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Instructions for use

THE ULTRASTRUCTURE OF THE MINERAL IN AND THE CONSTRUCTION OF THE CROSSED-LAMELLAR LAYER IN MOLLUSCAN SHELL

by

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and Yoshihiro Togo

(with 2 text-figures and 11 plates)

Introduction

Calcareous shell of molluscs is commonly composed of one or two plymorphic varieties of calcium carbonate, calcite, and aragonite, but the structure of the shell varies and several types can be recognized among different species. Of these types the crossed-lamellar structure is the most widespread in the shell of pelecypoda and gastropoda, and is restricted in the shell of aragonite, except in a few species. This structure has recently been reviewed by KOBAYASHI (1964), MACCLINTOCK (1967) and KENNEDY and TAYLOR (1969); and its fine structure has been elucidated to some extent. However, there is not seemingly full agreement about the overall ultrastructure of the mineral in and the construction of this layer.

In this paper the present writers report on an investigation of the ultrastructure of the mineral in and the construction of the crossed-lamellar layer using various methods of preparation for both optical and electron microscopes.

Materials and methods:

In the present paper the following species of recent and fossil molluscs were examined on the crossed-lamellar layer of their shells:
Pelecypoda
Glycymeris albolineata (LISCHKE): recent, Oga Akita-Pref.
Glycymeris yessoensis (SOWERBY): recent, Abashiri, Hokkaido.
Glycymeris yessoensis (SOWERBY): Pliocene, Setana formation, Hokkaido.
Glycymeris kyushuensis NAGAO: Eocene, Ashiya formation, Kyushu.
Anadara broughtoni (SCHRENK): Holocene, Nishinosato formation, Hokkaido.

Clinocardium californiense (Deshayes): recent, Saroma, Hokkaido.
Corbicula japonica Prime: recent, Jusangata, Aomori-Pref.
Meretrix lusoria (Roding): recent Hamada, Simane-Pref.

Gastropoda:
Neptunea arthritica (Bernardi): recent, Otaru, Hokkaido.
Neptunea bulbacea (Bernardi): recent, Hidaka, Hokkaido.
Neptunea polycostata Scarlata: recent, Hidaka, Hokkaido.
    These reagents were used for etching and decalcifying in preparation:

1) 0.5 M EDTA (ethylendiamintetrea acetic acid), Ph. 7.4
2) 6% sodium hypochlorite solution.

Preparations of mineral lamellae for the ordinary microscope: . . . Microscopic observations were carried out on undecalcified thin sections and polished sections, etched with 0.5 M EDTA.
   Preparations of mineral lamellae for the scanning electron microscope: . . . Specimens were cut to expose the crossed-lamellar layer, polished with Dp-diamond pastes, and then etched by 0.5 M EDTA for 2–10 minutes. Their surfaces were then sucessively coated with evaporated carbon and gold, and were studies in a JEM-S1 scanning electron microscope (Japan Electron Optics Lab. Ltd.)
   Preparations of conchiolin for the transmission electron microscope: . . . The methods employed for the electron microscope were essentially the same as those described in the previous paper (Uozumi and Iwata 1969). The crossed-lamellar layer was decalcified in an aqueous solution of EDTA, mentioned above. A portion of the sample was dehydrated sucessively by soluble epoxy resin (Durcupan), and embedded in Styren resins following the method of Kushida (1961). They were then cut into sections with a glass knife, using a Leitz microtome. The other parts of the samples were washed in tap water after decalcification, fixed with OsO_4, and dispersed by ultrasonic waves within the medium of water. The dispersed membranes were directly collected on a formvar coated mesh. The samples were then shadowed with Pt-Pd and studied in a transmission electron microscope, JEM-120U.
   Preparations of the mineral for the transmission electron microscope: . . . Shell with crossed-lamellae was loosely crushed in an agate mortar, and the mineral lamellae were isolated within the medium of water by ultrasonic waves. Some fragments were then collected on microgrids which were coated with carbon in order to prevent breaks in the microgrids during examination. They were studies in a transmission electron microscope with accelerating voltage up

to 100 kV.

The microstructure of the crossed-lamellar layer

The crossed-lamellar layer of shell consists of a series of nearly parallel rectangular lamellae, which, on the whole, are perpendicular to the shell surface. In the section parallel to the shell surface (H-section)* and the section normal to the growth trend (L-section), one can observe alternate light and dark coloured bands within the crossed-lamellar layer, as shown in Pl. 3, Fig. 2. Each of these bands is named first-order lamella in the crossed-lamellar layer, and has an average width of 10-30μ. They interdigitate with their adjacent lamellae, and commonly extend to the surface of shell interior normally. But they are frequently observed to be twisted about 90°, as shown in Pl. 2, Fig. 4. Observations under higher magnification show that each of the first order-lamellae is built up by several parallel sheets of small lamellae (the second-order lamella). In adjacent first-order lamellae, second-order lamellae are inclined in opposite directions but parallel to those of the next one, thus parallel in the alternate lamellae.
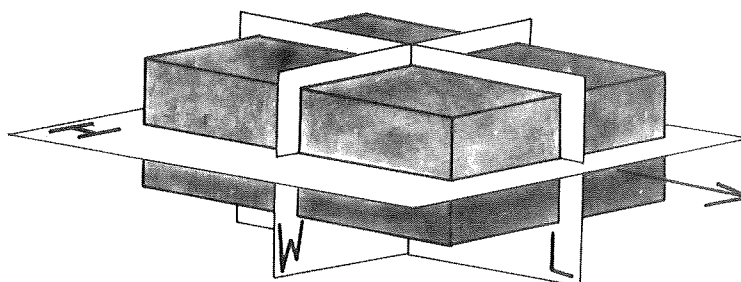

Fig. 1:
Three dimensional diagram of the H-, W- and L-profile.
H; showing the plane parallel to shell surface
L; showing the plane intersected at right angles to the growth line
W; showing the plane parallel to the growth line
Arrow: growth trend.

In profiles parallel to the growth line (W-section) one can observe flat parallel surfaces of the second-order lamellae. The second-order ones are inclined in opposite directions, alternately, but only two directions of inclination are present within a lamella of the first-order. In micrographs under a reflecting microscope, relationships of their orientation are clearly illustrated, as shown in Pl. 1, Fig. 1-4. The projection of flat-lying second-order lamellae with two different directions of inclination is observed as a bifurcated pattern

*Abbreviation for the direction of the section in Fig. 1. This abbreviation will be used throughout in this paper.

under an ordinary microscope as shown in Pl 2, Fig. 3. Further, smaller lamellae, the third-order lamellae, are distinguished within a lamella of the second-order. The dip angle of the third-order lamellae against the growth lines is 34-42°. And the third-order lamellae are parallel to the longitudinal axes of each other. They are observed as needle-like crystals, 0.3-2μ in width and 5μ + in length. In fact, the shape and orientation of second-order lamellae as stated above are controled by those of third-order ones.

Additive observations under an electron microscope make a more clear understanding possible of the relationships between third-order lamellae, and between third- and second-ones. That is to say, an apparent third-order lamella, which is seen as a needle-like lamella under an ordinary microscope, is an aggregate of numerous tiny lamellae, with longitudinal axes parallel to each other in an *en echelon* or bead-like arrangement. Also, a second-order lamella interdigitates with its neighbours, in the same manner of contact as first-order lamellae, mentioned above.

