STUDIES ON SHELL FORMATION

IX. An Electron Microscope Study of Crystal Layer Formation in the Oyster

NORIMITSU WATABE, D.Sc., and KARL M. WILBUR, Ph.D.

From the Department of Zoology, Duke University, Durham, North Carolina

ABSTRACT

Details of crystal growth in the calcitostracum of *Crassostrea virginica* have been studied with the purpose of analyzing the formation of the overlapping rows of oriented tabular crystals characteristic of this part of the shell. Crystal elongation, orientation, and dendritic growth suggest the presence of strong concentration gradients in a thin layer of solution in which crystallization occurs. Formation of the overlapping rows can be explained by three processes observed in the shell: a two-dimensional tree-like dendritic growth in which one set of crystal branchings creeps over an adjacent set of branchings; three-dimensional dendritic growth; and growth by dislocation of crystal surfaces. Multilayers of crystals may thus be formed at one time. This is favored by infrequent secretion of a covering organic matrix which would inhibit crystal growth. The transitional zone covering the outer part of the calcitostracum and the inner part of the prismatic region is generally characterized by aggregates of small crystals with definite orientation. Growth in this zone appears to take place in a relatively homogeneous state of solution without strong concentration gradients. Thin membranes and bands of organic matrix were commonly observed in the transitional zone bordering the prismatic region. The membrane showed a very fine oriented network pattern.

Recent electron microscope studies of the shell of the oyster *Crassostrea virginica* carried out by Tsujii *et al.* (6) and Watabe *et al.* (9) have given information concerning shell structure and crystal growth. The growing inner shell surface consists of two main areas: the prismatic region and the calcitostracum. The prismatic region is at the shell periphery and shows a tile-like arrangement of calcite crystals separated by thin layers of organic matrix. The calcitostracum occupies the remainder of the shell and has a pattern of overlapping rows of calcite crystals. The present investigation extends these studies and considers the following aspects: the habit of well developed calcite crystals and those undergoing dendritic growth in the calcitostracum; the mechanism of formation of the overlapping rows of the crystals present in the calcitostracum; and crystal form, arrangement, and growth in the transitional zone lying over the inner part of the prismatic region and the outer part of the calcitostracum.

MATERIALS AND METHODS

The methods employed for electron microscopy are essentially these described previously (6, 9). Two-step replicas of Formvar-silicon monoxide or Formvar-carbon were prepared from the growing inner surfaces of the shell of the oyster *Crassostrea virginica.* Replicas of vertical fracture surfaces of the calcitostracum were also made. The electron microscopes used were RCA EMU 2 and 3. Polarizing microscope observations were carried out on undecalcified and EDTA-etched petrographic thin sections of shell.

RESULTS

1. *The Calcitostracum*

Well Developed Crystals: The well developed crystals of the growing surface of the calcitostracum are characteristically tabular with polygonal, rounded, or irregular forms as described by Tsujii *et al.* (6) and Watabe *et al.* (9). Further details based on the examination of more than 800 micrographs can now be added. In a given area almost all crystals are elongated in the same direction or show a suggestion of elongation (Fig. 1; see also Figs. 8, 9 in reference 6, and Figs. 6, 7 in reference 9). The crystal length could not be determined because of overlapping but is estimated to be more than twice the width, which is about 2 μ.

The crystals are in side-to-side contact, forming a layer. Boundaries between adjacent crystals may be indistinct because of fusion of the side planes, or the crystals may remain separated because of the presence of conchiolin or the probable incorporation of impurities. The creeping of one crystal over another was sometimes observed as a result of continued growth after side-to-side contact. Two examples are shown in Fig. 2. Crystal *A* will be seen to have overlapped *B*. Crystals *C*, *D*, and *E* show the interesting situation in which parts of *C* and *D* have crept over *E* and fused, forming a bridge.

Replicas of vertical fracture surfaces showed the following features. (*a*) Elongation and creeping of crystals seen on the growing surfaces were confirmed. (*b*) Crystals were closely interlocked with one another. (*c*) The thickness of each crystal was about 0.2 μ on the average. The length was difficult to determine because of the indefinite orientation of the crystals in relation to the fracture surfaces. The longest crystal observed was 9 μ. (*d*) Basal planes often showed irregular surfaces. (*e*) Very few conchiolin membranes were observed. The detailed structure of vertical sections of the calcitostracum will be considered elsewhere.

Conchiolin membranes are found between all crystal layers in the nacreous region of aragonite shells (4, 7, 8). In contrast, conchiolin was not found between layers of calcite of the calcitostracum of *Crassostrea virginica.* However, a thin layer of conchiolin was secreted about every 30 crystalline layers. This striking difference in the amount of conchiolin between nacreous portions of aragonite shells and areas of calcitostracum in calcite shells is readily evident macroscopically on complete decalcification of the shells.

