

# Mineralogy, chemistry and ultrastructure of the external shell-layer in ten species of *Haliotis* with reference to *Haliotis tuberculata* (Mollusca: Archaeogastropoda)

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Mineralogy and ultrastructure of the external shell-layer are described in ten species of the Recent archaeogastropod genus *Haliotis*. In two species (*H. asinina*, *H. glabra*), this layer is exclusively aragonitic, whereas in two other species (*H. rufescens*, *H. kamtschatkana*), it is exclusively calcitic. In the remaining six species (*H. tuberculata*, *H. lamellosa*, *H. (Nordotis) cf. discus*, *H. iris*, *H. rotundata*, *H. fulgens*), the external shell-layer has a mixed aragonitic-calcitic composition. The aragonitic units are frequently entirely enclosed within the calcitic prisms which means that the mantle epithelium secretes simultaneously the two CaCO<sub>3</sub> morphs. The distributions of Mg, Sr, Na and S, analysed with the electron microprobe, do not show any significant differences between these morphs.

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Other <i>Haliotis</i> species .....	9	The shells of the great majority of gastropods consist of aragonite. Only a few gastropods, belonging to thirteen families (Bøggild, 1930) utilize cal-	
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cite, to form the whole shell (e.g. Patellidae), or separate layers. The separate layers are usually structurally and mineralogically uniform. However, in the archaeogastropod genus *Haliotis*, the external shell-layer may be exclusively calcitic or aragonitic, and in many species it contains both calcite and aragonite in proportionally varying mixtures. Moreover, in the latter case, the two morphs are so closely associated that the aragonitic units may be entirely enclosed within the calcitic units. However, they can be always recognized because of their distinctly different crystal habit. The ability of the haliolitid mantle epithelium to secrete, simultaneously, two CaCO<sub>3</sub> morphs has caused some confusion in the literature concerning the mineralogy of the external shell-layer.

The shell of *Haliotis* is of great importance for our understanding of the mechanism of shell secretion, the role of organic matter in this mechanism, and the causes which regulate the formation of the CaCO<sub>3</sub> morphs.

The present paper summarizes the results of our studies on the mineralogy and structure of the external layer in haliolitid shells, based on ten species from the different regions of the world. The mineralogy of this layer covers the entire range of variations which occur in this genus, to wit, calcite and aragonite, and various mixtures between these two morphs. In addition, aspects of the trace-element chemistry in these shells was studied by means of the electron microprobe.

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### Material and methods

The external shell-layer in *Haliotis tuberculata* Linné, and in its Mediterranean sub-species, *Haliotis lamellosa* Lamarck, was studied in our previous paper (Mutvei *et al.*, 1985). This species, together with its sub-species, is therefore chosen as a basis of the present comparative study. The material of *H. tuberculata*, here dealt with, comprises numerous shells of living and dead animals, obtained from the Station Biologique de Dinard, France, on the English Channel.

In addition, the following nine species were studied:

- H. asinina* Linné, Komodo Islands, Indian Ocean.
- H. fulgens* Philippi, Southern California, USA.
- H. rufescens* Swainson, north of San-Francisco, California, USA.
- H. glabra* Swainson, Java.
- H. iris* Martyn, New Zealand.
- H. rotundata* Perry, unknown locality.
- H. roei* Gray, unknown locality, but probably Australia.
- H. (Nordotis) cf. discus* Reeve, Japan.
- H. kamtschatkana* Jonas, San Juan Island, Washington State, USA.

### Methods

**Mineralogical composition.** Polished sections were cleaned by ultrasonic treatment and briefly etched in dilute hydrochloric acid, before staining with Feigl's solution. Aragonite is stained black, whereas calcite remains unstained. For SEM observations, the preparations were stained for a shorter period of time, in order to make the structural details discernible (Schneidermann & Sandberg, 1971).

Electron microprobe (energy dispersive X-rays) was also used for recognizing calcite and aragonite. The determination of the two morphs is based on the relative abundance of Sr, Na and K in aragonite, and Mg and Mn in calcite. The concentration of these elements is frequently at, or near, the limit of detection of this instrument. The analysis were made with a Link microprobe, using the AN 10000 program. The use of the ZPB method (Peak to Background) allowed us to study rough surfaces, caused by a slight etching of the polished preparations, made in order to reveal the structural details. The positions of the analyzed points with respect to the structural features are therefore precisely known. The latter is necessary, because of the imbrication between aragonite and calcite, and the small size of the individual aragonitic units within the calcitic prisms.

**Micro- and ultrastructures.** Ultra-thin sections (5–6 µm), studied in polarized light, allowed us to observe the shape and crystallographic orientation of the calcitic components; the aragonitic components are too small and can therefore only be observed with the SEM.

For studies of the structure of the mineral components with the SEM, the organic matter was removed either by enzymatic degradation (protease, chitinase), or bacterial activity. The protease was buffered at pH 7.8, to minimize the dissolution-effect of the buffer (Carriker, 1978).

Various methods for etching and decalcification were used. The most efficient methods were: (1) formic acid (5 %), to reveal the periodic growth-pattern of the calcitic prisms, but without any *in situ* preservation of the organic matter; and (2) a mixture of glutaraldehyde, acetic acid and Alcian blue at various concentrations and temperatures, for decalcification combined with fixation of the organic matter *in situ*. Most preparations treated by method (2) were critical-point dried.

Calcite and aragonite have different solubilities. But even in the case of identical mineralogical composition, the structural type of one and the same CaCO<sub>3</sub> morph influences the etching results. For example, the aragonitic nacre is dissolved more rapidly than the aragonitic prisms and spherulites. Calcitic prisms have the lowest solubility. The latter condition makes it difficult to observe the boundaries between different CaCO<sub>3</sub> morphs in etched sections in the SEM.

## Previous studies

### *H. tuberculata* and *H. lamellosa*

The first observation on the mineralogical variability of the external layer in *Haliotis* seems to have been published by Bøggild (1930, p. 300) who noted that "the shells of *Haliotis*. . . are rather uniform", but they "possess, in some instances, a calcitic layer inserted between two aragonitic ones. . . The calcite has a rather regularly prismatic structure with horizontal, radial prisms. . . As to the extension of this layer, the different species are very variable. . . in *H. tuberculata* there are only a few small spots of calcite".

Kessel (1935) showed that calcite in *H. tuberculata* and *H. lamellosa* seems to be located in the inner part of the external prismatic layer, whereas the aragonite occurs in the outer part. Aragonite and calcite are not separated into two distinct layers, but numerous small aragonitic spherulites are imbricated in calcitic prisms, and large calcitic spots occur in the aragonitic part. Kessel used a specific staining method to determine the CaCO<sub>3</sub> morphs.

Stolkowski (1951) found less than 1 % calcite in the entire shell of *H. tuberculata*. Sabatier (1953) noted that the external layer is characterized by "la présence simultanée de calcite et d'aragonite, en proportion sensiblement équivalente". Philippon (1974) found that the entire shell of *H. tuberculata*, comprising both the nacreous and the prismatic layer, is aragonitic. However, he described some

"éléments plus trapus qui pourraient bien être des prismes de calcite".

SEM studies were carried out on the shell structure in *H. tuberculata* and *H. lamellosa* by Mutvei *et al.* (1985). In agreement with the observations by Kessel (1935) and Sabatier (1953), it was found that calcite occurs abundantly in the inner part of the external shell-layer, where it forms large prisms, whereas aragonite occurs in the outer part of that layer as minute irregular radial units.

## Other *Haliotis* species

In its most restricted sense, the genus *Haliotis* comprises about 30 species, but only a few of them have been studied for their structural and mineralogical composition. Nakahara *et al.* (1982) noted that the external shell-layer in *H. rufescens* is completely calcitic. Iwata (1980) found that the larval shells of *H. discus hannai* consist of aragonitic spherulites.

Mutvei *et al.* (1985, tabl. 1, fig. 1) showed that the external shell-layer in *H. asinina* and *H. glabra* is aragonitic, but mixed aragonitic-calcitic in *H. cf. discus*, *H. fulgens*, *H. roei* and *H. rotundata*. In the latter group of species, aragonite occurs as small spherulites external to the large calcitic prisms.

## Structure of the external shell-layer

### A. Species with mixed aragonitic-calcitic external shell-layer

#### (1) *Haliotis tuberculata* and *H. lamellosa*

The external shell-layer is composed of both aragonite and calcite, the ratios of which largely vary in different individuals. As previously shown (Mutvei *et al.*, 1985), aragonite occurs as small prismatic and spherulitic units, located in the outer part of this layer, whereas calcite forms large prisms in the inner part of the layer, adjacent to the nacre (Plate 1, Fig. 1).

Attempts to relate the location of the calcite with respect to the shell ornament (radial costae and major growth lines), has not been possible. Therefore, in random sections, the calcite/aragonite ratios may exhibit considerable variation. The latter condition explains the contradictory results of the previous studies.

*Aragonitic part.* The main aragonitic units are minute and elongate, about 2 to 5 µm wide and 5 to 10 µm long. Their external shape is highly variable.

In places, they appear as single rods, particularly near the shell surface (Plate 1, Fig. 4). More frequently, they are larger and show an ovoid or prismatic shape with a more or less distinct costulate ornamentation (Plate 1, Figs. 5, 6). The aragonitic units do not show any preferred orientation, and they are not connected or fused to each other. Because of the small size of the aragonite units, it is difficult to obtain information on their internal organization. Transverse sections (Plate 2, Fig. 1) sometimes show parallel crystallographic planes which may be internal equivalents of the external costulae. In other preparations, particularly in polished and etched sections (plate 3, Figs. 1, 2), the aragonitic units consist of fascicles of diverging crystalline rods. Etching by means of bacterial activity demonstrates that the central part of these units is much more soluble than the outer cortical part (Plate 2, Fig. 3). The latter observation suggests that the outer and inner parts of these units have a different composition, although in transmitted polarized light they show a monocrystalline aspect.

After dissolving most of the mineral phase with a glutaraldehyde-acetic acid solution, delicate filaments of intracrystalline organic matter become visible (Plate 2, Fig. 2). It was not possible to establish the relationship between the crystallographic orientation of the aragonitic units and the organization of the intracrystalline organic matter. The decalcification process probably induces a progressive collapse of this organic matter which, without fixation, is soluble. We have not observed any indication that the aragonitic units are surrounded by an organic envelope.

*Calcitic part.* There is a considerable difference in the shape between the aragonitic and calcitic units in the external shell-layer. The calcite forms regular prisms elongate structures, polygonal in cross-sections, and with growth-surfaces more or less perpendicular to the axial direction. They are much larger than the aragonitic units, sometimes more than 1 mm in length and 100 to 200  $\mu\text{m}$  in width. They are arranged in complex and irregular groups (Plate 2, Fig. 4). Polished and etched sections show numerous horizontal growth-lines associated with abundant intracrystalline organic matter (Plate 2, Figs. 5, 6).

