of small elements (0.1 to 0.3 μm) that rarely show crystal faces. These elements are surrounded by and interlocked with organic shell material to a much greater extent than crystals are in other structures. These smallest units may be arranged in many different ways, but they always form a lamellar structure with lamellae strictly parallel to the growth surface. The thickness of lamellae varies from 0.1 to 15 μm. Lamellae are continuous over large stretches; we have rarely found a free end or a splitting. However, mineralized lamellae may turn abruptly into lamellar organic sheets. At the interface with one of the 2 other types of structure observed in *Spirula* and *Sepia,* lamellation sometimes continues a little into the other layer.

MUTVEI (1970, 1972a, 1972b) described the nacreous layer of *Nautilus* and *Mytilus* as being composed of small crystalline units that are very similar to those we find in the lamellar layer. The typical nacreous crystals are tabular and have a hexagonal outline; they are known from many species of gastropods, lamellibranchs and cephalopods (Recent *Nautilus,* fossil Nautiloids and Ammonoids). BANDEL (1977a) has shown that the smallest components of mature nacre are round or irregular rod-like elements, particularly visible on the sides of growing platelets. These smallest elements (0.2 μm) build platelets that have the optical properties of monocrystals. The nacre platelets described by MUTVEI (1972a) from the septum and the siphuncular tube of *Nautilus* have a highly variable intracrystalline structure. Variation is particularly great in the posterior end of the calcified septal neck, with platelets made of needles 0.1 - 0.2 μm in thickness, of dendritic crystallites or rods (MUTVEI, 1972a: plts. 15, 16). As in the lamellar layer, these crystallites are composed of smaller granules with a maximum diameter of 0.2 - 0.3 μm. But in contrast to the lamellar layer, these crystal laths build platelets with distinct margins. It is conceivable that the lamellar layer is a structure derived from nacre platelets of the type described by Mutvei from the posterior siphuncular tube of *Nautilus.*

Similar to what has been shown by MUTVEI (1970, 1972a, 1972b) and BANDEL (1977a) for nacre platelets, the lamellae of the lamellar layer may be composed of

needle elements lying parallel to each other, of dendritic crystallites with bifurcating branches, of rods parallel and perpendicular to the plane of lamellation, and of very small, irregular units. All components show the same basal unit with a size between 0.1 and 0.3 μm (as in different aragonitic layers of certain mollusc shells with crossed lamellae or with a helical structure; cf. BANDEL, 1977b).

In the hollow spines of the archaeogastropods *Guildfordia* and *Angaria,* BANDEL (1977a) found columnar nacreous structures that extend parallel into the central space of the spine. At their base, these pillars show mature nacre, followed by an increasingly coarse composition in subsequent platelets. In their uppermost part, the pillars are composed of coarse aragonite crystals. Lamellation continues through neighbouring pillars, and the lamellae are strictly parallel to each other. During their growth, the pillars are surrounded by liquids that are rich in calcium carbonate, and each nacre lamella continues to enlarge only according to its own structure, independently of the neighbouring lamellae. The closure of narrow spaces in the vicinity of these columnar structures during further growth is no evidence of the presence of membranes that should surround each lamella, thus restricting its type of growth to one specific structure, as postulated by the "compartment theory" (see ERBEN, 1974, and BANDEL, 1977a for a discussion of the two main hypotheses on the mode of shell formation in the molluscs). In an attempt to explain the difference in the respective type of crystal growth in the nacre of these pillars, from mature nacre to large crystals of inorganic fabric, BANDEL (*op. cit.*) suggested that lamellae of gelatinous matter, successively secreted by the epithelium, may form the medium in which the nacre platelets form. The chemical composition and the consistency of these gelatinous layers were thought to determine the composition and structure of the forming platelet.

Since the gelatinous lamellae may liquefy or be precipitated on the mineral shell components, they are generally not preserved by current fixation methods. However, our observations on the lamellar filling of newly formed chamber sections in the *Sepia* shell confirm the hypothesis of BANDEL (1977a) on the mode of formation of the lamellar structure in nacreous layers.

It must be emphasised, however, that the "chitinous" material which APPELLÖF (1893) observed in the last-formed chamber of cuttlebones is most likely the product of precipitation of the chamber liquid, rather than gelatinous layers not yet liquefied. These probably are liquefied in the upper layers of a chamber, separated to a large extent from the lower parts in formation by the horizontal organic sheets; so that only lower parts contain consistent gelatinous layers when the chamber floor is formed. In the last-formed chamber of alcohol-preserved cuttlebones, we have indeed observed contents that recall Appellöf's description.

Homology of the Shell Constituents in *Sepia* and *Spirula*

APPELLÖF (1893) apparently realized that the entire chamber zone of the cuttlebone should be considered as homologous to the siphuncular tube of *Spirula* and *Nautilus,* as the chambers of both have pillars at, or close to, the inner side of the septal neck (we shall return to his observations on *Nautilus* further below). In *Sepia,* the chamber zone, together with the fork, would represent a greatly modified siphuncular tube with an extremely enlarged dorsal part (= chamber zone) and an almost completely reduced ventral part (= fork).

We have shown above that neither the number of fork layers nor the total number of lamellae that make up these layers correspond to the number of chambers. According to Appellöf's idea of the development of the *Sepia* shell, earlier forms like *Belosepia* of the Eocene would have been followed by forms with flatter and broader shells, until the ventral part became completely "compressed" in the posterior portion of the cuttlebone in *Sepia.*

NAEF (1922) also considered *Belosepia* as a transitional form in the evolution of the *Sepia* shell. He suggested that the outer wall of the phragmocone ("Conothek") took its part in the formation of the fork. In Naef's reconstruction, the septal necks become flatter and the siphuncular tube widens until it is a shallow pit. The proostracum, still present in his reconstruction of *Belosepia,* disappears. According to Naef, *Belosepia* is derived from ancestors like *Spirulirostra* and *Belemnosella,* which had a proostracum and phragmocones that were longer than in *Belosepia.*

We have been able, thanks to the kindness of Dr. L. Jansen (Leiden), to examine a well preserved Miocene representative of *Spirulirostra.* This fossil form closely resembles *Spirula,* except for the large rostrum. We have not seen any particular similarity to *Sepia.* Although the gross morphology of the siphuncular tube of *Spirula* is very different from the siphuncular zone of *Sepia,* it has been shown by DENTON & GILPIN-BROWN (1971, 1973) that in both forms the siphuncular complexes are alike. The shell wall and the septa are always impermeable to sea water and other aqueous solutions; the only permeable zones lie in the siphuncular tube of the *Spirula* shell and in the siphuncular zone of the cuttlebone. In *Spirula,* the permeable zone is restricted to the posterior part of the siphuncular tube, and in the cuttlebone it is restricted to the rearmost part of what we call chamber in the *Sepia*

shell. Thus the only connection between the liquid inside a chamber and the living tissue of the siphuncle is through the permeable organic membranes of these specialized zones of the siphuncular wall.

From a newly closed chamber the liquid is actively extracted while gases slowly diffuse into the chamber; this exchange is prepared by the removal of salt from the chamber liquid (DENTON & GILPIN-BROWN, 1966). When gas appears in a new chamber of *Spirula,* the salt concentration of the remaining chamber liquid is only $\frac{1}{6}$ of that in the blood and sea water (DENTON & GILPIN-BROWN, 1971a). This osmotic difference that is actively maintained by the siphuncular tissue counteracts the hydrostatic pressure; the volume of the gas spaces can thus remain nearly constant despite an increasing outer pressure when the animal descends into deeper water, the salt concentration of the chamber liquid being lowered with increasing hydrostatic pressure.

With the appearance of a gas bubble in a newly closed chamber, the main volume of chamber liquid is "de-coupled" from the liquid that is in contact with the siphuncular wall, so that short-lasting changes in hydrostatic pressure do not imply an adjustment of the salt concentration in the entire chamber liquid (DENTON *et al.*, 1961).

