Metabolism of Radionuclides in a Cephalopod, IidakO, Octopus ocellatus

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Radionuclides/Metabolism/Cephalopods

Retention, distribution and excretion of radionuclides in a cephalopod, IidakO, Octopus ocellatus, were observed by administering radioisotopes (54Mn-, 59Fe-, 60Co-, 65Zn-chlorides and 57Co-cyanocobalamin) into the mantle cavity by injection. Whole body radioactivity of the octopus was measured periodically after the injection for one to ten weeks to obtain the retention curve. At different stages after the injection, the sacrificed octopus was dissected into eight parts to examine the distribution of radionuclides and its change with the lapse of time. For some organs of the octopus, gel filtration chromatography (GFC) with Sephadex G-75 was applied to elucidate the binding of radionuclides with the constituents of the octopus.

Excretion patterns consisted of two or three components for every nuclide except 57Co-cyanocobalamin which showed monophasic elimination. 54Mn was lost most rapidly whereas the longest biological half-life was shown by 59Fe. The most significant distribution of radioactivity was observed for 57, 60Co and 59Fe in the branchial heart of the octopus, while no specific accumulation of 54Mn and 65Zn was shown in this organ. The different accumulation mechanisms between each chemical form of cobalt and among the nuclides were suggested from the GFC elution profiles of radioactivity in the branchial heart and the liver.

INTRODUCTION

The behavior of radionuclides in marine ecosystems has been the subject of detailed studies with the development of nuclear industries. Among the marine organisms molluscs are well known to concentrate many induced radionuclides as well as their stable counterparts.1-8) Especially, the peculiar accumulation of radioactive or stable elements in specific organs or tissues of molluscs is frequently pointed out with their important roles as the biological indicators for water pollution or as critical organisms in the pathway of marine pollutants to man.9-13) In the case of certain cephalopods, for example, Madako, Octopus vulgaris, a small organ, the branchial heart, is known to show the potential con-
centrating power for certain radioactive and stable elements.\textsuperscript{14} - \textsuperscript{17}) In this study we report the results of laboratory experiments on the retention and tissue distribution of induced radionuclides in a species of cephalopod, Iidako, \textit{Octopus ocellatus}, to examine their metabolism in marine molluscs.

**MATERIALS AND METHOD**

The cephalopod, Iidako, \textit{Octopus ocellatus}, 15.4 - 62.2 grammes (average 42.8 g), were caught on the Pacific coast of Ibaraki Prefecture, Japan and four to eight individuals for each radionuclide were reared in 200 l or 40 l seawater tanks with circulation through sand filters and aeration by pumps without feeding at 18°C. The chemical forms and specific activities of the radioisotopes used were as follows; \textsuperscript{54}Mn (MnC\textsubscript{12}, carrier free), \textsuperscript{59}Fe (FeC\textsubscript{13}, 30.6 mCi/mg Fe), \textsuperscript{60}Co (CoC\textsubscript{12}, 135 mCi/mg Co), \textsuperscript{65}Zn (ZnC\textsubscript{12}, 6.8 mCi/mg Zn), and \textsuperscript{57}Co (220 mCi/mg cyanocobalamin).

About 0.1 to 0.2 ml of a physiological solution of sodium chloride containing 0.1 - 13.5 μCi of each radioisotope were injected into the mantle cavity of the octopus. Whole body radioactivity of the octopus was measured with a scintillation counter (Armac Scintillation Detector, model 446 with Tri-carb Scintillation Spectrometer, model 3001, Packard) periodically after the injection during one to ten weeks to obtain the retention curves.