Developing Crystals: We have shown in a previous paper (9) that crystal growth is initiated by the

---

Explanation of Figures

All the electron micrographs shown, except Fig. 17, were enlarged from original negatives. Shadows in the figures are white. Fig. 17 was enlarged from a reversed negative; shadows are black.

Figs. 1 to 5 are from Formvar–silicon monoxide replicas shadowed with chromium. Figs. 6 to 17 are from Formvar-carbon replicas shadowed with chromium.

FIGURE 1

Elongated crystals in the calcitostracum. × 11,250.

FIGURE 2

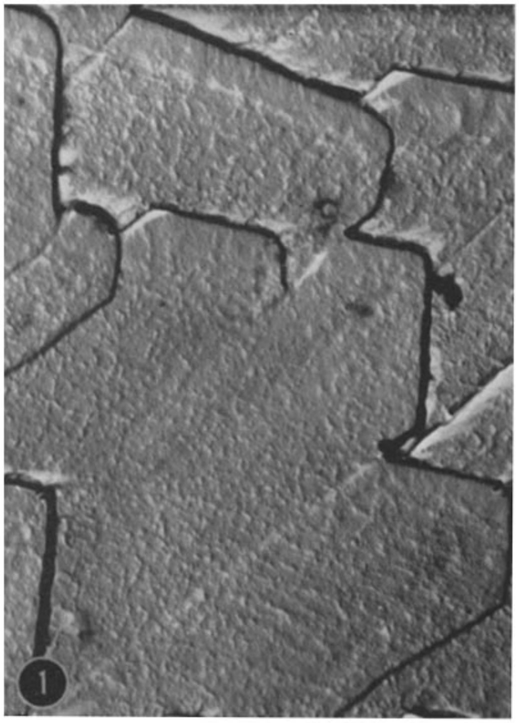
Creeping of crystals in the calcitostracum. Crystal *A* has overlapped crystal *B*. Parts of *C* and *D* have crept over *E* and fused, forming a bridge. × 13,500.

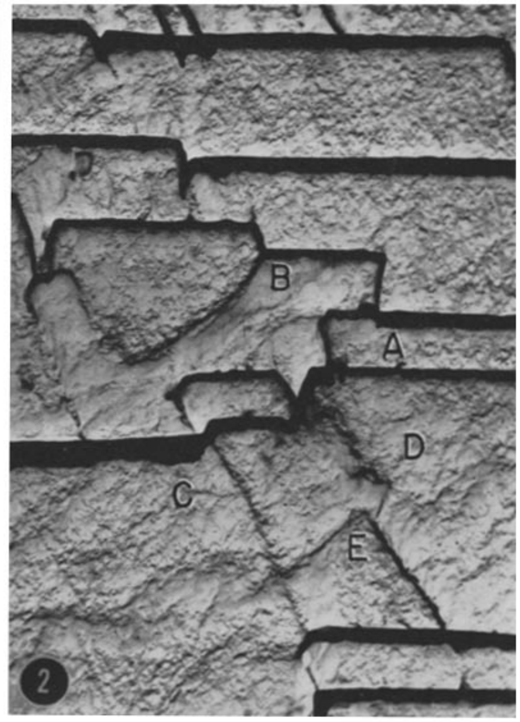
FIGURE 3

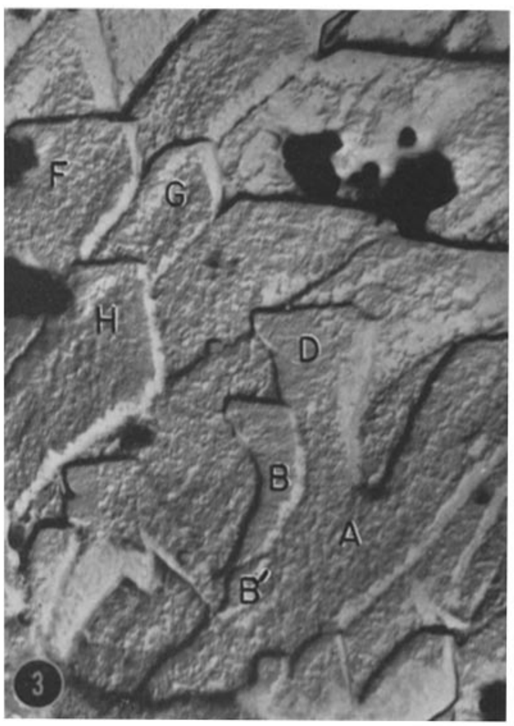
Crystals undergoing growth in the calcitostracum. The crystals are spear-shaped. Crystal *D* has branched from *A*. *B* and *B'* have branched from the basal plane of *A*. Growth on the edges and corners is seen in *F*, *G*, and *H* as raised areas. × 11,250.