Spirula normally swims head down, and in this position the main volume of chamber liquid is de-coupled from the permeable region as shown by DENTON *et al.* (1971). These authors cite a personal communication of Clarke, who had observed that *Spirula* can reverse this position for some time, and conclude: "Thus in *Spirula* the main body of liquid within a recently formed chamber may sometimes be brought directly against the permeable region of the siphuncular tube. It remains true, however, that when *Spirula* is in its normal swimming position, this liquid will be almost completely de-coupled from the permeable region."

This problem of course does not arise with the cuttlebone of *Sepia* where the main body of liquid is distributed in the form of a fluid film covering the greatly enlarged inner surface of the chamber into which gas has diffused. And even in the newly formed chamber, "the exchange of salts between the liquid just inside the siphuncular surface and that deeper within the cuttlebone will be limited by diffusion" (DENTON *et al.*, 1961).

Neutral buoyancy is thus achieved in the pelagic *Spirula* and in the nekto-benthic *Sepia* by a regulatory mechanism the structural elements of which are very similar despite the different shell form. Although we have no direct evidence of a common ancestor of *Sepia* and *Spirula,* there can be little doubt of the homology of these structural elements that compose the permeable siphuncular wall and the "de-coupling" zone. In the *Sepia* shell, this zone with its pillars and organic sheets has completely "replaced" the actual chamber of the form that is represented by the *Spirula* shell.

SURVEY OF THE SIPHUNCULAR SYSTEM OF ECTOCOCHLEATE CEPHALOPODS AND OF THE BELEMNITES

In a series of studies, DENTON & GILPIN-BROWN and DENTON *et al.* (see DENTON, 1974 for complete list of references) have analysed the structure and functioning of the buoyancy apparatus of *Nautilus, Spirula* and *Sepia,* and they suggest that the mechanism by which liquid is pumped out of a newly formed chamber and gas diffuses into it must have been the same in all cephalopods with chambered shells, including the fossil nautiloids, ammonoids and belemnites.

Nautiloidea

In *Nautilus,* the chalky and probably also the horny parts of the siphuncular tube are porous. The respective volume of chamber liquid diminishes from the last chamber to the older ones, most of the chambers containing very little liquid (DENTON & GILPIN-BROWN, 1966).

The complete extraction of liquid from a chamber in *Nautilus* depends on the physical properties of the pellicle that lines the chamber and the siphuncular tube. This pellicle makes the walls wettable, and the chalky siphuncular tube acts as a wick that draws liquid uphill towards the siphuncular epithelium (DENTON & GILPIN-BROWN, 1966). The chalky siphuncular tube (spherulitic-prismatic layer of MUTVEI, 1972a), in addition to acting as a wick also serves as a small reservoir of liquid close to the siphuncular epithelium. An additional space for liquid lies between the pillar-like structures, which are set on the nacreous central surface of the concave septal face and are covered by an organic pellicle. This pillar zone extends a little into the septal neck and there comes into contact with the porous chalky layer.

The structure of the siphuncular tube of *Nautilus* has been described in some detail by APPELLÖF (1893). He found that the nacreous septal neck is continuous with the horny tube. The latter is covered on the chamber side by a porous calcareous layer, the "chalky layer" of DENTON & GILPIN-BROWN (1966). Where this compound tube enters the aperture of the next older septum, it makes contact with a crystal structure in the form of pillars that are clearly separated by interstices. Appellöf also noted that only on the inner side of the apical-most portion of the

septal neck a solid (non-porous) calcareous inner layer is formed, in which this section of the siphuncular tube ends.

MUTVEI (1972a) studied the siphuncular tube of *Nautilus* again in great detail. He showed that the single organic sheets that compose the horny tube are calcified close to their apical end, *i. e.*, inside the septal neck of the next older septum. Thus they are solidly fused with the inner side of this septal neck.

Our observation on the siphuncular tube of *Nautilus pompilius* Linnaeus, 1758 indicate that the horny tube splits into thin organic sheets before it reaches the non-porous calcareous ridge mentioned above. These observations differ from those of MUTVEI (1972a: fig. 2), who figures the horny tube without a change in its structure up to the solid inner ridge. Only in contact with this ridge the horny tube is shown to split into single sheets that are incorporated in the ridge. A reconstruction similar to that of Mutvei has been presented by BLIND (1976: figs. 5, 7).

Our Figure 21 shows the attachment of the siphuncular tube within the septal neck of the previously formed septum, with the horny siphuncular tube splitting into many sheet-like, discontinuous and irregular organic membranes before it ends in the solid inner ridge. In this part, the organic tube is interspersed with the irregular prismatic crystals that are present all through the chalky layer of the siphuncular tube; it has a spongy structure therefore (Figure 90). This structure is very likely more permeable to liquids than the actual horny tube, which is rather solid (Figure 22). In the pillar zone inside the septal neck, the pillars are widely spaced (Figure 94). This zone is in contact with the spongy end of the siphuncular tube belonging to the following chamber. So we find an arrangement of pillars and an irregular roof made of crystal aggregates and organic sheets very similar to what we have seen in *Spirula*. The porous area is much shorter than in *Spirula*, however.

Behind this spongy part, the organic sheets unite again, partly covering and partly penetrating the solid ridge

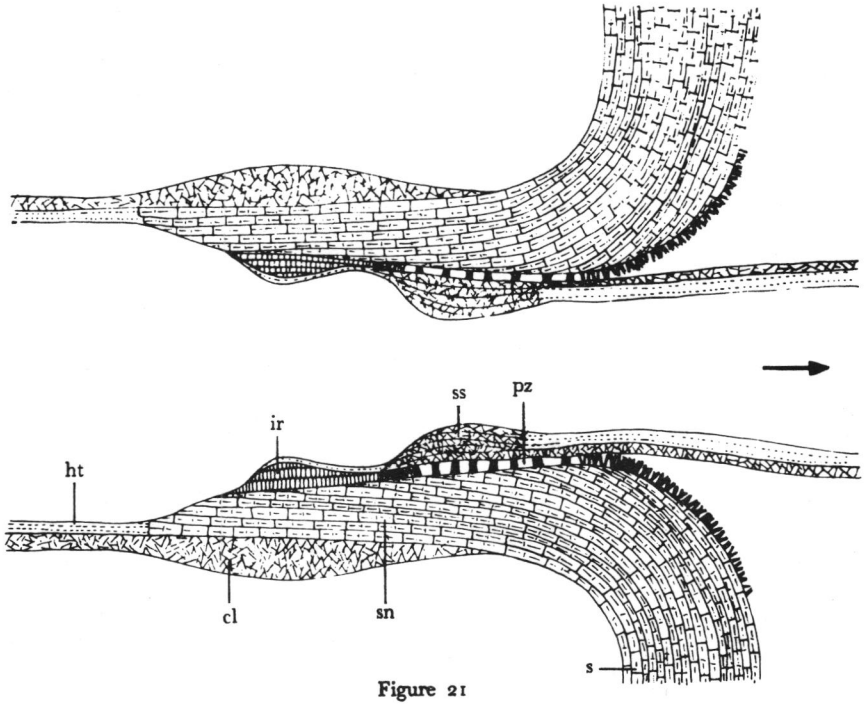

Figure 21

Longitudinal section of the siphuncular tube of *Nautilus pompilius*. The arrow points toward the aperture. The nacreous layers of the septum (s) continue into the septal neck (sn), the organic parts of which are continuous with the horny tube (ht). The septal neck and the horny tube are covered by the chalky layer (cl) which is a porous structure made of needle aggregates and organic sheets. The apical end of each section of the siphuncular tube is firmly attached to the inner side of the septal neck by a solid inner ridge (ir). In front of this lies a porous spongy structure (ss) made of many discontinuous organic membranes interspersed with elements of the chalky layer. This spongy structure brings the liquid contained in the pillar zone (pz) into contact with the siphuncular tissue