At different stages after the injection, the sacrificed octopus was dissected into eight parts for radioactivity measurements and the distribution of the radionuclides was compared among the different post-injection elapsed times. For some organs of the octopus a gel filtration chromatography (GFC) with Sephadex G-75 was applied to examine the existing state of radioisotopes in the organs and to deduce the mechanisms of accumulation and excretion for the radionuclides by the octopuses. Sephadex G-75 (Pharmacia Fine Chemicals, Sweden) was soaked in the tris buffer solution of pH 8.32 at room temperature and then packed into the column of 1.96 cm I.D. and 72 cm height. The each organ of the octopus was homogenized in the buffer solution and centrifuged at 10,000 RPM. The supernatant was added on the column and eluted with the buffer solution in a flow rate of 1.4 ml/min, maintained by a peristaltic pump. The contents of proteinous matter in the effluent were measured on a UV absorption monitor (UV-monitor, model CLC-5-UV and recorder, model QPD34, Hitachi) by absorbance at 254 nm, and aliquots fraction were collected continuously for subsequent radioactivity measurement with a scintillation counter (Auto Well Gamma System, model JDC-752, Aloka).
Table 1. Parameters for retention patterns of radionuclides in lidako, Octopus ocellatus.

<table>
<thead>
<tr>
<th></th>
<th>$^{54}$Mn</th>
<th>$^{59}$Fe</th>
<th>$^{65}$Zn</th>
<th>$^{60}$Co (chloride)</th>
<th>$^{57}$Co (cobalamin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short component</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_b$ (days)</td>
<td>3.2</td>
<td>10.3</td>
<td>9.6</td>
<td>6.5</td>
<td>—</td>
</tr>
<tr>
<td>$\beta_1$ (days$^{-1}$)</td>
<td>0.2186</td>
<td>0.0825</td>
<td>0.0748</td>
<td>0.1064</td>
<td>—</td>
</tr>
<tr>
<td>$f_1$</td>
<td>0.56</td>
<td>0.30</td>
<td>0.31</td>
<td>0.08</td>
<td>—</td>
</tr>
<tr>
<td><strong>Long component</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_b$ (days)</td>
<td>36.1</td>
<td>112.9</td>
<td>31.5</td>
<td>37.4</td>
<td>29.7</td>
</tr>
<tr>
<td>$\beta_2$ (days$^{-1}$)</td>
<td>0.0214</td>
<td>0.0215</td>
<td>0.0248</td>
<td>0.0189</td>
<td>0.0259</td>
</tr>
<tr>
<td>$f_2$</td>
<td>0.44</td>
<td>0.70</td>
<td>0.69</td>
<td>0.92</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$Q_t = Q_0 (f_1 e^{-\beta_1 t} + f_2 e^{-\beta_2 t})$

where,
- $Q_t$ : the whole body radioactivity at the time $t$ after injection.
- $Q_0$ : the whole body radioactivity just after injection.
- $f_1$, $f_2$ : the fractions of radioactivity lost with the coefficients $\beta_1$ and $\beta_2$, respectively.
- $\beta_1$, $\beta_2$ : loss coefficients of the fractions $f_1$ and $f_2$. ($\beta_{1, 2} = 0.693/T_{Te_{1, 2}}$)
- $T_{Te_{1, 2}}$ : effective half-lives of the fraction $f_1$ and $f_2$.
- $T_{b_{1, 2}}$ : biological half-lives of the fraction $f_1$ and $f_2$. 

Retension of radionuclides by the octopus:

The whole body retention of the radionuclides after the injection was shown in Figure 1 for each radionuclide. Except in the case of $^{57}$Co-cyanocobalamin which showed a monophasic retention curve, an almost similar elimination pattern with two or three components was observed for the all other nuclides examined. The retention curves were analysed in the first approximation by separating them into two components. The biological half-lives and loss coefficients were calculated by means of the least squares method and shown in Table 1. The most rapid excretion of radioactivity was observed on $^{54}$Mn but the loss of other radionuclides was slow with relatively large size and long biological half-lives of the long components. These values are a little longer than the results reported on other molluscs,\(^5,18-21\) but comparable to those observed in marine fishes,\(^22-27\) although the direct comparison is difficult among different species or different modes of uptake. The different accumulation and excretion between inorganic and organic forms of cobalt by shellfishes is reported,\(^28,29\) and high retention of $^{57}$Co-cyanocobalamin without the short component in the elimination curve is in agreement with those results but the bio-
Fig. 1. Whole body retention of radionuclides in Iidako, *Octopus ocellatus*. Each point represents the mean and standard deviation for four individuals.
logical half-life is somewhat shorter than that of $^{60}$Co-chloride in the long component suggesting a high turnover rate of organic cobalt in the octopus.