*Contact between aragonite and calcite.* The aragonite units are often embedded in calcitic prisms, without being separated from these by organic matter. It seems, therefore, that pre-formed compartments of the organic matter in which aragonite or calcite would be subsequently precipitated do not exist (compare e.g. with Nakahara & Bevelander, 1971).

A total mixture between the two  $\text{CaCO}_3$  morphs,

and the arrangement of the growth-lines, as seen in Plate 1, Figs. 2, 3, indicate an unusual secretory function of the mantle cells. These cells must have secreted simultaneously both aragonite and calcite. At successive growth-stages, the amounts of these two morphs show large variations (Plate 1, Fig. 2). This means that one and the same part of the secretory epithelium is capable of secreting both categories.

*Transition between the aragonitic external layer and the nacreous layer.* At the boundary to the nacreous layer, rods in the aragonitic units become elongate and oriented transversally to this boundary (Plate 3, Fig. 1). The distal parts of the rods are traversed by horizontal parallel organic sheets which are discontinuous, and variably spaced (Plate 3, Fig. 2). In the nacreous layer, these organic sheets become continuous and regularly spaced, forming the interlamellar organic sheets (right-lower corner in Plate 3, Fig. 2). Thus, there is a gradual transition from the prismatic-spherulitic structure of the external layer to the nacreous structure. In some nacreous tablets at this boundary, one may still recognize the crystalline rods which are directly continuous from the external layer.

*Transition between calcitic external layer to the nacreous layer.* A direct contact between calcitic prisms and true nacre has not been observed. The basal parts of the calcitic prisms are always lined by aragonitic rods (Plate 2, Fig. 5). It seems that in the present species, a production of aragonitic rods is needed before the secretion of the organic interlamellar sheets which are characteristic for the nacreous layer.

## (2) *Haliotis (Nordotis) cf. discus*

As is the case in *H. tuberculata*, the external shell-layer is here composed of an inner calcitic and an outer aragonitic part (Plate 3, Fig. 3).

*Aragonitic part.* The aragonitic units of *H. cf. discus* and *H. tuberculata* show a similar shape and distribution: small crystalline rod-shaped units occur near the shell surface, and larger ovoid units, with costulate ornament, occur in the rest of the layer (Plate 3, Fig. 4).

In oblique fracture-planes, the ovoid units show a thick outer cortical part, composed of large crystalline rods, and an inner part, composed of parallel or slightly diverging crystalline laminae (Plate 3, Fig. 6). These laminae, which are elongated along the major axis of the unit, consist of parallel crystalline rods of smaller size. In some specimens, the ovoid units lack the outer cortical part. In polished and etched sections, the latter units often appear as

diverging crystalline rods arranged in fascicles (Plate 3, Fig. 5). The regularly shaped ovoid units are composed of three to four fascicles (Plate 4, Fig. 1), whereas the irregularly shaped units consist of a single fascicle. After prolonged etching with glutaraldehyde-acetic acid solution, numerous organic filaments were seen to appear between the crystalline rods. The arrangement of these filaments is independent of the orientation of the crystalline rods.

*Calcitic part.* The variations in the shape of the calcitic prisms, and their complex arrangement are shown in Plate 4, Fig. 2. In contrast to *H. tuberculata*, the calcitic prisms in the present species have strongly protruding basal ends which are in direct contact with the succeeding nacreous layer. The first deposited nacreous laminae are therefore secreted between the protruding basal ends of the prisms. The deposition of the nacreous layer remains interrupted at these places until the nacreous laminae completely fill up the interspaces between the basal ends of the prisms (Plate 4, Figs. 3, 4).

### (3) *Haliotis roei*

In this species, the outer aragonitic parts and the inner calcitic part of the external shell-layer are approximately of equal development.

*Aragonitic part.* The aragonitic units have about the same shape and arrangement as in the two species described above. Thus, the size of the units decreases from the nacreous boundary towards the outer shell surface. Most of the larger units have a regular ovoid shape and a regular hexagonal outline in axial view (Plate 4, Figs. 5, 6). They are composed of parallel, prismatic sub-units of polygonal cross-sections (Plate 4, Fig. 5). After treatment with enzymatic solutions, these sub-units reveal a complex structure (Plate 5, Fig. 2). In other preparations, treated with enzymes, the internal region of the ovoid units shows a composition of small, distinct granular elements, sometimes arranged radially (Plate 5, Fig. 1).

These observations show that (1) the ovoid units are, in fact, aragonitic prisms; this concept (e.g. Philippon, 1974) is in agreement with their monocrystalline optical characteristics when studied in ultra-thin sections under polarized light; and that (2) the frequent radial arrangement of the crystalline rods indicate a spherulitic nature of the ovoid units (e.g. Kessel, 1935); the spherulitic structure is often seen in polished and etched sections. Consequently, both terms, the prismatic and the spherulitic, can be applied to be ovoid units. This condition demonstrates the inadequacy of current crystallographic terminology.

*Calcitic part.* The complex polycrystalline structure of the large calcitic prisms is clearly visible (Plate 5, Figs. 3, 4). After enzymatic treatment, the intracrystalline organic matter is removed and the growth lamellation of the prisms becomes distinct.

### (4) *Haliotis iris*

The aragonitic units in the outer part of the external layer show similar features to those in the species dealt with above.

The growth surfaces of the calcitic prisms at the shell-margin are similar to those in *H. (Nordotis) cf. discus*. They have a rhomboid shape and an imbricated arrangement (Plate 5, Figs. 5, 6; Plate 6, Figs. 1, 2). The lateral faces of the prisms have a regularly spaced growth-lamellation (Plate 6, Figs. 3, 4). The prisms enclose isolated aragonitic units, some of which are large and have a radial structure.

### (5) *Haliotis fulgens*

As shown in Plate 7, Figs. 1, 2, the structure of the external shell-layer in this species agrees to that in the species dealt with above, both in the shape and arrangement of the calcitic prisms and the aragonitic units.

### (6) *Haliotis rotundata*

The external shell-layer in this species differs from that in the species dealt with above, in the following respects: (1) this layer is predominantly aragonitic; in each aragonitic units, the crystalline rods are often arranged parallel to each other, but the orientation is different in adjacent units (Plate 6, Figs. 5, 6); and (2) the calcitic prisms occur only occasionally in the inner part of the layer (Mutvei *et al.*, 1985).

## B. Species with an exclusively aragonitic external shell-layer

### (1) *Haliotis asinina*

This is the type species of the genus *Haliotis*. In contrast to other species dealt with here, the outer shell-surface is green and devoid of encrusting organisms. In the numerous sections made of four specimens, the external shell-layer is entirely ara-

gonitic, except for a minute calcitic spot observed in one specimen.

The size of the aragonitic units decreases towards the shell-surface. Most units differ from those in the species dealt with above in having a much more regular structure: the crystalline rods are arranged parallel to each other, or they form fasciculate bundles, oriented almost perpendicular to the surface of the shell (Plate 7, Figs. 3, 4, 5). Decalcified sections show thin organic filaments, the orientation of which is independent of that of the crystalline components (Plate 7, Fig. 6). After a prolonged decalcification with glutaraldehyde-acetic acid solution, organic sheets composed of these filaments can be occasionally seen.

At the transition to the nacreous layer, the crystalline rods of the aragonitic units continue into the adjacent nacreous tablets (Plate 7, Figs. 4, 5).

## (2) *Haliotis glabra*

The crystalline rods in the aragonitic units are oriented approximately perpendicular to the shell-surface, in the same manner as those in *H. asinina* (Plate 8, Figs. 1, 3, 4). It is only in some units that the orientation of the crystalline rods can instead be seen to be approximately parallel to the shell-surface (two units in the middle height of Plate 8, Fig. 1, one of which is magnified in Plate 8, Fig. 2).

## C. Species with an entirely calcitic external shell-layer

### (1) *Haliotis rufescens*

In all preparations, the external shell-layer is composed entirely of calcitic prisms (Plate 8, Fig. 5). On the growth surface at the shell margin, the prisms form large imbricated blocks, similar to those described by Nakahara *et al.* (1982). The growth surface of each block shows parallel, diamond-shaped edges.

In polished vertical sections, etched for several days with glutaraldehyde-acetic acid solution, the calcitic prisms show a distinct subdivision into growth lamellae. Each lamella consists of vertical crystalline rods arranged in fasciculate bundles (Plate 9, Figs. 1, 2, 3). The etching also reveals that the consecutive crystalline growth lamellae are separated by parallel, perforated organic sheets (Plate 9, Fig. 3). Such continuous organic sheets do not appear if the period of etching is short.

### (2) *Haliotis kamtschatkana*

As in *H. rufescens*, the external shell-layer is here entirely composed of calcitic prisms (Plate 9, Fig. 4). The size of the prisms decreases towards the outer shell surface (Plate 9, Fig. 5). All prisms show a distinct growth lamellation in polished vertical sections etched with glutaraldehyde-acetic acid solution. Etching also reveals abundant intracrystalline organic matter.

The boundary between the calcitic prisms and the adjacent nacreous layer displays a variable pattern of development. In some places, aragonitic rods are formed between the basal parts of the calcite prisms and the nacreous tablets. In other places, the prisms are in direct contact with the nacreous layer, as in *H. (Nordotis) cf. discus* (plate 9, Fig. 6).

## Chemical composition

Each shell-layer (aragonitic prismatic-spherulitic, calcitic prismatic, and aragonitic nacreous), was studied with regard to the concentration of some minor elements. We have obtained data on the following thirteen elements: Na, Mg, Al, Si, P, S, Cl, K, Mn, Fe, Sr, Ba, and Pb. The concentrations of four of them (Mg, Na, Sr, S) are dealt with below.

The elementary composition of gastropod shells has been given little attention in the extant literature. The review of Milliman (1974), including previous results from Vinogradov (1953) to Seger *et al.* (1971), is one of the most extensive and precise for both taxonomical and numerical data. It concerns mainly aragonitic shells. Unfortunately, in shells with mixed mineralogy, analyses have been made without separating the different shell-layers. For this reason, we have had to compare our data with those recently published by Masuda & Hirano (1980) for Bivalvia. These authors present comprehensive results with details on the mineralogy and microstructure of the analyzed shell-layers. The data for bivalves on the relationship between the environmental parameters and the elementary composition of the shell are, moreover comprehensive than for gastropods (e.g. Rosenberg, 1980; Carter, 1980).

