Biochemical Studies on Pearl. VIII. Occurence of Calcite, Aragonite and Dolomite in Pearl and Shell

By Shozo TANAKA, Hiroyuki HATANO* and Osamu ITASAKA**

(Received July 20, 1959)

Among many kinds of bivalved molluscs which are available in the pearl culture industry, *Pinctada martensii* (Dünker), the Japanese pearl oyster, is one of the most important species in Japan. In the previous papers of biochemical studies on pearl, physiological properties of the pearl oyster were reported to have a marked resemblance to the ordinary oyster[1,2] and the chemical mechanism of pearl formation was discussed from a view-point of comparative biochemisrty upon the calcification of invertebrata and vertebrata[3-5].

Several analytical results on the pearl and the shell materials of the pearl oyster have been reported[1-10], but few observations have been made on the nature of chemical constituents in the pearl and the shell.

In the present work, investigations have been made on the chemical compositions of the nacre of pearl, the nacreous portion of the shell or "mother-of-pearl", which forms an innermost layer of the shell, and the prismatic substance of an outer layer of the shell (Fig. 1). Furthermore, crystalline forms of calcium carbonate in the pearl and the shell were determined by the X-ray diffraction method. Also, a minute amount of magnesium was found in the shell and occurrence of dolomite was ascertained by a chemical method.

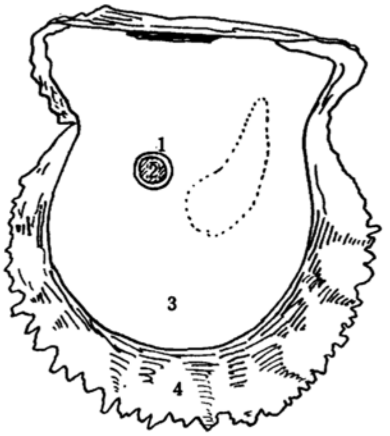

Fig. 1. A schematic diagram of the pearl and the shell of the pearl oyster.
1) The nacre of the pearl; 2) The nuclei of the pearl; 3) The nacreous layer of the shell; 4) The prismatic layer of the shell.

Experimental

Materials.—Cultured pearls used in the following experiments were collected from three-year old Japanese pearl oysters, while shell samples were obtained from pearl oysters of two winters***.

The cultured pearls were separated into nacres and nuclei mechanically, the latter being put aside. By scraping off the outer layers with a grinding-wheel, a nacreous substance of the inner layers of shells was prepared. A prismatic substance of the outer layers was obtained mechanically from thin posterior shell margins. These materials were first crushed into pieces by means of a porcelain mortar and, then, pulverized after drying at room temperature for a few days.

Calcium carbonate crystals for the X-ray diffraction analysis were prepared from the pearls and the shell substances by immersing the pulverized materials in one per cent sodium hypochlorite solutions for a few hours to remove organic substances.

Methods.—The chemical compositions of the

---

* A part of this study was carried out by the author, at Kobe University, Kobe.

** Present address: School of Chemistry, Shiga University, Otsu.

1) S. Tanaka and H. Hatano, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **73**, 870 (1952).

2) S. Tanaka and H. Hatano, ibid., **74**, 74 (1953).

3) S. Tanaka and H. Hatano, *Publ. Seto Mar. Biol. Lab. Kyoto Univ.*, **II** (2), 341 (1952).

4) S. Tanaka and H. Hatano, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **76**, 602 (1955).

5) S. Tanaka and H. Hatano, *Proc. 1st Symposium on Isotopes. Japan*, **B-1**, 307 (1956).

6) U. Okuda and K. Takeuchi, *J. Chem. Soc. Japan* (*Nippon Kwagaku Kwaishi*), **38**, 819, (1919).

7) J. Tsutsumi, *Mem. Coll. Sci. Kyoto Univ., Ser. A.* **11**, 217, 401 (1928); **12**, 199 (1929).

8) J. Ogushi, "Studies on Pearl (Shinju no Kenkyu)", Ito Bunshindo, Tokyo (1938), p. 143.

