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FURTHER STUDIES ON STRUCTURE OF THE ORGANIC COMPONENTS IN MOTHER-OF-PEARL, ESPECIALLY IN PELECYPODS (PART I),

by Charles GRÉGOIRE (Liège).

(With 5 plates.)

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FURTHER STUDIES ON STRUCTURE OF THE ORGANIC COMPONENTS IN MOTHER-OF-PEARL, ESPECIALLY IN PELECYPODS (PART I) (1),

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(With 5 plates.)

As reported in previous studies (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1949, 1955), ultrasonic irradiation cleaves and breaks the organic membranes of decalcified mother-of-pearl (nacreous conchiolin) into fragments of leaflets. In the electron microscope, these fragments appear perforated by openings or pores and assume a reticulated lace-like texture.

Subsequent studies on replicas of inner surfaces of normal shells, of fragments of cleavage produced by fracture and of polished surfaces before and after etching (GRÉGOIRE, 1957), have shown that these reticulated structures belong to the system of organic membranes or sheets which run as continuous formations in between adjacent stratified mineral lamellae of mother-of-pearl.

Three patterns of structure (nautiloid, gastropod and pelecypod), based on differences in the size, in the shape and in frequency distribution of the pores in the three classes of molluscs, were provisionally recognized in nacreous conchiolin. Measurements of these details of structure suggested that the differences recorded in the patterns were statistically characteristic at the class level of taxonomy.

In part I of the present study, measurements have been performed on reticulated sheets from 36 species of Gastropods and of Pelecypods. This

(1) An abstract of preliminary results appeared in *Arch. Intern. Physiol. Bioch.*, 1958, *66*, 667.

material includes 26 species not investigated previously, some from the most primitive *(Trigoniidae)*, others from the most recent groups *(Aetheriidae)* of Pelecypods, pearls from different origins and shell material from archaeological sites. The differences in the patterns were appreciated statistically.

In some preparations, the effects of protracted exposition of the sheets to the ultrasonic beam have been studied and compared with the alterations, described previously (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955), induced in the structures of the organic components by successive extractions of chemical fractions.

Part II of the present study, dealing with specific peculiarities in the topographical connections between organic and mineral components of mother-of-pearl, especially in pearls, will be reported elsewhere.

MATERIAL AND METHODS.

Material. — The following species have been used in the present study :

GASTROPODA :

ANGARIIDAE : *Angaria delphinus* LINNÉ (*laciniata* LAMARCK).

STOMATIIDAE : *Stomatella papyracea* CHEMNITZ; *Stomatia rubra* LAMARCK.

TURBINIDAE : *Astraea rugosa* LINNÉ, *Astraea olivacea* WOOD.

UMBONIIDAE : *Umbonium vestiarium* LINNÉ.

PELECYPODA :

MYTILIDAE : *Mytilus crenatus; Brachyodontes bilocularis* LINNÉ.

PTERIIDAE : *Pinctada (Pteria, Meleagrina) margaritifera* LINNÉ (n° 1 : from Persian Gulf; n° 2 : from Margarita Islands, Venezuela); *Pinctada (Pteria, Meleagrina) vulgaris (fucata)* GOULD (from Ceylon); *Pinctada galtsoffi* BARTSCH; *Pinctada mazatlanica* HANLEY; *Pinctada martensi* DUNKER (double, triple and baroque japanese cultured pearls; shell); *Pteria pengin* LINNÉ (half pearls).

PINNIDAE : *Pinna nobilis; Pinna nigra* CHEMNITZ ≠ 1 and ≠ 2 (*Atrina nigra* DURHENN, from Caroline Islands).

TRIGONIIDAE : *Trigonia lamarckii* REEVE.

MARGARITANIDAE : *Margaritana margaritifera* LINNÉ.

UNIONIDAE : *Grandidieria burtoni* WOODWARD; *Caelatura (Zairia) elegans* de ROCH; *Caelatura (Caelatura) bakeri* H. AD.; *Quadrula undulata* BARNES (pig shoe mussel from Mississippi, used as nucleus in the japanese pearls of culture); *Hyriopsis schlegeli* VON MARTENS : pearls; *Unio rectus* LAMARCK; *Unio* sp. (Holocene, see GRÉGOIRE, 1959); *Obovaria retusa* LAMARCK; unidentified specimen (possibly *Elliptio* sp.) : shell fragment collected in the archaeological site of Tikal, Guatemala, from Maya Period I.

MUTELIDAE : *Anodontites trapezialis* LAMARCK; *Aspatharia (Spathopsis) Wismanni* VON MARTENS; *Mutela langi* PILSBRY and BEQUAERT; *Iridina (Cameronia) spekii* S. P. WOODWARD; *Iridina spekii* S. P. WOODWARD (Holocene, see GRÉGOIRE, 1959).

AETHERIIDAE : *Aetheria elliptica* LAMARCK (undetermined forma); *forma typica* AETCH; *forma caillaudi* FERUSSAC; *forma tubifera* LAMARCK;

Aetheria elliptica (*bourguignati* DE ROCHEBRUNE, var. *tubifera*); *Bartlettia stefanensis* MORLEY.

ANATINIDAE : *Pandora cuningii* HANLEY; *Pandora trilineata* SAY.

Preparation of the conchiolin membranes for electron microscopy.

The inner nacreous part of the shells or fragments of mother-of-pearl were decalcified by saturated aqueous solutions of the disodium salt of ethylene-diamine tetra-acetic acid : sequestrene NA 2, Alrose Chemical Corporation, Providence, Rhode Island; titriplex III, Merck, Darmstadt, Germany).

The soft iridescent membranes, freed from the mineral substance, were washed by repeated centrifugation in bidistilled water. A part of the material was immersed for 24 hours in a 2 per cent aqueous solution of osmic acid and afterwards washed in several changes of bidistilled water.

Aqueous suspensions of the membranes were placed in glass tubes, about 2 cm in diameter, closed at one end by cellophane windows, which were fastened to the outer part of the tubes by strings glued with collodion.

The tubes, partly immersed in a tank in which cold water was running continuously, were exposed to the converging beam of ultrasounds, focused at the centre of the cellophane drums.

The source of ultrasonic radiations consisted of a generator S. C. A. M., type B, associated to a projector type L (Frequency : 960 Kcy/sec.; 90-100 V, 45-50 mA; intensity : 14-20 W/sec./cm 2).

After a period of time varying greatly with the species, the suspensions became iridescent, while fragments of intact membranes were still floating. Samples of the material were collected at this stage, then the suspensions of membranes were exposed again to a protracted ultrasonic irradiation until these membranes were thoroughly disintegrated.

Small drops of the suspensions were deposited on to formvar-coated (0.1 per cent solution in ethylene dichloride) copper screens, then drained and dried. Most preparations were shadow-cast with palladium at angles varying from 10 to 25°.

The background granularity of the supporting formvar films, especially visible in shadow-cast preparations, frequently interferes with detection of the fine details of structure of the reticulated sheets. In several preparations, supporting carbon films (WATSON, 1956), smoother than formvar films, and supporting nets (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955, SJÖSTRAND, 1956) were used instead of formvar films.

Measurement of the size, of the shape and of distribution frequency of the pores in the reticulated sheets.

The method used for the measurements has been adapted by Messrs. P. BOURGEOIS and R. COUTREZ (from the Observatoire Royal) in the study of the organic nacreous material from a procedure employed in astronomy for numeration of stars.

All the measurements in the present work were performed on prints of micrographs of desiccated, unfixed, reticulated sheets lying on supporting films and shadow-cast with palladium.

The micrographs were enlarged to a standard final magnification of 40.000 diameters.

Fragments of sheets obviously shrunk, stretched, torn or folded were discarded from the measurements.

By means of this procedure, already used in a previous study (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955), the average number of pores per unit of print surface, the average diameter of the pores on print, and the relative surface occupied by the pores in the sheets, have been calculated.

A metallic frame, enclosing a window 10 cm by 10 cm in size, and divided into 100 squares by a meshwork of thin threads, was placed at random on to the enlarged picture of a sheet.

1. All the pores detected on the surface delimited by the window were numbered. The operation was repeated on different fields of the same and of other reticulated sheets of the specimen.