## Mean values

The mean values (Figs. 1,2) of Mg, Sr, Na and S contents in the shells of all ten analyzed haliotis species are in good agreement with the means ob-

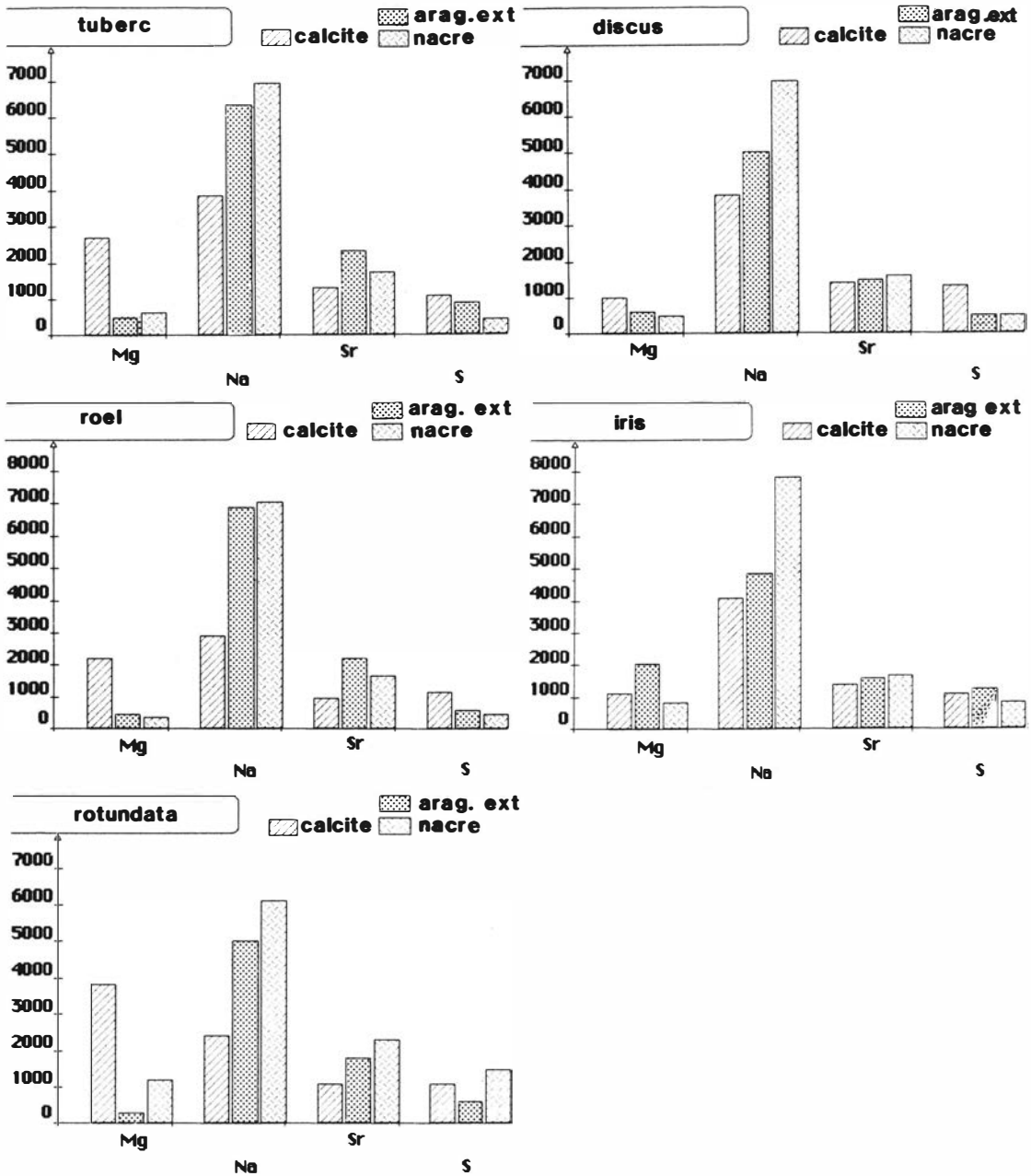


Fig. 1. Histograms of the mean values (in ppm) of the contents of Na, Mg, Sr and S, in the aragonitic nacreous layer (nacre) and the mixed aragonitic-calcitic external layer (arag. ext, calcite): *H. tuberculata*, *H. (Nordotis) cf. discus*, *H. roei*, *H. iris*, and *H. rotundata*.

tained by Masuda & Hirano (1980) for the corresponding structural types in bivalves. The content of Mg in the haliolitid calcitic layer is similar to that of the bivalvian fibrous calcite, whereas the Na content

is closer to the bivalve prismatic calcite. However, the haliolitid aragonite (external spherulitic-prismatic and nacreous layers) shows a higher Mg-content than the aragonitic layers in bivalves. The dif-

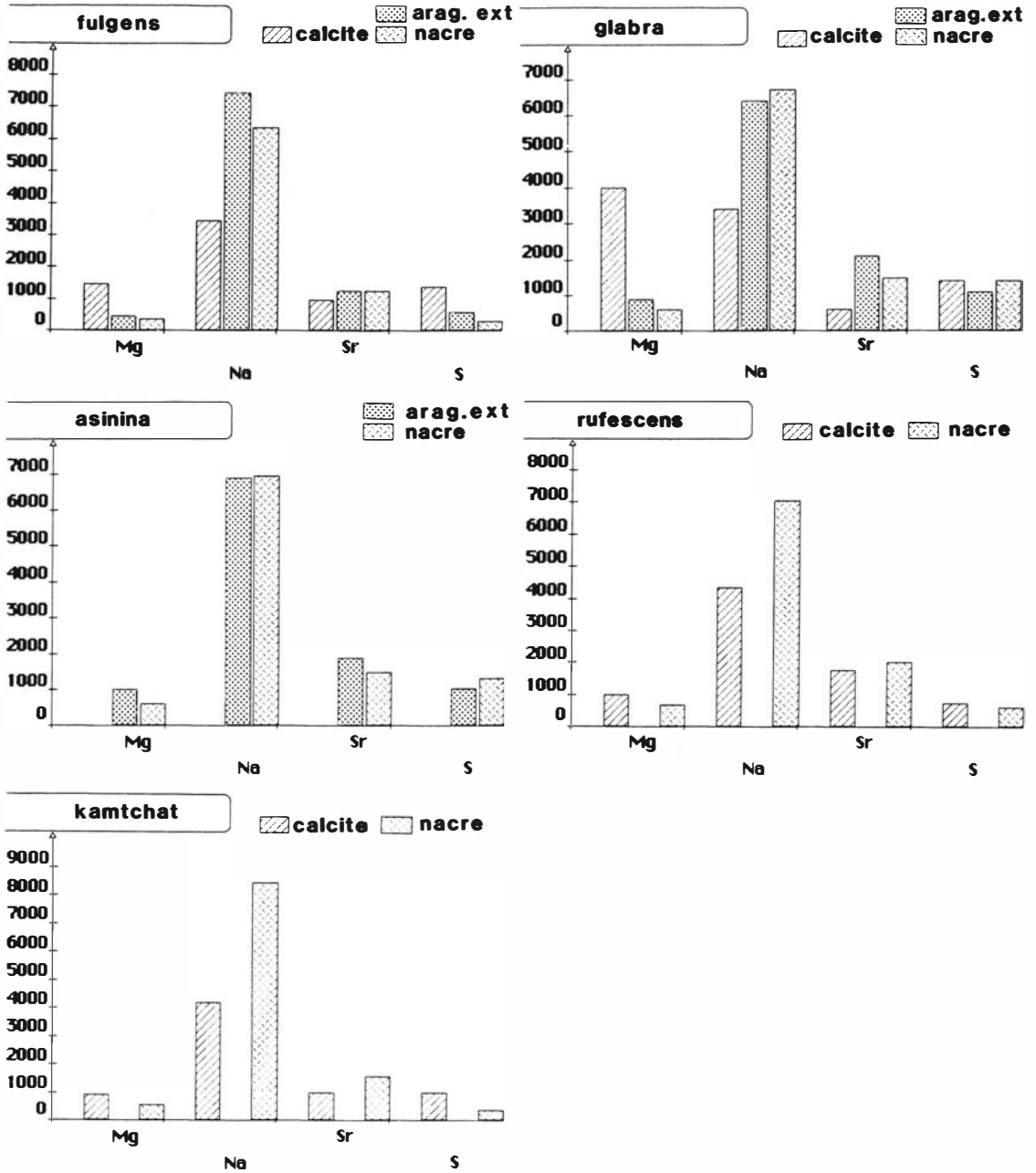


Fig. 2. Histograms of the mean values (in ppm) of the contents of Na, Mg, Sr and S, in the aragonitic nacreous layer (nacre) and the mixed aragonitic-calcitic external layer (arag. ext., calcite): *H. fulgens* and *H. glabra*; in the aragonitic nacreous layer (nacre) and the aragonitic external layer (nacre, arag. ext): *H. asinina*; and in the aragonitic nacreous layer (nacre) and the calcitic external layer (calcite): *H. rufescens* and *H. kamtschatkana*.

ferences in the means do not indicate any obvious interpretation, and have to be explained by a more detailed examination of each shell-layer.

For each analyzed shell-layer, we present first the

possible significance of the direct values, then the ratios between the minor elements, as they have been supplied by various authors (e.g. Loreau, 1982).



Table 1. Summary of the mean composition (in ppm) and ratios in all analysed species of *Haliotis*. Mg/Ca, Sr/Ca = weight ratios  $10^{-2}$ ; mMg/mCa, mSr/mCa = atomic ratios  $10^{-2}$ ; mSr/mMg = ratio defined by Loreau (1982) as substitution rates of  $\text{Sr}^{++}$  and  $\text{Mg}^{++}$  to  $\text{Ca}^{++}$ ; K'A Mg = partition coefficient of Mg  $10^{-3}$ ; K'A Sr = partition coefficient of Sr  $10^{-2}$ .  
a: aragonitic nacreous layer; b: aragonitic external layer; c: calcitic external layer.

