Effects of Chemical Forms and Intake Pathways on the Accumulation of Radioactive Cobalt by the Abalone *Haliotis discus*

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Although contamination of the abalone by radioactive cobalt is caused by its accumulation through the 2 intake pathways (from sea water and from food algae), the contribution of each pathway to the total accumulation of the element remains unknown. While, the effect of chemical forms of the element is not solved yet. Therefore, we examined the uptake of cyanocobalamin (^{57}Co) or ionic cobalt (^{60}Co) by the abalone from sea water and from 3 species of marine algae *Ulva pertusa*, *Eisenia bicyclis* and *Chondrus ocellatus*. The excretion was also examined.

The abalone accumulated cyanocobalamin (^{57}Co) from sea water approximately 5 times more than ionic cobalt (^{60}Co) at the equilibrium and also it absorbed cyanocobalamin (^{57}Co) 6-16 times more than ionic cobalt (^{60}Co) from the food algae. Cyanocobalamin (^{57}Co) stayed in the abalone about 2 times longer than ionic cobalt (^{60}Co). Whereas, the abalone accumulated much more cobalt in both chemical forms from sea water than from food algae except *Ulva pertusa*. Therefore, it was concluded that when the abalone lived mainly on *Eisenia bicyclis* which was its favorite food, the influence of food on the accumulation of radioactive cobalt by the abalone could be negligible.

Cobalt-60 is one of the currently marked collision products released from nuclear power stations to the coastal sea and reported to be accumulated remarkably in marine invertebrates.¹⁾ While, some species of mollusca have physiological peculiarity of radioecological importance. For example, the cephalopoda has branchial hearts which have never identified in other marine organisms and the kidney of the giant clam *Tridacna* sp. is very well developed. Those organs were recently reported to concentrate large amount of cobalt in sea water.^{2,3)} The abalone is also important from the radioecological point of view, because it ingests marine algae which accumulate various radionuclides up to relatively high level and because its inhabiting area is extremely limited in comparison to the fish. Therefore, we conducted an experiment on the accumulation of radioactive cobalt by the abalone.⁴⁾ Especially, in this report we investigated effects of different chemical forms and uptake pathways of radioactive cobalt on its accumulation by the abalone. Chemical forms of radioactive cobalt in sea water turn up to have pretty large influence on the concentration factor of a bivalve.⁵⁾ So,

we examined not only the accumulation of ionic cobalt (CoCl_2) by the abalone, but also the accumulation of the element in the form of cyanocobalamin (Vitamin B_{12}) which was reported to exist in sea water at the level of several nano grams per liter.⁶⁾ Whereas, considering that the radioactive cobalt accumulated from food occupied a considerable part in the total radioactive cobalt accumulated by the fish,⁷⁾ we examined the contribution of the food algae to the accumulation of radioactive cobalt by the abalone.

Materials and Methods

Accumulation of Radioactive Cobalt from Sea Water

Two abalones (*Haliotis discus*, average body weight: 190 g, shell length: 11-12 cm) were reared for a week in 12 liters of filtered sea water (Filter: Millipore filter Type GS, pore size: 0.22 μm) containing 100 μCi of ionic Co-60 (Specific activity: 93 mCi/mg of Co). The experimental condition was as follows: Sea water temperature: 15°C, Air supply: Aerating system without water filtration equipment.

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Fig. 1. Uptake of radioactive cobalt from sea water and its excretion by *Halotis discus*.

In 7 days, abalones were taken out of the rearing sea water and then they were put into non-radioactive fresh sea water for the following long-term excretion experiment with periodical renewal of the rearing sea water.

Another experiment began with laying one abalone in 6 liters of filtrated sea water containing about 20 μCi of cyanocobalamin Co-57 (Specific activity: 220 $\mu\text{Ci}/\mu\text{g}$ of cyanocobalamin) from which ionic cobalt had been removed in advance by a Chelex-100 column ($10 \times 1 \text{ cm}$). At the 7th day after the beginning of the experiment, the abalone was transferred from the contaminated sea water to non-radioactive fresh sea water and kept in it for 46 days. Sea water was periodically renewed during the excretion experiment. During the uptake and the excretion experiment, radioactivity in the abalone was measured periodically in the "Armac" 4π -Scintillation counter (Packard 446) and the radioactivity was corrected against the physical decay of those radionuclides (Physical half-life: 5.27 years for ^{60}Co and 271 days for ^{57}Co).