The total number of the pores counted (Σ), divided by the number (x) of cm^2 involved in the counts, gave the number of pores per cm^2 of print, or the average pore frequency ($n^\star$) in the reticulated sheets of the specimen :

$$\frac{\Sigma}{x\ \mathrm{cm}^2} = n^\star$$

2. In each field covered by the window, the pores of different diameters were numbered separately.

3. The total number of pores of each diameter (Σn), collected from the measurements performed in all the fields used, was multiplied by that diameter ($\Sigma n \times \varnothing$), which provided the sum of all the diameters of the pores of that diameter.

4. All the diameters of the pores of all sizes were then summed up : $\Sigma (\Sigma n \times \varnothing)$.

5. This sum was then divided by the total number of pores counted in all the fields involved in the measurements. The latter value $\Sigma (\Sigma n)$ or N was obtained by adding the numbers of pores of all sizes counted.

6. The average diameter of the pores in the organic sheets of the specimen was calculated by dividing the sum of all the diameters of the pores of all sizes, $\Sigma (\Sigma n \times \varnothing)$, by the total number of pores counted in all the fields (N) :

$$\frac{\Sigma (\Sigma n \times \varnothing)}{N}$$

7. The relative surface occupied by the pores in the reticulated sheets, expressed in per cent, was calculated by multiplying the pore frequency (n^*) by π (3.14) and R^2 (square of the average radius of the pores).

In the table, the statistical significance of the differences reported has been established, when necessary, by calculation of the probable error of the differences of the means and with the « u » test of Student. The value $1 - P$ corresponds, in the method used, to the value P generally reported in literature. For values of $1 - P > 0.100$, the differences were considered as not significant, and for values of $1 - P > 0.050$, as of doubtful or of moderate significance. In a few border line instances, the degree of significance of the differences of the means was expressed as the number of chances against one (reported in the text in brackets : *x* ag. 1) that the differences of the means are not accidental.

All the preparations were examined with a RCA, type EMU-2, electron microscope. No aperture was used in the objective (Hillier and Ramberg, 1947).

OBSERVATIONS.

Reactions of nacreous conchiolin to the ultrasonic radiations.

Resistance of conchiolin membranes from decalcified mother-of-pearl to dislocation into thin leaflets induced by the ultrasonic beam varied greatly in the different specimens.

Development of an iridescent fog in the liquid of suspension was selected as an indication that breakage and cleavage of the membranes into leaflets thin enough for examination with the electron microscope had taken place.

With the generator functioning at its highest intensity (90-100 V, 45-50 mA; 14/20 W/sec/cm^2), and focussed on to the center of the drum containing the suspensions of membranes, the following gradations were observed in the speed of dissociation of the unfixed membranes :

Immediate dissociation. — *Stomatella papyracea, Unio* sp. (Holocene), *Iridina spekii* (Holocene), unidentified *Unionidae* (*Elliptio* sp.?) from Tikal (see Material).

Rapid dissociation. — *Angaria delphinus, Umbonium vestiarium, Pinna (Atrina) nigra, Grandidieria burtoni, Obovaria retusa, Mutela langi, Aspatharia wismanni, Aetheria elliptica* (slow in some samples).

Slow dissociation. — *Stomatia rubra, Mytilus crenatus, Brachyodontes bilocularis, Trigonia lamarckii* (rapid in some samples), *Caelatura elegans, Caelatura bakeri, Pinna nobilis, Hyriopsis schlegeli, Pandora cuningii, Pandora trilineata.*

No iridescence visible in the suspensions, even after protracted treatment. — *Astraea rugosa, Pinctada martensi* (pearls); *Pteria pengin* (pearls), *Quadrula undulata, Bartlettia stefanensis.*

Membranes immersed for 24 hours in a 2 per cent aqueous solution of osmic acid before being exposed to the irradiation, were generally dissociated more rapidly than the unfixed material.

ELECTRON MICROSCOPY.

The organic lace-like reticulated sheets.

The characteristic structural features of the leaflets obtained after ultrasonic fragmentation of the nacreous conchiolin membranes in different families and the results of the measurements are reported in table I.

Representative pictures of the reticulated sheets (plates 1 to 5) were selected from 1.600 micrographs.

GASTROPODS.

Except for subsidiary variations, the patterns of the sheets collected from mother-of-pearl of the species used in the present study, conformed to the Gastropod pattern, characterized, as previously reported (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955), by large pores of uniform size, evenly dispersed in the sheets and separating cylindrical or strand-like trabeculae (Pl. I, fig. 1 and 2). This arrangement gives to the sheets in Gastropods a sieve-like appearance.

TABLE 1.

Mother-of-Pearl. Organic reticulated sheets.

Material	Pores (average values)			Comments
	Frequency, per cm², on prints	Diameter in mm, on prints	Relative surface, in per cent	
GASTROPODA :				Round pores of large size, of uniform shape, evenly scattered in the sheets. Short cylindrical or ribbon-like trabeculae, embedding fibrillar material, and studded with hemispherical protuberances. (Pl. 1, fig. 1, fig. 2.)
ANGARIIDAE :				
Angaria delphinus LINNÉ *(laciniata* LAMARCK)	5.4	2.88	35.10	
STOMATIIDAE :				
Stomatella papyracea CHEMNITZ	5.1	2.20	19.38	
Stomatia rubra LAMARCK	8.0	2.16	21.67	
UMBONIIDAE :				
Umbonium vestiarium LINNÉ	7.5	2.13	26.62	
Average *(GASTROPODA)*	6.5	2.34	25.69	
PELECYPODA :				Condensed texture, in which a few large pores *(Brachyodontes)* and numerous small pores are obliterated by shrinkage in many preparations. Thin fibrillar trabeculae, studded with tuberosities. (Pl. 1, fig. 3, fig. 4.)
MYTILIDAE :				
Mytilus crenatus	11.0	1.00	8.63	
Brachyodontes bilocularis LINNÉ	11.8	1.10	9.26	
Average (MYTILIDAE)	11.4	1.05	8.94	

TABLE 1.

Mother-of-Pearl. Organic reticulated sheets. (Continued.)

Material	Pores (average values)			Comments
	Frequency, per cm², on prints	Diameter in mm, on prints	Relative surface, in per cent	
PTERIIDAE :				Thick leaflets with sparse large pores of uniform size.
Pinctada (Pteria, Meleagrina) margaritifera LINNÉ	3.6	1.81	9.37	Strand-like trabeculae, embedding fibrils.
Pinctada vulgaris (fucata) GOULD ...	4.5	1.58	8.76	Round protuberances scattered on the trabeculae.
Pinctada galtsoffi BARTSCH	4.0	1.62	8.03	
Pinctada mazatlanica HANLEY	3.9	1.70	8.71	
Pinctada (Pteria) martensi DUNKER (pearls)	4.5	1.50	8.02	
Pteria pengin (half pearls)	2.0	2.29	8.29	
Average (PTERIIDAE)	3.7	1.75	8.53	
Probable error	0.25	0.07	0.14	
Differences between PTERIIDAE and :				
UNIONIDAE (1 — P)	0.000	0.003	0.069	
AETHERIIDAE (1 — P)	0.023	0.121	0.138	
PINNIDAE :				Condensed texture.
Pinna nobilis	3.3	1.16	3.51	Small pores of uniform size, sparsely distributed in the sheets.
Pinna nigra CHEMNITZ (n° 1)	4.6	1.04	3.90	Fibrillar trabeculae on which round granules appear frequently alined.
Pinna (Atrina) nigra DURHENN (n° 2)	4.5	1.03	3.72	(Pl. 2, fig. 5, fig. 6.)
Average (PINNIDAE)	4.1	1.08	3.71	

TABLE 1.

Mother-of-Pearl. Organic reticulated sheets. (Continued.)