a	tuber.	discus	roei	iris	rotun.	fulgens	asinina	glabra	rufes.	kamtsch.
Na	6900	6900	7100	7900	6100	6400	6900	6700	7100	8400
Mg	600	500	400	800	1200	400	600	600	700	600
Sr	1700	1600	1700	1700	2300	1300	1500	1500	2000	1600
S	400	500	500	800	1500	300	1300	1400	600	400
Mg/Ca	0.15	0.13	0.10	0.20	0.30	0.10	0.15	0.15	0.18	0.15
Sr/Ca	0.43	0.40	0.43	0.43	0.58	0.33	0.38	0.38	0.50	0.40
mMg/mCa	0.25	0.21	0.33	0.33	0.50	0.17	0.25	0.25	0.29	0.25
mSr/mCa	0.19	0.18	0.19	0.19	0.26	0.15	0.17	0.17	0.23	0.18
mSr/mMg	0.78	0.88	0.58	0.58	0.53	0.89	0.69	0.69	0.78	0.73
K'A Mg	0.48	0.40	0.64	0.64	0.96	0.32	0.48	0.48	0.56	0.48
K'A Sr	0.23	0.21	0.23	0.23	0.31	0.17	0.20	0.20	0.27	0.21

b	tuber.	discus	roei	iris	rotun.	fulgens	asinina	glabra
Na	6300	5000	6900	4900	5000	6900	6900	6400
Mg	500	600	500	2100	300	600	1000	900
Sr	2300	1500	2200	1600	1800	2300	1900	2100
S	900	500	600	1300	600	600	1100	1100
Mg/Ca	0.13	0.15	0.13	0.53	0.07	0.15	0.25	0.23
Sr/Ca	0.58	0.38	0.55	0.40	0.45	0.58	0.48	0.53
mMg/mCa	0.21	0.25	0.21	0.87	0.12	0.25	0.42	0.37
mSr/mCa	0.26	0.17	0.25	0.18	0.20	0.27	0.22	0.24
mSr/mMg	1.26	0.69	1.21	0.21	1.64	1.05	0.52	0.64
K'A Mg	0.40	0.48	0.40	1.69	0.24	0.49	0.80	0.72
K'A Sr	0.31	0.20	0.29	0.21	0.24	0.31	0.25	0.28

c	tuber.	discus	roei	iris	rotun.	fulgens	glabra	rufes.	kamtsch.
Na	3800	3800	2900	4200	2400	3500	3400	4400	4200
Mg	2700	1000	2200	1200	3800	1500	4000	1000	900
Sr	1300	1400	1000	1400	1100	1400	600	1800	1000
S	1100	1300	1200	1200	1100	1000	1400	800	1000
Mg/Ca	0.68	0.25	0.55	0.30	0.95	0.37	1.00	0.25	0.23
Sr/Ca	0.30	0.35	0.25	0.35	0.28	0.35	0.15	0.45	0.25
mMg/mCa	1.12	0.42	0.92	0.50	1.58	0.62	1.67	0.42	0.37
mSr/mCa	0.14	0.16	0.11	0.16	0.13	0.16	0.07	0.21	0.11
mSr/mMg	0.12	0.38	0.12	0.32	0.08	0.26	0.04	0.49	0.30
K'A Mg	2.17	0.80	1.77	0.96	3.05	1.19	3.21	0.80	0.72
K'A Sr	0.16	0.19	0.13	0.19	0.15	0.18	0.08	0.24	0.13

### (1) Aragonitic nacreous layer

**Numerical values.** In bivalves the highest concentrations of the minor elements are those of Na (6700 ppm) and Sr (1400 ppm). In haliotids, we obtained only slight differences in the content of these elements from those in bivalves (see Table 1a). The content of Sr is in closer agreement with the general values of molluscan aragonitic layers, to wit, between 1500 and 2100 ppm, but without any clear correlation with the concentration of Na.

For other elements, our analysis shows relatively high contents of Mg and S in *H. rotundata*. A com-

parison of the concentrations of these two elements for the ten analysed haliotid species seems to show a rather good correlation. Very low in some species (*H. fulgens*: 200/300 ppm), the Mg and S concentrations rise simultaneously to 400/600 ppm (*H. rufescens*, *H. tuberculata*, and others), and reach 1000 to 1200 ppm in *H. rotundata*.

**Ratios.** The content of Na and Sr in the nacreous layer of *H. tuberculata* is most similar to the values given by Masuda & Hirano (1980) for the nacre of bivalves. Since the Mg-content is generally higher in *Haliotis*, all the ratios based on the Mg-content are different from those obtained from the data pub-

lished by those authors. However, the Mg-content of the aragonite in bivalves, given in Loreau (1982), without any distinction between structurally different layers, is higher than that of Masuda & Hirano (1980), and lower than that in *Haliotis*. Large variations in the Mg-content have also been noted by Kinsman (1970) in experimental studies on aragonites.

Comparative diagrams on all analyzed species show that the content of Sr is relatively stable, and that *H. tuberculata* displays average values (Table 1a). The two extreme values are represented by *H. iris* (the lowest) and *H. rotundata* (the highest). No clear differences can be noted between the species with uniform and mixed mineralogy. Diagrams based on Mg confirm the large variations of the content of Mg. *H. fulgens* shows the lowest content, whereas *H. rotundata* shows the highest content. All the ratios of mSr/mCa : mMg/mCa, which indicate the substitution rates of Sr<sup>++</sup> and Mg<sup>++</sup> to Ca<sup>++</sup>, are clearly lower than those obtained by Masuda & Hirano (1980) and Loreau (1982).

## (2) Aragonitic prisms and spherulites in the external layer

**Numerical values.** In the aragonitic units of the external layer, Na is also the most abundant of the minor elements (Table 1b). It is in some cases very close in concentration to the succeeding nacreous aragonite (*H. asinina*, *H. glabra*, *H. roei*), but in other cases markedly different (*H. iris*). In one species only, the Na-content in the external layer appears to be higher than in the nacre (*H. fulgens*).

The content of Sr represents approximately the mean value for this element in molluscan shells in general: i.e. between 1000 and 2000 ppm (Table 1b).

**Ratios.** The content of Na, Sr and Mg in the aragonitic units of *H. tuberculata* are slightly higher than those in the aragonitic fibrous layer of bivalves (Masuda & Hirano, 1980). The Loreau ratios based on our data and those of Masuda & Hirano are similar, and lower than the average ratios given for the aragonite in bivalves by Loreau (1982).

The Sr-content in all haliotid species reported on here is more stable than that of Mg. *H. fulgens* and *H. tuberculata* show a high content of Sr; *H. discus* has the lowest value. *H. iris* has the highest Mg-content and the lowest Loreau ratio. Concerning the latter ratio, *H. roei* is similar to *H. tuberculata* and to bivalvian fibrous aragonite. Diagrams of the Loreau ratios show large irregularities in comparison with the nacreous layer. The two aragonitic

layers, the spherulitic-prismatic and the nacreous, differ not only in structure but also in the content of minor elements. Both layers have a high content of Mg in comparison with bivalvian aragonite. The entirely aragonitic and the mixed aragonitic-calcitic external shell layers cannot be distinguished on the basis of their Sr- and Mg-contents.

## (3) Calcitic prisms in external layer

**Numerical values.** As we have seen for the mean values, the content of Mg in the calcitic prisms of *H. tuberculata* is between that of bivalvian prismatic and fibrous calcite, whereas the content of Na is close to bivalve prismatic calcite (1420 ppm). The content of Sr in the calcitic prisms is similar to that of bivalvian fibrous and prismatic calcite (see Table 1c).

**Ratios.** The Mg/Ca ratio in *H. rufescens*, *H. kamtschatkana*, *H. fulgens*, *H. iris* and *H. discus* is similar to that in the fibrous calcite of bivalves, whereas in *H. glabra* and *H. rotundata*, it is the same as that in the prismatic calcite of bivalves. *H. tuberculata* and *H. roei* occupy an intermediate position. The ratio of Sr/Ca is more stable, and with the exception of *H. glabra*, it is higher than that in the bivalvian fibrous and prismatic calcite. Thus, the Loreau ratio is extremely variable and more than that of the aragonitic prisms-spherulites. The calcitic prisms in *H. glabra* and *H. rotundata* are similar to the bivalvian calcitic prisms with regard to this ratio, whereas in *H. kamtschatkana* and *H. fulgens*, they are similar to the fibrous calcite of bivalves. There is no clear difference in this ratio between species with entirely calcitic, and mixed aragonitic-calcitic external shell-layers.

## Discussion

We describe here the mineralogy and structure of the external shell-layer in ten *Haliotis* species. In six of these species: *H. tuberculata*, *H. lamellosa*, *H. (Nordotis) cf. discus*, *H. iris*, *H. rotundata* and *H. fulgens*, the external layer has a mixed aragonitic-calcitic composition; in two species, *H. asinina* and *H. glabra*, this layer is exclusively aragonitic, and in two species, *H. rufescens* and *H. kamtschatkana*, it is exclusively calcitic. In species with a mixed aragonitic-calcitic external shell-layer, the mantle epithelium secretes simultaneously the two CaCO<sub>3</sub> morphs. Consequently, there exists in *Haliotis* a

unique opportunity to study the factors which influence the precipitation of these two morphs.

In previous papers, it has been suggested that some inorganic ions may promote the precipitation of aragonite or calcite (for a review see, for example, Wilbur, 1972). Therefore, our studies on the distribution of minor elements in haliolitid shells are of importance for elucidating this question. The distributions of Mg, Na and Sr allow us to conclude that there do not seem to exist any significant differences in the contents of these elements between the aragonitic and calcitic shell-layers in *Haliotis*. Our results confirm those of the previous analysis on molluscan shells. Moreover, no distinct difference could be found between the external layer composed of aragonite or calcite, and that composed of mixed aragonite-calcite. Much more information is still needed on the distribution of trace-elements and minor elements, particularly in gastropod shells. Such information should be associated with the mineralogical and structural composition of the shells.