Accumulation of Radioactive Cobalt by the Abalone from Food Algae

To prepare food algae labelled by ionic Co-60, several grams of the green alga *Ulva pertusa*, the brown alga *Eisenia bicyclis* and the red alga *Chondrus ocellatus* were held for a week in 12 liters of filtrated sea water containing about 100 μCi of ionic Co-60 under the condition that the water temperature was $15 \pm 2^\circ\text{C}$ and the illumination was about 6000 lux. Then the labelled food

algae (0.5–1.5 g) were given to individuals of the abalone kept in separate aquariums with 10 liters of non-radioactive sea water and the periodical radioactivity measurement of the abalone was continued for about 60 days with the scintillation counter mentioned above. The decay correction of the radioactivity was also carried out.

The same procedure under the same condition was repeated on cyanocobalamin Co-57 except that the marine algae were held in 2 liters of sea water containing about 10 μCi of cyanocobalamin Co-57.

Results and Discussion

Accumulation of Radioactive Cobalt from Sea Water

The radioactivity (cpm/g) of the abalone in the uptake experiment from sea water and in the excretion experiment was shown in Fig. 1. The higher radioactivity of ^{60}Co than ^{57}Co in the abalone was due to the higher concentration of ionic Co-60 than cyanocobalamin Co-57 in the rearing sea water. The excretion curves of ^{60}Co and ^{57}Co could be approximated to the different lines on a semi-logarithmic graph and were expressed as follows:

$$y = 65000e^{-0.0059t} \quad \text{for ionic cobalt } (^{60}\text{Co})$$

$$y = 16000e^{-0.0032t} \quad \text{for cyanocobalamin } (^{57}\text{Co})$$

Where, y : radioactivity of the abalone (cpm/g)

e : exponential function

t : days after the beginning of the excretion experiment

Biological half-lives were calculated from the

Fig. 2. Estimated accumulation curves of cobalt for *Haliotis discus*.

turnover rates (0.0059 and 0.0032) as shown in Table 1. Cobalt accumulated from sea water in the form of cyanocobalamin stays approximately 2 times longer in the abalone than the element absorbed as ionic. The biological half life of ionic cobalt (^{60}Co) in the abalone showed 2–5 times longer value than those in marine fishes,⁸⁾ bivalves⁹⁾ and algae,⁹⁾ and that of cyanocobalamin (^{57}Co) in the abalone was also a little longer than those in bivalves.⁹⁾ In the above sentence, the phrase: the biological half-life of ionic cobalt (^{60}Co) or cyanocobalamin (^{57}Co) means the biological half-life of cobalt which was accumulated by the abalone from sea water in the form of ionic cobalt (^{60}Co) or cyanocobalamin (^{57}Co) and for the convenience, the same meaning was given to all the biological half-lives, turnover rates, concentration factors and absorption rates which were used in this report.

By substituting the actual data obtained in the uptake experiments for Y and t of a theoretical uptake formula: $Y = A(1 - e^{-\beta t})$, A was calculated as follows:

$$A = 6300 \quad \text{for cyanocobalamin } (^{57}\text{Co})$$

$$A = 1300 \quad \text{for ionic cobalt } (^{60}\text{Co})$$

Where, Y : concentration factor (CF) of radioactive cobalt for the abalone from sea water.

β : turnover rate.

t : days after the beginning of the uptake experiment.

