Studies on the Production of Black Pearls by Irradiation with Radioactive-rays—III. Relationship between the Coloration Obtained by γ -Ray Irradiation and the Manganese Contents in the Shells of Several Shellfishes*

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As a cause for the coloration of pearls by irradiation with radioactive-rays, the existence of trace elements in them, especially manganese, has already been pointed out by the author¹⁾. Also, in the previous work in this series,^{2,3)} it was demonstrated that the manganese content in the pearl layer in pearl oysters could be increased by the administration of manganese compounds and that the pearl layer of marine pearls containing a high level of manganese content is colored by means of γ -ray irradiation. However, information on shell-coloration by irradiation with radioactive-rays was obtained only a few instances in two species of fresh-water shellfishes.

Further study on several fresh-water and marine shells was carried out by comparing their manganese contents and the degrees of coloration obtained by γ -ray irradiation. The data obtained showed that the assumption offered by the authors²⁾ may be applied universally in all 9 species of fresh-water shells used in this experiment.

Materials and Methods

Materials. Shells of fresh-water shellfishes: "Nigger head",*** "Pig toe",*** *Quadrula obliqua* (LAMARCK), and "Dove shell",*** *Quadrula undulata* (BAROES), from North America; "Kankoonbiki"**** and "Kyukohiragai"**** from China; fresh-water mussel, *Hyriopsis schlegelii* (V. MARTENS), "Otokotateboshi",**** *Inversidens renuana*, "Setaishigai",**** *Inversidens hirasei*, and "Setashijimi",**** *Corbicula sandai* REINHARDT, from Lake Biwa, were prepared, respectively.

Shells of marine shellfishes: Pearl oyster, *Pteria (Pinctada) martensii* (DUNKER), from Matoya Bay, Mie Prefecture and black-lip shell, *Pteria (Pinctada) margaritifera* (LINNE), from Kagoshima Prefecture.

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*** Local name in North America; unfortunately the scientific name of "Nigger head" is obscure.

**** Japanese name.

Shell of brackish-water shellfish: "Yamatoshijimi", *Corbicula japonica* PRIME, from Mie Prefecture.

Analytical methods. Total nitrogen of the shell, after the periostracum was eliminated, was measured by the micro Kjeldahl method⁴⁾. Manganese²⁾, free iodine released in the KI-HCl-shell system¹⁾, and coloration³⁾ were measured by the methods described in the previous papers.

Results and Discussion

Measurement of the total nitrogen and manganese contents in the shells, and of free iodine released in the KI-HCl-shell system. The quantitative results of these elements obtained from the shells of 12 species of shellfishes are shown in Table 1.

Table 1. Quantitative comparison between the manganese and free iodine released in the KI-HCl-shell system in the shells of several fresh-water and marine shellfishes irradiated with γ -rays.

Shells	Nitrogen (%)	Manganese (γ per g)	Free iodine released in the KI-HCl-shell system	
			After 20 days from irradiation** (γ per g)	After 16 months from irradiation (γ per g)
(Setashijimi) <i>Corbicula sandai</i> REINHARDT	0.040	9.0, 29.0	11	15
Nigger head* (Kyukohiragai)	0.195	109.2	214	
(Otokotateboshi) <i>Inversidens renuana</i>	0.243	182.5	240	
(Setaishigai) <i>Inversidens hirasei</i>	0.236	199.6	472	448, 460
(Setaishigai) <i>Inversidens hirasei</i>	0.209	202.9	375	360
Pig toe* <i>Quadrula obliqua</i> (LAMARCK)	0.098	216.1	251, 283	223
Dove shell* <i>Quadrula undulata</i> (BAROES)	0.074	230.0	255	241
Fresh-water mussel <i>Hyriopsis schlegelii</i> (V. MARTENS)	0.243	235.6	364	359, 381
(Kankoonbiki) (Yamatoshijimi) <i>Corbicula japonica</i> PRIME	0.147	420.5	385	385
(Yamatoshijimi) <i>Corbicula japonica</i> PRIME	0.057	12.5	0	0
Pearl oyster <i>Pteria (Pinctada) martensii</i> (DUNKER)	0.406	15.8	0	0
Black-lip shell <i>Pteria (Pinctada) margaritifera</i> (LINNÉ)	0.304	31.7	35, 61	40, 56

* Local name in North America. Names in parentheses are Japanese.

** Shells are irradiated with a 2×10^7 r dose of γ -rays.

Non-irradiated shells did not release iodine in the KI-HCl-shell system.

Prior to the analysis, the periostracum containing more organic matter and trace elements than the pearl layer was removed from the shells to be studied.

As shown in Table 1, the manganese contents in the shells of marine shellfishes are only about 10 to 30 γ per g and those in the shells of fresh-water shellfishes, except the shell of *Corbicula sandai* REINHARDT containing 9 to 27 γ , are about 100 to 420 γ .

Some information on the manganese contents in the shells of marine and fresh-water shellfishes suggests that the different manganese contents in the shells results from the difference in the environmental water in which they live, but the shell of *Corbicula sandai* REINHARDT has an exceptionally low manganese content compared with the other 8 species of fresh-water shells. According to ŌYAMA⁵⁾, *Corbicula sandai* REINHARDT originally belonged to the marine mollusks, but recently migrated to fresh-water estuaries. Therefore its manganese contents are the same as marine mollusks.