On the other hand, it should be emphasized that crystals of third-order lamellae are pached into a compartment of organic matrix (conchiolin), as shown in Pl. 5 and 6. ; and other kinds of organic membranes have not been observed along the boundary between second-order and first-order lamellae.

The ultrastructure of the conchiolin membrane

The ultrastructures of crossed-lamellar organic matrices of the shells of the following families have been examined: Arcidae, Glycymeridae, Corbiculidae, Cardiidae, Tellinidae, and Mactridae. It could be summarized that the conchiolin of crossed-lamellae is markedly different in the shell of different families. Furthermore, compared with the conchiolin in other structural layers of shell, this conchiolin is essentially different in appearance. This fact will be reported on in detail at a future opportunity. Only general characteristics of the conchiolin membrane of crossed-lamellae are described briefly in the present article.

The conchiolin membrane of crossed-lamellae is composed of one kind of sheet, and shows roughly homogeneous and monotoneous ultrastructure. Compared with other shell structures, structures of crossed-lamellae seem to show a complicated nature of construction and optical properties as stated above. Nevertheless, it is noticed that the conchiolin of crossed-lamellae is composed of only one kind of membrane within one species. Of course, there are some differences in the surface ornamentation and fibrous arrangement in different species, as shown in Pl. 7, Fig. 2 and 3. For example, the one of *Clinocardium* shows a smooth and homogeneous membrane with a carpet-like

appearance. The frills on this membrane are illustrated as lines of high electron density, parallel to each other, and have two widths, $0.5 - 1\mu$ and $1.5 - 3\mu$. Also, one can observe another kind of dense lines with a different orientation, which are oblique to the lines mentioned above. The thickness of this membrane is estimated at about 300 Å, low relief and fibril arrangements on this membrane are observed to be nearly parallel to the frills.

The conchiolin membrane of *Meretrix* shell is clearly different from that of *Clinocardium*, although their thicknesses are similar. The former shows a bark-like ornamentation with many hollows on the overall membrane. The hollows are disposed parallel or slightly oblique to the frills.

The conchiolin membrane of *Corbicula* is rather flat and has no relief as in the membrane of *Meretrix*, but ultrastructures of the surface of a membrane, and arrangements of fibrils may be seen scattered on this membrane.

The conchiolin membrane of *Glycymeris* is homogeneous as a whole, and fibrous as compared to that of others.

The ultrastructures of the mineral of crossed-lamellae

Aragonite crystals were isolated by ultrasonic waves within the medium of water from third-order lamellae of the crossed-lamellar layer. Their sizes are small than the resolution of an optical microscope and they are not visible; but of course, are directly visible under an electron microscope. However, one can not always decide with complete certainity whether the radiation images seen on the fluorescent screen of an electron microscope represent mineral crystals or structures of similar morphology but of different chemical composition. Comparative observations of aragonite crystals from lamellae of various shell suggest that crossed-lamellae crystals are not similar in different structure lamellae, except in a few other layers.

As already point out by GREGOIRE (1962) and MUTVEI (1971), aragonite crystals in nacreous layers have predominantly hexagonal shapes, being elongated along the a-axis. In those crystals, one can clearly observe that aragonite laths are parallel to the a-axis. From the present investigation, shapes of crossed-lamellae crystals are entirely different from those of nacreous crystals, although both crystals are aragonite, determined by the use of x-ray diffraction. Crystals of crossed-lamellae are rectangular in shape, and have a wide range of variation in width. Generally speaking, the following size crystals can be observed most frequently: $0.2 - 0.5\mu$ in thickness, $0.3 - 2\mu$ in width, and 10μ + in length. In these crystals, one can observe that dark and light bands alternate and are parallel to the longitudinal axis of each lamella developed within a crystal. The widths of these bands are from 50–800 Å. Comparative examinations of aragonite crystals of different layers of shell, suggest that such

bands are only to be seen in aragonite crystals of crossed-lamellae, containing the complex- and psedo-crossed-lamellae.* Furthermore, aragonite crystals in various crossed-lammellae of pelecypoda (*Glycymeris, Anadara* and *Arca*), gastropoda (*Neptunea*) and Scaphoda (*Dentalium*) have been examined. As a result of examination, light and dark bandings of those crystals do not differ essentially in different species and cenozoic fossil shell. Also, electron micrographs of these crystals do not always show the same electron density throughout. The nature of the zones of high density within crystals is not decided yet, but determining the nature of these zones in crystals of crossed-lamellae is important.

Highly magnified micrograph of interiors of crossed-lamellar crystals is illustrated in Pl 11, Fig. 1. These crystals show series of dark stripes with periodic distance of 7.8 Å, which are interpreted as diffraction images. These dark stripes seem to obliquely intersect the longitudinal axes of the crystals. Similar dark stripes can be also observed in crystals of complex lamellae of *Glycymeris,* and they intersect at about 55° to the longitudinal axes of the crystals. They do not seem to indicate structural features which result by incorporation of organic substances nor imperfections of crystals structures, such as twinning or stacking faults. Such systems of stripes are due to diffraction phenomena, the same as those observed in human enamel crystals (hydroxy-apatite), and are supposed to reflect the lattice structures of aragonite crystals. However, internal structures of crystals are not able to be determined from electron micrographs alone. Fine electron beam diffractions and other techniques are necessary before we can draw any definite conclusions.

Summary and tentative conclusions

Based on the observations under ordinary microscopes and electron microscopes, the following conclusions are given regarding the ultrastructure of the mineral, and the construction and organic matrix of the crossed-lamellar layer in molluscan shell.

1) The smallest structural units in these structures are so-called third-order lamellae, which are surrounded by a rather homogeneous and monotonous organic membrane (conchiolin). Crystals of third-order lamellae show two types of minute structures within themselves: one is characterized by bands of high electron density, which are parallel to the longitudinal axes of the crystals, and the other is shown by series of dark stripes with periodic distances of 7.8 Å. The former characteristic is observed occasionally in aragonite crystals of crossed and complex crossed-lamellae of Cenozoic fossils of *Anadara* and *Arca.*

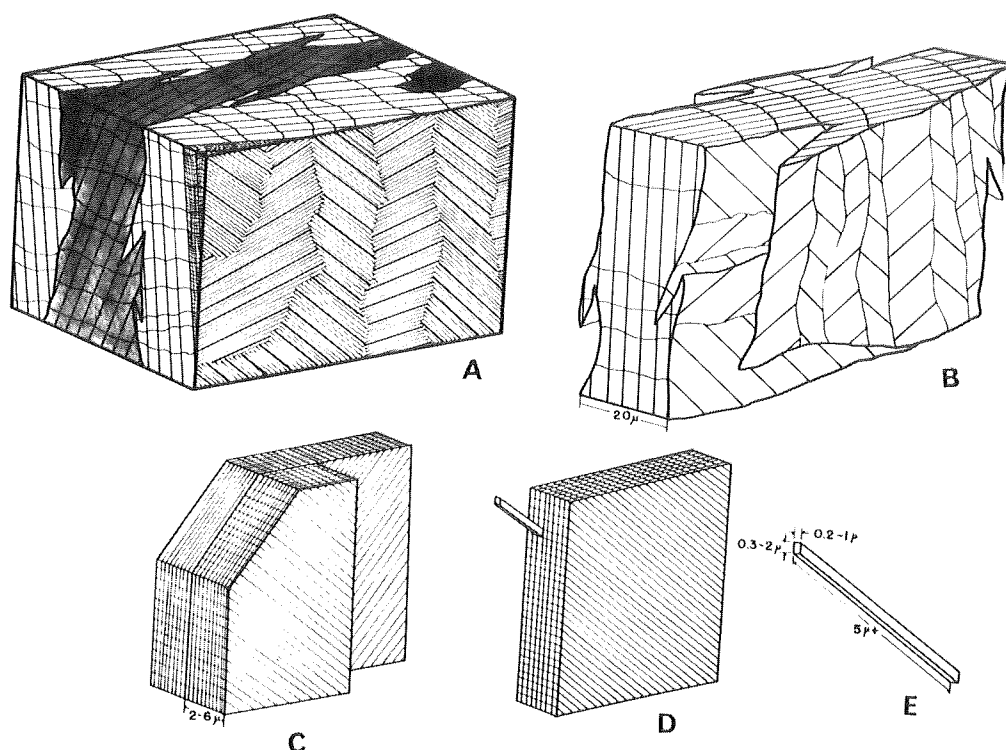*Aragonite crystals of the homogeneous structure are similar to those of crossed-lamellae.