Material	Pores (average values)			Comments
	Frequency, per cm², on prints	Diameter in mm, on prints	Relative surface, in per cent	
TRIGONIIDAE :				
Trigonia lamarckii REEVE	9.3	1.17	9.93	Pores of round shape and of uniform size, evenly scattered in the leaflets. (Pl. 2, fig. 8). Pores frequently disposed in parallel lines which are separated by sturdy trabeculae bulging with protuberances of various sizes. (Pl. 2, fig. 7.)
MARGARITANIDAE :				
Margaritana margaritifera (LINNÉ) ...	(6.8)	(1.73)	(15.80)	Considerable variations in the pattern recorded in the different preparations of this specimen. The values, reported in brackets, are questionable.
UNIONIDAE :				Round pores evenly distributed in the sheets. Cylindrical or strand-like trabeculae, embedding fibrillar material, and studded with protuberances. Thick leaflets in pearl material. (Pl. 3, fig. 14; Pl. 4, fig. 17.) In preparations of *Grandidieria burtoni,* pores disposed in parallel lines, separated by straight trabeculae coated with rows of tuberosities of various sizes, in several preparations. (Pl. 3. fig. 12; Pl. 4, fig. 15.)
Grandidieria burtoni WOODWARD	9.9	1.23	11.50	
Caelatura (Zairia) elegans de ROCH ...	7.6	1.17	8.11	
Caelatura (Caelatura) bakeri H. AD. ...	7.7	1.16	8.22	
Quadrula undulata (BARNES)	6.3	1.46	10.48	
Hyriopsis schlegeli von MARTENS (pearls)	6.4	1.41	9.85	
Unio rectus LAMARCK	6.5	1.54	12.04	
Obovaria retusa LAMARCK	5.5	1.37	7.94	
Average (UNIONIDAE)	7.1	1.33	9.73	
Probable error	0.36	0.03	0.42	

TABLE 1.

Mother-of-Pearl. Organic reticulated sheets. (Continued.)

Material	Pores (average values)			Comments
	Frequency, per cm², on prints	Diameter in mm, on prints	Relative surface in per cent	
Differences between UNIONIDAE and :				
MUTELIDAE (1 — P)	0.282	0.001	0.000	
AETHERIIDAE (1 — P)	0.077	0.117	0.424	
Unio sp. (Holocene, see GRÉGOIRE, 1959)	7.2	1.30	9.49	
Unidentified specimen (UNIONIDAE, possibly *Elliptio* sp.) : fragments of mother-of-pearl and iridescent dust (Tikal, Maya period I)	5.3	1.43	8.32	Mostly round pores of uniform size, evenly scattered in the sheets. Fibrils embedded in the trabeculae.
MUTELIDAE :				Round pores of large size, of uniform shape, evenly distributed in the sheets, especially in *Mutela langi*. (Pl. 5, fig. 20.) Cylindrical trabeculae, in which fibrillar material is visible. The texture of the sheets resembles the gastropod pattern, especially in *Mutela langi*. (Pl. 5, fig. 20.) However, in MUTELIDAE, the pores are of smaller size, more sparsely, and, in general, less evenly distributed in the sheets than in gastropods.
Anodontites trapezialis LAMARCK	4.7	2.06	15.64	
Aspatharia (Spathopsis) wismanni von MARTENS	5.8	1.80	14.75	
Mutela langi PILSBRY & BEQUAERT ...	7.7	1.74	18.37	
Iridina (Cameronia) spekii S. P. WOODWARD	8.0	1.54	14.82	
Average (MUTELIDAE)	6.5	1.78	15.89	
Probable error	0.52	0.07	0.57	
Differences between MUTELIDAE and :				
AETHERIIDAE (1 — P)	0.210	0.073	0.005	

TABLE 1.

Mother-of-Pearl. Organic reticulated sheets. (Continued.)

Material	Pores (average values)			Comments
	Frequency, per cm², on prints	Diameter in mm, on prints	Relative surface in per cent	
Iridina spekii S. P. WOODWARD (Holocene, see GRÉGOIRE, 1959)	7.5	1.47	12.48	
AETHERIIDAE :				Thick sheets. Pores of various sizes and shapes. Sturdy trabeculae, embedding substantial fibrillar material. (Pl. 4, fig. 19.)
Aetheria elliptica LAMARCK, unidentified forma	5.6	1.56	10.72	
forma typica AETCH.	5.4	1.58	10.51	
Aetheria bourguignati de ROCHEBRUNE var. *tubifera*	5.7	1.52	10.38	
(bracketed mean of the three above)	5.6	1.55	10.54	
Bartlettia stefanensis MORLEY	5.2	1.42	8.16	
Average (AETHERIIDAE)	5.4	1.48	9.35	
Probable error	0.13	0.04	0.79	
ANATINIDAE :				Sheets in the shape of flattened membranes with a thinly granular and fibrillar appearance. Except for scattered groups, pores absent or not visible in the sheets. (Pl. 4, fig. 16; Pl. 5, fig. 21.)
Pandora cuningii HANLEY				
Pandora trilineata SAY				
PELECYPODA (general average) ...	6.1	1.47	9.90	

PELECYPODS.

Compared with the gastropod pattern, the pelecypod pattern is characterized by a denser reticulum, in which numerous pores of various sizes, mostly small, with larger ones scattered, separate thinner, frequently fibrillar, trabeculae.

After collapse of the pores by shrinkage, these sheets assume occasionally a deceptive appearance of homogeneous granular or fibrillar membranes.

As shown in the table, the general average diameter and the relative surface of the pores are significantly lower than in Gastropods.

Differences between families of Pelecypods in the pattern of the reticulated sheets are obvious if f.i. *Mytilidae, Pinnidae, Anatinidae* are compared with *Pteriidae, Unionidae, Aetheriidae* and *Mutelidae.*

In the four latter groups, the patterns of the reticulated sheets differ significantly from each other (*Pteriidae - Unionidae, Pteriidae - Aetheriidae, Pteriidae - Mutelidae, Unionidae - Mutelidae, Aetheriidae - Mutelidae,* but not *Unionidae - Aetheriidae)* by one, two, or by the three criteria selected for the measurements.

The differences between *Pteriidae* and *Unionidae* are highly significant with regard to the frequency and to the diameter of the pores, and moderately (15 ag. 1) significant concerning the relative surface occupied by the pores in the reticulated sheets.

The differences between *Pteriidae* and *Aetheriidae* are highly significant with regard to the frequency of the pores, and of doubtful significance with regard to the diameter (8 ag. 1) and to the relative surface (7 ag. 1) of the pores.

The differences between *Mutelidae* and *Aetheriidae* are highly significant with regard to the relative surface of the pores and moderately significant (14 ag. 1) concerning the diameter of the pores.

The differences between *Unionidae* and *Mutelidae* are highly significant concerning the average diameter of the pores and the relative surface occupied by these pores.

On the other hand, the differences between *Unionidae* and *Aetheriidae* are of moderate or of doubtful significance with regard to the frequency (13 ag. 1) of the pores. The differences between the two other criteria are not significant.

The patterns recorded in the reticulated sheets of *Pteriidae* and of *Mutelidae,* characterized by relatively large round pores, evenly distributed (f. i. *Mutela langi*) in the sheets, show some resemblance to the gastropod pattern (compare Pl. I, fig. 1 and 2 with Pl. 5, fig. 20).

However, these two families of Pelecypods differ significantly from the Gastropods by the smaller relative surface occupied by the pores perforating the reticulated sheets, by the smaller size (in *Mutelidae*) and by the sparser distribution (in *Pteriidae*) of the pores.

Reticulated sheets from subfossil Pelecypods, and from material found in archaeological excavations.

In the reticulated sheets from *Unio* sp. (Holocene, 500 to 15.000 years old, see GRÉGOIRE, 1959 pl., 8, fig. 30), the values reported in table I with regard to the pore frequency, to the diameter and to the relative surface of the pores are identical to the average figures reported for *Unionidae*.

In *Iridina spekii* (Holocene, see GRÉGOIRE, 1959, pl. 8, fig. 33), shrinkage of the sheets and of the pores is probably responsible for the differences noticed between the recent and the subfossil specimen.

In the reticulated sheets from an unidentified specimen (possibly *Elliptio* sp., *Unionidae*) collected in the excavations at Tikal (Maya period I, approximately 1.400-1.600 years old), the appearance of the lace-like structure (not shown) is closely similar to that of *Unionidae* and the values recorded, though different from the average figures of the group, approach those of individual species reported in the table.

Particular structures.

In mother-of-pearl of *Aetheria elliptica*, two kinds of organic membranes have been observed : thick brown sheets and thin white membranes.

The thin membranes, freed by decalcification of mother-of-pearl, consisted of soft, transparent and iridescent veils, with bluish hues, easily disrupted into fragments by ultrasonic irradiation. These fragments exhibited the characteristic lace-like pattern illustrated in pl. 4, fig. 19.