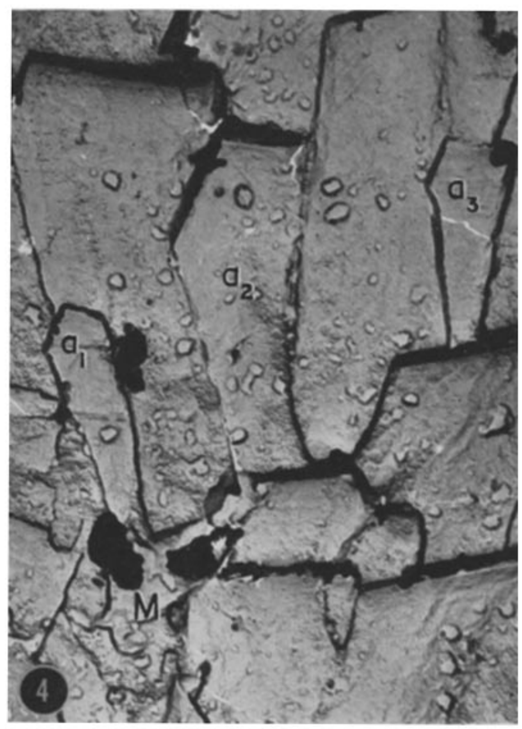
FIGURE 4

Tree-like dendritic growth in the calcitostracum. Dendrites a_1, a_2, and a_3 have branched from the main trunk *M*. × 12,400.


1


B
A
D
C
E
2


F
G
H
D
B
A
B′
3


a3
a2
a1
M
4

deposition of seeds on or in conchiolin or on the surface of well developed crystals. It is interesting that in the main part of the calcitostracum, seeds deposited on crystals always fused with the surface and never grew as independent isolated crystals. Such isolated crystals were found only near the transitional zone. The following configurations were commonly observed in the central region of the calcitostracum. Spear-shaped crystals apparently undergoing growth were often seen on larger crystals (Fig. 3). The parallelism of corresponding edges and the general crystal form indicate that these are a stage of the large crystals. The crystals are wide at the apices, and the other edges are irregular. They are not in side-to-side contact. The shape of the crystals indicates that the most rapid growth is at the apices. Growth of daughter crystals also takes place (Fig. 3, *D* from *A*). Branching from the basal planes, which represents growth in a third dimension, was also observed (*B* and *B'* from *A*). Growth of the basal planes along the vertical axes also occurred in most crystals as seen on the edges and corners of crystals *F*, *G*, and *H*.

Figs. 4 and 5, illustrating typical tree-like dendritic growth, offer important clues in an analysis of the growth processes and layer formation in the calcitostracum. Both figures show main trunks (*M*, or M_1 and M_2) from which branches develop at right angles. By reference to Fig. 5, we can now suggest processes leading to completion of the layer. From what we know of the nature of dendritic growth, M_1 and M_2 have probably grown from a main stem not shown in the picture. Crystals a_1, a_2, and a_3 are, in turn, branches of M_1, and b_1 and b_2 are branches of M_2. Crystals a_1 and b_1, in turn, have branches a_1' and b_1'; and b_2 has a branch, b_2'. With continuation of growth, spaces between the crystals a_1, a_2, b_1, b_2, and so on, will be filled in. Also a_1, a_2, and a_3 will cover b_1 and b_2. Thus, rows of parallel overlapping crystals will be formed.

2. *Transitional Zone*

The term transitional zone is applied to that narrow shell layer which covers the boundary between the prismatic region and the calcitostracum and slightly overlaps both. This zone shows structures remarkably different from the two regions yet retaining some of the characteristics of both.

Zone Covering the Calcitostracum: In general, the area is covered by wide crystalline sheets and small crystals undergoing growth. Fig. 6 shows the details of such a sheet made up of aggregates of granular blocks about 0.8 μ in width which are formed by the fusion of minute crystals. These minute crystals first develop along the edges and corners of the large regular crystals of the surface of the calcitostracum which underlies the transi-

FIGURE 5

Tree-like dendritic growth in the calcitostracum. Crystals a_1, a_2, and a_3 have grown from the main trunk M_1, and b_1 and b_2 from M_2. × 7,000.

FIGURE 6

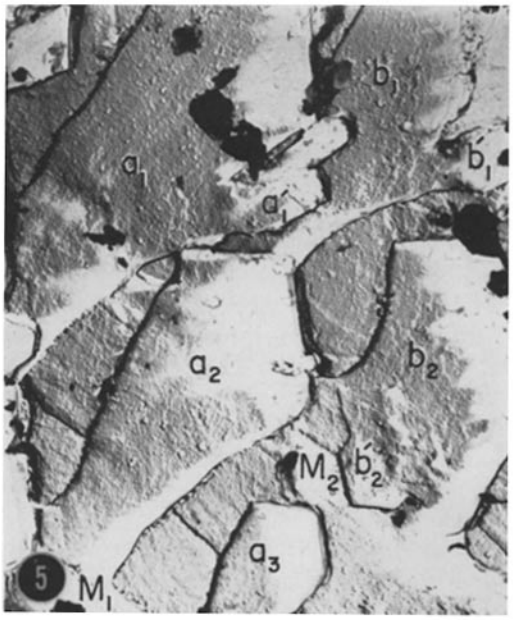
Crystalline sheets composed of small granular blocks in the transitional zone covering the calcitostracum. The granular blocks are formed by fusion of minute crystals, which first developed along the edges and corners of the large regular crystals of the calcitostracum underlying the transitional zone (see right bottom of the figure). × 7,300.