Fig. 2:
Schematized reconstruction of the crossed-lamellar structure of molluscan shell.
A: Four first-order lamellae of the crossed-lamellar structure; B: One isolated first-order lamella, C: Two second-order lamellae on one of which lies obliquely the other, D: One second-order lamella composed of many third-order ones; E: One third lamella, smallest unit of the crossed-lamellar structure.

2) Third-order lamellae are arranged parallel to each other, resembling *en echelons* or bead-like patterns and united to construct second-order lamellae.

3) First-order lamella is built up from several sheets, 2–6μ thick, of second-order lamellae which are parallel to each other in H- and L- profiles. Internal orientations of first-order lamella are determined by arrangements of second-order lamellae within first-order lamella. Borders of first-order lamellae contact each other without any perceptive walls, but internal orientations of adjacent ones are not parallel to one another. Namely, layered structures of first-order lamellae intersect each other obliquely in H- and L- profiles. Light and dark alternation patterns in these sections of this lamellae (see Pl 3, Fig. 2) are due to different internal orientations between first-order lamellae; and such optical phenomena are similar to those seen in polysynthetic twin lamellae of certain minerals. No organic membrane exists along the border surfaces of first-order lamellae. One notices that the organic matrix (conchiolin) is

composed of only one kind of membrane in crossed-lamellae of shell of one species.

4) Based on the present observations, schematized reconstruction of the crossed-lamellar structure is shown in Text-figure 2. The optical and morphological features of the corssed-lamellar structure are possibly inclusively interpreted by this model.

However, a few problems are not yet clarified:

i)   the nature of septa-like patterns across first-order lamellae as seen in H- and L- profiles.

ii)   the nature of distinct lines seen on etched surfaces of second-order lamellae in W- profiles.

These two patterns seem to be related to each other to some extent. Further detailed studies are required in this regard.

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(Manuscript received August 31, 1971)

Explanation of Plate 1

Fig. 1-4: Reflecting micrographs of the crossed-lamellar layer of *Neptunea bulbacea*, showing the alternately crossed relationship of very thin rectangular lamellae, each of which is built up of small and numerous lamellae. Fig. 1, 3 and 4, polished and etched surfaces: W-section, X 300, X 1,000, X 600; Fig. 2: fracture surface; W-section; X 300. (All are under an oblique illumination.)
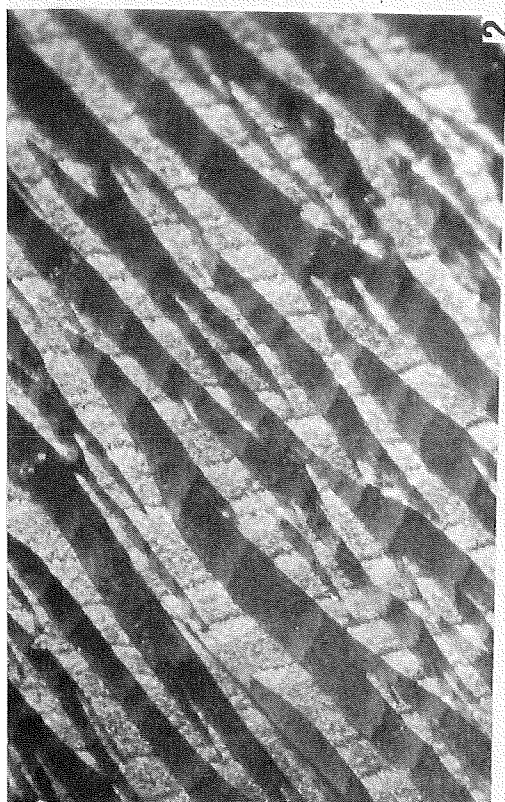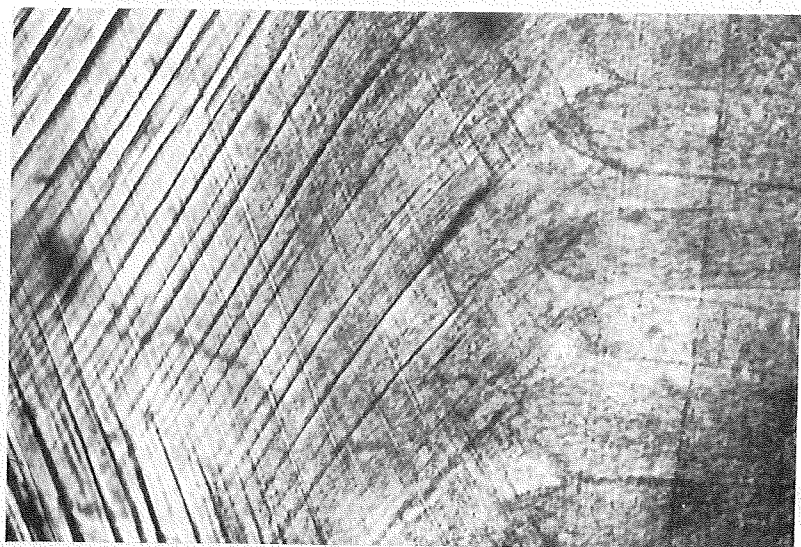
plate 1

458

plate 2

Explanation of Plate 3

Fig. 1: Optical micrograph of the crossed-lamellae of *Neptunea arthritica*, showing two patterns, crossed in upper and banded in lower part. Such patterns suggest that each blocks differ their orientation reciprocally. L-section; under an oblique illumination, X 180.

Fig. 2: Reflecting micrograph of the crossed-lamellae of the same species, showing dark and light alternated patterns on adjacent lamellae, H-section, X 300.