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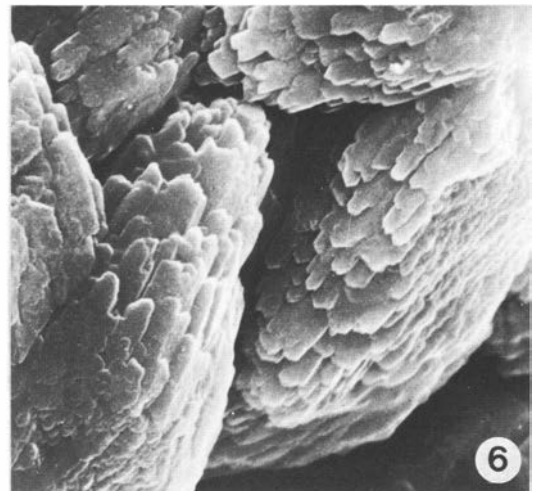
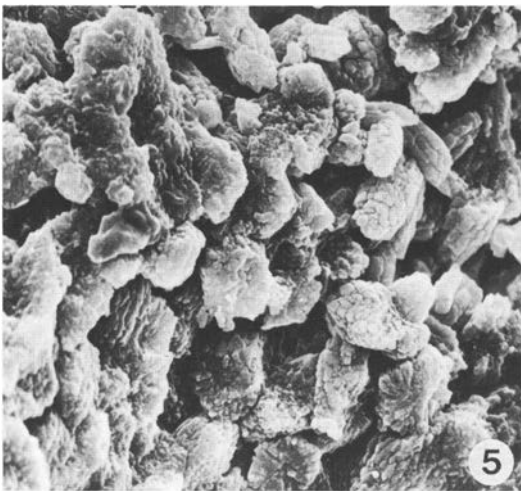
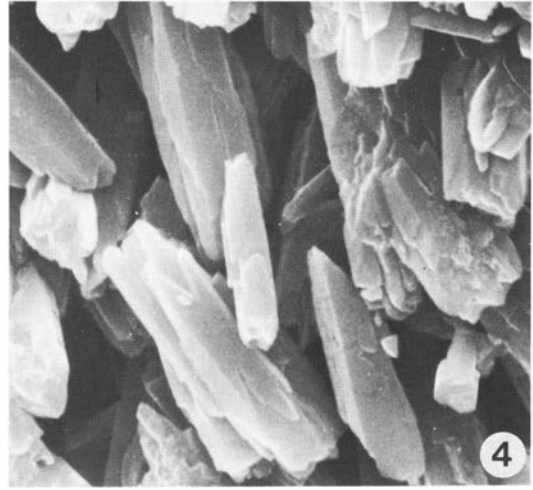
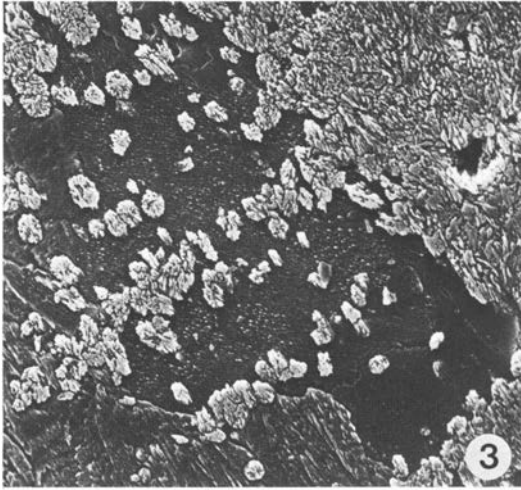
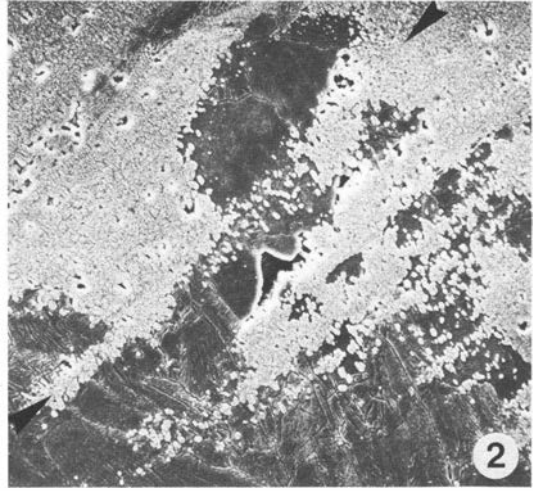
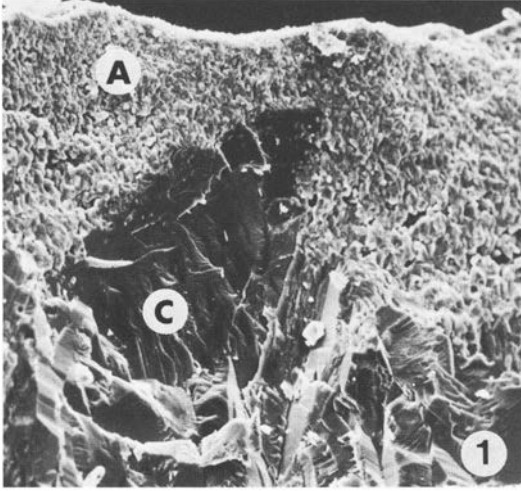


# PLATES

**Plate 1**

*Haliotis tuberculata*

1. Vertical fracture-plane of the external shell-layer; outer aragonitic part (A) and inner calcitic part (C) are distinctly separated; untreated. × 355.
2. Vertical polished and etched section to show highly mixed distribution of small aragonitic units and large calcitic prisms; direction of growth increments indicated by arrows. × 150.
3. Detail of Fig. 1 to show inclusions of aragonitic units (white) in calcitic prisms (dark). × 550.
4. Rod-shaped aragonitic units near the outer shell-surface; untreated. × 5500.
5. Ovoid aragonitic units in the inner part of the external shell-layer, to show their irregular arrangement; untreated. × 1200.
6. Details of ovoid aragonitic units and their rod-like sub-units. × 5500.

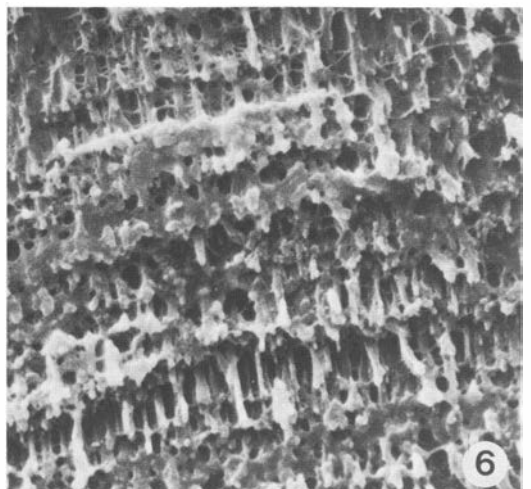
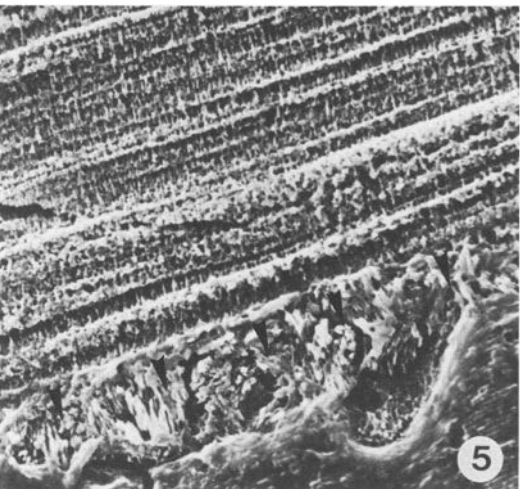
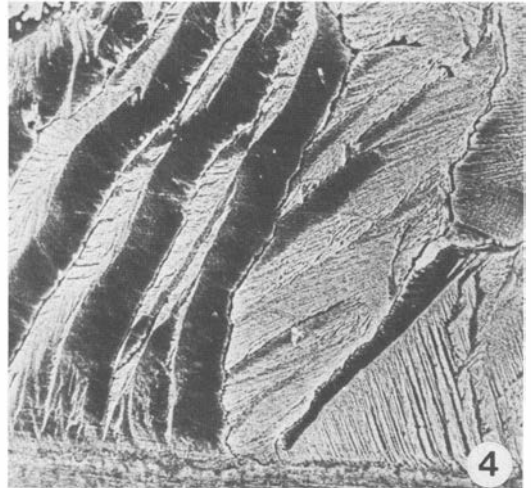
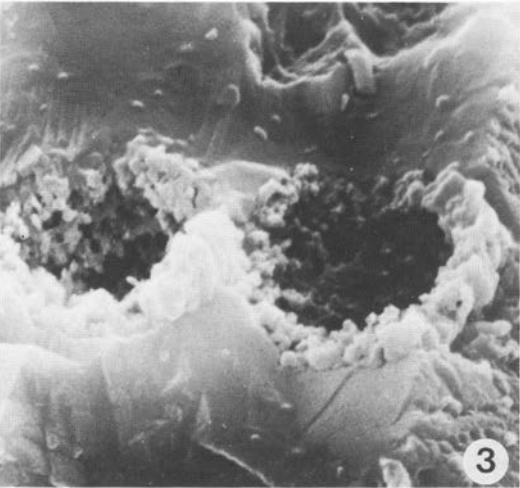
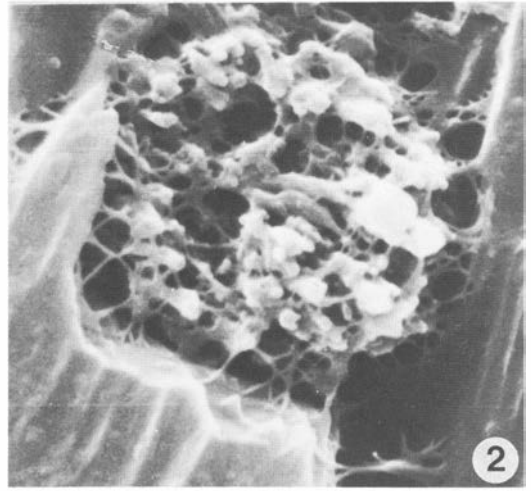
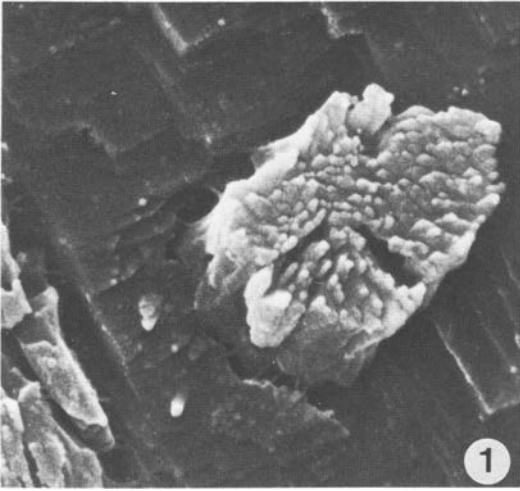


## Plate 2

### *Haliotis tuberculata*

1. Slightly etched oblique section of an aragonitic unit, enclosed within a calcitic prism; note sector-like arrangement of the crystalline sub-units.  $\times 10400$ .
2. Similar aragonitic unit as in Fig. 1, after prolonged etching with glutaraldehyde-acetic acid solution; note distinct filaments of organic matter within the unit and the absence of an organic envelope around the unit.  $\times 13000$ .
3. Aragonitic units etched by bacterial action; note that the central part is more soluble than the outer cortical part.  $\times 12000$ .
4. Vertical polished and etched section of the calcite prisms in the external shell-layer.  $\times 185$ .
5. Vertical section of a calcite prism and its boundary to the succeeding nacreous layer, etched with glutaraldehyde-acetic acid solution; note the occurrence of parallel horizontal growth lamellae in the prism; arrows indicate a thin layer of prismatic aragonite at the boundary between the calcite prism and the nacre.  $\times 2500$ .
6. Detail of the vertical section in Fig. 5, to show vertical crystalline rods associated with organic matter.  $\times 11000$ .





### Plate 3

#### *Haliotis tuberculata*

1,2. Vertical polished and etched sections to show the gradual transition between the aragonitic units in the external layer and the nacreous layer; note that the crystalline rods in the aragonite units become elongated and traversed by parallel organic sheets which, in the nacre, form interlamellar sheets.  $\times 1200$  and  $\times 3300$ , respectively.