**Distribution of radionuclides in the octopus:**

The distribution of radioactivity in the octopuses at the different stages of elimination is shown in Table 2 and 3 with the percentage of the weight of each organ or tissue of the octopus. The distribution ratio of $^{60}$Co-chloride decreased in most of organs or tissues during the elimination period between a week and ten weeks after the injection, while increasing in the liver and the branchial heart suggesting the transfer of radiocobalt to those organs from other parts of the octopus. Especially, the branchial heart retains more than 40 per cent of whole body radioactivity in spite of its very small weight percentage. It shows higher concentration by an order of magnitude than that in the liver. The remarkable accumulation by the branchial heart was also observed in the case of $^{57}$Co-cyanocobalamin but the relatively wide occurrence of radioactivity also in other parts of the octopus such as the gonads or arms suggested a different behavior in metabolic processes from inorganic cobalt as previously supposed in the whole body retention patterns of both forms, although the difference of

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>Suggesting a high turnover rate of organic cobalt in the octopus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elapsed time after injection (Weight per cent)</td>
<td>1 week</td>
</tr>
<tr>
<td>Liver</td>
<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>Gill</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Branchial heart</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Gonad</td>
<td>6.1 ± 3.2</td>
</tr>
<tr>
<td>Viscera*</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>Mantle</td>
<td>18.1 ± 3.6</td>
</tr>
<tr>
<td>Arms</td>
<td>64.6 ± 6.1</td>
</tr>
</tbody>
</table>

*Visceral mass other than liver, branchial heart, kidney and gonad.
Table 2. Distribution of $^{54}$Mn, $^{59}$Fe and $^{65}$Zn in lidako, *Octopus ocellatus*, at the different stages after injection. Each value represents the mean and standard deviation for four individuals. (%)

<table>
<thead>
<tr>
<th>Radionuclides</th>
<th>$^{54}$Mn</th>
<th>$^{59}$Fe</th>
<th>$^{65}$Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organs or tissues</strong></td>
<td>1 week</td>
<td>5 weeks</td>
<td>1 week</td>
</tr>
<tr>
<td>Liver</td>
<td>17.4 ± 3.5</td>
<td>4.9 ± 0.5</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Gill</td>
<td>5.3 ± 1.5</td>
<td>6.5 ± 1.0</td>
<td>1.9 ± 1.2</td>
</tr>
<tr>
<td>Branchial heart</td>
<td>2.1 ± 0.7</td>
<td>1.2 ± 0.5</td>
<td>40.4 ± 16.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.5 ± 0.2</td>
<td>8.1 ± 1.7</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Gonad</td>
<td>20.1 ± 6.7</td>
<td>7.2 ± 6.5</td>
<td>32.5 ± 12.1</td>
</tr>
<tr>
<td>Viscera*</td>
<td>5.5 ± 1.0</td>
<td>12.7 ± 4.8</td>
<td>10.5 ± 8.0</td>
</tr>
<tr>
<td>Mantle</td>
<td>17.5 ± 3.3</td>
<td>11.5 ± 1.5</td>
<td>10.2 ± 5.9</td>
</tr>
<tr>
<td>Arms</td>
<td>29.8 ± 6.1</td>
<td>47.9 ± 10.1</td>
<td>3.0 ± 1.2</td>
</tr>
</tbody>
</table>

*Visceral mass other than liver, branchial heart, kidney and gonad.
specific activity between the isotopes of cobalt should be taken into account at the same time.