Therefore, concentration factor curves of the abalone for these chemical forms were expressed

by the following formulas:

$$Y = 6300(1 - e^{-0.0082t})$$

for cyanocobalamin (^{57}Co)

$$Y = 1300(1 - e^{-0.0059t})$$

for ionic cobalt (^{60}Co)

As shown in Fig. 2, the estimated concentration factor of cyanocobalamin (^{57}Co) for the abalone was nearly 5 times greater than that of ionic cobalt (^{60}Co) at steady state and the difference may be owing to the fact that the constituents of the abalone liver combined with cyanocobalamin (^{57}Co) differed from those combined with ionic cobalt (^{60}Co).⁴⁾

Accumulation of Radioactive Cobalt by the Abalone from Food Algae

Decreasing curves shown in Fig. 3 indicate the retention (%) of radioactive cobalt in abalones fed separately with the 3 species of marine algae labelled in sea water containing cyanocobalamin (^{57}Co) or ionic cobalt (^{60}Co). Much difference was observed between the retention curves of cyanocobalamin (^{57}Co) and those of ionic cobalt (^{60}Co), though there was not so clear discrepancy among retentions of radioactive cobalt in the 3 species of food algae. Retention curves descended steeply during early 3 or 4 days and then showed gentle slopes. This rapid decrease of radioactivity can be considered to result from the loss of radioactive cobalt accompanied with the excrements of undigested food algae, whereas the following slow decrease will be attributed to the excretion of radioactive cobalt through the metabolic route

Fig. 3. Retention (%) of radioactive cobalt in marine algae by *Haliotis discus*.Table 1. Turnover rates, biological half-lives and absorption rates of *Haliotis discus*

	The chemical form added in the experimental sea water					
	Cyanocobalamin (^{57}Co)			Ionic cobalt (^{60}Co)		
	Turnover rate (β) (day $^{-1}$)	Biological half-life (day)	Absorption rate (%)	Turnover rate (β) (day $^{-1}$)	Biological half-life (day)	Absorption rate (%)
From sea water	0.0032	220	—	0.0059	120	—
From food						
<i>Ulva pertusa</i>	0.0034	200	84	0.0086	81	52
<i>Eisenia bicyclis</i>	0.0044	160	97	0.0073	95	40
<i>Chondrus ocellatus</i>	0.0029	240	83	0.0094	74	44

of the abalone (metabolic excretion). Therefore, retention (%) on the 3rd or the 4th day was regarded as an absorption rate by the abalone for radioactive cobalt in algae. Those absorption rates were corrected against the physical decay of these radionuclides (^{57}Co and ^{60}Co) and shown in Table 1. Absorption rates of cyanocobalamin (^{57}Co) were approximately 2 times higher than those of ionic cobalt (^{60}Co) and the clear difference between these absorption rates suggested the different existence of cobalt in food algae.

Absorption rates of cyanocobalamin (^{57}Co) were realized to be extremely high values never observed in other marine organisms before and those of ionic cobalt (^{60}Co) showed approximately one magnitude higher values than ever reported on some species of the fish.^{10,11} However, NAKAHARA *et al.* reported as comparable absorption rates as ours on abalone and clam.^{9,12} Therefore, shellfishes will generally absorb such an element as cobalt through their digestive tracts at much higher absorption rates than marine fishes. The relatively high absorption rates of ionic cobalt (^{60}Co) by the abalone may be caused by the physiological property of abalone's digestive

organs or some possible change of its chemical form in algae from inorganic to organic one.¹³ On the metabolic excretion curves in Fig. 3, turnover rates of cobalt from food algae in the abalone were calculated according to the approximate formulas induced from an exponential regression analysis, as mentioned above and shown in Table 1. Difference was not definitely observed among the 3 species of food algae but it was confirmed that turnover rates of cyanocobalamin (^{57}Co) were smaller than those of ionic cobalt (^{60}Co) and inversely, biological half-lives of cyanocobalamin (^{57}Co) were 2–3 times longer than those of ionic cobalt (^{60}Co).

Comparison of the Accumulation from Sea Water with that from Food Algae

In order to compare the accumulation of cobalt by the abalone from food algae with that from sea water, the accumulation from food algae had to be expressed with the concentration factor (CF) against the sea water based on the conception that after the concentration of cobalt in the alga attained to the equilibrium in sea water of a constant cobalt concentration, the abalone took the

alga at a constant daily intake up to the equilibrium of the cobalt concentration in the abalone. Namely, the concentration factor from food algae was calculated according to the following process.

