Accumulation of Co by Abalone I—Effect of chemical form

T. UEDA,1) Y. SUZUKI,1) R. NAKAMURA,1) M. NAKAHARA1) and C. SHIMIZU2)

1) Division of Marine Radioecology, National Institute of Radiological Sciences, Nakaminato, Ibaraki, 311-12; 2) Fisheries Laboratory, Faculty of Agriculture, University of Tokyo, Maisaka, Shizuoka, 431-02.

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The appearance of radioactive Co in the liver of abalone from sea water was examined to consider the effect of chemical forms of Co(CoCl2 and cyanocobalamin; vitamin B12) in sea water upon the metabolisms in marine organisms. Organic 57Co(cyanocobalamin) from sea water appeared in the liver of abalone combining with a constituent with a molecular weight of 4 x 10^4. The constituent had the activity of vitamin B12, while inorganic 60Co(CoCl2) appeared combining with three constituents with molecular weights more than or equal to 1.5 x 10^6, 7 x 10^3 and less than or equal to 1.5 x 10^3 which did not show the activities of vitamin B12. The effect of chemical forms of Co in sea water is significant in its accumulation by some species of marine organisms.

INTRODUCTION

On clarifying the mechanisms of radioactive contamination of marine organisms, the following items should be taken into account,

1) Environment factors.
   level of radioactivity in sea water, sediment and food, pH, salinity and temperature of water,
   coexisting substances, physico-chemical form of radionuclide in water, light, among others

2) Biological factors
   species of organisms, morphology, growth stage, sex, migratory habits,
   feeding habits, among others

3) Metabolism of radionuclide in marine organisms

Heretofore, mainly the environmental factors and biological factors have been investigated. And concerning the metabolism of radionuclides, only limited data are available.1-4)
Accordingly, we have studied the metabolism of radionuclides in marine organisms together with other factors, and already reported the combining of radionuclides with constituents of organisms using gel filtration.\textsuperscript{5,6} In this paper, the different appearance of Co in the liver of abalone due to the different chemical forms of Co in sea water will be described utilizing the results of gel filtration. The chemical forms used are \(^{57}\text{Co}(\text{cyanocobalamin})\), an organic form and \(^{60}\text{Co}(\text{CoCl}_2)\), an inorganic one.

**MATERIALS AND METHODS**

(1) Different appearance of radioactive Co in the liver of abalone due to chemical forms

The specific activity of \(^{60}\text{Co}\) and \(^{57}\text{Co}\) was 93 mCi/mg of Co and 220 µCi/µg of cyanocobalamin, respectively. The solution of \(^{57}\text{Co}\) was passed through a Chelex-100 column (10 x 1 cmφ) and the effluent was used in the experiment.

An abalone (\textit{Haliotis discus}, 50 g) was reared for 3 days in one liter of sea water containing 40 µCi of \(^{60}\text{Co}(\text{CoCl}_2)\). Another individual weighing 68 g was reared in sea water containing 5 µCi of \(^{57}\text{Co}(\text{cyanocobalamin})\). These abalones were artificially propagated at The Fisheries Experimental Station of Ibaraki-Ken. This experiment was conducted at 20°C. No food was given to them to avoid the intake of radioactive Co through food contaminated by radioactive Co in sea water. After 3 days' exposure, the livers of the abalone were removed. The liver samples (2 g) were homogenized with 10 ml of 0.025M Tris-acetate buffer solution (pH 8.4) by a high speed homogenizer (2 x 10\(^4\) rpm) and centrifuged at 1 x 10\(^4\) rpm for 30 minutes. The supernatant was applied to a Sephadex G-75 gel column (75 x 2 cmφ) which could separate materials with a molecular weight (MW) ranging from 8 x 10\(^4\) to 3 x 10\(^3\). The gel filtration was performed at a flow rate of 40 ml/hour using the same buffer solution. Each 5 ml of the effluent was fractionated and measured by a well-type γ-ray counter with an automatic sample changer (Aloka auto-well γ-system, JDC-752). Sixty per cent of the radioactivity in the liver sample of the abalone was found in the supernatant for \(^{57}\text{Co}\) and 66% for \(^{60}\text{Co}\). The recovery of the radioactivity in the gel filtration was more than 90%. After measurement of the radioactivity, the content of protein in each fraction was monitored at 280 nm with a spectrophotometer (Hitachi Model 124 DB).

The experiment was repeated twice.

In order to know the more precise molecular weight of the constituents combined with \(^{60}\text{Co}\), the effluents around each radioactivity peak in the gel filtration profile of \(^{60}\text{Co}\) were collected, respectively, and concentrated under reduced pressure. Then further gel filtrations on Sephacryl S-300 and Sephadex G-50 were applied to the constituents considering their molecular weight.