9) K. Wada, *Bull. Natl. Pearl Res. Lab. Japan*, **1**, 1, 7 (1956); **2**, 74 (1957).

10) Y. Sawada, ibid., **2**, 68 (1957).

*** The authors wish to express their hearty thanks to Mr. K. Isowa, the president of the Nippon Pearl Co., at Matoya Bay near Kashikojima in Mie-ken for his kind presentation of these materials.

pearl and the shell materials were determined by the methods reported in the previous papers[1,2]; moisture was determined by measuring the decrease in weight on drying at 105°C for about 24 hr. until the weight came to a definite constant; total nitrogen was determined by a semi-micro-Kjeldahl's technique; total ashes were determined by igniting any sample to a constant weight at approximately 700°C and making a correction for the ignition loss of carbonate. Inorganic constituents in the ashes were determined as follows: phosphate as ammonium phosphomolybdate, calcium as calcium oxalate, sulfate as barium sulfate, and magnesium as ammonium magnesium phosphate after being separated from calcium, respectively; the carbonate content was calculated from the amount of carbon dioxide determined by the Conway's microdiffusion method[11].

Conchiolin, a screloprotein contained in the pearl and the shell, was estimated from the amount of residue remaining after the inorganic constituents were dissolved in dilute hydrochloric acid[12].

X-ray diffraction patterns of crystalline calcium carbonate in the pearl and the shell were recorded by the Norelco's X-ray diffractometer and were compared with those of the standard crystals of calcite and aragonite.

The state of chemical combination of a minute amount of magnesium which was found in the pearl as well as the shell was determined by the chemical method established by Kitano[13]. The principle of his method is as follows. When a small amount of magnesium is contained in calcareous shells, it is expected to be present in one of the only two possible forms, i. e. dolomite $CaMg(CO_3)_2$ and a solid solution of calcite and dolomite. Now, when carbon dioxide gas is passed into mixed suspensions of calcium

TABLE I. AMOUNTS OF CALCIUM AND MAGNESIUM DISSOLVED INTO SUSPENSIONS WHICH CONTAIN MAGNESIUM COMPOUNDS, UNDER THE CONDITION OF BEING SATURATED WITH CARBON DIOXIDE[a]

Suspension					Dissolved quantity		Ratio[e]	
$CaCO_3$[b] g.	Shell[c] g.	$CaMg(CO_3)_2$[d] g.	Ca mg.	Mg mg.	Ca mg./200 ml.	Mg	Ca %	Mg %
*1.61	—	0.89	841	117	91	1.0	10.9	0.9
*1.97	—	0.53	904	70	99	0.8	10.9	1.1
*2.06	—	0.44	920	58	91	0.5	9.9	0.8
*2.33	—	0.17	967	23	90	0.3	9.3	1.4
*2.39	—	0.11	980	14	100	0.2	10.2	1.1
**2.5	0.0	—	838	101	76	6.8	9.0	6.6
**2.0	0.5	—	870	81	71	5.7	8.2	7.0
**1.0	1.5	—	935	41	68	2.6	7.3	6.3
**0.5	2.0	—	968	20	67	2.3	6.9	12
**0.2	2.3	—	987	8.1	68	2.3	6.9	29

a) Into a suspension containing 2.5 g. of magnesium preparation in 200 ml. of distilled water, purified carbon dioxide was passed for 25 min. After being allowed to stand for 10 min., the suspension was filtered through a sheet of Toyo Roshi No. 6 filter paper. Total quantities of calcium and magnesium ions in the filtrate were determined by the volumetric method using EDTA, while the amount of calcium ion was estimated as calcium oxalate, and then the amount of magnesium ion was calculated from these two values, or was determined directly by the volumetric method using oxim reagents.

b) Extra pure reagents of calcite and aragonite prepared by Y. Kitano were used in this experiment.

c) As a standard preparation of a solid solution of calcite and aragonite, were used shells of four kinds of sea urchins, in which the magnesium had been found to be present in the form of a solid solution by X-ray diffraction techniques.

d) A dolomite preparation found at Kuzuo, Tochigi-ken, was used. The analytical results are as follows: CaO, 33.80; MgO, 19.37; clay, 0.22%.

e) [g. of $Ca^{2+}(Mg^{2+})$ dissolved into 200 ml. of a suspension]×100/[g. of $Ca^{2+}(Mg^{2+})$ in 2.5 g. of a suspension].