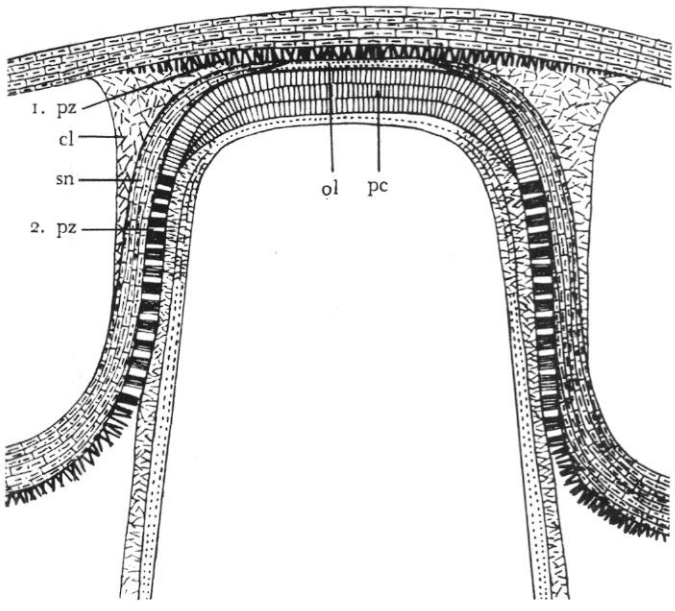

Figure 22

Section through the beginning of the siphuncular tube of *Nautilus pompilius*. The siphuncle makes contact with the inner side of the shell at the site of the earliest embryonic shell (protoconch). Between the nacre of the shell wall and the blind end of the siphuncular tube lies a porous, prismatic first pillar zone (1st pz). In the central part, this zone is covered by an organic layer (ol), and in the peripheral parts by a chalky layer (cl) made of organic sheets and prismatic crystallites. The nacreous septal neck (sn) extends to the porous first pillar zone, through which the liquid of the first chamber is pumped out before more shell material is deposited with the formation of the following section of the siphuncular tube. The latter forms a solid prismatic cap (pc) closing the porous end of the siphuncular tube. The second pillar zone (2nd pz) corresponds to what is formed in later parts of the tube

(Figures 21; *91, 92*). DENTON & GILPIN-BROWN (1966) showed that the siphuncular tube of living *Nautilus* is porous. Their experiments were carried out in such a way that more or less porous zones would not be differentiated along the tube. The structure of the siphuncular tube now clearly shows that the most porous zone lies at the apical end of each tube section. There the liquid that is in close contact with the siphuncular tissue is retained in the interstices between the pillars; thus it is de-coupled from the main body of chamber liquid.

The beginning of the siphuncular tube is shown in Figures 22 and *89*. Different reconstructions of this feature have been presented in the literature. MUTVEI (1964, text fig. 26; plt. 14) has found an outer spherulitic-prismatic layer corresponding to the outer chalky layer of the siphuncular tube of later chambers. In his reconstruction, a continuous nacreous layer, which is thin in the earliest part of the siphuncle, underlies the outer chalky layer.

ERBEN, FLAJS & SIEHL (1969: fig. 8; plt. 11, figs. 4, 5) reconstructed the blind beginning of the siphuncular tube as consisting of an outer organic layer and a solid, continuous nacreous wall beneath it. In the explanation of their figure 1 on plate 13, however, they note that the apical portion of this nacreous layer is very rich in organic material. BLIND (1976) found that the outermost hull of the initial cap of the siphuncular tube consists of irregular crystalline elements and of organic sheets, thus confirming both the observations of Mutvei and those of Erben, Flajs and Siehl. Blind stated that the nacreous layer that makes up the apical cap of the siphuncular tube seems to have a prismatic structure. In his figure 3 the wall of the cap is presented as a solid structure that would be impermeable to liquids.

Since the first chamber of *Nautilus pompilius* has been shown to contain gas, this chamber must have been pumped out through the initial part of the siphuncular tube. This seems difficult with a structure as represented by the reconstructions of MUTVEI (1964: text fig. 29), ERBEN, FLAJS & SIEHL (1969: fig. 8) and BLIND (1976: fig. 3). They all show a solidly calcified initial cap the layers of which are continuous with the nacre of the septum. This agrees with Appellöf's assumption that the initial siphuncular tube is solidly mineralized.

However, the layers seen in the cap-like beginning of the siphuncular tube have not all been deposited one immediately after the other; they belong to 2 different phases of secretion (cf. Figure 22). In the first phase, crystal growth starts with the formation of pillar-like crystallites on the nacreous inner wall of the shell apex. Then irregular crystals and sheet-like, discontinuous organic membranes form the chalky layer on the sides of the siphuncle that is now differentiated (Figure *89*). Only next to the shell wall purely organic sheets are formed; they cover the porous prismatic layer of the first pillar zone. At the apical margins, growth of irregular crystals and deposition of organic sheets continue when the nacreous septal neck and the septum grow. Thus, the short initial siphuncular tube is very similar to the sections formed later on, with the only exception that the actual horny tube is missing and the nacreous neck is followed by the spongy complex of organic sheets and irregular, loosely packed crystallites. In contrast to later portions, the ending of the organic tube is fused to form the initial cap.

At the end of nacre growth, the first siphuncular tube is functional, and the liquid of the first chamber can be pumped out. When the second chamber forms, a loosely prismatic layer (pillar zone) grows on the sides of the first siphuncular tube, and the new section of the siphuncular tube is fused into the old blind portion. Then a solid calcareous ring forms near the apex of the siphuncular tube. This ring fuses at its apical side and thus becomes a solid prismatic cap that seals the first chamber. In this newly formed, impermeable prismatic cap, the organic layers of the new section of the siphuncular tube are firmly attached. In all other features this second section of the siphuncular tube is like those formed later on.

A pillar zone serving as a fluid reservoir did not exist in the siphuncular system of *Pseudorthoceras* from the Carboniferous period (MUTVEI, 1972a). A spherulitic-prismatic layer (chalky layer) near the septal neck was absent in this form and the prismatic layer on the inner side of the foremost part of the septal neck is solid; there are no pillars or pores. The organic posterior part of the siphuncular tube contains some needle aggregates; it must have been permeable for gas and liquid (MUTVEI, 1972c). As far as we know to date, little space was available in *Pseudorthoceras* for liquids that were not in direct contact with the main body of chamber liquid. This situation would have impeded an extensive vertical mobility.

DENTON (1974: plt. 17) figures the siphuncular tube of the endoceratoid *Dideroceras*. In this animal, the calcareous portion of the siphuncular tube extends into the siphuncular tube formed before, passing halfway through the next older chamber. The permeable part would be confined to the small region lying between the septal neck and the calcareous tube, very much as in the Recent *Spirula*. It is not known whether there were any pillar structures or organic portions as a continuation of the calcareous siphuncular tube, as there are no traces of such formations in this fossil form. But this form shows that among orthocone cephalopods from Paleozoic times, siphuncular structures different from the situation known in *Pseudorthoceras* have existed, which resemble those of the Recent *Spirula*.

Ammonoidea

According to LEHMANN (1976), MUTVEI (1967), ERBEN, FLAJS & SIEHL (1969), ERBEN & REID (1971) and BIRKELUND & HANSEN (1968) the siphuncular tube of the ammonoids has a structure similar to that of *Pseudorthoceras*. Again there is no chalky porous layer, and the posterior part of the siphuncular tube is organic. BIRKELUND & HANSEN (*op. cit.*) think that the structure of the septa and the siphuncular tube described from *Saghahalinites* and *Hypophylloceras* (ammonoids from the upper Cretaceous) indicate that the hydrostatic apparatus of nautiloids and ammonoids are much more similar to each other than MUTVEI (*op. cit.*) suggested, but they do not present any data to demonstrate this.