FIGURE 7

Transitional zone covering the calcitostracum. A more advanced stage of crystal growth than that shown in Fig. 6. The surface of each crystal block is partially smoothed out by fusion, but many spaces still remain unfilled. Two sheets having different orientation are shown in the upper and lower parts of the figure. × 7,300.

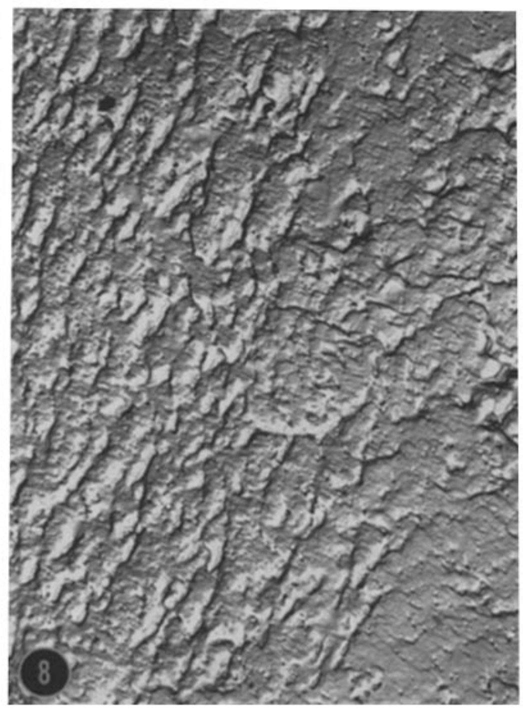
FIGURE 8

Transitional zone covering the calcitostracum. The final stage of growth. At the right a relatively homogeneous crystalline sheet has been completed. At the left, hollows are being filled in. × 7,300.


5
b_1
b_1'
a_1
a_1'
a_2
b_2
M_2
b_2'
a_3
M_1


6


7


8

tional zone. On further deposition and growth the small crystals form aggregates and finally cover the entire surface of the large crystals. The arrangement of the small crystals is relatively regular, probably owing to the orientation provided by the underlying crystals. Parts of large crystals of calcitostracum which have not yet been covered are shown at the lower right in Fig. 6. Although the large crystals form overlapping rows, the aggregates of smaller crystals deposited on them do not form this type of structure, but cover the entire area without forming distinct steps. Fig. 7 indicates a more advanced stage. Here the surfaces of each block are partially smoothed out by fusion. However, many spaces still remain unfilled. Two sheets having different orientations are shown in the figure, one in the upper part and the other in the lower. At the boundary of the two sheets the crystals of each sheet are fused. In this region the substrate crystals are completely masked.

Fig. 8 shows the final stage in growth. At the right a relatively homogeneous crystalline sheet has been completed, and at the left, hollows are being filled in. A similar configuration is present in Fig. 9, in which the ground mass is somewhat uneven and shows diagonal markings running obliquely from the upper right to lower left, probably representing depressions like those at the left side of Fig. 8. Small rectangular crystals (0.4 to 0.9 μ in length, and 0.2 to 0.5 μ in width), generally oriented in one direction, probably represent new deposition. The large crystalline blocks labeled *A* have resulted from fusion of smaller crystals. It is interesting that the large idiomorphic crystals characteristic of the calcitostracum are not present in the transitional zone, but instead the area is covered by broad sheets of crystalline aggregates. Rarely, a few comparatively large rhombohedral crystals develop from the ground mass.

Zone Covering the Prismatic Region: The prismatic region at the edge of the shell is made up of polygonal chambers composed of calcite within frameworks of conchiolin. Toward the transitional zone, the chambers become elliptical or round; the outlines gradually disappear; and the structure becomes that of the crystalline sheet of the transitional zone (Fig. 10, right side). The entire surface shows small regular grooves and elevations. Schmidt (5) found a similar structure in *Pinna* shells and thought these might represent impressions made by the mantle epithelial cells. However, the grooves and elevations have an appearance very similar to that shown in Fig. 8 for transitional zone of the calcitostracum. It is considered that the configurations have developed from aggregates of small crystals. A continuous sheet is formed which covers the underlying large rounded chambers of the prismatic region.