The amounts of free iodine released in the KI-HCl-shell system were measured. If the coloration of shells caused by the irradiation with radioactive-rays is due to the formation of colored material which was produced by oxidizing the manganese compounds containing in it, because of the chemical property whereby some manganese oxides release iodine from potassium iodide in a weakly acidic solution, then colored shells may be expected to show the action of iodine release.

The amounts of released iodine in the fresh-water shells having a high level of manganese content (about 100 to 420 γ per g) are about 200 to 472 γ per g. The amount from the shell of *Corbicula sandai* REINHARDT with a low level of manganese (9 to 27 γ) is only about 11 γ , despite the fact that all are shellfishes native to Lake Biwa whose water seems to be having a uniform level of manganese.

This result, despite a small quantity of conflicting evidence, practically supports the assumption described above. However, it is desirable to consider not only the manganese contents but also other substance, such as conchiolin, whose denaturated damages by irradiation was noted by HATANO *et al.*⁶⁾

Table 2. Relationship between the dose of γ -rays for the shell of "Nigger head" and the yields of free iodine released in the KI-HCl-shell system.

Shell	Dose of γ -rays (r)	Amount of iodine released in the KI-HCl-shell system (γ per g)
Nigger head	2.5×10^5	7
"	2.0×10^6	61
"	5.0×10^6	172
"	2.0×10^7	214

In the shells of "Nigger heads", the amounts of released iodine are increased in proportional to the dose of γ -rays within certain limits.

Measurement of the degrees of the coloration of shells irradiated with γ -rays. The shells described in the preceding item were irradiated by ^{60}Co at the dose of 2×10^7 r, and the degrees of coloration were compared by their reflectances measured by the automatic reflectospectrometer. A part of the results are shown in Fig. 1 (A-I).

In the manganese-rich fresh-water shell group (such as the shells of "Nigger head", "Setaishigai", "Pig toe", and "Kankoonbiki", manganese content: 109–420 γ per g), manganese-poor fresh-water shell ("Setashijimi": 9–29 γ), manganese-poor brackish shell ("Yamato-shijimi": 12.5 γ), and manganese-poor marine shell group (such as the shells of pearl oyster and of black-lip shell: 16–32 γ), the degrees of the shell coloration before and after the irradiation with γ -rays have been compared with their reflectances.

Fig. 1. A—D

From these reflection curves, it is recognized that the degrees of the shell coloration on many species of shellfishes which live individually in different environmental conditions were approximately proportional to their manganese contents and amounts of free iodine. However, the changes of the reflection curves on a few fresh-water shells used was not directly proportional to their manganese contents and amounts of free iodine. For example, excess yield of free iodine and lower degree changes of reflection curves compared with their manganese contents were found in several fresh-

Fig. 1. E—H

Fig. 1. I

Fig. 1. Reflection curve for several shells of fresh-water and marine shell-fishes before and after the irradiation with a $2 \times 10^7 \gamma$ dose of r-rays.

water shells having a native blackish color, such as the shells of "Otokotateboshi" and "Setaishigai". The shells of "Setaishigai" having a different degree of coloration (reflectance) before the γ -ray irradiation ((A) and (B) in Fig. 1, B) gave the same level of reflection curve (not the difference of reflectances at before and after irradiation) with the same dose of γ -rays despite the fact that they have a similar amount of manganese. This fact seems to help explain the few disagreements between the manganese contents and reflectances on the shells described above.

Measurement of the stability of the colored material produced in the irradiated shell. The black pearls produced from pearl oysters tend to discolor, while color in the black pearls produced by means of γ -ray irradiation is quite stable.

Fig. 2. Influence of heating on the stability of the colored material produced in the irradiated shell of *Quadrula undulata* (BAROES).

As shown in Fig. 2, the free iodine is approximately proportional to the degrees of coloration, and in a fresh-water shell irradiated with γ -rays it is not changed within the limits of experimental error for 16 months at room temperature. Heating at 100°C produced a decrease of free iodine and discoloration. The mechanism of this phenomenon caused by heating is not explained satisfactory, but the fact of the proportionable decrease between the coloration and the amounts of free iodine in the shell by heating suggests that it is part of the mechanism of pearl coloration described above. In general, the pearls are not placed in temperature over 50°C. Therefore, the color of marine black pearls produced by γ -ray irradiation is semi-permanently stable like the color of the black-lip shell at the general condition of custody.

Summary

1. To confirm the universality of the assumption that the coloration of pearls by irradiation with radioactive-rays is due to the coloration of manganese contained in

them, the shells of 9 species of fresh-water shellfishes with various contents of manganese were irradiated with γ -rays. The relationship among the manganese contents, amounts of released iodine, and coloration in their shells was discussed.

2. Coloration of the shells of fresh-water and marine shellfishes by irradiation with γ -rays, as shown in Fig. 1 (A—I) and Table 1, is proportional to their manganese contents and to amounts of released iodine in a reasonable accuracy. A few shells give conflicting results.

3. The shell of "Setashijimi", *Corbicula sandai* REINHARDT, was the only one having a small amount of manganese and hardly colored by the γ -ray irradiation among the shells of 9 species of fresh-water shellfishes.

4. The colored material which was produced in the shell irradiated with γ -rays was stable for 16 months or more at room temperature, but it is comparatively unstable and discolorable in high temperature over 50°C.

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