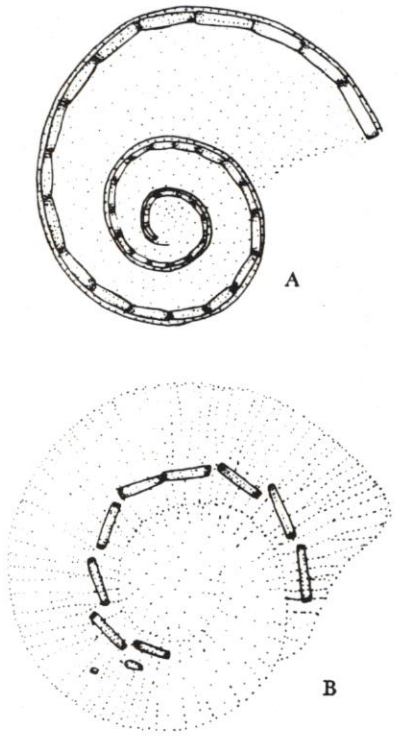

Figure 23

Jurassic ammonoids that had been decalcified before being fossilized in limestone. A: *Glochiceras* with the siphuncular tube in its original position. B: *Subplanites* in which sections of the siphuncular tube have separated and shifted towards the center before the onset of fossilization

The siphuncular tube of the Ammonoidea and its attachment to the septum differ in several respects from those of *Nautilus* and *Spirula*.

1. The beginning of the siphuncular tube (caecum) extends into the first ovoid chamber; it is similar to what we have seen in *Spirula*. But this blind ending is hemispherical; the thickness of its wall does not change (BRANCO, 1880; MILLER & UNKLESBAY, 1943; ERBEN, 1962: fig. 1; ERBEN & REID, 1971: plt. 1, fig. 4). This bulbous structure is continuous with

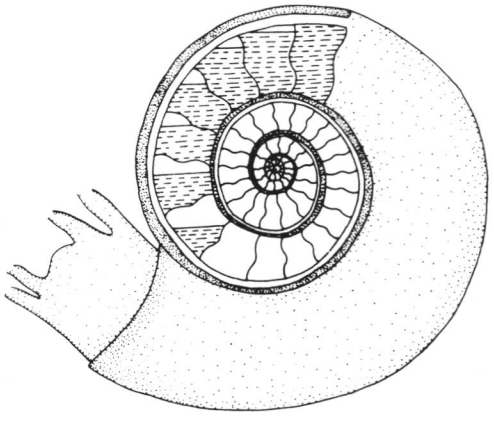

Figure 24

Hypothetical swimming position of an ammonoid, with the presumed distribution of liquid in the last chambers

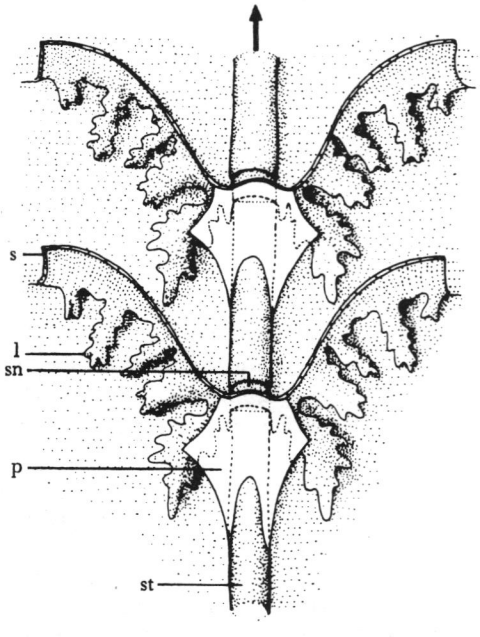

Figure 25

The siphuncular tube (st) of a Jurassic ammonoid, with the septa (s) cut above the folded lobes (l) by which they are attached to the shell wall. The arrow points towards the aperture of the shell. On the apertural part of each section of the siphuncular tube a pellicle (p) or a series of sheets form a cavity apart from the actual chamber. The septal necks (sn) are turned towards the apertural side of the shell

an organic sheet that resembles that observed in *Spirula*; it may also be branched, however (ERBEN, FLAJS & SIEHL, 1969).

2. The structure of the siphuncular tube in the earliest ammonoids reminds one of the tube of nautiloids, for the septal necks are drawn out in the apical direction. But for the majority of late Paleozoic and Mesozoic ammonoids this situation reigns only at early developmental stages (retrosiphonate, retrochoanitic). At the later growth stages, the septa first develop apertural projections where they make contact with the siphuncle, and eventually are entirely drawn out to the apertural side (prosiphonate, prochoanitic) (BRANCO, 1880: plt. 9, fig. 9; MILLER & UNKELSBAY, 1943). Thus the last septal neck of the semi-adult and adult ammonoid always points towards the living chamber (cf. Figure 26).

3. Each portion of the siphuncular tube has the same length as the corresponding chamber (Figure 25). The walls of the tube are not continuous with the septal necks (Figure 26). It has been thought that the siphuncular tube consisted of phosphatic material (ARKELL, 1957; GRANDJEAN, 1910), but recent observations indicate that it is made of organic material similar to the horny tube of the *Nautilus* siphuncle (MUTVEI, 1967; ERBEN, FLAJS & SIEHL, 1969; ERBEN & REID, 1971).

4. The individual portions of the siphuncular tube are not fused with one another, so as to form one continuous organic tube; instead they are connected by calcareous material (MUTVEI, 1967, 1975; BOEHMER, 1936; GRANDJEAN, 1910). Fossil ammonoids (*Subplanites, Glochiceras*) from the "Mörnsheimer Schichten" (lower Tithonian, upper Jurassic) of the Horstberg near Mörnsheim (South Germany) were completely decalcified before they became fossilized (Figures 23a, 23b). Only the outer ornamentation of the shell is found together with the non-calcareous siphuncular tube. Some specimens show this tube in its original dorsal position (Figure 23a); in others it has been shifted to a more central position (Figure 23b). This shifting of the tube must have occurred before the decalcified shells were compacted with the muddy sediment around them. In the original position, the individual tube portions are connected to one another in such a way that the apical part of each tube segment is narrower than the apertural part of the segment formed before (Figure 26). In a decalcified shell not filled with sediment the tube segments may easily have broken apart as soon as the

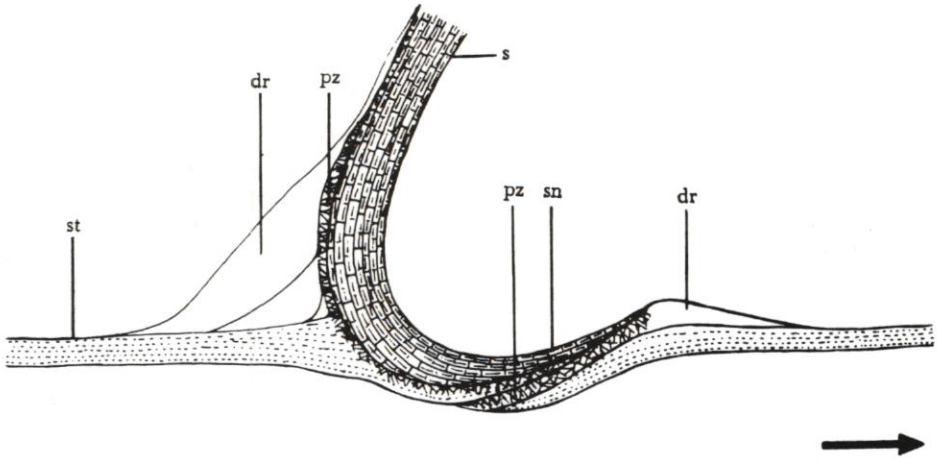

Figure 26

A reconstruction of the septal neck (sn) and the attachment of the siphuncular tube to the septal neck in adult ammonoids. The nacreous septal neck is a continuation of the septum (s). In addition to the de-coupling room (dr) and porous prismatic zone (pz) between the rear end of a tube section and the septal neck turned towards the aperture of the shell (arrow), each chamber has large de-coupling rooms and a porous zone in the anterior part

calcareous connecting material was dissolved, so that the siphuncular tube was free to shift inside the shell.