The molecular weight of the constituents was estimated on the basis of a
calibration curve which was obtained by the same treatment of materials such as Blue Dextran 2000 (MW: 2 x 10^6), Thyroglobulin (MW: 6.7 x 10^5), Catalase (MW: 2.3 x 10^5), Ovalbumin (MW: 4.3 x 10^4), Ribonuclease A (MW: 1.37 x 10^4), Cytochrome C (MW: 1.24 x 10^4), Insulin (MW: 6 x 10^3) and Cyanocobalamin (MW: 1.36 x 10^3).

(2) The activity of vitamin B₁₂ accumulated by abalones

An abalone was reared in 3 litres of sea water reinforced with non-radioactive CoCl₂ (44 mg of Co) and another was reared in sea water reinforced with non-radioactive cyanocobalamin (44 mg of Co) for 2 days without food for the easy detection of the activity of vitamin B₁₂ by the bioassay. The gel filtration on Sephadex G-75 was applied to those livers using the procedure previously described. Referring to the gel filtration profile of the experiment (I), 15 ml of the effluents corresponding to each of 3 radioactivity peaks were separately collected. The bioassay for vitamin B₁₂ was performed on them using Lactobacillus leichmannii ATCC-7830. To each 2 ml of the effluents, 2 ml of 0.2M acetate buffer solution (pH 4.5), 0.1 ml of KCN (0.5 mg/ml) and 5 ml of distilled water were added and the mixed solution was autoclaved at 120°C for 5 minutes. After cooling, 0.2 ml of meta phosphoric acid (10%) was added and the solution was centrifuged at 3000 rpm for 15 minutes. The supernatant was adjusted to pH 7 with alkaline solution and diluted to 50 ml with distilled water. The diluted solution was used for the bioassay of vitamin B₁₂.

RESULTS AND DISCUSSION

(1) Different appearance of radioactive Co in the liver of abalone due to chemical forms

The profiles of ⁵⁷Co and ⁶⁰Co using gel filtration of the liver on Sephadex G-75 are shown in Fig. 1, accompanied with the curve of optical density at 280 nm. The profile of ⁵⁷Co showed one peak (peak I) between 25—80 ml of the effluent. But the profile of ⁶⁰Co showed a dominant peak between 25—50 ml (peak II) and 150—200 ml (peak III) which coincided with the peaks of optical density at 280 nm. It was estimated from the calibration curve of the materials described above that ⁵⁷Co combined with a constituent which has a molecular weight of 4 x 10^4 and also with the same constituent as peak II, because the profile of ⁵⁷Co had a shoulder on the left side which correspond to the peak II. Furthermore the constituent with a molecular weight of 4 x 10^4 combined with ⁵⁷Co but not ⁶⁰Co. This constituent is probably a kind of proteins although it does not clearly show a peak of optical density at 280 nm. The molecular weight of the constituent combined with ⁶⁰Co could not be estimated from the profile on Sephadex G-75 gel which could separate constituents with molecular weights of 8 x 10^4 — 3 x 10^3. Then further gel filtration on Sephacryl S-300 was done for the effluent
of 25–50 ml (peak II) and on Sephadex G-50 for that of 150–200 ml (peak III) after concentration. In Fig. 2, the further gel filtration profiles are presented. The molecular weight at peak II was considered to be more than or equal to 1.5 x 10^6. The constituent at peak III in Fig. 1 consisted of two constituents with molecular weight of 7 x 10^3 and less than or equal to 1.5 x 10^3.

The difference in gel filtration profiles due to the chemical forms of Co in sea water was also observed in the experiment using another species of bivalve (Gomphina melanaegis) but not clearly observed in the case of octopod (Octopus vulgaris) by us. Thus, it could be said that the chemical forms of Co in sea water have affected the metabolism of Co in some species of marine organisms. In other words, the accumulation and excretion of Co by some species of marine organisms would be varied by the chemical forms of Co.

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**Fig. 1.** Gel filtration profile on Sephadex G-75 of radioactive Co accumulated in liver of abalone

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- —— ^{97}Co, —— ^{60}Co, ——— optical density
(2) The activity of vitamin B12 accumulated by abalone

The activity of vitamin B12 at peak I-III was examined. The effluent of 15 ml at peak I showed the activity of 107 ng of vitamin B12/ml. Therefore, the majority of vitamin B12 (MW: \(1.36 \times 10^3\)) accumulated by the liver of abalone was incorporated in the constituent with a molecular weight of \(4 \times 10^4\) (peak I) and the minority was in the constituent with a molecular weight more than or equal to \(1.5 \times 10^6\). The constituent at peak II and III had no activity of vitamin B12, suggesting that ionic Co was not varied to cyanocobalamin in sea water in 3 days. The results are summarized in Fig. 3. The constituent at peak II had the ability to combine with both organic and inorganic Co.

![Fig. 2. Furhter gel filtration on Sephracryk S-300(A) and Sephadex G-50 of \(^{60}\)Co accumulated in liver of abalone](image)

![Fig. 3. Flux of Co to liver of abalone from sea water](image)
Thus, the chemical forms of radionuclides and probably heavy metals in sea water will affect not only quantitative difference on the accumulation of them\(^9\), but also the metabolism in some marine organisms.

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