The transitional zone, together with the pris-

FIGURE 9

Transitional zone covering the calcitostracum. A configuration similar to that shown in Fig. 8. The diagonal markings running obliquely from the upper right to lower left probably represent depressions like those at the left side of Fig. 8. Small rectangular crystals oriented in one direction represent new deposition. Large blocks (*A*) have resulted from fusion of small crystals. × 8,100.

FIGURE 10

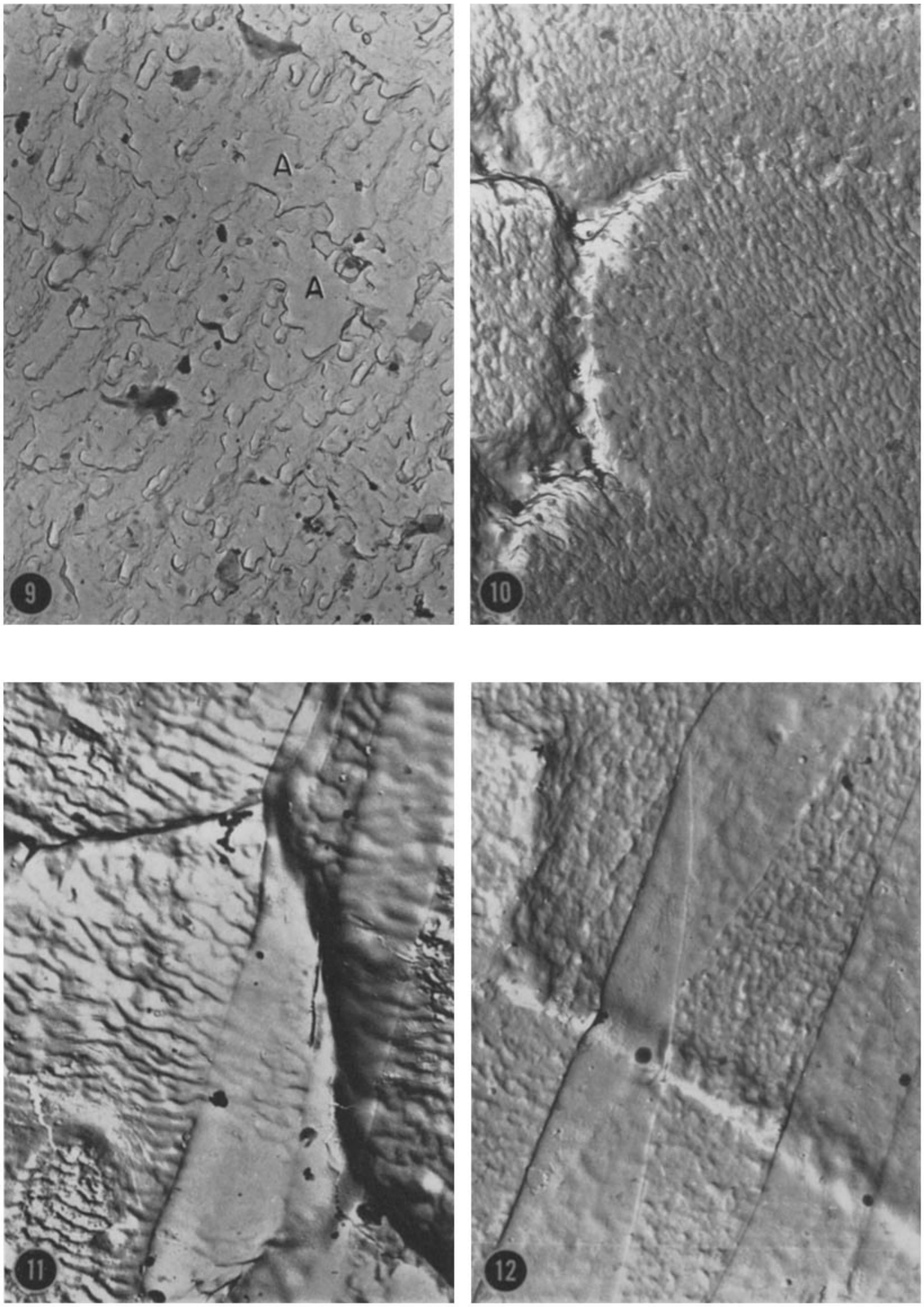
Transitional zone covering the prismatic region. At the left, a rounded chamber of the prismatic region is seen. The outline of chambers disappears at the right, and the structure resembles that of the crystalline sheet at the right in Fig. 8. × 7,000.

FIGURE 11

Conchiolin bands deposited in the transitional zone covering the prismatic region. Conchiolin membrane is also seen at the center of the picture. × 10,400.

FIGURE 12

A folded conchiolin band in the transitional zone covering the prismatic region. At the right, two bands are overlapped. × 7,800.