Fig. 3: Optical micrograph of the crossed-lamellae of the same species, showing very thin lamellae which are themselves built up of much thinner lamellae, W-section; X 180.

plate 3

462
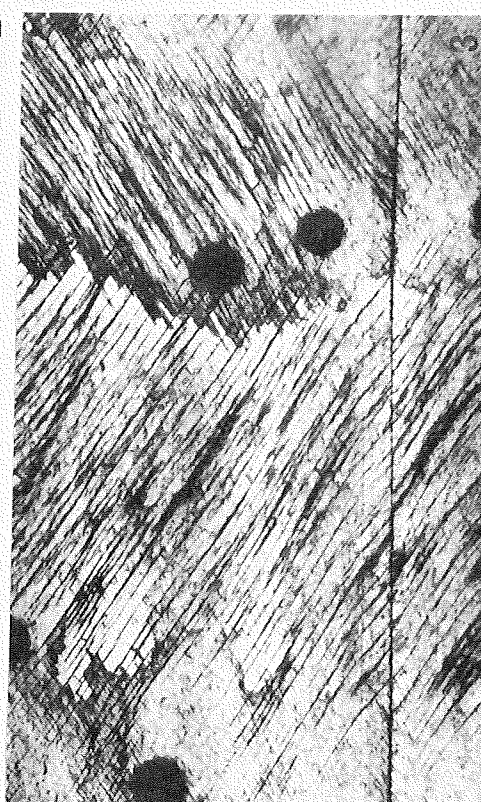
Explanation of Plate 4

Fig. 1: Scanning electron micrograph of the crossed-lamellae of *Neptunea arthritica* showing obliquely arranged first-order lamellae; L-section; X 3,000.

Fig. 2: Scanning electron micrograph of the crossed-lamellae of the same species, showing obliquely arranged second-order lamellae on the left and first-order lamellae on the right, W-L-section, X 1,000.

Fig. 3: Scanning electron micrograph of the crossed-lamellae of *Glycymeris yessoensis* showing obliquely arranged second-order lamellae, W-section; X 300.
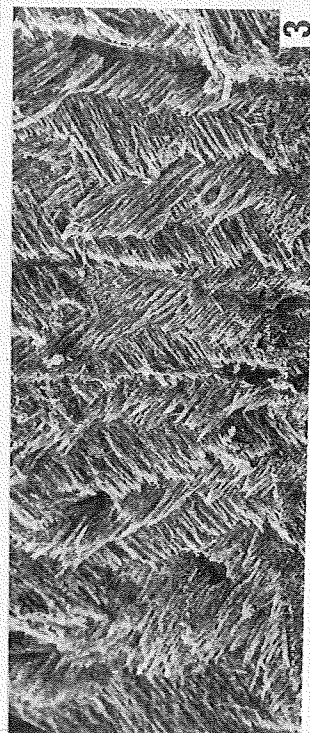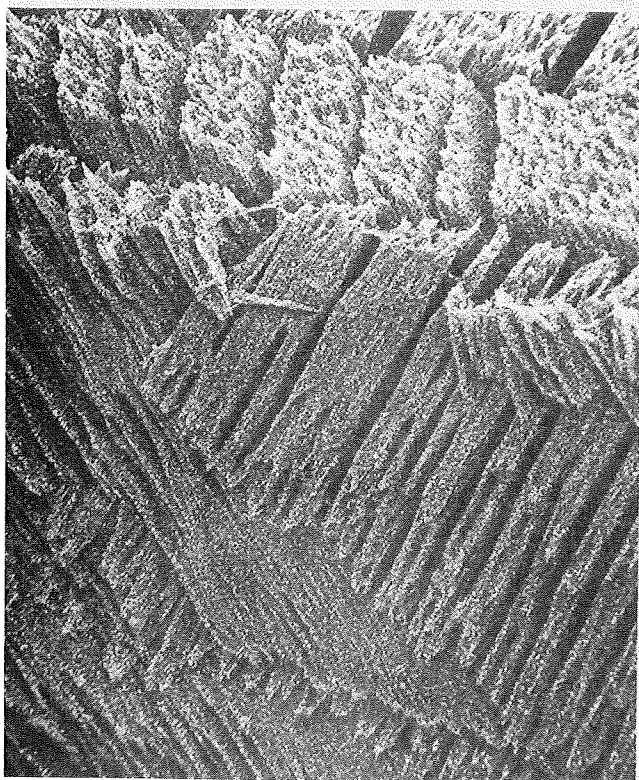
plate 4

Explanation of Plate 5

The organic matrices of the demineralized crossed-lamellae of *Corbicula japonica* (Fig. 1), and that of *Clinocardium californiense* (Fig. 2). Note two different zones in the vertical direction of the picture. In W-section (Fig. 1), internal structures of zones are in oblique relation and show bifurcated pattern around the contacts, while in nearly W-section (Fig. 2), they cross at nearly right angles alternately. Fig. 1: X 10,000; Fig. 2: X 7,500.

plate 5

1 μ

1

0.5 μ

2
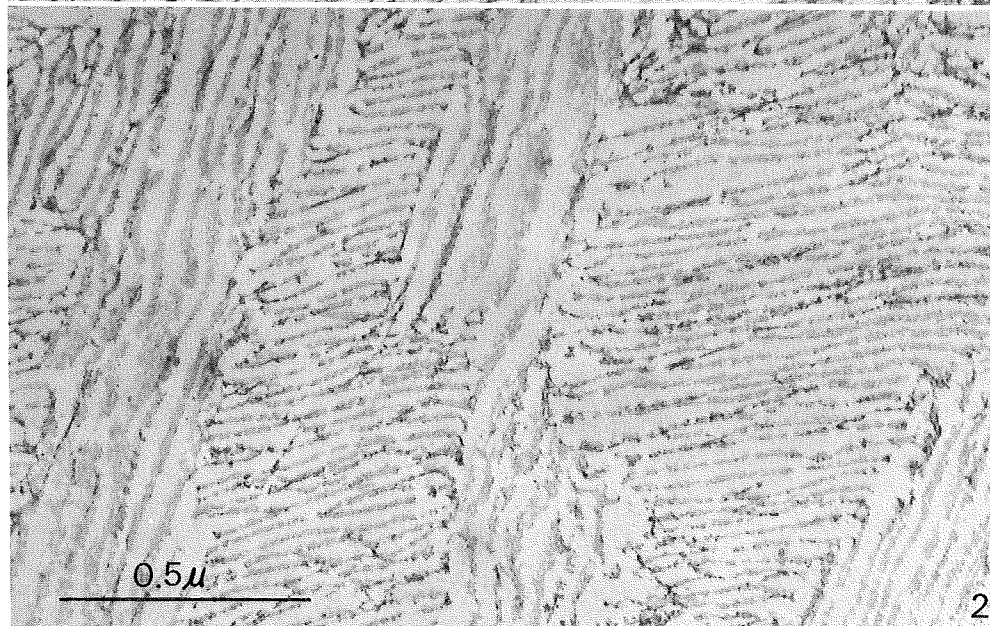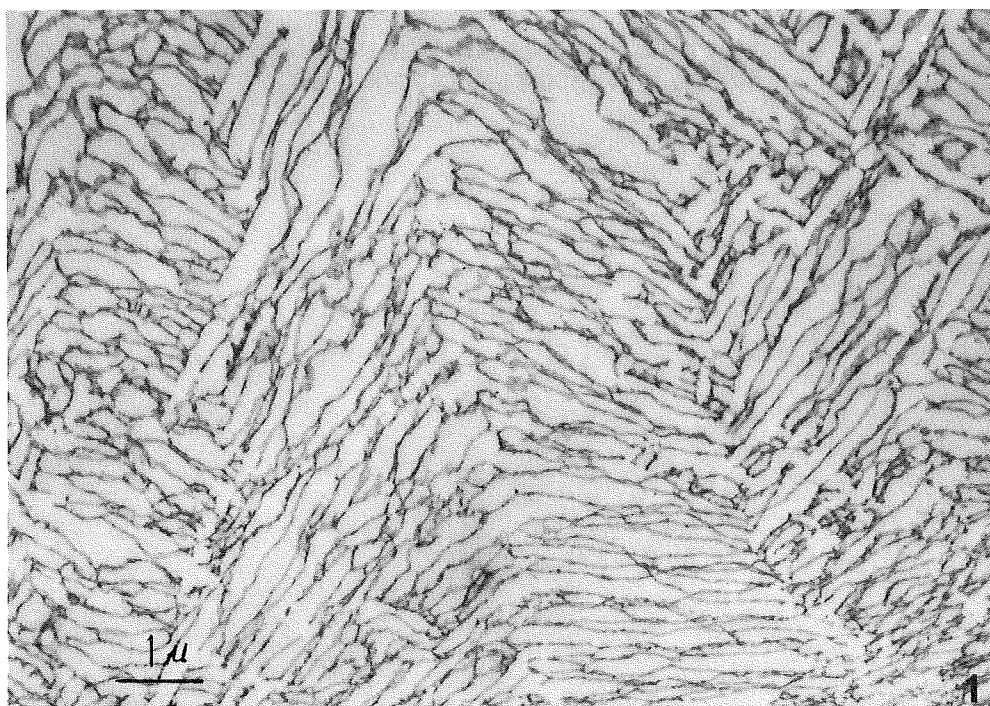
Explanation of Plate 6