* See Ref. 13.

** Y. Kitano, unpublished data (1958).

---

[11] E.J. Conway, "Microdiffusion Analysis and Volumetric Error", Crosby Lockwood & Son, Ltd., London (1950), p. 87; E. J. Conway and A. Byrne, *Biochem. J.*, **27**, 419 (1933).

[12] S. Tanaka et al., *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **74**, 193 (1953).

[13] Y. Kitano, ibid., **77**, 211 (1956).

carbonate with dolomite or into those of calcium carbonate with a solid solution of calcite and dolomite, the quantities of magnesium dissolved into the suspensions are not the same for two cases. The data obtained by Kitano[13] are given in Table I. On the basis of these results, therefore, the state of chemical combination of magnesium present in calcareous shells can be determined by measuring the quantity of magnesium dissolved in water saturated with carbon dioxide.

Results and Discussion

Chemical Composition. — The chemical compositions of the pearl and the shell of the pearl oyster are shown in Table II, which indicates that the pearl as well as the shell contain about ninety per cent of calcium carbonate and a few per cent of conchiolin. It seems to be noticeably characteristic that, contrary to various calcareous tissues of vertebrata, there are little or only minute amounts of phosphate and sulfate in the pearl and the shell of the pearl oyster. Less content of conchiolin and a larger amount of calcium carbonate in the nacre than in the prismatic substance are observed. Scant amounts of pigments are present in the pearl and the nacreous oyster, while plenty of coloring substances can be found in prismatic substances[14]. Compared with the case of the nacre, relatively large amounts of magnesium are contained in the prismatic layer of the shell.

TABLE II. THE CHEMICAL COMPOSITIONS OF THE NACRE OF THE PEARL AND OF THE SHELL VALVES OF THE JAPANESE PEARL OYSTER, *Pinctada martensii* (DÜNKER)*

	Cultured	Shell	
	pearl nacre %	Nacreous layer %	Prismatic layer %
Total nitrogen	0.43	0.53	1.01
Conchiolin	2.72	2.83	9.61
Pigments	±	±	+++
Reducing substance	0.00	0.00	0.02
Total ashes	80.60	89.60	76.00
Calcium	27.71	29.35	27.47
Magnesium	0.12	0.12	0.59
Carbonate	56.95	58.19	56.41
Phosphate	0.01	0.06	0.06
Sulfate	0.66	0.59	1.09

* Free from water content.

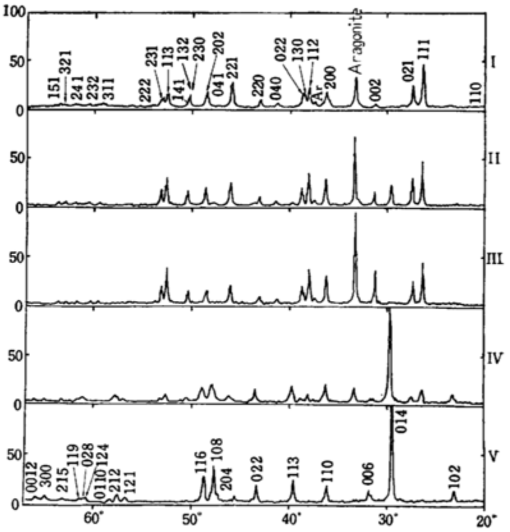

Fig. 2. X-ray diffraction patterns of calcium carbonate crystals in the nacre of pearl and in the shell material of the pearl oyster, *Pinctada martensii*. Pattern I: standard aragonite crystals; II: crystals in the nacre of the pearl; III: crystals in the nacreous layer of the shell; IV: crystals in the prismatic material; V: standard calcite crystal.