The concentration factor is equivalent to the quotient of the uptake rate (u) by the turnover rate (β) and the uptake rate could be expressed as follows:

$$u = \frac{\text{Radioactivity absorbed daily by the unit weight of the abalone}}{\text{Radioactivity in sea water (W}_0\text{)}}$$

In the accumulation from the food algae:

$$\begin{aligned} u &= \frac{\left(\frac{\text{Amount of the alga taken}}{\text{daily by the abalone}} \right) \times \left(\frac{\text{Radioactivity}}{\text{of the alga}} \right) \times \left(\frac{\text{Absorption}}{\text{rate}} \right)}{\text{Weight of the abalone (W}_A\text{)}} \times \frac{1}{W_0} \\ &= \frac{(W_A \times \text{Daily intake}) \times (\text{CF of the alga} \times W_0) \times (\text{Absorption rate})}{W_A} \times \frac{1}{W_0} \\ &= (\text{Daily intake}) \times (\text{CF of the alga}) \times (\text{Absorption rate}) \end{aligned}$$

Therefore, the concentration factor from the food alga

$$= \frac{(\text{Daily intake}) \times (\text{CF of the alga}) \times (\text{Absorption rate})}{\text{Turnover rate } (\beta)}$$

Concentration factors from food algae at steady state were calculated as shown in Table 3, using the absorption and the turnover rates (Table 1) and the concentration factors for algae (Table 2). As a daily intake, 3% of abalone's weight was employed in the calculation, presumed from the report by KIKUCHI *et al.*¹⁴⁾ Cyanocobalamin (^{67}Co) offered 6–16 times higher concentration factors than ionic cobalt (^{60}Co) and the predominance of cyanocobalamin (^{67}Co) in the concentration factors was considered to originate mainly in the high concentration factors of cyanocobalamin (^{67}Co) for algae and the high absorption rates of cyanocobalamin (^{67}Co) in algae by the abalone.

On the other hand, in regard to the intake pathways, concentration factors from food algae, except only *Ulva pertusa*, were by far lower than that from sea water for both chemical forms of cobalt. In comparison among species of algae, regardless of the chemical forms, concentration factors decreased in order of *Ulva pertusa*, *Chondrus ocellatus* and *Eisenia bicyclis*, and the exceptional high concentration factors of the

Table 2. Concentration factors of algae

	Cyanocobalamin (^{67}Co)	Ionic cobalt (^{60}Co)
<i>Ulva pertusa</i>	3700	920
<i>Eisenia bicyclis</i>	80	50
<i>Chondrus ocellatus</i>	210	270

abalone from *Ulva pertusa* were considered to be obliged to the very high concentration factor of the alga as shown in Table 2. It is interesting that the accumulation of radioactive cobalt showed the least value when the abalone took *Eisenia bicyclis* which was known to be a favorite of the abalone.

Although the concentration factor of cobalt for the abalone was discussed so far separately as to the 2 intake pathways, the actual accumulation of cobalt by the abalone occurs simultaneously through both pathways in the sea. Therefore, contribution (%) of the accumulation from food algae to the total accumulation through both pathways was estimated according to the following formula and shown in Table 3.

Table 3. Concentration factors of *Haliotis discus* and contribution (%) of food algae to the accumulation of radioactive cobalt by the abalone

	The chemical form added in the experimental sea water				$\frac{A}{B}$
	Cyanocobalamin (^{67}Co)		Ionic cobalt (^{60}Co)		
	CF (A)	Contribution (%)	CF (B)	Contribution (%)	
From sea water	6300		1300		5
From food					
<i>Ulva pertusa</i>	27000	81	1700	57	16
<i>Eisenia bicyclis</i>	500	7	80	6	6
<i>Chondrus ocellatus</i>	1800	22	250	16	7

Contribution (%)

$$= \frac{\text{CF from the food alga}}{(\text{CF from the food alga} + \text{CF from sea water})} \times 100$$

If an abalone feeds on *Ulva pertusa* only, influence of the food alga on the accumulation of radioactive cobalt by the abalone will dominate that of sea water. However, in case that the abalone lives mainly on *Eisenia bicyclis* in the natural environment, the contribution of the algae will be very small compared with that of sea water, that is, it can be reasonably said that the most of radioactive cobalt in the abalone comes directly from sea water through the gill and the surface skin of the abalone in the natural ecosystem.

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