5. The descriptions and figures presented by BOEHMER (1836), MILLER & UNKLESBAY (1943), ERBEN, FLAJS & SIEHL (1969) suggest that the organic portion of the siphuncular tube is continuous with the nacreous septal neck as in *Nautilus,* as long as the septal neck points to the apical side. Among the ammonoids, in general, this is only so at very early ontogenetic stages. Representatives of the Paleozoic *Agathiceras* are an exception to this rule in that the septal necks may even increase in length during the individual development until their length is $\frac{5}{8}$ of the chamber length (MILLER & UNKLESBAY, 1943). In this genus we thus find septal necks similar to those of the Recent *Spirula.* The siphuncular tube of juvenile individuals of many Mesozoic ammonoid species has a central or sub-central position. It gradually "migrates" to the outer part of the chamber and becomes marginal by the time when the third volution is reached (cf. ERBEN, FLAJS & SIEHL, 1969: plt. 1).

6. With the change in the orientation of the septal necks during the ontogenesis of most ammonoids the siphuncular tube becomes independent of the septum; it can only form after the septum is completed. The nacreous layers of the septal neck are therefore no longer continuous with the horny siphuncular tube. The latter is now attached to the septal neck by secondary calcareous deposits. These may have been porous as in *Nautilus.* Figure 26 is a reconstruction of the attachment of the siphuncular tube to the septal neck of an adult ammonoid. The drawing is based on data presented by MILLER & UNKLESBAY (1943: fig. 6 H; plt. 5, fig. 5) from species of the genera *Eoasianites* and *Perrinites* (both ammonoids from the late Paleozoic), and by GRANDJEAN (1910: fig. 3) and MUTVEI (1967: plt. 14, fig. 2; 1975: fig. 2) from species of the genera *Ludwigia, Eleganticeras* and *Promicroceras* from Jurassic strata. In the course of their ontogenesis the ammonoids thus developed an additional porous zone in the siphuncular tube; when the septal necks changed from a retro- to a pro-siphonate arrangement, the chamber liquid could be drained at both ends of the tube segment crossing the chamber.

7. The siphuncular tube of the ammonoids is covered by an organic pellicle that separates from the tube near

its end (BRANCO, 1880; BOEHMER, 1936; ERBEN & REID, 1971; BAYER, 1975). The siphuncular tube is thus attached to the ventral shell wall. What is particularly important is that this pellicle encloses spaces near the end of each tube segment that are not in direct contact with the actual chamber (Figure 25). Liquid could be held there independent of the main body of chamber liquid. The living chamber of different species of ammonoids varies greatly in length, but usually amounts to more than ½ of a whorl (ARKELL, 1957). Thus the position of the last-formed chamber, which is still completely filled with liquid, is different from that of a new chamber in the Recent *Nautilus*. The liquid contents would have been in a position roughly as that shown in Figure 24. This is a situation very different from that shown to exist in *Nautilus* (DENTON & GILPIN-BROWN, 1966).

8. In the majority of the ammonoids, the margins of the septum are corrugated and fluted (Figure 25). Thus many small indentations are formed between the inner face of the outer shell wall and the septum. In chambers only partially filled with liquid this morphological differentiation of the septal sides may have assisted in the de-coupling of the chamber liquid from the liquid contained in the pouches near the end of each segment of the siphuncular tube. This would have an effect similar to what we have seen in the cuttlebone of *Sepia*.

HEPTONSTALL (1970) suggested that the individuals of the genus *Buchiceras,* which in their life have been encrusted with oysters, have been able to maintain neutral buoyancy by removing liquid from the chambers. This author states that in all ammonoids a considerable amount of water would have been kept in the chambers for maintaining neutral buoyancy. Experimental studies by MUTVEI & REYMENT (1973) carried out with plastic shell models confirmed that ammonoid shells in general have been more buoyant than *Nautilus* shells and that in order to maintain neutral buoyancy they must have had more liquid in their chambers. The model presented in our Figure 24 thus probably comes close to reality.

In conclusion we may say that the ammonoids in general have developed a "typical" siphuncular system allowing rapid disposal of the chamber liquid for buoyancy regulation with an effective de-coupling of the main body of chamber liquid from the liquid in closer contact with the siphuncular tissue.

Belemnoidea

MUTVEI (1971) described the siphuncular tube in representatives of the Aulacocerida and Belemnitida ftom Jurassic strata. His pictures show that the structure of the pillar zones and the course of the siphuncular tube are strikingly similar to the Recent *Spirula*. The pillar zone extends a little further into the next older siphuncular tube, as it is slightly longer than one chamber. In contrast to *Spirula,* however, the siphuncular tube is organic except for the septal neck.

MUTVEI (1971) thought that the structural features of the belemnite siphuncular tube were neither represented in the Recent *Nautilus* and *Spirula,* nor in any known group of fossil cephalopods. The close relationship that actually exists between the siphuncular tube of *Spirula* and that of the belemnites, in terms of both their structure and functioning (cf. Figure 27), was therefore not yet emphasized by DENTON (1974). Referring to Mutvei's study, Denton suggested that this rather complicated structure might form a connection for liquid movements between one chamber and its neighbours.

MUTVEI (1971) suggested that the aulacocerids and the belemnitids had no need for porous layers on the wall of the siphuncular tube and that the whole wall of the tube was permeable. The liquid of the last-formed chamber would thus have been pumped across the 2 organic layers and the "semi-prismatic layer," as Mutvei calls the pillar zone. He comes to the conclusion that belemnites, at least at juvenile stages, could not descend into deep water as *Nautilus* and *Spirula* do.

In contrast to MUTVEI's (1971) statement, but in accordance with his figures 1 and 2 and with his plates, the siphuncular tube of the last-formed chamber in belemnites did not consist of a double-walled organic tube with pillars between the 2 organic layers, but of a single tube the anterior part of which continues into the posterior pillar zone, as in the siphuncular tube of *Spirula.* A double tube can be found only in the second-to-the-last chamber. Considering the situation described from *Spirula* by DENTON & GILPIN-BROWN (1971a), where only the last chamber is filled entirely or to a large extent with liquid, it seems likely that similar conditions existed in the belemnites, *i. e.*, that the wall of the mainly organic tube was largely or entirely impermeable to liquid, so that contact between the chamber liquid and the siphuncular tissue was made through the pillar zone, as in *Spirula;* and that the chambers next to a double siphuncular tube were already empty.