The difference in distribution of radioactivity observed among other radionuclides examined is shown in Table 3. The high accumulation in the branchial heart was shown on $^{57}$Fe but not observed on $^{54}$Mn and $^{65}$Zn, whereas the distribution of these nuclides was fairly uniform throughout the octopus. The occurrence of iron in the branchial heart of the octopus is reported by Fox et al. $^{30}$ and others $^{31,32}$ but the concentration of iron in the branchial heart is not significant compared to cobalt according to the report of Ueda et al. $^{33}$ They also reported the difference in the order of $10^2$ in the concentration of cobalt in the branchial heart among four species of cephalopods. Differences in trace elements distribution from species to species might be considered with other variabilities caused by the source of pollution or the period of metabolic processes as pointed out by Amiard et al on cobalt-60. $^{34}$

**The binding of radionuclides to the octopus tissue:**

The results of GFC on the extracts of branchial hearts of the octopuses are shown in Figure 2 and 3. Most of the radioactivity of $^{60}$Co-chloride distributed in the branchial heart was eluted in the region of lower molecular weight and only five per cent of the radioactivity was hypothesized to be bound with high molecular weight proteinous matter in the branchial heart. On the contrary, nearly forty per cent of the radioactivity was eluted in the high molecular weight region in the case of $^{57}$Co-cyanocobalamin and rest of radioactivity was eluted in wide ranges including the region of the molecular weight of cyanocobalamin. From the elution profile of some standard molecular weight markers, ovalbumin (av. M.W. 45,000), chymotrypsinogen (av. M.W. 23,000), cytochrome-c (av. M.W. 13,000), and bacitracin (av. M.W. 1,450) shown in the lowest graph in Figure 2, the molecular weight of the two substances combined with radiocobalt in the branchial heart were thought to be $\geq 50,000$ and $1,000 \sim 10,000$, respectively. As referred to before, $^{30-32}$ the potential accumulating power of the branchial heart of the octopus, *Octopus vulgaris*, for certain radioactive or stable elements is explained by the predominant combining of these elements to the glandular pigment in the branchial heart, adenochrome, with a minimum molecular weight of about 1,200. The main fraction of $^{60}$Co-chloride eluted at the elution volume around 200 ml suggests the combining of cobalt to those low molecular weight substance but the binding to different substances was suggested from the elution profile of $^{57}$Co-cyanocobalamin. For the other three radionuclides in the branchial heart, similar GFC patterns were observed as shown in Figure 3. Although the distribution to the branchial heart was quite different among the nuclides examined, any clear difference was not observed in the existing states of them in the branchial heart.
Fig. 2. GFC elution profile of radionuclides and proteinous matter in the branchial heart of Iidako, *Octopus ocellatus* with a reference of standard samples.
Fig. 3. GFC elution profile of $^{54}$Mn, $^{59}$Fe and $^{65}$Zn and proteinous matter in the branchial heart of Iidako, *Octopus ocellatus*. 
The results of GFC on the liver of the octopus are shown in Figure 4 and 5. The difference between inorganic and organic cobalt is clear in the elution curves showing the dominant combining of organic cobalt with high molecular weight proteinous matter identical to the results of the branchial heart. $^{60}$Co-chloride in the liver is thought to be bound with three kinds of substances with different molecular weights though the identification of these materials is to be done in further experiments. Figure 5 shows different elution profiles among three radionuclides in the liver. Especially, the different combining states between $^{54}$Mn and $^{65}$Zn was inferred although both nuclides showed higher distribution to the liver. The distribution to the branchial heart seemed to be more element-

![GFC elution profile of radioactive cobalt and proteinous matter in the liver of Iidako, Octopus ocellatus.](image)
Fig. 5. GFC elution profile of $^{54}$Mn, $^{59}$Fe, $^{65}$Zn and proteinous matter in the liver of Iidako, *Octopus ocellatus*. 
specific than the liver but the different existing states in the octopus tissue was expressed more clearly in the latter. Based on the different physiological functions of the two organs, the different roles of the proper constituents to combine trace elements are thought to be in the metabolic processes of the octopus, but further studies on the physiological ground are necessary to draw a conclusion.

The high accumulation of radioactive iron and cobalt by the branchial heart of the octopus, Octopus ocellatus, was observed by the aquarium experiments with radioisotope tracers. Although the organ is quite small in proportion to its body weight compared with Octopus vulgaris, the biological concentration of marine pollutants by the octopus is worthy of further investigation from the view point of its popularity as sea food.

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