The organic matrices of the demineralized crossed-lamellae of *Corbicula japonica.* (nearly L-section) Fig. 1: X 3,000, Fig. 2: X 4,500; Fig. 3: X 7,000

plate 6 467

468
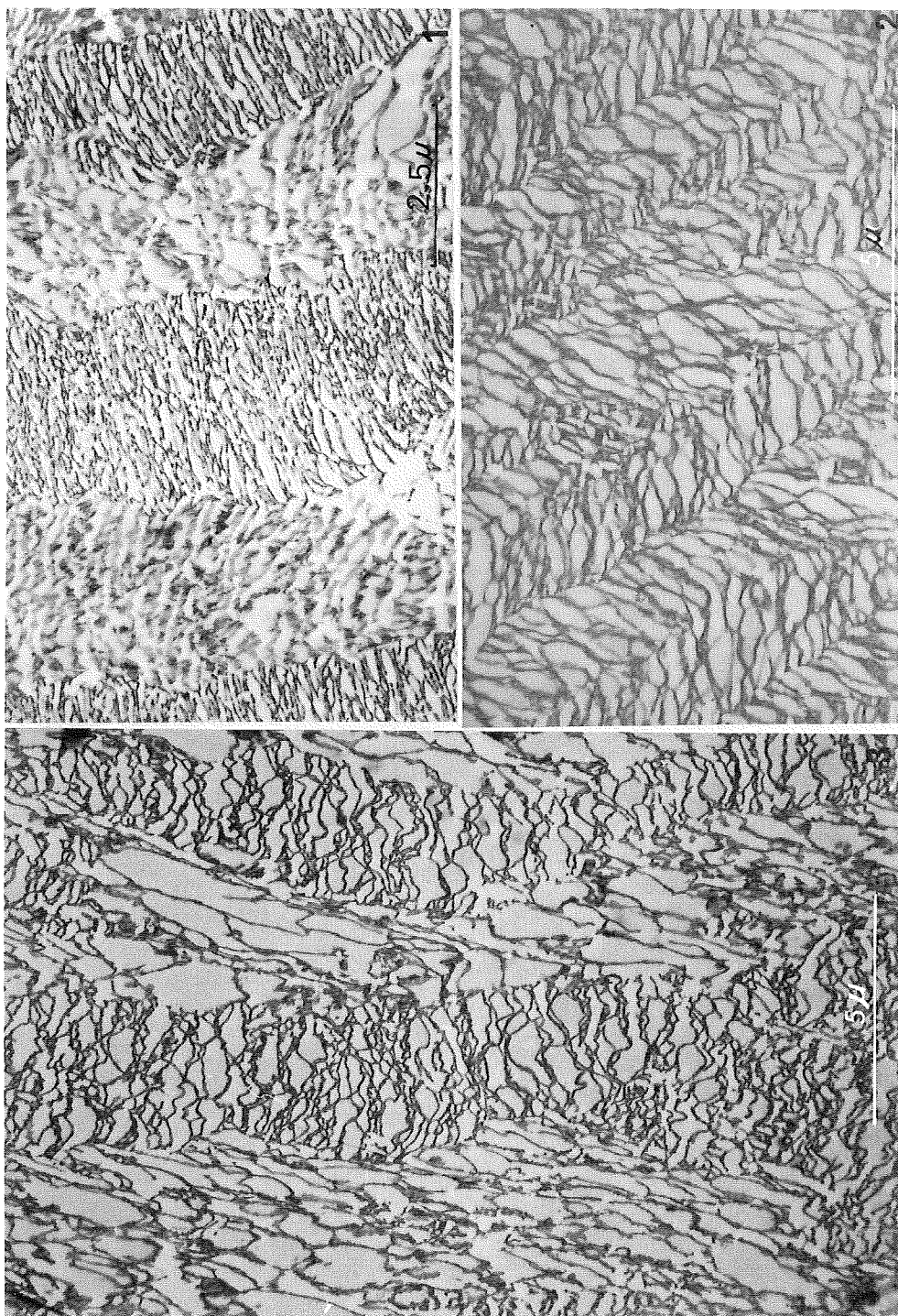
Explanation of Plate 7

Fig. 1: Organic matrix of the demineralized crossed-lamellae of *Corbilula japonica.* (nearly H-section) Transmission electron micrograph. X 5,000.

Fig. 2: Conchiolin membrane of the crossed-lamellae of *Glycymeris albolineata.* Pt-Pd shadowing. Transmission electron micrograph. X 10,000.

Fig. 3: Conchiolin membrane of the crossed-lamellae of *Corbicula japonica.* Pt-Pd shadowing. Transmission electron micrograph. X 30,000.