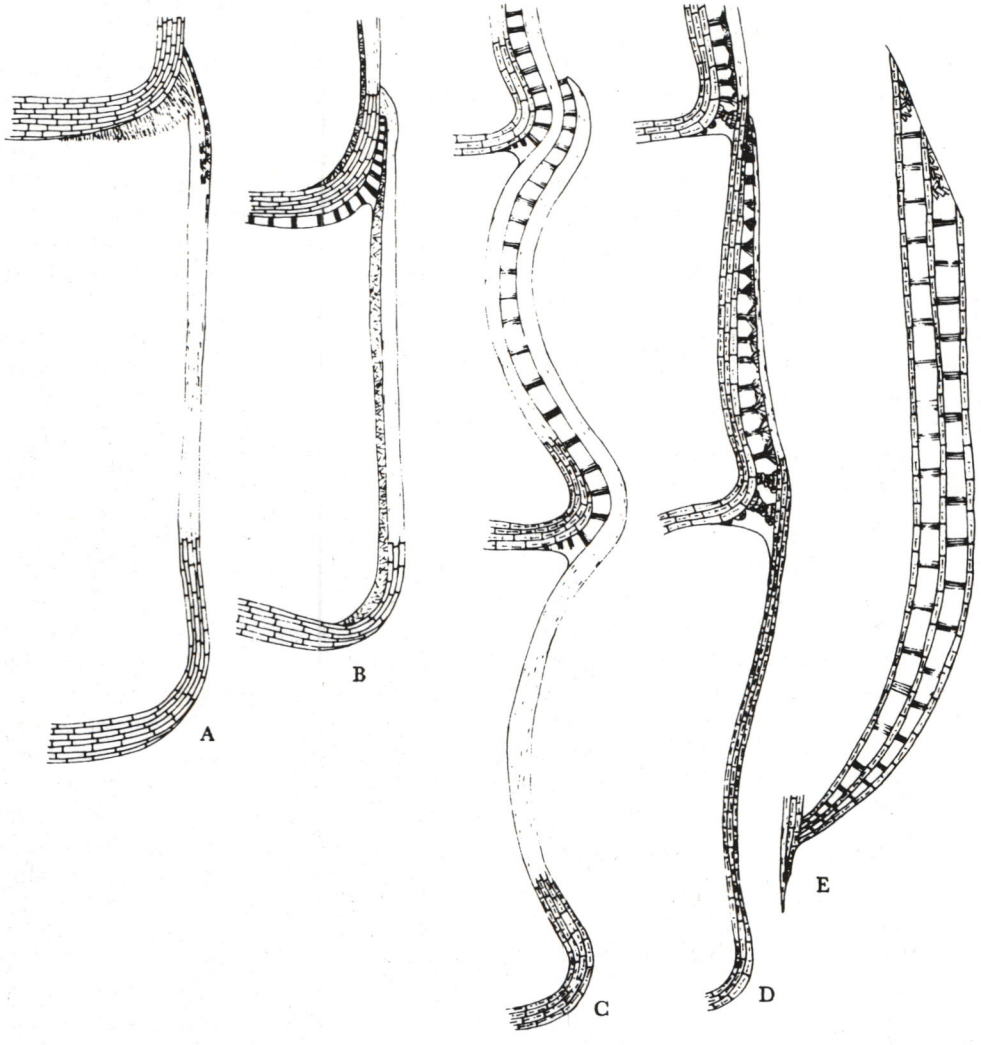

Figure 27

A comparison of the siphuncular systems of fossil and Recent cephalopods. A: *Pseudorthoceras* (redrawn from MUTVEI, 1972). B: *Nautilus*. C: belemnoids (redrawn and re-interpreted after MUTVEI, 1971). D: *Spirula*. E: *Sepia*. Note the enlargement of the pillar zone from B to E. In *Pseudorthoceras* no pillar zone is found, as in the ammonoids which have developed different means of de-coupling liquid from the main body of chamber liquid. In *Nautilus* the pillar zone is small in comparison to the chamber volume. In the belemnites and in *Spirula*, the pillar zone is enlarged, but still chambers of considerable volume are present. In *Sepia* finally the pillar zone alone remains and fulfills the chamber function.

Spirula is known to make extensive vertical migrations; it lives mainly below 200 m, but usually does not descend deeper than 1000 m. CLARKE (1970) showed that during the day *Spirula* stays at depths around 600 - 700 m. Since the siphuncular tube of the belemnites appears to be rather strong, a vertical mobility similar to that of *Spirula* can be presumed. The pillar zone represents a large space for liquid, apart from the main body of liquid in newly formed chambers, so that high osmotic pressures may have been established, enabling the belemnites to maintain neutral buoyancy at great depths.

CONCLUSIONS ON THE PHYLOGENETIC DEVELOPMENT OF THE SIPHUNCULAR SYSTEM

The siphuncular system of the only living cephalopods that have a chambered shell is a very conservative structure. This undeniable fact is partly obscured by the differences in the gross morphology of the shells and their respective siphuncular systems in *Nautilus*, *Spirula* and *Sepia*.

As we have seen that the structural elements of these 3 modifications of a common type of siphuncular system are very similar, it remains to be seen whether their formation is also similarly timed with the formation of a new chamber.

MUTVEI (1972a) assumed that in *Nautilus* the lamellar pillars ("prismatic layer") on the apertural side of the septum are formed as the final layer of a new septum before secretion of calcareous material ceases. It seems more likely, however, that the pillars are formed just before the pellicle that will cover them is secreted, and that these 2 steps initiate the withdrawal of the mantle, which is accompanied by secretion of body fluid into the new chamber now forming. In the "state of rest," the epithelium of the mantle would thus closely join the smooth surface of nacre. In chronological terms, formation of pillars would thus be the earliest of the events in the formation of a new chamber, similar to the sequence found in *Spirula* and *Sepia*.

In *Nautilus*, the structure of the pillars is transitional to the columnar structures that are formed by nacre platelets piled up to pyramid-like complexes (MUTVEI, 1972a) (Figures *93, 94*). It is conceivable that such pillars have existed in the Paleozoic orthocone cephalopods, between the walls of successive calcareous siphuncular tubes (cf. DENTON, 1974: plt. 17).

In the belemnites (MUTVEI, 1971) and in *Spirula*, the lamellar pillars are consistent with the lamellar structure, not with the nacreous structure. During the evolution of the endocochleate shell, the nacreous layer must have been transformed into the lamellar layer. To our knowledge, all ectocochleate cephalopods show only a nacreous structure. Structures transitional between nacreous and lamellar are present, however, in the septal neck of *Nautilus* and *Pseudorthoceras* (MUTVEI, 1972a, 1972c).

Spirula and the belemnites have a wide zone of the siphuncular tube occupied by the pillars. This pillar zone is distinct from the actual chamber. In *Sepia*, however, the actual chamber is lost, and the pillar zone is opened up into a broad blade. The organic sheets, confined to the anterior part of the pillar zone in *Spirula*, now extend throughout the "chambers" of the cuttlebone. No significant difference has been found between the structure of the lamellar pillars of *Spirula* and those of *Sepia*. Also the septa of *Sepia* and the anterior siphuncular tube of *Spirula* show the same composition of their lamellar structure.

The cuttlebone can withstand high external pressure. *Sepia officinalis* is known to live in coastal waters and to descend to a depth of about 150 m. DENTON (1974) has shown that cuttlebones of *Sepia officinalis* implode at pressures around 20 atmospheres, which corresponds to a depth of about 200 m. Other species of *Sepia* live at greater depths, however. *Sepia elegans* and *S. orbignyana* have been found on bottoms as deep as 450 m (cf. MANGOLD-WIRZ, 1963). It is not surprising then that the strong shell of *Nautilus* can withstand pressures of 60 to 70 atmospheres (DENTON & GILPIN-BROWN, 1966), but the rather more delicate shell of *Spirula* regularly withstands pressures twice as high, and occasionally pressures corresponding to depths of more than 2000 m (DENTON & GILPIN-BROWN, 1971a).

Since the structure of the siphuncular tube of the ammonoids and belemnoids known to date resembles so much the structures in *Nautilus* and *Spirula*, it seems reasonable to assume that these fossil cephalopods were able to descend into deep waters.

DISCUSSION

The only cephalopod with a chambered shell of which we know the embryonic development is *Sepia*. Although this particular development cannot be taken as representative in every detail for other cephalopods, Recent or fossil, that have a calcified, chambered shell, it is at any rate interesting to consider very carefully all the features of *Sepia* embryos that might present signs of ancestral features.

Setting aside the question of whether or not the closure of the shell sac in the embryo of Recent Coleoidea is a recapitulation of the process by which the external shell of ectocochleate cephalopods has been surrounded by the pallial integument to become the internal shell of the endocochleate type, one wonders what the phenomena of folding and stretching of the shell sac epithelium at the early stages of shell formation in *Sepia* embryos signify.