The second type of organic membranes appeared in the shape of dark brown, lustreless, rigid and papyraceous sheets, which alternated in the shell structure with several layers of nacreous lamellae of varying thickness. These sheets assumed the colour and consistency of the tobacco leaves in a cigar.

This rigid material could not be dissociated by ultrasonic irradiation, even after protracted exposition : its aqueous suspensions remained transparent without development of iridescent fogs. In the preparations examined by electron microscopy, dispersed fibrils were found together with opaque fragments.

A more detailed description of this organic papyraceous material and of its relations with the other organic and mineral components of the shells in the genus *Aetheria* will be reported in part II of the present study.

Substructure of the reticulated sheets.

In many fragments of reticulated sheets exposed to moderate irradiation by ultrasounds, fibrils frequently protruded at the edges of the fragments of leaflets.

Similar fibrils were also observed in the trabeculae of intact fragments of sheets, in which they appeared as if they were embedded in some coating material (Pl. 1, fig. 1).

Protracted ultrasonic irradiation of suspensions of conchiolin membranes gave rise to progressive and finally thorough disintegration of the reticulated sheets.

The successive stages of that disintegration were studied especially in *Grandidieria burtoni, Trigonia lamarckii* and *Aetheria elliptica.*

The first stage of dislocation of the characteristic lace-like pattern consisted of separation of the trabeculae, probably by rupture of lateral bridges of substance. The isolated trabeculae appeared coated with chiefly granular material, including spherical and pebble-like bodies of various sizes, frequently alined in rows on the trabeculae (Pl. 2, fig. 7; Pl. 3, fig. 12). These bodies correspond probably to the hemispherical protuberances, visible especially on the surface of the trabeculae in the still intact reticulated sheets (Pl. 1, fig. 1 and 2; Pl. 2, fig. 8; Pl. 3, fig. 12 and 14; Pl. 4, fig. 15; Pl. 5, fig. 20).

In preparations exposed to a longer irradiation, numerous fibrils were freed from the reticulated sheets, and appeared either singly or associated in bundles still attached to the intact fragments of typical lace-like leaflets (Pl. 2, fig. 9; Pl. 3, fig. 10, 11 and 13; Pl. 4, fig. 18).

Most of these fibrils were naked and no longer coated with granules or pebble-like bodies. The latter structures had been washed away during rinsing or they appeared scattered and agglutinated at random in small clusters on the background (Pl. 2, fig. 9; Pl. 3, fig. 10, 11 and 13).

The diameter of the fibrils, measured in preparations from 14 species, varied between 6 and 8 m μ. Fibrils of 5 m μ in diameter were observed in the thinly fibrillar reticulated sheets of *Mytilidae* and of *Anatinidae*. On the other hand, fibrils of 10 m μ in diameter and more, were recorded in Gastropods *(Astraea rugosa)*.

DISCUSSION.

Patterns of structure of the organic reticulated sheets in nacreous conchiolin and taxonomy.

The present results support former conclusions about statistically significant differences in the organic nacreous patterns, at the class level, between Gastropods and Pelecypods.

Statistically significant differences in the pelecypod pattern at a subclass level (family), appreciated by means of the three criteria selected for the measurements — frequency distribution and diameter of the pores, relative surface occupied by the openings in the reticulated sheets — were recorded in the present investigation between *Mytilidae, Pteriidae, Pinnidae, Unionidae, Mutelidae, Aetheriidae* and *Anatinidae* (2) (3).

Absence of significant difference, recorded in the present study between *Unionidae* and *Aetheriidae,* is not surprising : the results are in agreement with the well known close taxonomical relationships between these families (SIMROTH, 1894; ANTHONY, 1904, 1905; ADEMSAMER, 1925).

On the other hand, as shown in table I, the phenotypic variations which characterize the shells of *Aetheriidae* do not seem to affect the pattern of the reticulated sheets in the different ecological forms investigated in a genus *(Aetheria)* of that family.

In *Trigoniidae* and in *Margaritanidae,* scarcity in the material available did not permit a statistical appreciation of the differences with the other families.

Substructure of the reticulated sheets.

As shown previously (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955), extraction by water and by a borate buffer of water-soluble constituents (nacrin) from the protidic material of nacreous conchiolin, did not induce distinct alterations in the lace-like configuration of the reticulated sheets.

On the other hand, after subsequent extraction by sodium hydroxide of a second fraction, scleroprotidic in its composition (nacrosclerotin), the lace-like texture had disappeared and the residues consisted of bundles of polypeptidic fibrils (nacroin), composed chiefly (83,6 %) of alanine and of glycine.

It has been assumed that nacreous conchiolin would consist of a core of polypeptidic fibrils, surrounded by a double coating of scleroprotidic substance (extracted by sodium hydroxide) and of soluble proteins (extracted by water and by the borate buffer).

In agreement with these conclusions, the following observations in the present study give evidence that the core of the trabeculae in the reticulated sheets consists actually of fibrils :

(2) The discrepancies between the values recorded in the present study and those published formerly (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955) with regard to a same sp. (f.i., *Pinctada margaritifera)* are caused chiefly by differences in the procedures of preparation of the material, by broad individual variations in the structure of fragments of leaflets chosen at random, and by errors of measurements.

(3) In the same previous paper, it had been wrongly reported that the innermost layer of the shell in *Grandidieria burtoni* WOODWARD did not consist of mother-of-pearl but of calcite (Calcitostracum).

The characteristic lake-like pattern illustrated in fig. 9, 12, 13 and 15 of the present plates had not been observed in the fragments then available.

1. In the reticulated sheets exposed to moderate action of the ultrasonic irradiation, which did not disintegrate the lace-like textures, fibrils protruded in many samples at the edge of the broken leaflets of conchiolin.

2. In several preparations, fibrils appeared embedded in the trabeculae of the reticulated sheets, studded with hemispherical protuberances, frequently disposed in rows on the fibrils.

3. In the sheets of nacreous conchiolin from several species (especially *Trigonia lamarckii* REEVE, *Grandidieria burtoni* WOODWARD and *Aetheria elliptica* LAMARCK), on which action of ultrasonic irradiation of high intensity has been prolonged beyond the stage of cleavage of the intact lace-like leaflets, the first step to disintegration consisted of separation of the trabeculae from each other into filaments coated with muffs of substance containing protuberances and bulging rounded bodies of various sizes.

This step was followed by liberation of naked fibrils and of the muff of coating substances in the shape of scattered granules and pebble-like bodies.

4. Spontaneous disintegration of the sheets into fibrils was recorded in shells, approximately 1.400-1.600 years old, collected in archaeological excavations. Similar alterations had been formerly observed in mother-of-pearl from eocene and from oligocene fossils (GRÉGOIRE, 1959).

The present results suggest that the patterns in the organic reticulated sheets (thickness and shape of the trabeculae, size, disposition and shape of the pores), are determined essentially by differences in size (see p. 14) and in arrangement of the polypeptidic fibrils (nacroin), which form the core of the trabeculae, and perhaps also by differences in the spatial combination between the fibrils and the substances coating these fibrils (nacro-sclerotin + nacrin), which appear, at least in part, in the shape of tuberosities.

The fibrillar core and the coating substances might constitute the actual structures involved in the taxonomical differences recorded statistically between different families in the organic reticulated sheets of mother-of-pearl (4).

(4) As described in the present and in previous studies (see GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1955; GRÉGOIRE, 1957, 1959), the trabeculae of the organic lace-like structures appear as knobby cords, studded with hemispherical protuberances of various sizes. These tuberosities give to the trabeculae a resemblance with « iris rhizomes » (see GRÉGOIRE, 1957, disc. p. 804 and Pl. 255, fig. 15; plate 2, fig. 8 of the present study).

In many preparations, the size of these protuberances is distinctly different from that of the formvar granulations which cover the background.

In the lace-like organic reticulated sheets, disclosed in replicas of etched nacreous surfaces, the tuberosities were found exclusively on the trabeculae, and were absent from the adjacent mineral surfaces (GRÉGOIRE, 1957).

As pointed out previously, these observations seem to discard the possibility that the tuberosities represent a general contamination of the structures by small particulate impurities, such as droplets of pump oil. Such oil films would cover uniformly the background and all the structures.