Plate 7   Uozumi, Iwata and Togo/ Ultrastructure of crossed-lamellar layer
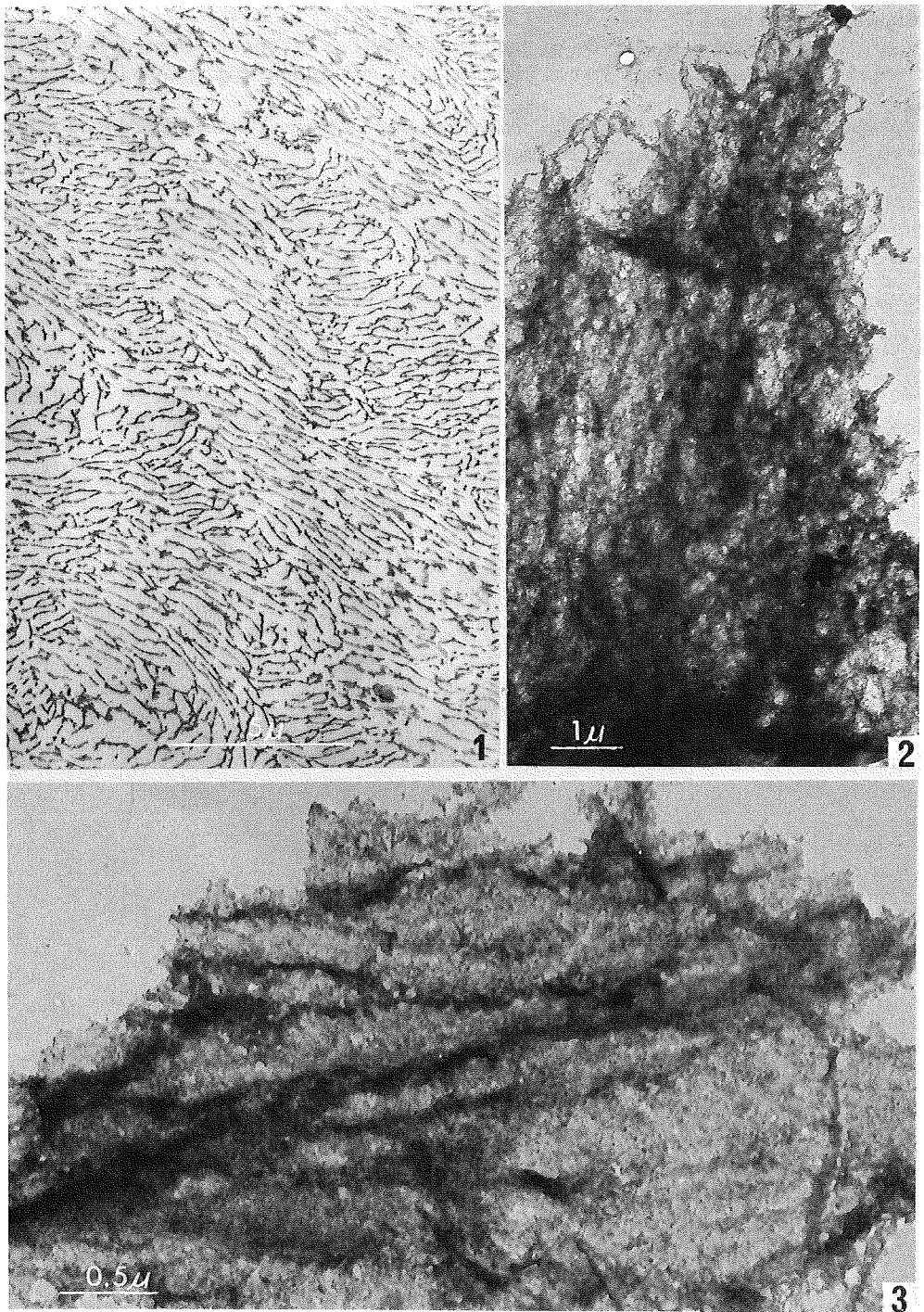
plate 7

Explanation of Plate 8

Fig. 1, 3.: Conchiolin membrane of the crossed-lamellae of *Clinocardium californiense.* Pt-Pd shadowing. Transmission electron micrograph. Fig. 1: X 6,000; Fig. 3: X 30,000.

Fig. 2: Conchiolin membrane of the crossed-lamellae of *Meretrix lusoria.* Pt-Pd shadowing. Transmission electron micrograph. X 25,000.

472
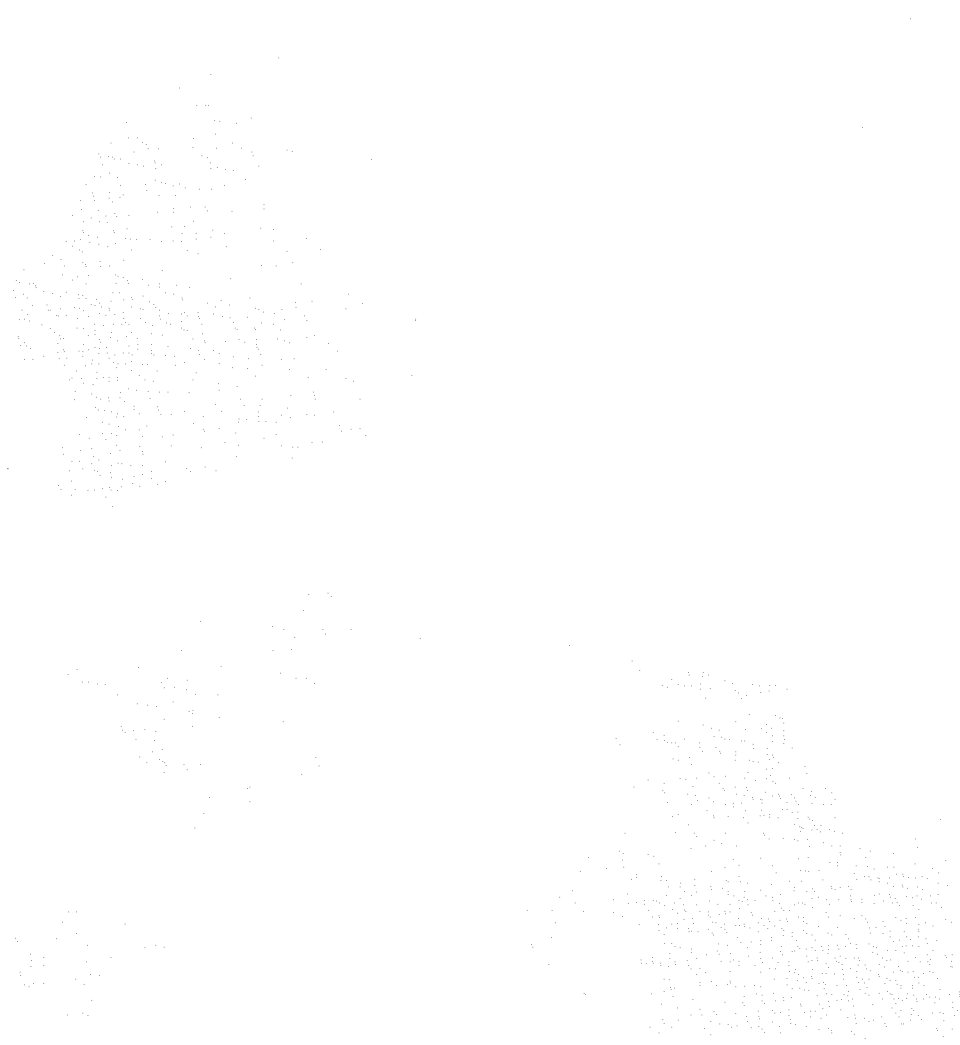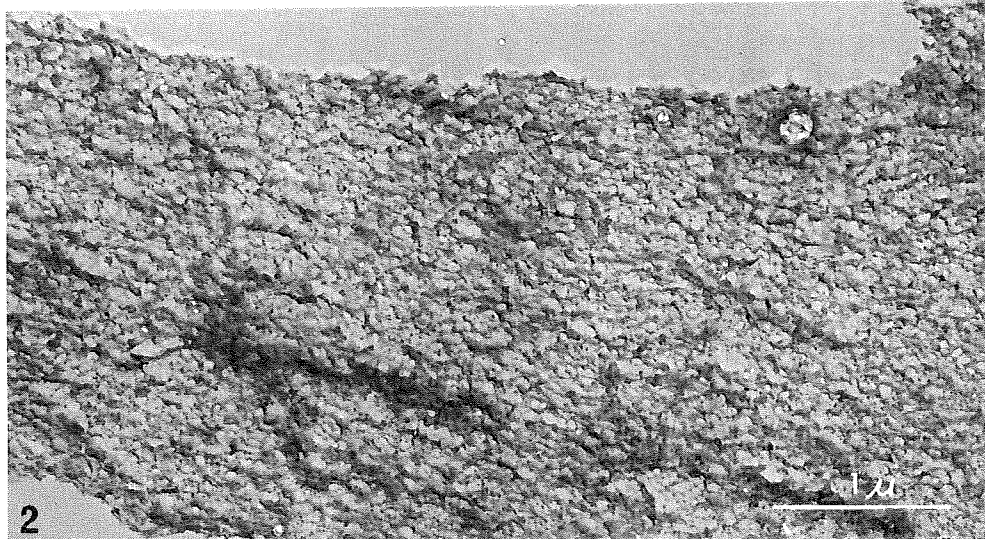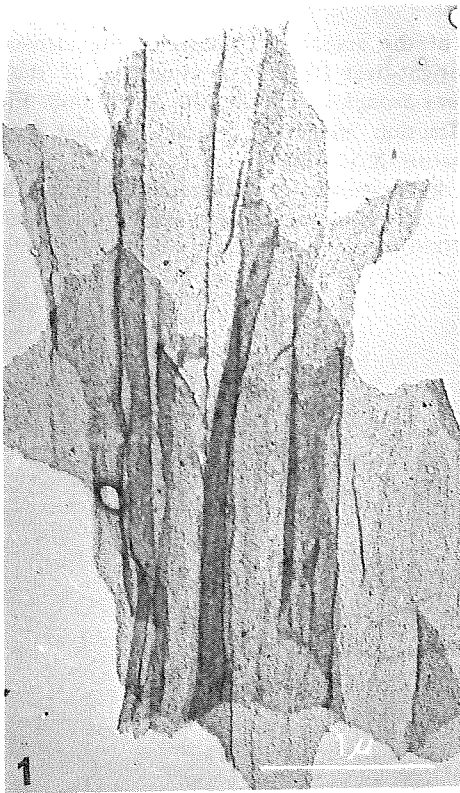
Explanation of Plate 9