One wonders whether these phenomena are related to the formation of the so-called "cicatrix" in the shell of the Recent *Nautilus* and of certain fossil Nautiloidea. The morphological features of the *Sepia* protoconch closely match the definition of the cicatrix as given by ERBEN & FLAJS (1976). The presence of such a structure in the

protoconch cannot be taken, however, as an indication of the presence or absence of a post-embryonic larval phase.

Considering the morphological relationship of the fins to the shell complex, one may formulate several hypotheses on the origin of these special locomotory organs. For example, it is conceivable that the cephalopod fins are derived from originally larval "appendages" that already served locomotion. This hypothesis could lead to the construction of a larva that had some similarity with a typical veliger, but in which the locomotory appendages (equipped with cilia) were part of the pallial complex, in contrast to the cephalic vela.

If some or all ammonoids had true larvae, these larvae may have been anything but veligers of, *e. g.*, a gastropod-like appearance as figured by ERBEN (1964) (cf. also JÄGERSTEN, 1972).

Before one attempts to interpret the sequence of calcareous formations deposited on the early organic shell of *Sepia,* one must of course be certain that the steps considered are well distinguished. Looking through the literature, one finds a great deal of contradiction and confusion as to the first appearance of certain shell structures. Thus KOELLIKER (1844) correctly stated that the first embryonic shell of *Sepia* is not mineralized. APPELLÖF (1893) doubted that these observations were correct, as he had found only calcified shells in the embryos he dissected. Koelliker also correctly observed that in the earliest embryonic chambers of the cuttlebone, the pillars are more regularly columnar than in the later chambers, whereas Appellöf made no differentiation between the pillars of the earliest and those of the later chambers.

However, APPELLÖF (1893) correctly stated that the fork is not yet formed in the embryonic cuttlebone, whereas NAEF (1928) was convinced he saw the fork layer corresponding to each embryonic chamber. SPIESS (1972) also thought that all parts, including the spine, were well differentiated in the embryonic cuttlebone, but he found no horizontal organic sheets in the embryonic chambers. These are present, however, whereas the spine begins to form only towards the end of embryonic development in *Sepia officinalis.* If the fork and the spine were ancestral features, one would indeed expect to find them among the earlier differentiations of the shell.

As we do not yet know the embryonic development of *Spirula,* we are again restricted to hypotheses. These will necessarily be misleading if the structural properties of the initial chambers are not considered in every detail.

Although APPELLÖF's (1893) idea of the formation of the first chamber of *Spirula* was hampered by his assumption that this chamber is originally filled with soft chitin, his description of the first chamber is correct. NAEF (1928), however, saw a double-layered structure in the outer wall of the first chamber, and a rudimentary septum continuous with the septal neck. But the ridge-like constriction of the inner apertural wall of the first chamber has no structural similarity with the actual septa, and it is not continuous with the septal neck. Furthermore, NAEF (*op. cit.*) noted a feeble calcification of the "prosipho," and he mentions a transversal supporting rod ("Sagittallamelle") lying between the "prosipho" and the ventral chamber wall (NAEF, *op. cit.*: text fig. 279a). Such an additional lamella does not exist, and the "prosipho" sheet shows no trace of calcification.

The morphology and the fine structure of the first chamber in *Spirula,* with its blindly ending siphuncular tube, is very similar to the first chamber of ammonoids with the so-called caecum. A comparison of the first chamber of *Spirula,* opened up (Figure *64*), and the first chamber of the ammonoid *Eleganticeras* (LEHMANN, 1976) will show this. SCHINDEWOLF (1933) suggested that a caecum of the type known from *Spirula* and the ammonoids represents the primitive situation, and that the siphuncular end as it now exists in the first chamber of *Nautilus* is the result of a secondary differentiation.

The shell structure of the first chamber (ERBEN, FLAJS & SIEHL, 1968, 1969; KULICKI, 1975) and of the proseptum and flange in ammonoids also shows great similarity between different ammonoid species on the one hand, and *Spirula* on the other. There is one important difference, however, in that *Spirula* shows a constriction between the first and second chamber that is much stronger than subsequent ones, whereas the ammonoid shell presents an even spiral growth from the beginning. Only in some ammonoideans of the early Devonian age, when the coiling of the first whorl was still evolute, as it is in *Spirula,* a constriction reminiscent of the early *Spirula* shell has been observed (ERBEN, 1964); a similar feature has also been noted in longicone nautiloids (SCHINDEWOLF, 1933).

In the literature we find two opinions as to the development of the first septum in the ammonoids. One is based on the observations of ERBEN (1964) and ERBEN *et al.* (1968, 1969); it claims a free larval life for the young. The other is expressed by BIRKELUND & HANSEN (1968, 1974), DRUSHITS & KHIAMI (1970), and by KULICKI (1974, 1975); it suggests that the ammonoids had a direct development, corresponding to what is known of all the Recent coleoid cephalopods so far studied (cf. BOLETZKY, 1974b).

In the first theory, three phases similar to those of gastropod and lamellibranch metamorphosis are postulated. In the first phase, the early embryonic shell gland produces a shallow, bowl-shaped protoconch or a simple organic cap. This early formation is enlarged until a shell of nearly one complete whorl is formed. With this

shell, the animal hatches as a larva similar to a praeveliger or trochophora. In the shell, this stage would be marked by a constriction and a condensation of growth lines.

In the second phase, a free swimming, planktonic veliger or veliger-like larva adds shell material to the aperture in such a way that a ventral indentation forms, reflecting the ventral position of the velum. The secondary walls of the embryonic shell are then added. Only shortly before the end of the larval phase, the "flange" and the "proseptum" are formed (the flange is the first prismatic addition to the ventral inner side of the shell; the proseptum is the constriction of the aperture of the first chamber). The siphuncle now differentiates.

The end of metamorphosis and the beginning of the third phase is documented by the so-called nepionic constriction, a distinct mark in the shell wall; the prismatic shell layers wedge out, or the direction of their growth is suddenly inverted, apparently due to a temporary retreat of the mantle edge. The first nacreous layers are formed and the buoyancy apparatus becomes functional with the formation of the first primary septum that closes the second chamber. This first septum may have a prismatic (ERBEN, FLAJS & SIEHL, 1968, 1969) or a nacreous structure (BIRKELUND & HANSEN, 1974; KULICKI, 1975).

The second theory was formulated by KULICKI (1974, 1975). According to this theory, the embryo first forms a cup-shaped prismatic shell, the aperture of which is then reduced by the addition of shell material on the ventral inner side, which forms the flange. Immediately after this, another constriction (proseptum) is formed next to the flange. During further growth, the soft tissue filling the initial chamber is withdrawn, and the epithelium separates from the wall of the first embryonic shell. Only a minute part of it remains attached at the end of what will become the "prosipho" (organic sheet between the end of the siphuncular tube and the chamber wall). Along with this, the wall of the proseptum grows to become an annular constriction (as a continuation of the initial ventral ridge in front of the flange that is also ventral). After the proseptum, the prosipho and the caecum take shape as soon as the tissue is withdrawn from the first chamber. In the ammonoid genus *Quenstedtoceras,* a newly hatched animal would have 3 complete chambers, if the nepionic constriction marks the stage of shell growth reached at hatching. TRUEMAN (1940) reports on shells with 3 septa and a diameter of 0.5 to 0.6 mm, which he considers to be the shells of newly hatched *Arnioceras.*

In *Spirula,* the construction of the shell wall has been described in different ways (cf. DAUPHIN, 1976). APPELLÖF (1893) noted 2 layers, the inner primary and the outer secondary. BØGGILD (1930) found that the inner shell wall consists of regular prisms, whereas the outer part would be homogeneous. It is very likely that Bøggild observed the layers of the primary wall only. MUTVEI (1964a) stated that a periostracum is not known from the *Spirula* shell. NAEF (1928) gave a description that comes close to the actual structure of the *Spirula* shell. He found 3 layers, of which the thickest is the middle layer that consists, according to him, of a somewhat irregular nacre (lamellar layer!). Our observations make it clear that a lamellar layer makes up the bulk of the shell wall, with an underlying prismatic layer. This innermost layer shows only the structure that was thought to compose the entire primary wall, according to Mutvei's description. If the central layer with its lamellar structure is considered to be similar to the nacreous layer of *Nautilus,* the shell wall of *Spirula* certainly appears very similar to the wall of the *Nautilus* shell. In contrast to Mutvei's assumption that the superior part of the epithelium lining the "living chamber" of *Spirula* can produce only prismatic layers (outer wall), whereas the posterior parts produce 4 different layers of the septum and the siphuncular tube, we can now state that the epithelium lining the "living chamber" can produce similar aragonitic deposits independent of their position in the chamber.