An additional support to this interpretation has been obtained by using a procedure decreasing the background granularity and increasing at the same time the size of the tuberosities on the trabeculae of the reticulated sheets (see description in legend of Pl. 3, fig. 15).

SUMMARY.

1. — The thin fragments of leaflets resulting from ultrasonic irradiation of the organic membranes of conchiolin freed by decalcification of mother-of-pearl by chelating agents, have been studied with the electron microscope in six species of Gastropods and in 30 species of Pelecypods. The material includes shells found in archaeological excavations and subfossil species.

2. — In all the specimens, the fragments appear in the shape of lace-like reticulated sheets, perforated by openings or pores separated by cylindrical or strand-like trabeculae.

3. — The average diameter, the frequency, and the relative surface of the pores in the reticulated sheets, have been measured.

Previous conclusions about statistically significant differences in the patterns at the class level between Gastropods and Pelecypods have been supported by the present observations.

In Pelecypods, the present results have brought evidence of significant differences between the patterns at a subclass (family) level.

4. — The successive steps of disruption of the reticulated sheets and of their trabeculae by protracted ultrasonic irradiation have been observed in several species (including *Trigonia lamarckii, Grandidieria burtoni* and *Aetheria elliptica*).

5. — The findings suggest that the trabeculae consist of a core of fibrils, coated in normal conditions by sheets or muffs of another material which appears in part in the shape of hemispherical protuberances of various sizes.

The similarity of these mechanical effects of ultrasonic irradiation with modifications induced in previous studies in the organic reticulated sheets of mother-of-pearl by biochemical fractionation is discussed.

It is assumed that differences in the size and in the arrangement of the fibrils forming the core of the trabeculae, and that variations in the spatial relations between fibrils and coating substances, might account for the taxonomical differences recorded between species with regard to the pattern of the organic lace-like reticulated sheets.

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EXPLANATION OF PLATES.

The figures are micrographs of fragments from organic reticulated sheets of decalcified mother-of-pearl (nacreous conchiolin).

These sheets, unfixed (fig. 2, 3, 5, 6, 7, 10, 11, 14, 16, 17, 18, 19, 20, 21) or fixed by immersion in a 2 % solution of osmic acid for various lengths of time (fig. 1, 4, 8, 9, 12, 13, 15) are shown at various stages of their disintegration by ultrasonic irradiation (duration of irradiation in brackets).

Drops from aqueous suspensions of fragments of these sheets were deposited on to copper mesh screens, previously coated with films of formvar (fig. 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21) or with carbon films (fig. 1, 4, 8, 15), and shadowcast with palladium, at angles varying from 11 to 22°.

All the pictures are negative prints.

PLATE 1:

Fig. 1. — *Angaria delphinus* LINNÉ (*laciniata* LAMARCK) (*GASTROPODA*, ANGARIIDAE).

(297 D-28-10-59-3 B) (6 min). Round pores of large size, evenly scattered in the sheet. Short ribbon-like or cylindrical trabeculae, sprinkled with hemispherical protuberances. Embedded fibrils are visible in several trabeculae. (× 40,000).

Fig. 2. — *Angaria delphinus* LINNÉ.

(297 D-2-2-59-1 B) (3 min). Numerous protuberances of various sizes scattered on the trabeculae. Thin fibrils are visible in the reticulum (bottom centre) (× 40,000).

Fig. 3. — *Mytilus crenatus* (*PELECYPODA*, MYTILIDAE).

(412-3-7-59-1 A) (17 min). Condensed reticulum, appearing as a granular membrane. Thinly fibrillar trabeculae, studded with protuberances of various sizes. A fibril, coated with granular material, is shown protruding at the edge of the fragment (bottom right). (× 40,000).

Fig. 4. — *Brachyodontes bilocularis* LINNÉ (*PELECYPODA*, MYTILIDAE).

(331-28-10-59-8 D) (19 min). Condensed reticulum. Thin trabeculae consist chiefly of fibrils with irregular shapes, and are sprinkled with blisters or tuberosities of various sizes (× 40,000).

PLATE 2:

Fig. 5. — *Pinna nobilis* (*PELECYPODA*, PINNIDAE).

(278-25-6-59-2 C) (33 min). Reticulated sheets with a condensed texture. Small round pores of uniform size(in black on the micrograph), are sparsely distributed in the sheets. The trabeculae consist of thin fibrils, coated with hemispherical elevations of various sizes. Fibrils sprinkled with rows of small granules appear disposed in parallel arrays (× 40,000).

Fig. 6. — *Pinna nigra* CHEMNITZ (*PELECYPODA*, PINNIDAE).

(277-9-7-59-4 E) (15 min). Fragment of a reticulated sheet appearing as a fibrillar network perforated by holes (black round spots). Small tuberosities, mostly hemispherical in shape, are scattered on the fibrillar trabeculae (× 40,000).

Fig. 7. — *Trigonia lamarckii* REEVE (*PELECYPODA*, TRIGONIIDAE).

(294 B-4-2-58-5 A) (7 min.). Sheet probably stretched, showing some degree of dislocation of the reticulum and incomplete separation of the trabeculae by rupture of lateral bridges of substance. The trabeculae are coated with a muff of tuberosities of various sizes (× 40,000).

Fig. 8. — *Trigonia lamarckii* REEVE (*PELECYPODA*, TRIGONIIDAE).

(294-26-10-59 A) (20 min). Trabeculae, sprinkled with tuberosities of various sizes (iris rhizome aspect), separate round pores (black spots) (× 40,000).

Fig. 9. — *Grandidieria burtoni* WOODWARD (*PELECYPODA*, UNIONIDAE).

(287-23-10-59-5 B). Protracted ultrasonic irradiation (72 min. instead of 40 to 60 sec.). Dislocation of the organic reticulated sheets into smaller fragments of various sizes and into fibrils, either singly or disposed in bundles. Fragments of material coating the fibrils (tuberosities) are scattered at random on the background (× 28,000).

PLATE 3:

Fig. 10 and fig. 11. — *Hyriopsis schlegeli* von MARTENS (*PELECYPODA*, UNIONIDAE).

(329-22-9-59-1 D). Protracted ultrasonic irradiation (30 min.). Organic material from a decalcified pearl. Dislocation of the perforated reticulated sheets into naked fibrils and into small globular fragments scattered on the background. The latter structures are tuberosities detached from the fibrillar core of the trabeculae. Bundles of fibrils appear especially on fig. 10 (top left) (× 24,000).

Fig. 12. — *Grandidieria burtoni* WOODWARD (*PELECYPODA*, UNIONIDAE).