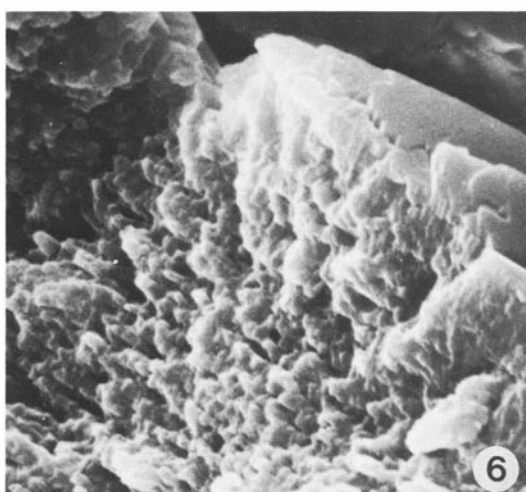
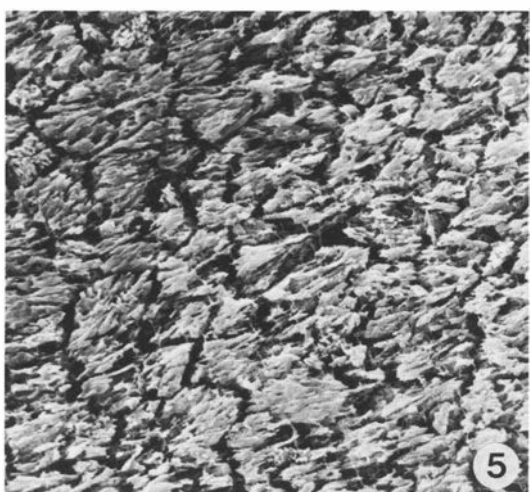
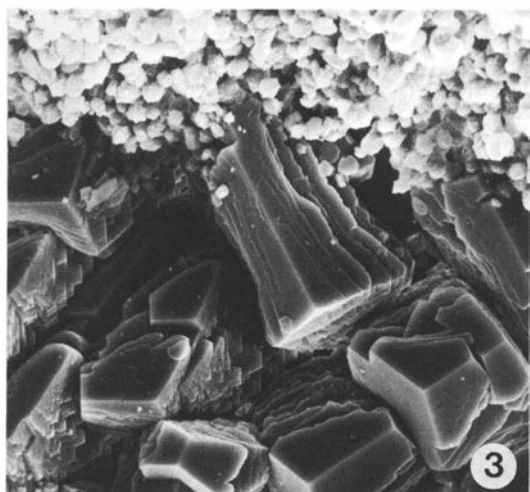
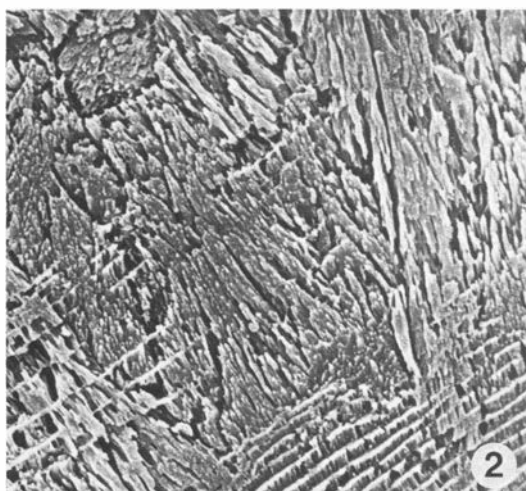
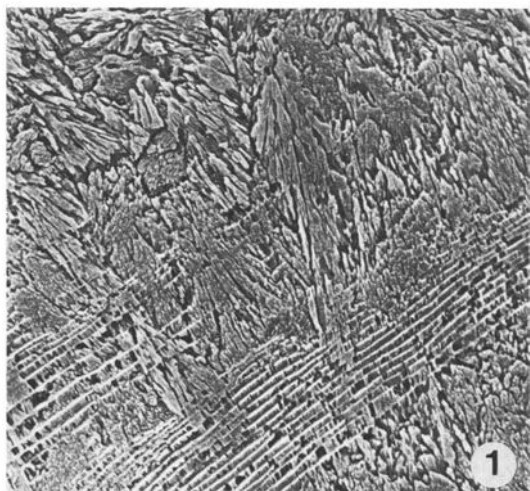
#### *Haliotis (Nordotis) cf. discus*

3. Growth-surface of the external layer at the shell margin; lower part shows irregularly shaped and sized calcitic prisms, upper part shows small aragonitic units; untreated.  $\times 600$ .

4. Prismatic aragonitic units composed of parallel subunits with costulate ornament; untreated.  $\times 300$ .

5. Vertical polished and etched section of the outer part of the external layer, to show fascicles of diverging crystalline rods in the aragonitic units.  $\times 1500$ .

6. Oblique fracture-plane through a prismatic aragonitic unit; note that the outer cortical part is made up of large crystalline rod-shaped subunits, whereas the inner part consists of parallel or slightly diverging lamellae of smaller crystalline rods.  $\times 10500$ .



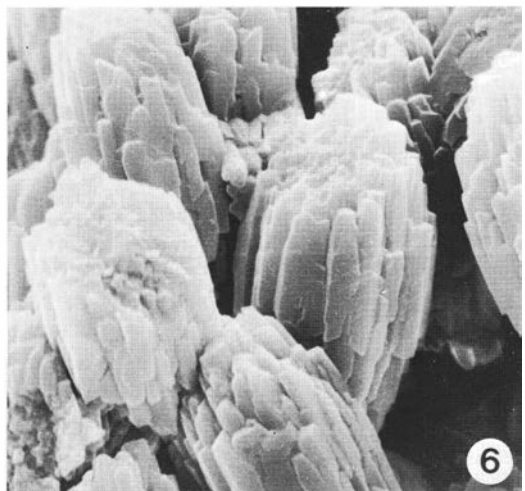
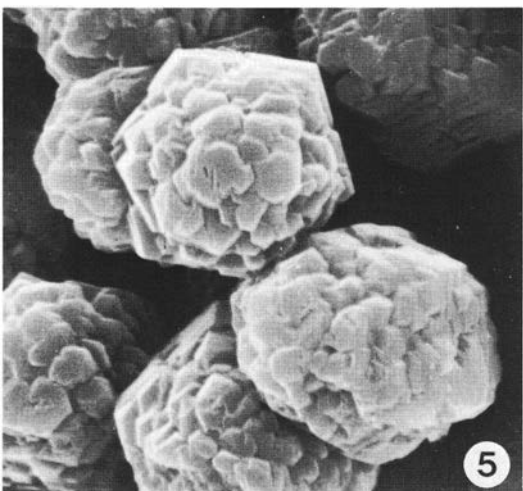
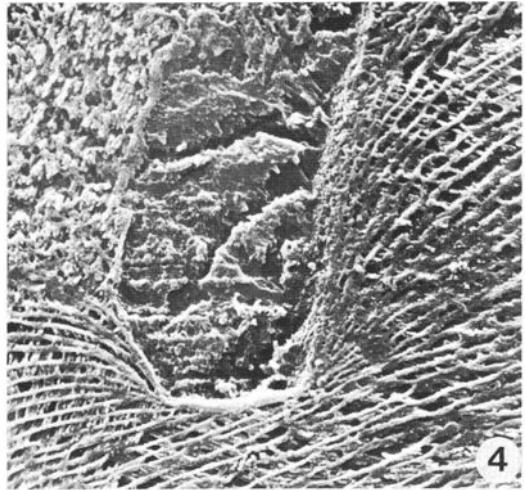
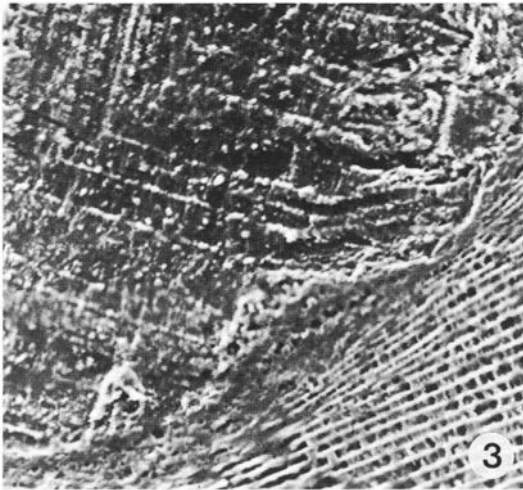
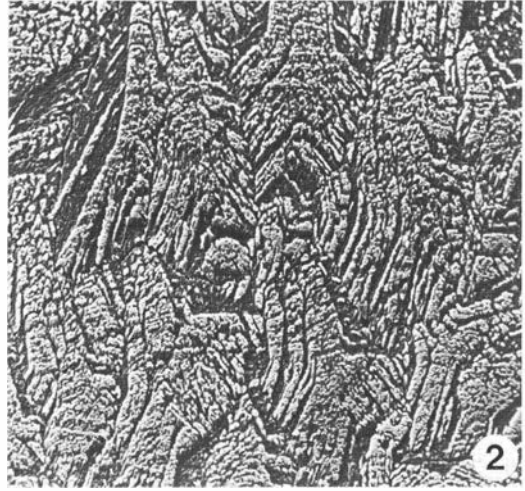
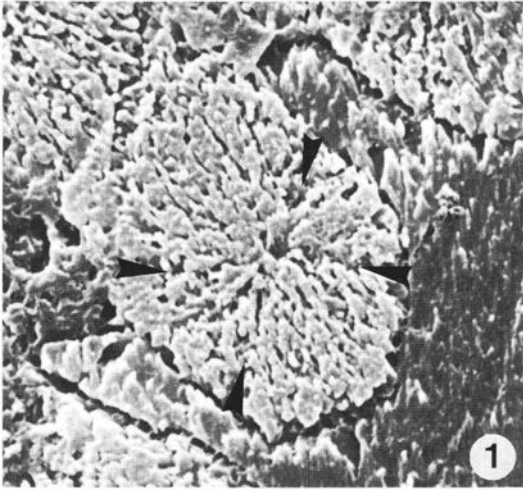
**Plate 4**

*Haliotis (Nordotis) cf. discus*

1. Polished and slightly etched section of a well defined aragonitic unit which shows a complex radial structure.  $\times 4500$ .
2. Polished and etched section of calcitic prisms to show their imbricated arrangement.  $\times 800$ .
- 3, 4. Vertical polished and etched sections at the boundary between a calcite prism and the adjacent nacreous layer; note the protruding distal ends of the prisms, and their direct contact with the nacreous layer.  $\times 4700$  and  $\times 4300$ , respectively.

*Haliotis roei*

- 5, 6. Regularly developed prismatic aragonitic units in the external shell-layer; untreated.  $\times 6500$  and  $\times 3500$ , respectively.