A
A
9
10
11
12

matic region, is characterized by abundant deposition of organic matrix material (conchiolin). This takes the form of a thin membrane (Fig. 11, center) or bands (Fig. 11, right). The bands may be folded (Fig. 12) and overlapped (Figs. 11 and 12), or grouped in a complex manner (Fig. 13). Combinations of bands and membrane were seen. Sometimes conchiolin is deposited as ridges (Fig. 14, top) or as a membrane covering small crystal aggregates (Fig. 14, center). The wide bands running parallel to the bottom of Fig. 14 are composed of narrow bands.

Crystal formation on or in the conchiolin membrane in the transitional zone begins in the same way as in the other regions, that is, with the deposition of crystal seeds (Fig. 15). The seeds develop into regular hexagonal or rounded crystals and are scattered randomly without any definite orientation. Fusion of neighboring crystals was observed. With continued formation and growth, the membrane on which the crystal seeds are deposited would come to be covered with aggregates of crystals. Finally the surface would show the texture illustrated in Fig. 16. Attention is called to the conchiolin which in the upper part of Fig. 16 covers the crystalline material as a relatively thick sheet and which also takes the form of broad bands in the lower half of the figure. These bands are of interest in that they have a superficial resemblance to the conchiolin which separates the crystalline aggregates in the prismatic region but which in the transitional zone is deposited on the surface of the aggregates.

The conchiolin membrane at higher magnification exhibited a very fine network pattern showing a regular orientation (Fig. 17). The dimensions of the network as measured from replicas will not be used here because of the limitations of this method. However, examination of decalcified thin sections of the matrix resolved a similar structure with spacings which average 60 A in one direction and 80 A in the other. These findings will be reported separately.

CONSIDERATIONS CONCERNING POSSIBLE MECHANISMS OF SHELL FORMATION

The Structure of the Central Part of the Calcitostracum: The central part of the calcitostracum is characterized by aggregates of well formed crystals which overlap in successive rows. In an earlier paper (9) it was pointed out that these crystals develop from seeds deposited on conchiolin and that the growth

FIGURE 13

Conchiolin bands grouped in a complex manner in the transitional zone covering the prismatic region. × 7,800.

FIGURE 14

Combination of very thin conchiolin membrane (center) and bands (top and bottom) deposited on small crystals in the transitional zone close to the calcitostracum. The bands at the bottom are composed of narrow bands. × 7,000.

FIGURE 15

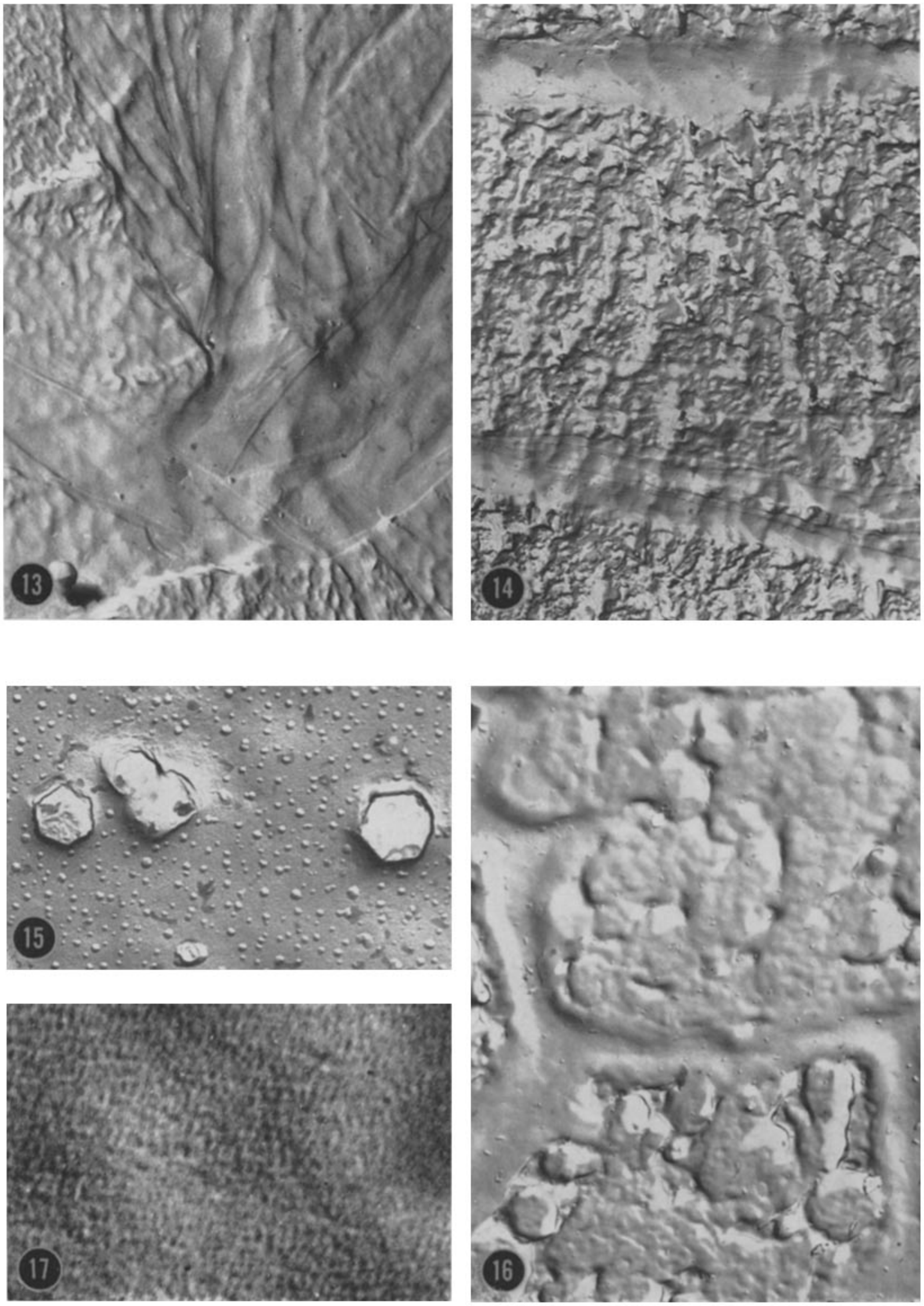
Transitional zone. An early stage of crystal growth on or in the conchiolin membrane showing randomly scattered crystal seeds and small hexagonal crystals developed from them. Fused crystals are also seen. × 9,600.