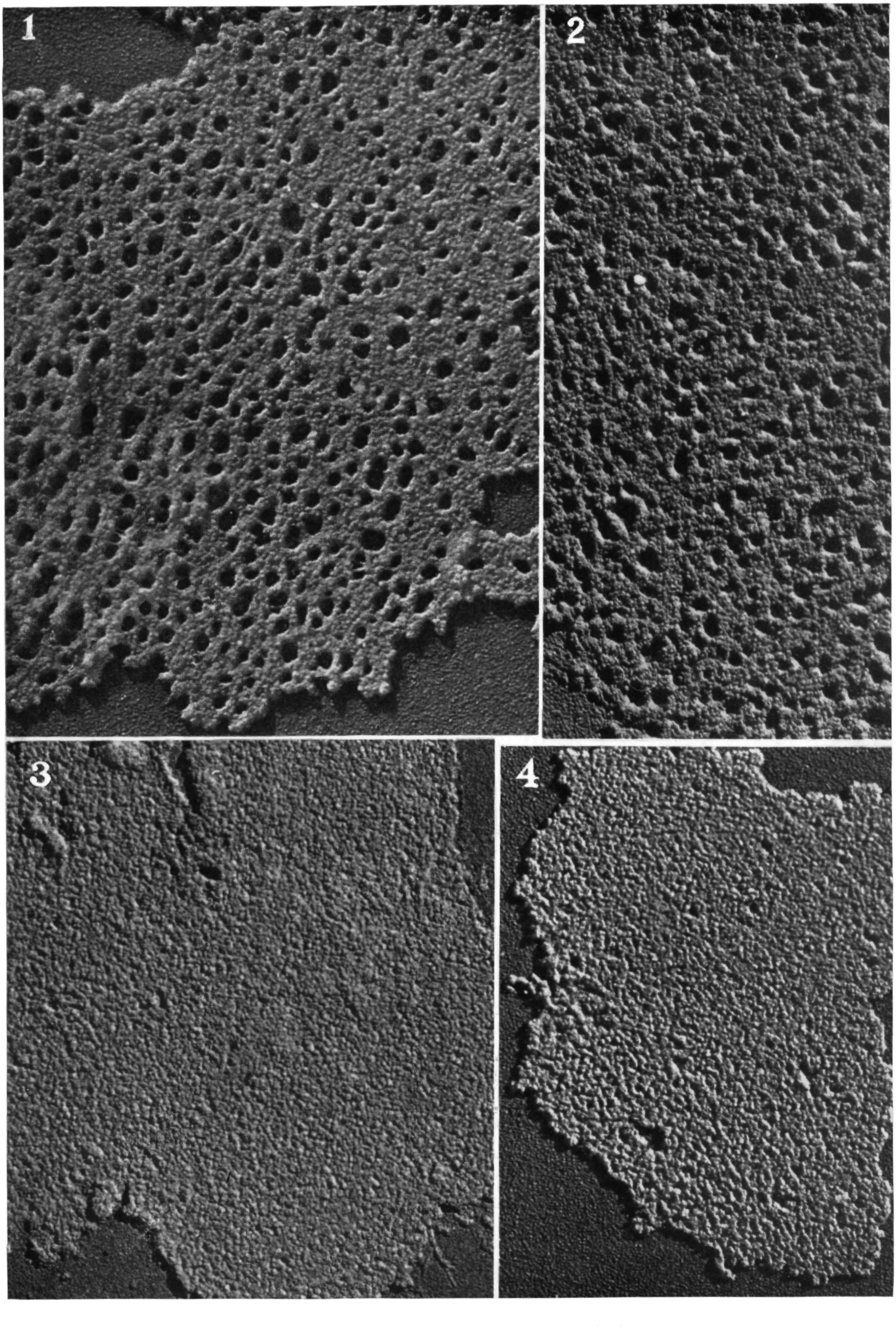
(287-4-11-59-5 E) (1 min.). In this material, fixed with osmic acid, disintegration of the leaflets took place rapidly. The picture shows fragments of still intact reticulated sheets perforated by holes, together with the first stage of the dislocation of these sheets, consisting of isolation of the trabeculae into parallel granular strands by disruption of lateral connections. These strands are made of a core of fibrils, coated with a muff which includes the tuberosities visible on the trabeculae of the intact sheets. Protracted ultrasonic irradiation disintegrates these strands into naked fibrils and a granular pebble-like material (see fig. 9, 10, 11, 13 and 18) (× 24,000).

Fig. 13. — *Grandidieria burtoni* WOODWARD (*PELECYPODA*, UNIONIDAE).

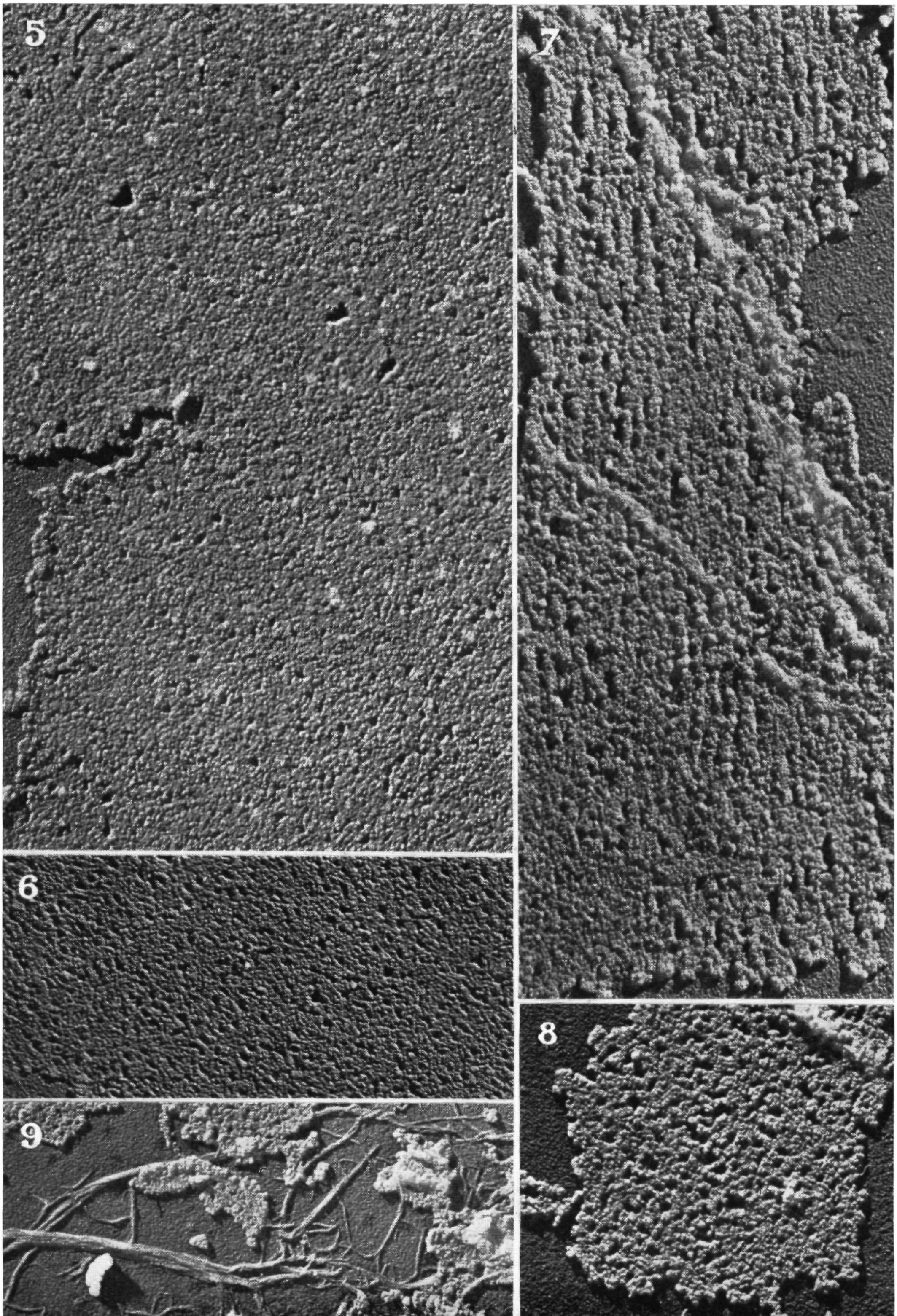
(287-23-10-59-5 A). Protracted ultrasonic irradiation. (72 min.). See legend of fig. 9. Numerous naked fibrils appear mixed with fragments of reticulated sheets and small aggregates of granular material. This material corresponds to the tuberosities which coat the fibrils in intact reticulated sheets (× 28,000).

Fig. 14. — *Caelatura (Caelatura) bakeri* H. AD. (*PELECYPODA*, UNIONIDAE).

(416-6-7-59-4 C) (12 min.). Fragment of a reticulated sheet perforated by round pores. Strand-like, somewhat flattened, trabeculae, on which numerous small granular tuberosities are scattered. Fibrils do not appear clearly in the trabeculae of this preparation, but their presence is occasionally revealed by orientation in rows of the tuberosities scattered upon these fibrils (× 40,000).



Ch. GREGOIRE. — Further Studies on structure of the organic components in Mother-of-Pearl, especially in Pelecypods (Part I).