Fig. 1—3: Transmission electron micrographs of the aragonite crystals of the crossed-lamellae. Dark-field (Fig. 1) and bright-field (Fig. 3) micrographs of those isolated from Holocene fossil, *Anadara broughtoni*. X 25,000; X 100,000. Fig. 2: Bright-field micrograph and diffraction image of crystals, isolated from Pliocene fossil shell, *Glycymeris yessoensis*. X 50,000.

Fig. 4: Transmission electron micrograph of the aragonite crystals, isolated from the semi-decalcified nacreous layers of *Nautilus*. For comparision with the crossed-lamellae crystals. Pt-Pd shadowing, X 25,000.

plate 9

474
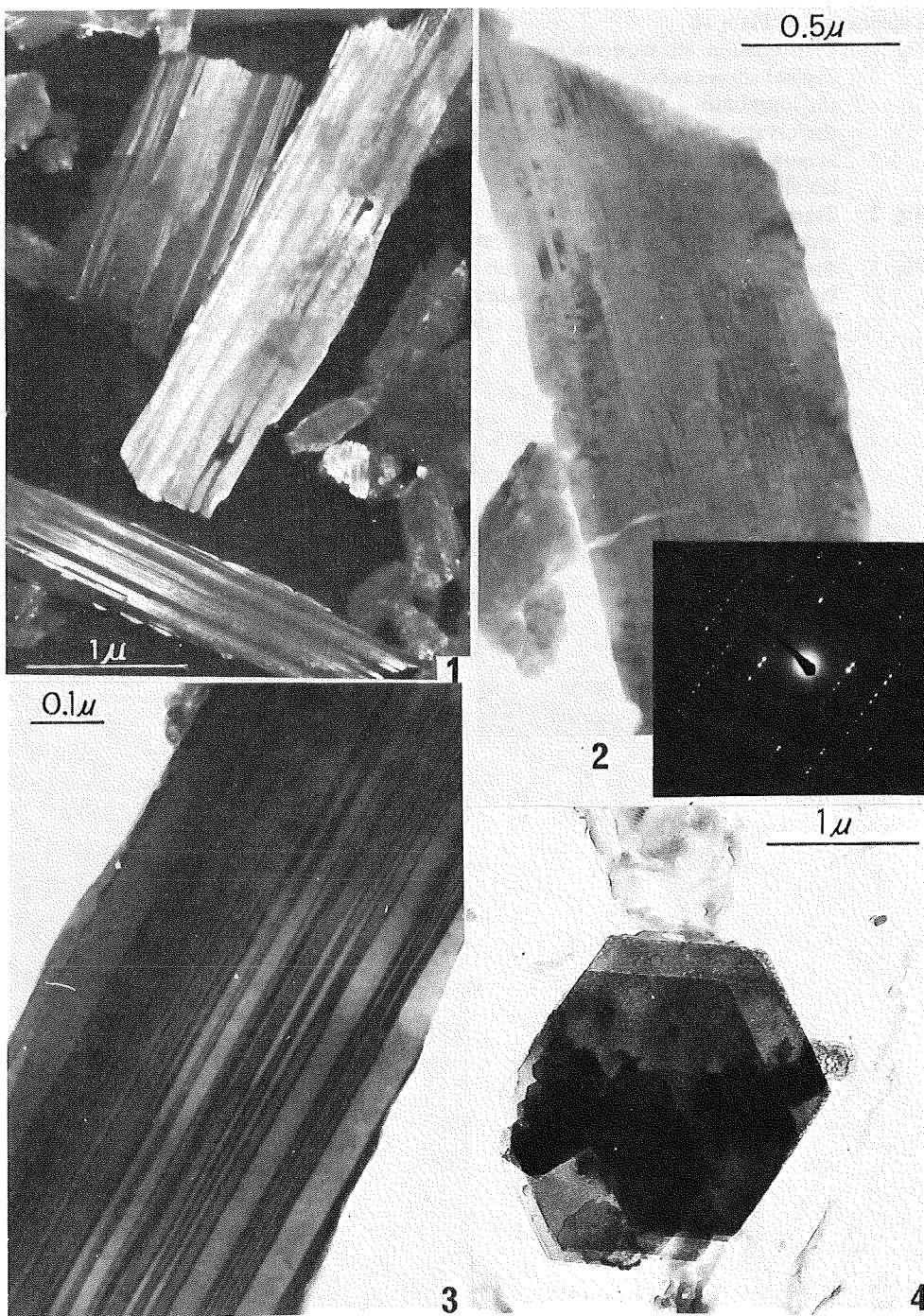
Explanation of Plate 10

Fig. 1: Transmission electron micrograph of aragonite crystal of *Glycymeris albolineata*. Crystal shows rectangular in shape. The dark and light bands which are parallel to the longitudinal axis of crystal develop on the crystal. Bands of high density within the crystal are of an undermined nature, but this feature appears to be confined to aragonite crystals consisting of crossed-, complex-crossed and pseud-crossed lamellae. unstained. X 30,000.

Fig. 2: Transmission electron micrograph of aragonite crystal of the crossed-lamellae of recent *Glycymeris albolineata*. X 50,000.

Fig. 3: Transmission electron micrograph of aragonite crystal of the crossed-lamellae of Paleogene fossil, *Glycymeris kyushuensis*. X 100,000.