FIGURE 16

Transitional zone. More advanced stage than in Fig. 15. Crystals are combined. New conchiolin has been deposited over the crystals as a relatively thick sheet in the upper part of the figure and as broad bands in the center and lower part. × 7,800.

FIGURE 17

Fine regular network pattern in the conchiolin membrane. From a reversed negative. × 80,000.


13
14
15
17
16

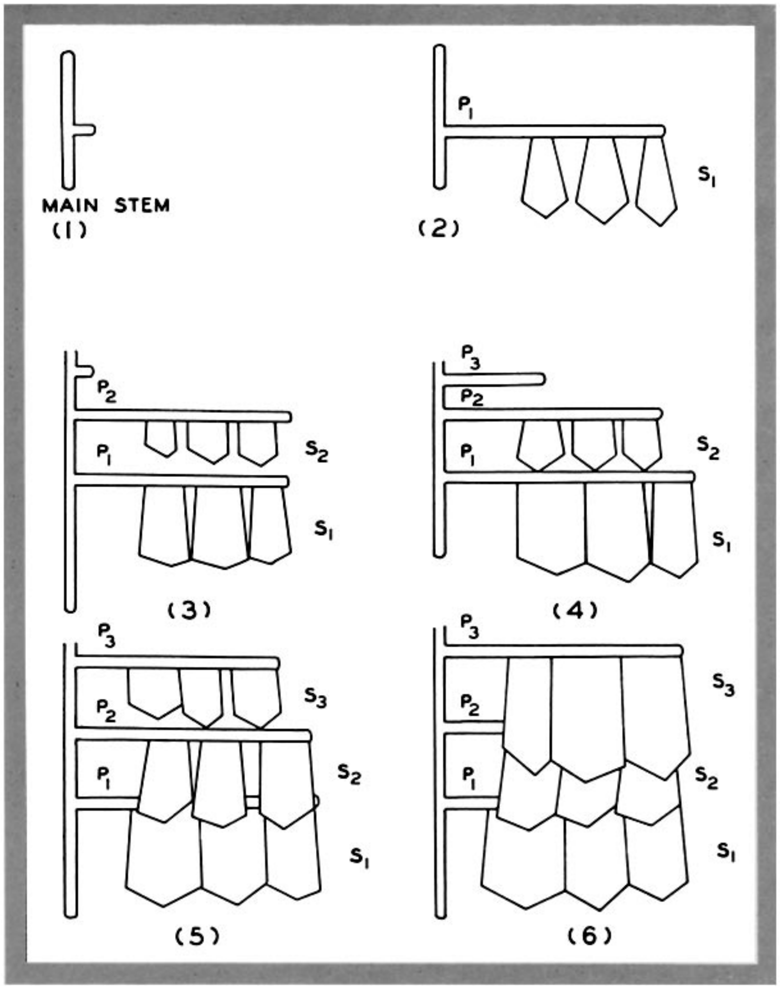


FIGURE 18

Schematic representation of dendritic growth of crystals.

is of a dendritic nature. However, the process of layer formation of successive rows was left unexplained. The present study has demonstrated (*a*) typical tree-like dendritic growth of crystals, (*b*) elongation of well developed crystals in a common direction, and (*c*) the creeping of crystals over adjacent crystals. These findings make possible an explanation of the structure of the calcitostracum.