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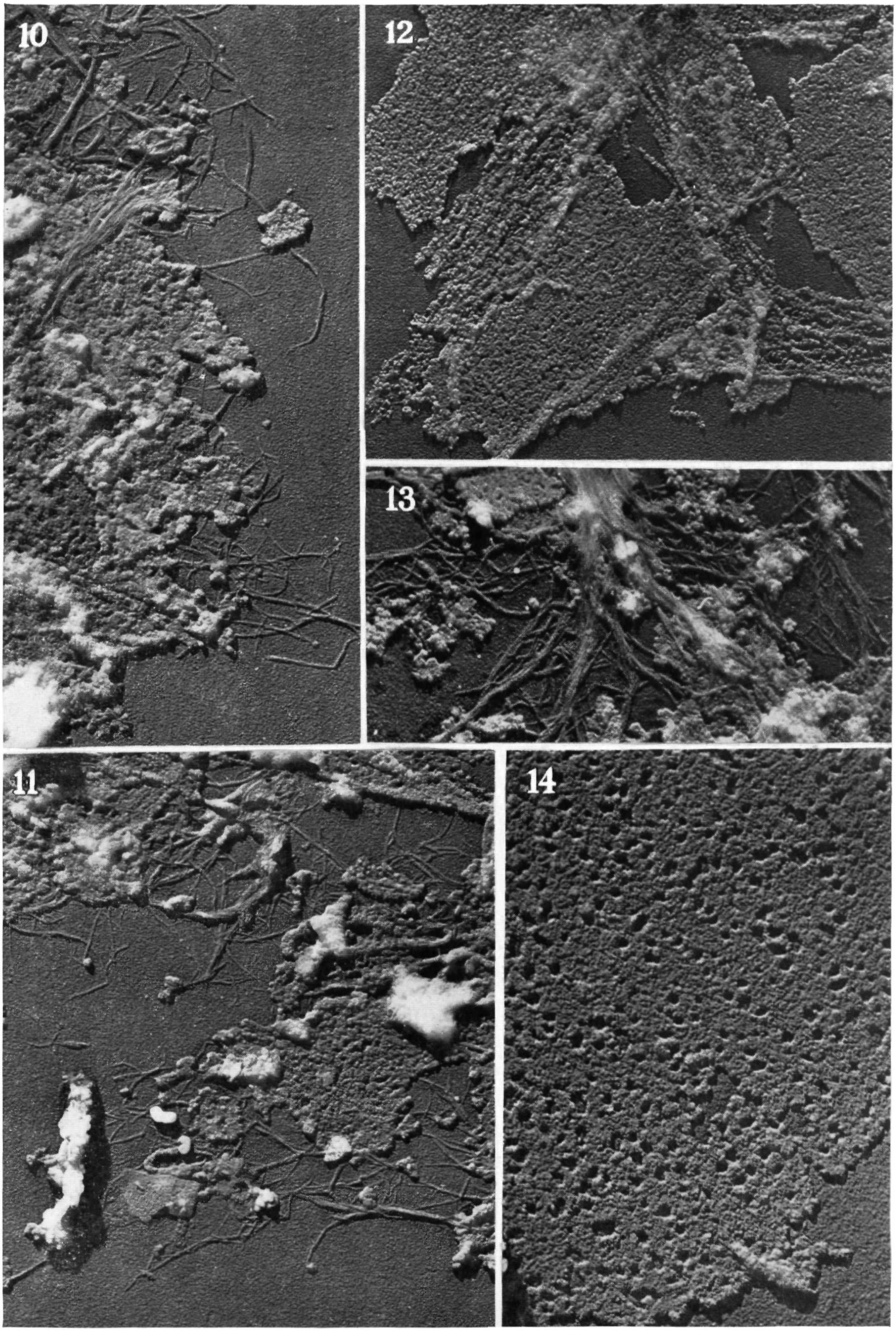

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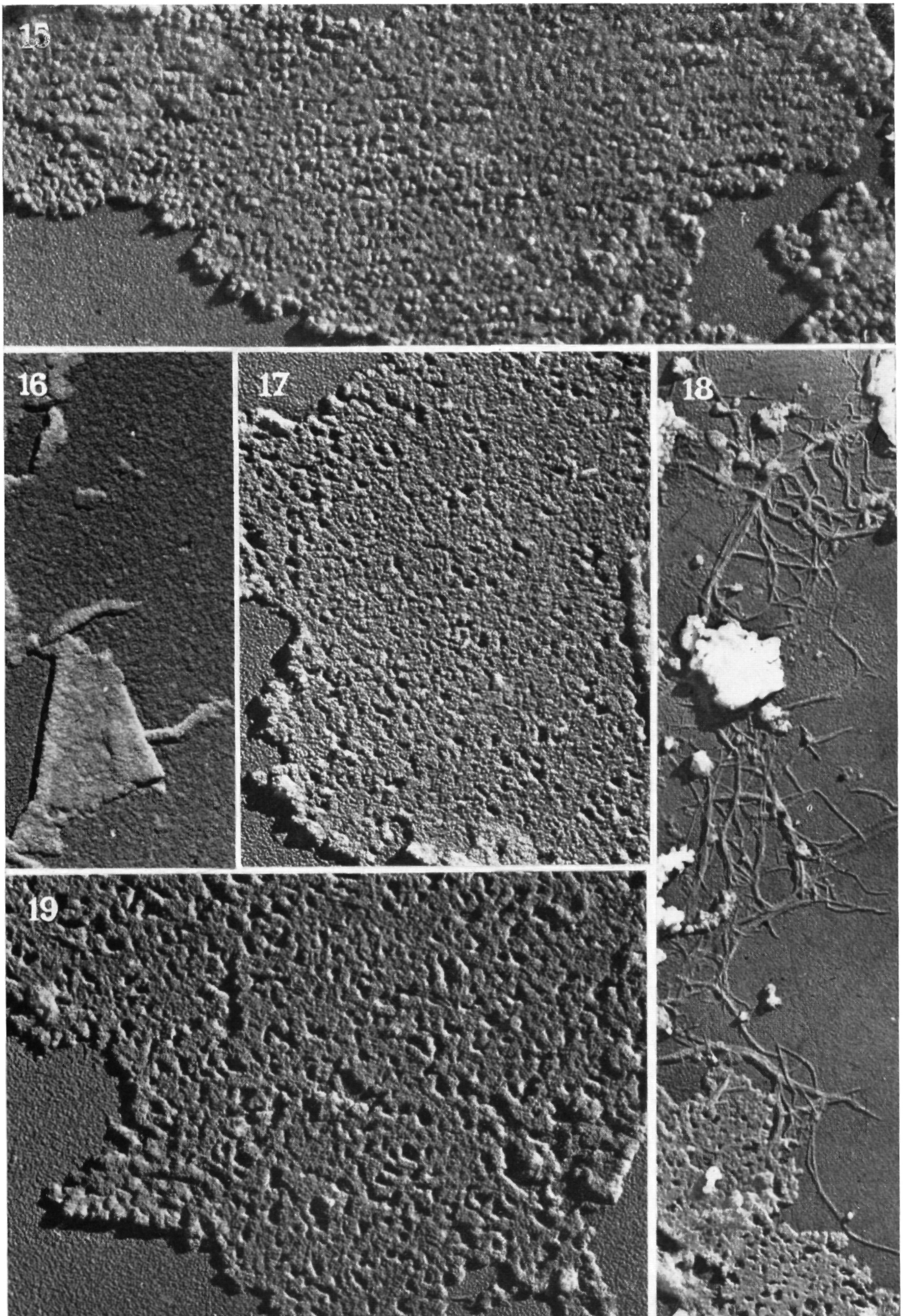

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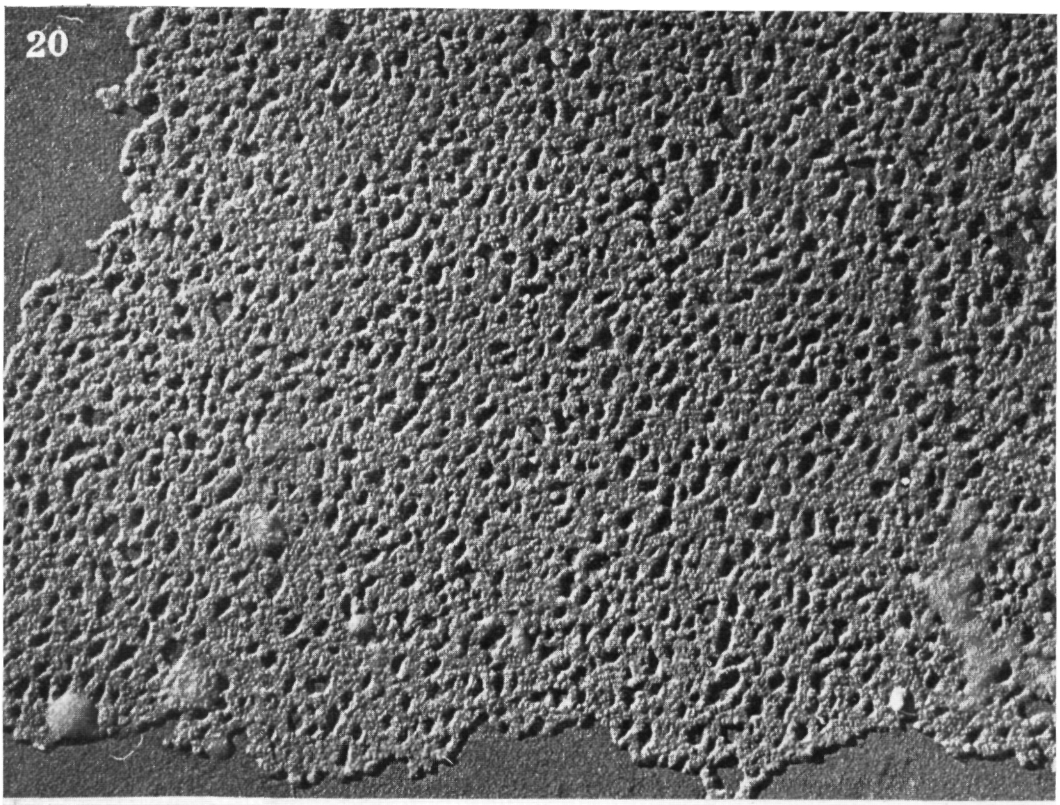