The curves shown in Fig. 2 indicate the X-ray diffraction patterns, recorded by Norelco's apparatus****, of crystalline preparations of calcium carbonate from the pearl and shell materials. The crystals of calcium carbonate existing in the nacre of pearl and in the nacreous layer of shell were identified as aragonite crystals of a rhombohedral lattice, and those in the prismatic layer as calcite crystals of a rhombic lattice.

Calcite is known to form the most stable lattice of calcium carbonate while aragonite is less stable under usual crystallographical conditions. For aragonite crystal to be produced, therefore, certain special conditions should be given such as higher temperature, higher pH value of surrounding media, or a larger growing rate of crystals due to sudden supply of materials[15,16]. In connection with these requirements, it may be considered that the presence of relatively large amounts of magnesium ion is favorable to the formation of aragonite crystals.

Magnesium preparations prepared from the pearl and the shell were treated by the same procedure and the results obtained

---

[14] Y. Takagi and S. Tanaka, ibid., **76**, 406 (1955).

**** The authors wish to express their thanks to the operating committee of this apparatus in Osaka University, for these measurements.

[15] Y. Kitano and M. Nishimura, ibid., **76**, 581 (1955).

[16] Y. Kitano, *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **59**, 1346 (1956).

TABLE III. RESULTS SHOWING THE OCCURRENCE OF DOLOMITE IN THE PEARL AND THE SHELL MATERIALS OF THE PEARL OYSTER*

Preparation	Suspension			Dissolved quantity		Ratio	
	Sample mg.	Ca mg.	Mg mg.	Ca mg./200 ml.	Mg mg./200 ml.	Ca %	Mg %
Pearl, nacre	952.2	240.8	1.14	20.0	0.01	8.3	0.9
Shell, nacreous substance	954.6	287.0	1.14	16.3	0.0	5.7	0
Shell, prismatic substance	973.8	267.5	5.84	14.3	1.72	5.3	29.3

* The procedure of this analysis is quite identical with that in Table I.

are presented in Table III. Comparing these results with the data in Table I, it has been determined that magnesium in the nacre of pearls and in the nacreous layer of shells exists as dolomite and that in the prismatic layer as a solid solution of calcite and dolomite.

Several experiments have been carried out on the formation of calcareous skeletons in marine organisms and it is demonstrated that the temperature is one of the most effective factors on differentiation of the composition of calciferous skeleton in some marine invertebrates[17,18]. Any other possible factors may be expected to alter the modification and the chemical composition of a crystal.

According to the experimental data in Table III, the fact that magnesium carbonate is difficult to crystallize in a rhombic lattice owing to a geometrical correlation of constituent atoms, seems to have some relations with the mode of crystallization of calcium carbonate in the pearl oyster.

Summary

The chemical compositions of the pearl and the shell of the Japanese pearl oyster were investigated from a crystallographical and biochemical point of view.

By the X-ray diffraction analysis, crystals of calcium carbonate in the nacre of pearl and in the nacreous layer of shell were determined to be aragonite, while those in the prismatic layer were shown to be calcite.

The state of chemical combination of minute amounts of magnesium present in the pearl and the shell materials was examined by a chemical method. In the nacreous substances, magnesium is present as dolomite, while in the prismatic material it exists as a solid solution of calcite and dolomite.

The state of crystals of calcium carbonate and magnesium carbonate occurring in the pearl oyster has been discussed with respect to the formation of the pearl and the shell of the pearl oyster.

The authors wish to express their gratitude to Dr. Y. Kitano, Nagoya University, Nagoya, for his kind permission to quote his unpublished data in Table I and also for his advice which was given throughout the course of this research.

Department of Chemistry
Faculty of Science
Kyoto University
Sakyo-ku, Kyoto

---

17) H. A. Lowenstam, *J. Geol.*, **62**, 284 (1954).
18) K. E. Chave, ibid., **62**, 266, 587 (1954).