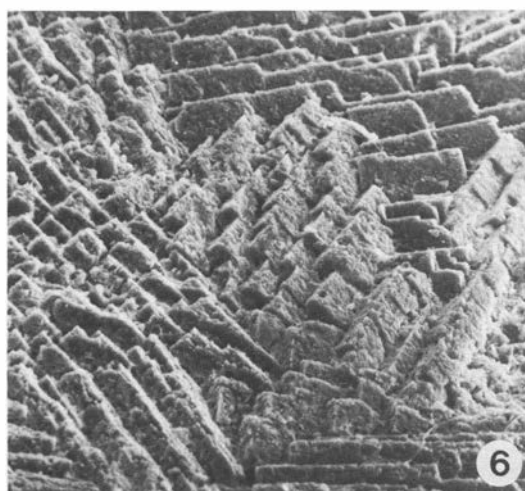
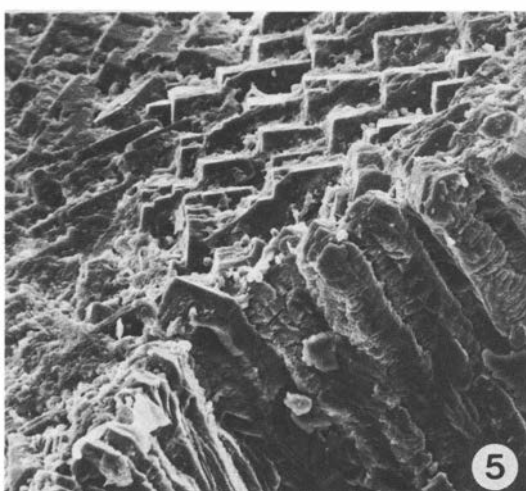
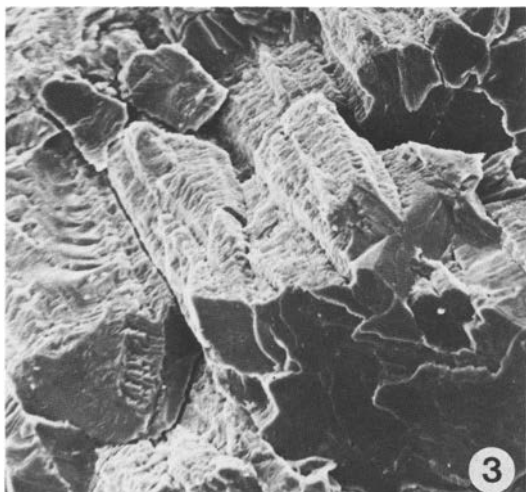
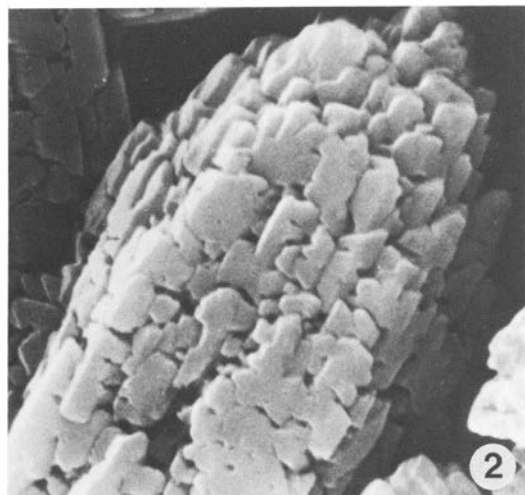
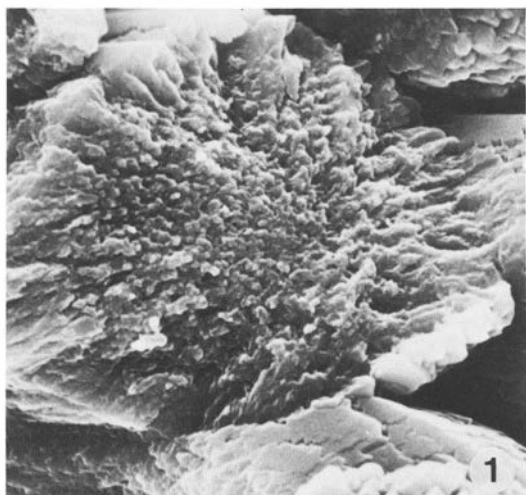
## **Plate 5**

### *Haliotis roei*

1. Etched oblique fracture plane of an aragonitic unit to show the structural difference between the outer cortical part and the inner part.  $\times 5200$ .
2. Lateral view of an aragonitic unit after etching with enzymatic solutions; note that the outer surface is composed of numerous polygonal plates.  $\times 9500$ .
- 3, 4. Fractured calcitic prisms to show their imbricated and complex structure.  $\times 410$  and  $\times 700$ , respectively.

### *Haliotis iris*

- 5, 6. Growth-surface of the calcitic prisms at the shell margin; note that the adjacent prismatic groups in Fig. 6 have a different crystallographic orientation.  $\times 350$ .



**Plate 6**

*Haliotis iris*

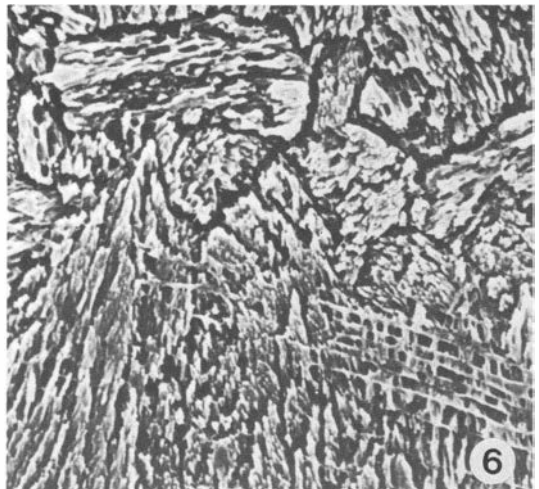
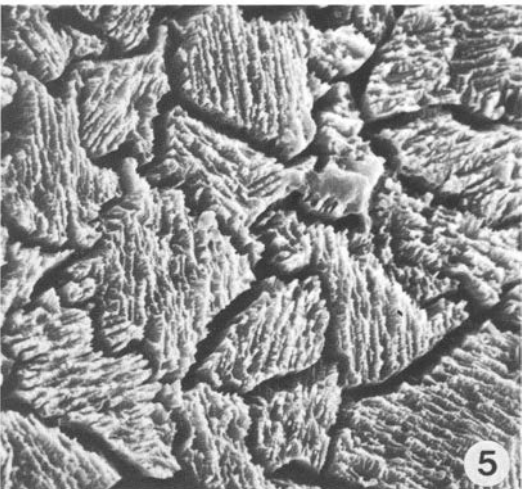
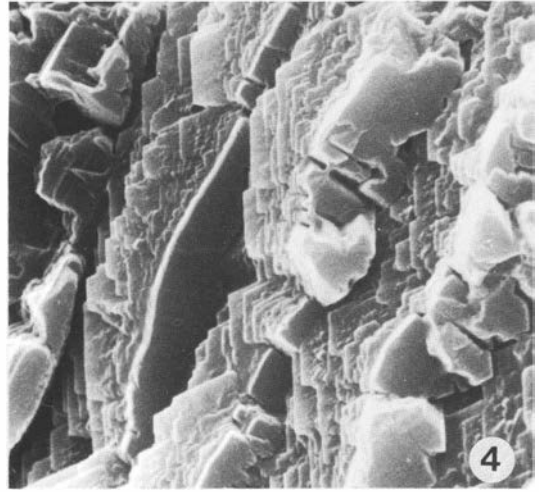
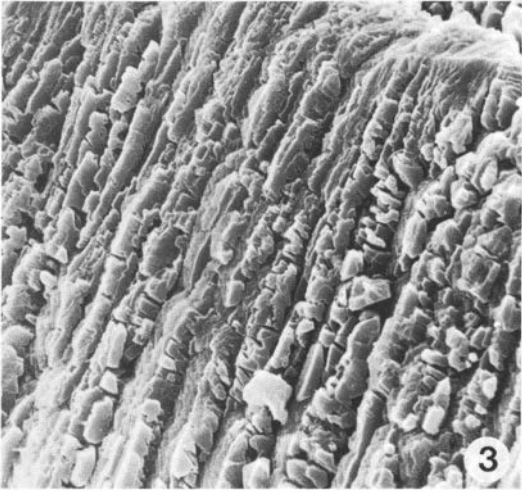
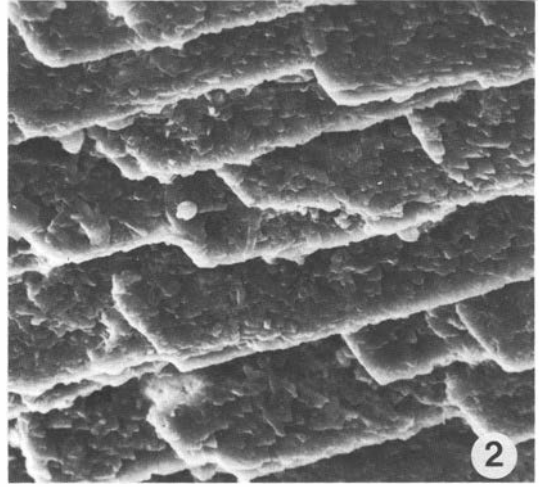
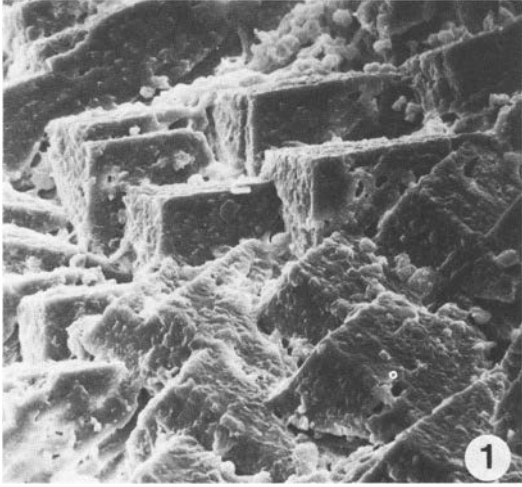
1, 2. Growth-surfaces of the calcitic prisms at the shell margin in two specimens; note the parallel arrangement of the rhombohedral faces of the prisms; untreated.  $\times 600$  and  $\times 825$ , respectively.

3, 4. Lateral surface of a calcite prism to show distinct growth-lamellae; untreated.  $\times 1300$  and  $\times 6800$ , respectively.

*Haliotis rotundata*

5, 6. Vertical polished and etched sections of the external layer; the aragonitic units are composed of numerous crystalline rods; close to the nacreous layer (Fig. 6) the rods are in places traversed by parallel, horizontal, organic sheets.  $\times 2300$  and  $3200$ , respectively.