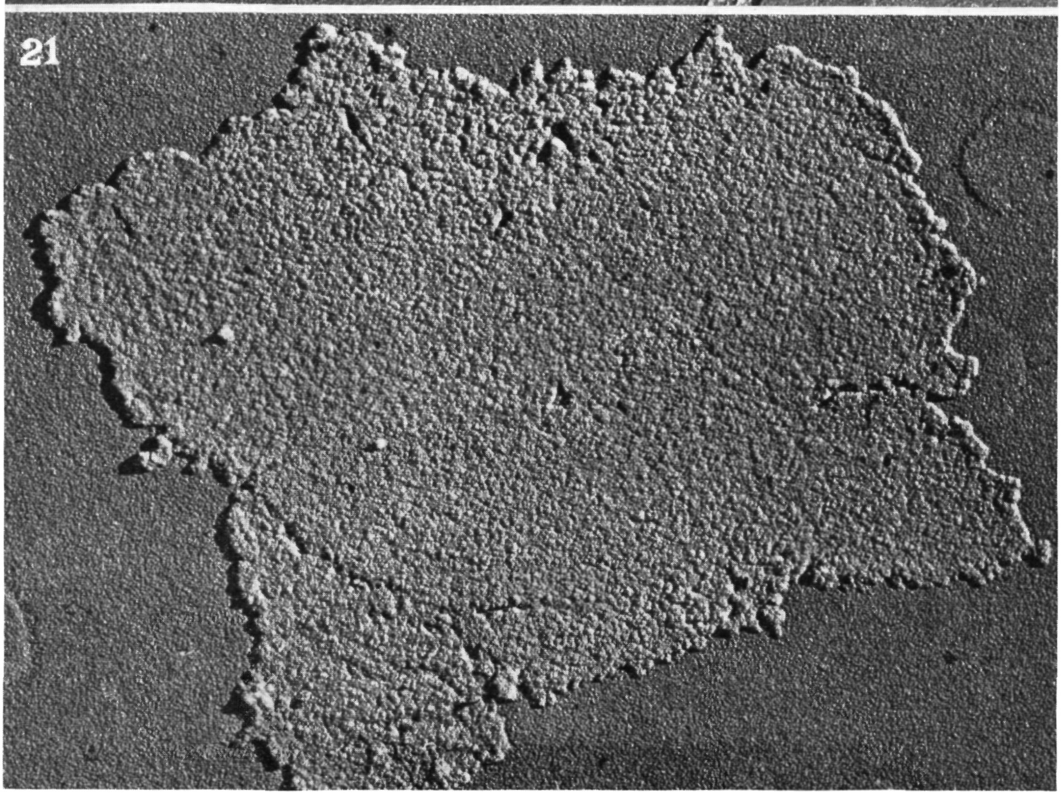

Ch. GREGOIRE. — Further Studies on structure of the organic components in Mother-of-Pearl, especially in Pelecypods (Part I).

Plate 4 :

Fig. 15. — *Grandidieria burtoni* Woodward (*PELECYPODA*, UNIONIDAE).

(287-2-11-59-4 A). Protracted ultrasonic irradiation (72 min.). In this fragment of an altered, but not yet disintegrated reticulated sheet, rows of rounded, pebble-like tuberosities appear disposed in parallel arrays. In the other fields of the preparation (not shown) dislocation of the sheets took place as illustrated in fig. 9 and 13.

The preparation of the material shown in this picture differed from the standard procedure : suspensions of fragments of sheets were deposited on to formvar-coated screens and dried, as in the standard procedure. A carbon film was then evaporated normally to the screens. The carbon-coated screens were subsequently shadow-cast with palladium at a low angle (12-13°). The formvar support underlying the preparation was then dissolved by immersing the screens in ethylene dichloride.

The reticulated sheet shown in fig. 15 is actually hanging below the double metallic coating (carbon and palladium films superimposed), in contrast with all the other sheets illustrated in the plates, which are actually lying upon the formvar or carbon films.

Use of this method resulted in a decrease in the granularity of the background (compare fig. 15 with fig. 17, formvar background, same magnification) and in an increase in the size of the tuberosities, induced by the double metallic coating of the structures. The swollen appearance of the tuberosities in this picture is therefore an artifact of preparation. However, the procedure gives evidence that the tuberosities, scattered on the trabeculae of the reticulated sheets and observed in most preparations examined so far, are not artifacts, such as overlying layers of small particulate impurities (f.i. droplets of pump oil), but belong to the actual structure of the material (× 40,000).

Fig. 16. — *Pandora cuningii* Hanley (*PELECYPODA*, ANATINIDAE).

(380-28-5-59-3 A) (20 min.). Fragment of an organic sheet in the shape of a thinly granular membrane, with folds (× 40,000).

Fig. 17. — *Obovaria retusa* Lamarck (*PELECYPODA*, UNIONIDAE).

(384-26-5-59-2 C) (16 min.). Fragment of a reticulated sheet perforated by scattered round pores. Fibrils are visible in the trabeculae, which are studded with mostly thin granular tuberosities (× 40,000).

Fig. 18. — *Aetheria elliptica* Lamarck (*PELECYPODA*, AETHERIIDAE).

(280 B/2-1-7-57-2 C) (22 min.). Small fragments of lace-like reticulated sheets perforated by holes of irregular shape. Naked fibrils resulting from dislocation of the sheets and scattered debris of tuberosities are shown. In the lower part of the picture, the connection between fibrils, still partly coated with a granular material (tuberosities) and the still undissociated perforated leaflet, is visible (× 24,000).

Fig. 19. — *Aetheria elliptica* (*PELECYPODA*, AETHERIIDAE).

(280 A-11-3-58-2 A) (20 min.). Fragment of a reticulated sheet perforated by pores of various sizes and shapes. In this preparation, fibrils appear only incidentally (centre right) in the trabeculae, on which thin hemispherical tuberosities are scattered (× 36,000).

Plate 5 :

Fig. 20. — *Mutela langi* Pilsbry and Bequaert (*PELECYPODA*, MUTELIDAE).

(415-7-7-59-3 E (7 min.). Representative picture of the lace-like pattern in *Mutelidae* : round pores of relatively large size, of rather uniform shape, are evenly scattered in the sheets. Short thick trabeculae, in which parallel fibrils appear incidentally, are sprinkled with hemispherical protuberances. Except for the smaller size of the pores, their sparser and less even distribution in the sheets, the texture of these sheets in *Mutelidae* resembles the Gastropod pattern (compare fig. 20 with fig. 1 and 2, same magnification) (× 40.000).

Fig. 21. — *Pandora trilineata* SAY (*PELECYPODA,* ANATINIDAE).

(381-28-5-59-5 A) (27 min.). Fragment of a reticulated sheet appearing as a thinly granular membrane. Pores absent or scattered in small groups. Condensed texture in which thin fibrillar trabeculae are revealed by rows of hemispherical protuberances, which coat the fibrils and are visible in various parts of the fragment (× 40,000).

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