After the deposition of seeds on or in a conchiolin membrane, the larger seeds grow at the expense of smaller ones. We shall now make the assumption that crystallization is occurring in a supersaturated solution and that the degree of saturation is usually not uniform even in a limited field. This condition might well be produced by the close apposition of the mantle to the shell with a resulting lack of circulation in the very thin layer of solution. Further, it is reasonable to expect that the supersaturation would become metastable in one limited area and labile in another, with a resulting strong concentration gradient. Crystal growth will then proceed rapidly from the metastable into the labile region. Thus the main stem of the dendrite will be formed (Fig. 18). Then, following the characteristics of dendritic growth (Buckley, 1), branching would occur at right angles to the main stem (P_1), and this would be followed by secondary branchings (S_1). (In Fig. 5 secondary branches are represented by crystals b_1 and b_2.) Meanwhile, another primary (P_2) and another secondary (S_2) branch are formed. By further growth the apices of S_2 reach P_1. If the growth rate is very rapid, S_2 will creep over P_1 and onto S_1. Likewise S_3 will cover P_2 and S_2, and so forth. Overlapping rows will result. Overlapping of crystals which belong to the same primary branch is less likely in that the growth rate of the side planes would be less than that of the apices. In other words, lattice formation on the side planes is slower and accordingly the probability of the formation of a common lattice shared by two crystals would be increased. Fusion would result. If the formation of a common lattice fails because of the presence of conchiolin or impurities, creeping may take place as shown in Fig. 2.

The above explanation represents the simple case. Three-dimensional dendritic growth is also to be expected. Moreover, daughter crystals which branch from the surface of the dendrites may also form overlapping rows. The branching which results in a form of daughter crystals is considered to be due to edge dislocation which occurs at geometrical faults in the crystal lattice (see review by Frank, 2). Gorodetsky and Saratovkin (3) have recently shown that foliated crystals of cadmium iodide are formed by successive dislocation, indicating that overlapping rows can be formed by this means. Vertical sections of this material showed a brick wall type of structure like that present in the calcitostracum. Through such dendritic growth and dislocation multilayers of crystals will be formed at one time. The amount of conchiolin is very small in the calcitostracum of the oyster, and the formation of multilayers is favored by the infrequent secretion of the organic matrix, which would inhibit crystal growth.

In the central parts of the calcitostracum, crystal seeds were observed previously (9) and in the present study, but well developed isolated crystals have not been seen. The absence of isolated crystals is probably to be explained as a result of fusion of the seeds with the surface of the crystals on which they rest, or of the dissolving of the seeds in the presence of strong concentration gradients.

The Structure of the Transitional Zone: The transitional zone, with the exception of the area where crystals are deposited on or in the organic matrix, is characterized by a definite orientation of crystals which are smaller than those of the neighboring prismatic and calcitostracum areas. Growth in the transitional zone proceeds toward the center of the shell as indicated by a continuous layer of crystals neighboring the prismatic region and by individual crystals more centrally located.

Growth of small crystals resting on the large crystals is similar to that in the central areas of calcitostracum in which new crystals have the same orientation as those on which they lie (9). However, the final stage of growth in the transitional zone is an aggregate of small crystals of almost uniform size. This indicates that a number of seed crystals survive without undergoing any considerable dissolution and grow until they come into contact and the layer is completed. This type of growth without the formation of large crystals is apparently due to a relatively homogeneous state of solution without strong concentration gradients. The calcitostracum differs from the transitional zone and prismatic region in showing evidence of strong concentration gradients. The gradients must derive from unequal rates of transfer of calcium or carbonate or both by the mantle and presumably by specialized mantle areas. Whether the parts of the mantle covering the more peripheral transitional zone and prismatic region transfer calcium and carbonate more uniformly or whether gradients are prevented by mixing due to mantle movements is unknown.

This work was aided by a grant from the Office of Naval Research, United States Navy.

Received for publication, September 20, 1960.

BIBLIOGRAPHY

1. Buckley, H. E., Crystal Growth, New York, J. Wiley and Sons, Inc., 1951, 479.
2. Frank, F. G., *Discussions Faraday Soc.*, 1949, **5,** 48.
3. Gorodetsky, A. F., and Saratovkin, D. D., *Rep. 1st Conf. Crystal Growth* (*English translation*), 1958, 151.
4. Grégoire, C., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 797.
5. Schmidt, W. J., *Mikrokosmos*, 1924–1925, **12,** 73.
6. Tsujii, T., Sharp, D. G., and Wilbur, K. M., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 275.
7. Wada, K., *Bull. Nat. Pearl Research Lab.*, 1957, **2,** 86.
8. Watabe, N., *J. Fuji Pearl Inst.*, 1952, **2,** 31.
9. Watabe, N., Sharp, D. G., and Wilbur, K. M., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 281.