The dark and light bands within crystal of the crossed lamellae are also preserved in fossil.

plate 10

1

1μ

2

0.5μ

3

0.1μ

476
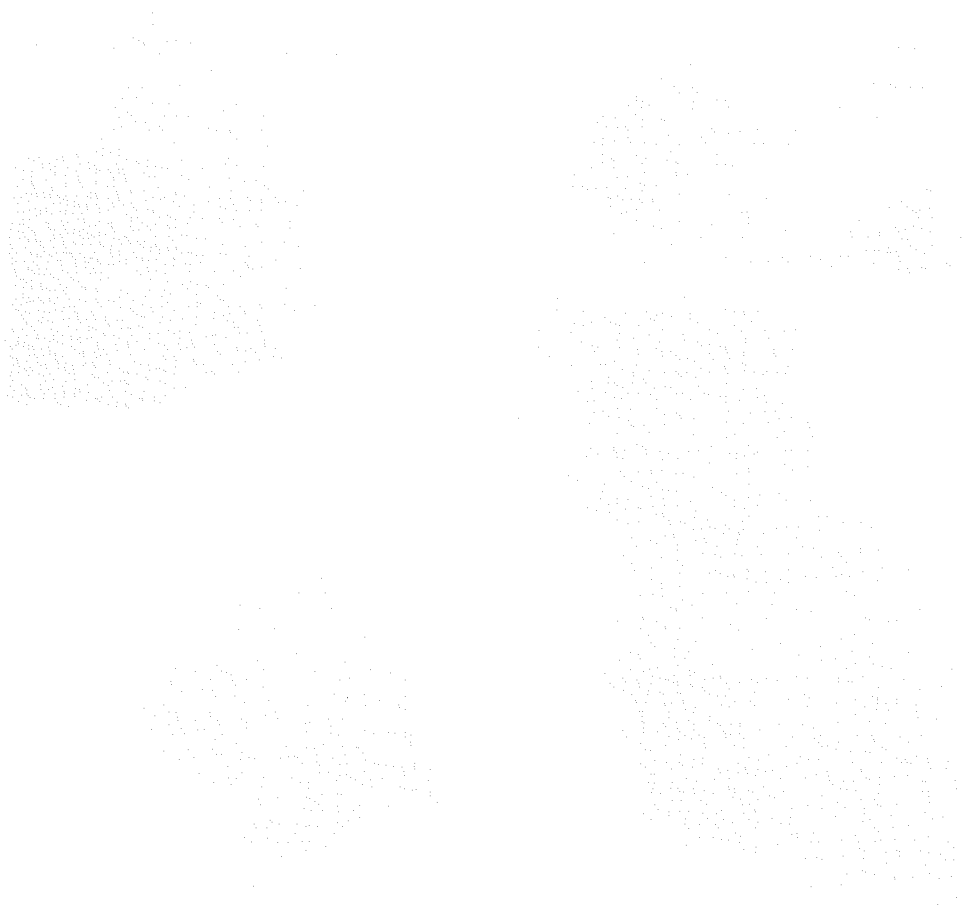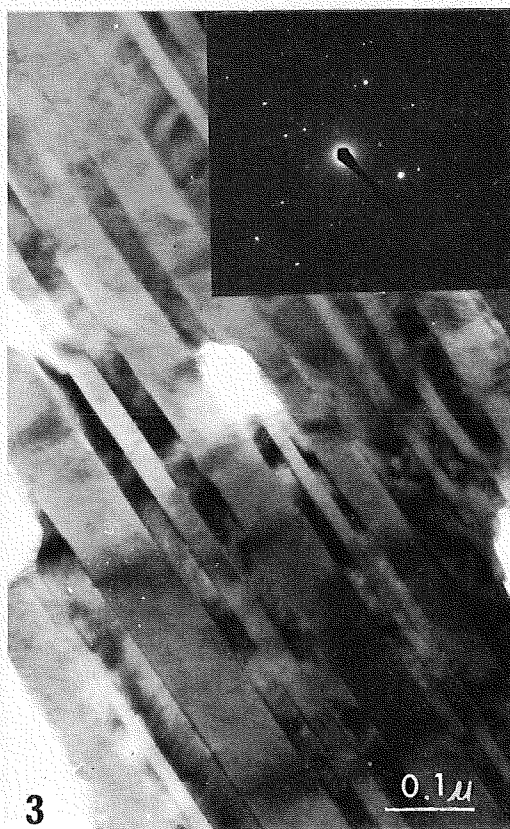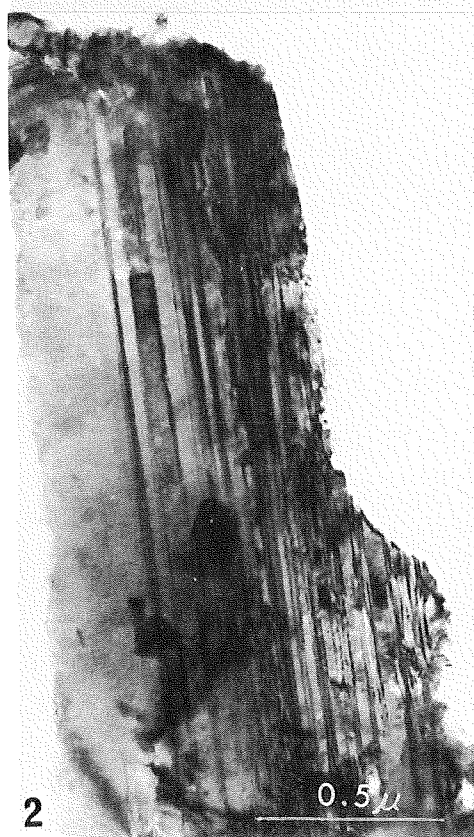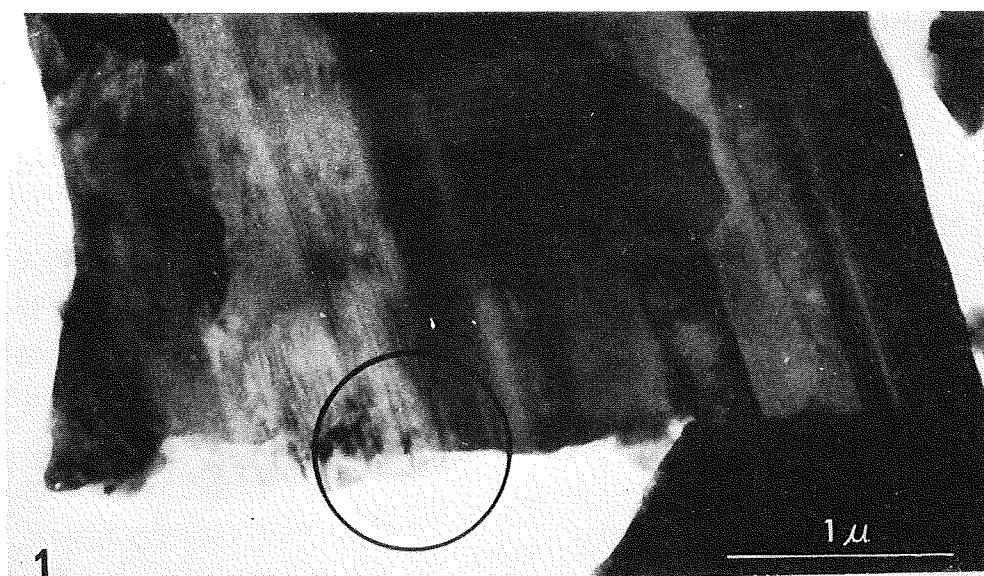
Explanation of Plate 11

Fig. 1: A higher magnification, a part of the crystal of Fig. 1 (black circle) in Pl. 10.
The crystal shows series of dark stripes with periodic distance of 7.8 Å. Those
stripes are seemed to obliquely intersect the longitudinal axis of the crystal.
X 1500,000.

plate 11   477