In his analysis of the *Spirula* shell, MUTVEI (1964a) found that the septal and siphuncular deposits retained their original structure, being made up of the same 4 layers as the shell of *Nautilus.* Mutvei observed that these 4 layers are visible in the newly formed siphuncular tube only; in the posterior part of the tube which he thought is formed later, these deposits are reduced and consist of a single layer, the spherulitic-prismatic layer. In Mutvei's view, the posterior parts of the siphuncular tube of *Spirula* are at an advanced stage of reduction as compared to the corresponding formations of *Nautilus.* The septum and the anterior part of the siphuncular tube are assumed to be made of an outer conchiolin layer (organic layer), a spherulitic-prismatic layer, a nacreous layer, and an inner semi-prismatic layer. We can not confirm these observations of Mutvei. Depending on the part of the shell, one finds different sequences of layers (cf. Figure 7).

In a diagrammatic presentation of the shell and its epithelium, MUTVEI (1964: text fig. 28) figures a newly formed septum with the epithelium and the siphuncular tube that extends through 1½ chambers. On the anterior part of the siphuncular tube, which has just been formed, pillars are present. Mutvei states that the semi-prismatic layer (pillar zone; "Pfeilerchen" of Appellöf) forms a continuous coat on the ventral face of each septum and that it also invests the inner face of the siphuncular tube. Our observations indicate that this reconstruction is er-

roneous, as the pillars in fact are formed along with a new septum and anterior part of the siphuncular tube.

APPELLÖF (1893) described the annulations of the pillars and the organic sheets between the anterior pillars in *Spirula* and compared them to the corresponding structures of the *Sepia* shell. He also noted the coarse structure of the pillar apices, especially near the posterior end of the pillar zone, where pillars and needle aggregates are interlaced, and he compared this area with the posterior part of the siphuncular area in *Sepia*, where crystal aggregates are also present. These very clear similarities were not recognized by NAEF (1928) who differentiated between a calcareous tube ("Kalkdüte"), into which an organic tube ("Conchindüte) were fitted.

The lamellar structure that forms, according to MUTVEI (1964a, 1964b, 1970), the type of nacre found in belemnoids and in *Spirula* differs in one important point from the nacre of gastropods, pelecypods and of *Nautilus*. In the lamellar layers, there are no concrete tabular plates of a certain size that is characteristic of each shell section, because the small components of each platelet may vary in size. Interlamellar partitions of organic shell material are not distinct in the lamellar layers, so that they do not present, in a section, the appearance of brickwork or stacks of coins that is so typical of nacre. Although the lamellar structure may have developed from the nacreous structure, or vice versa, they are clearly distinct structures. This is also indicated by the different composition of the organic septal material in *Nautilus* and in *Spirula* (GRÉGOIRE, 1961, 1962).

DENTON & GILPIN-BROWN (1961b) showed that the oldest (embryonic and early post-embryonic) chambers of the *Sepia* shell are almost completely filled with liquid at the adult stage. These authors found that nevertheless these oldest chambers can again be pumped out. In large individuals of *Sepia officinalis* and *S. orbignyana*, the siphuncular zone of the oldest chambers is often covered with a secondary calcareous deposit. When this is present, the respective chambers can probably no longer be pumped out by the siphuncular epithelium. In *Sepia pharaonis*, younger individuals already show this kind of mineral cover on the siphuncular zone of the oldest chambers, with an additional lamellar, mainly organic deposit uppermost (Figure 4). Thus, the oldest chambers, which are refilled with liquid, are completely sealed up.

Although we know very little of the function at the cellular level of the different parts of the shell sac epithelium, it is clear that the epithelium of the siphuncular zone fulfills very different tasks, according to the different phases of shell formation. It first takes a part in the formation of the chamber layers, then acts as a "pumping organ" when the chamber is emptied, and finally part of it secretes the calcareous and organic components of these last shell deposits on the ventral side of the cuttlebone. The great change in the histological aspect of the epithelium during the first two steps has been described by DENTON & GILPIN-BROWN (1961a).

Finally, it can be conjectured that the secondary calcareous deposits on the posterior part of the siphuncular zone of the *Sepia* shell correspond to the intra-siphuncular deposits that are observed in many Paleozoic cephalopod shells. Deposits that are not calcareous, however, are found in the siphuncular tube of the first chamber of *Spirula* (Figure 66), and also in the first-formed parts of the siphuncular tube of *Nautilus*. These translucent organic deposits completely fill the tube so that the siphuncular tissue is no longer in contact with the first chambers.

SUMMARY

1. The structural composition of the shell is essentially alike in *Sepia* and *Spirula*, notwithstanding their very dissimilar form. The lamellar structure composes the septa and the greater part of the wall in the cuttlebone and in the *Spirula* shell. The inner prismatic layer of the *Spirula* shell is homologous to the inner layer of the *Sepia* shell. The external spherulitic-prismatic layer that is produced by the secondary shell epithelium is alike in both types of shell.

2. The early embryonic shell or protoconch of *Sepia* is different from that of *Spirula*. The former shows similarities to the protoconch of *Nautilus* and many fossil Nautiloidea, whereas the shape and composition of the early *Spirula* shell resemble very much that of the Ammonoidea and Belemnoidea, including the first prismatic apertural constriction (proseptum) and the end of the siphuncular tube (caecum) with its sheet-like extension that is fixed to the shell wall. *Sepia* and *Nautilus* both show a groove in the initial organic shell cap, the so-called cicatrix. The similarity of these structures suggests an identical configuration of the respective parts of the primary epithelium. So far, the embryonic development of *Sepia* only is known.

3. The lamellar structure is composed of 0.1 - 0.3 μm units, similar to the composition of other biocrystal structures of higher organisation known from the molluscs: nacre, crossed lamellae and the helical structure. Like the closely related nacreous structure, the lamellar structure is elaborated in gelatinous lamellae that have the same thickness as the crystalline lamellae.

4. The siphuncular system is essentially alike in *Sepia* and *Spirula*, as the so-called chambers of the cuttlebone

represent the siphuncular system and septal necks of the *Spirula* shell. The actual chamber of *Spirula* is not represented in the *Sepia* shell. The septal neck (lamellar structure) of *Spirula* is homologous to the septum of *Sepia,* and the pillars of the siphuncular wall of *Spirula* are homologous to the chamber pillars of the cuttlebone. The irregular crystals and crystal aggregates in the posterior part of the siphuncular zone of each cuttlebone chamber are homologous to the very similar structures found in the posterior part of the siphuncular tube in *Spirula.*

5. The function of the siphuncular system has probably never changed in the chambered shells of cephalopods. With the suppression of the actual chamber in the *Sepia* shell, the "main body of liquid," which in the chambers of other shells is "de-coupled" from the small part of liquid in contact with the siphuncular wall, is no longer a distinct component of the buoyancy apparatus; rather the innermost parts of the cuttlebone chambers are fluid reservoirs that are **analogous** to the actual chamber as a reservoir.

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