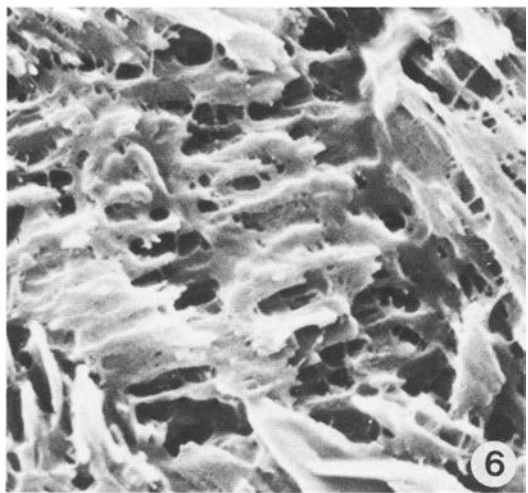
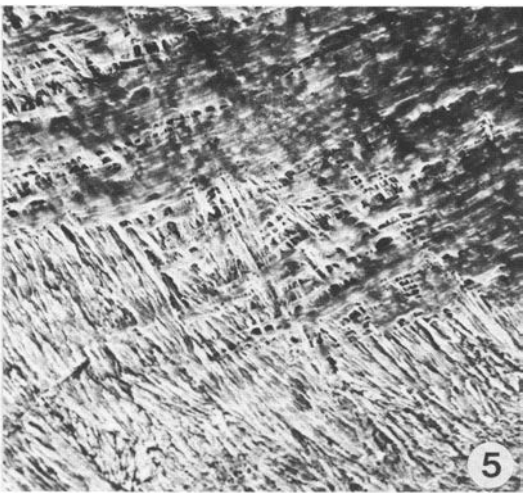
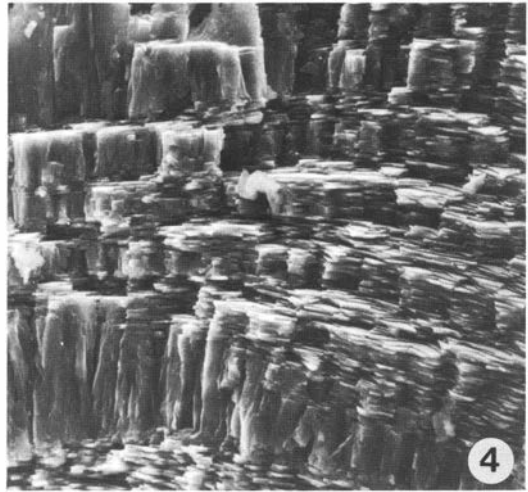
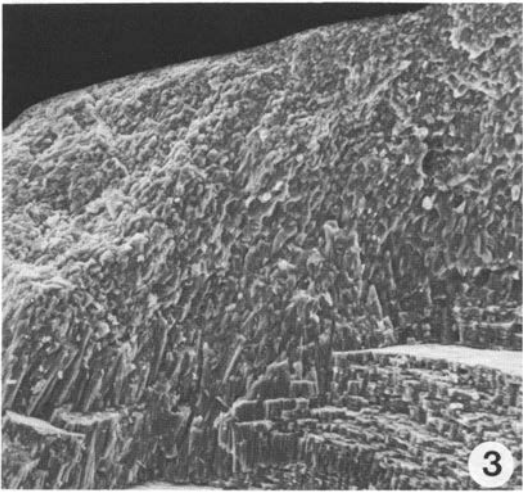
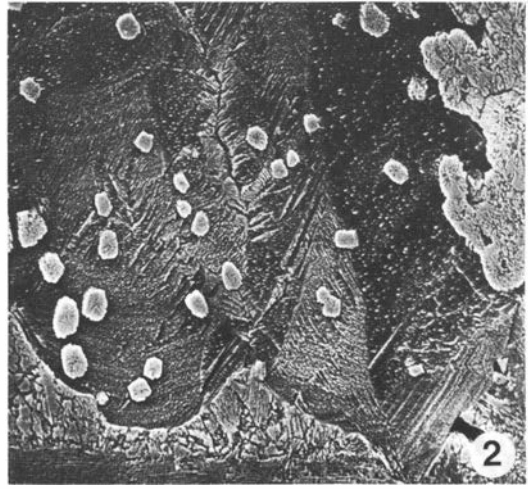
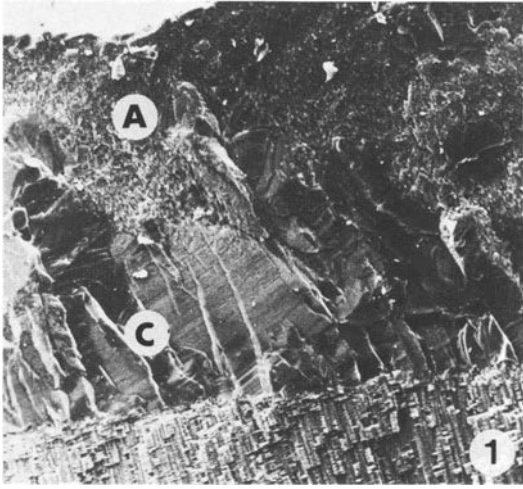
**Plate 7**

*Haliotis fulgens*

1. Vertical section of the external layer to show distribution of the aragonitic units (A) and calcitic prisms (C). × 80.
2. Vertical polished and etched section (formic acid) to show numerous aragonitic units within the calcitic prisms. × 850.

*Haliotis asinina*

3. Vertical fracture-plane of the external layer, composed exclusively of aragonitic units; untreated. × 80.
4. Vertical fracture-plane at the boundary between the external layer and the nacreous layer; note the gradual appearance of the nacreous laminae (right hand side) within the prismatic external layer; untreated. × 850.
5. The same shell region as in Fig. 4 in vertical polished section, etched with chromium sulphate. × 1200.
6. Detail of Fig. 5; the aragonitic rods, partially dissolved by etching, are embedded into an organic matrix. × 11000.



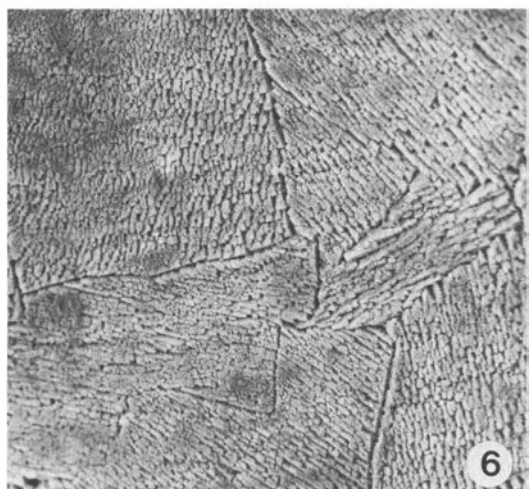
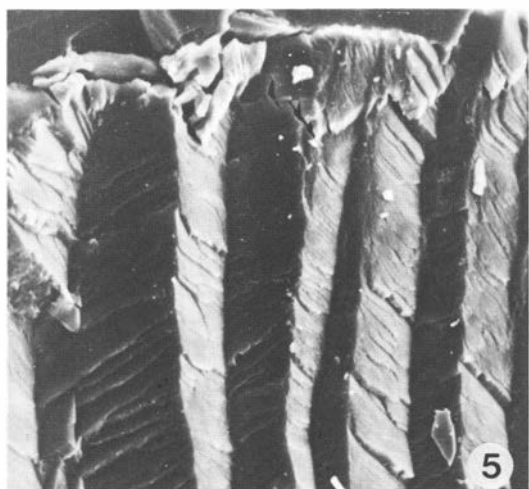
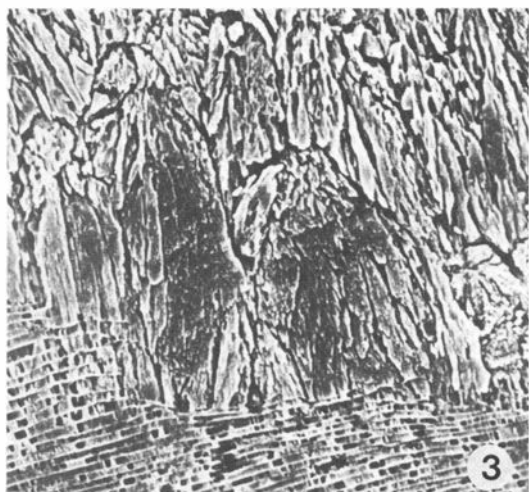
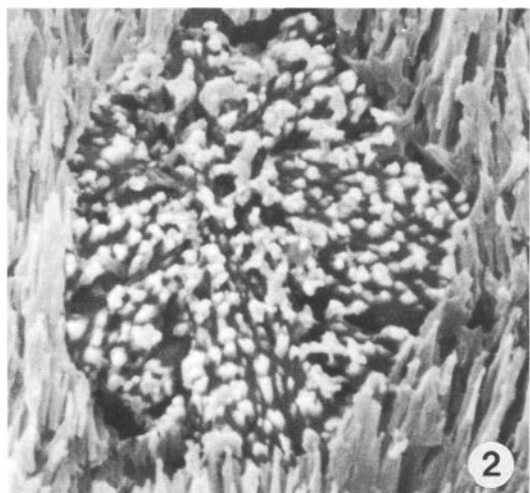
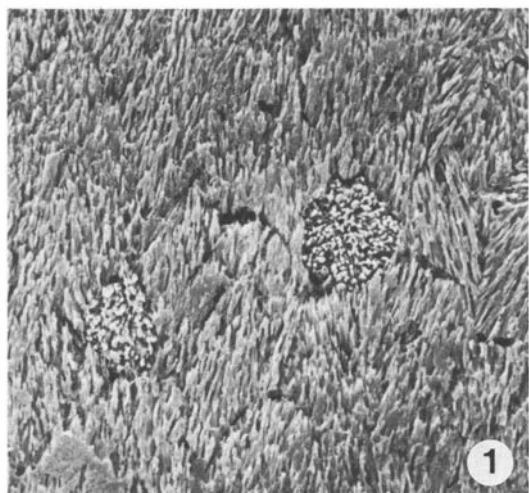
**Plate 8**

*Haliotis glabra*

1. Vertical polished and etched section of the external layer; note the vertical orientation of the crystalline rods in the aragonitic units, except in two units.  $\times 2500$ .
2. Detail of the aragonitic unit in the central position in Fig. 1.  $\times 8300$ .
- 3, 4. Vertical polished and etched sections to show the gradual transformation between the external layer and the nacreous layer.  $\times 2300$  and  $\times 3400$ , respectively.

*Haliotis rufescens*

5. Vertical fracture-plane of the calcitic prisms to show their growth-lamellation.  $\times 100$ .
6. Growth-surface of the calcitic prisms at the shell margin; note that the adjacent prisms are imbricated; untreated.  $\times 260$ .



**Plate 9**

*Haliotis rufescens*

1, 2, 3. Vertical polished sections of the external layer, etched with glutaraldehyde-acetic acid solution; note that the organic matrix appears as filaments and regularly spaces sheets within the calcitic prisms.  $\times 2200$ ,  $\times 1100$  and  $\times 4300$ , respectively.

*Haliotis kamtschatkana*

4. Vertical fracture-plane of the external layer, composed exclusively of calcitic prisms, and the succeeding nacreous layer; untreated.  $\times 120$ .

5. Oblique vertical polished and etched section of the calcitic prisms showing their decreasing size towards the outer shell-surface.  $\times 350$ .

6. Vertical fracture-plane at the boundary between the calcitic prisms (bottom) and the nacreous layer (top).  $\times 4200$ .

