Binding of Radionuclides to Proteins in Fish*1

Yuzuru SUZUKI*2, Ryoichi NAKAMURA*2, Motokazu NAKAHARA*2, and Taishi UEDA*2
(Received November 4, 1980)

Radioisotope tracer experiments on binding of radionuclides to proteins in fish were carried out in order to gain further information on biochemical behavior of radionuclides in marine fish. The radionuclides, which were bound to proteins in fish through sea water or food, were extracted with a Tris-acetate buffer solution and separated by gel filtration on Sephadex G-75.

Most of $^{137}$Cs in the fish liver were bound only to a peptide with a molecular weight of 1,100-1,300. The most remarkable feature of $^{60}$Co in the profiles of the gel filtration was the presence of two clear radioactivity peaks and the radioactivity appeared to transfer from a low molecular weight protein to a high molecular weight protein in the uptake, and the reverse phenomenon was observed in the excretion. Therefore, this suggested that these proteins had each inherent turnover rate for $^{60}$Co. The profiles of the gel filtration of $^{65}$Zn varied widely among species of fish, tissues or organs even in the same fish and pathways of the uptake.

Thus, it was considered that there existed some different rules peculiar to each radionuclide in the binding of radionuclides to proteins in fish.

Radionuclides in the marine environment are introduced into marine fish through sea water and food organisms. We have already reported on roles of sea water and food organisms in the accumulation of some radionuclides by marine fish1, and found that the role varied widely with the radionuclides. The investigation on radionuclides in the fish have been mainly carried out for quantitative values including concentration and distribution. Thus, we need information on the biochemical behavior of radionuclides that entered into the fish. It is important to gain information more on the fundamental metabolism of radionuclides for elucidation of the mechanism of radioactive contamination of marine fish. And this is necessary also for more accurate estimation of radiation effect on man, who takes marine fish as food.

The experiments reported here were designed to examine the binding of radionuclides to the protein and/or peptides which have been known to be principal constituents in fish during the uptake and excretion.

Materials and Methods

Marine fish used for this experiment are Lateolabrax japonicus (average body length: 23 cm, average body weight: 160 g), Seriola purpurascens (14 cm, 46 g), Acanthogobius flavidus (15 cm, 38 g), Navodon modestus (13 cm, 53 g) and Seriola quinqueradiata (14 cm, 39 g) collected from the coastal sea of Nakaminato, Ibaraki Pref. during Oct. 1978 to Apr. 1979. Each species of these fish was divided into six groups.

Three groups of the fish were contaminated by $^{137}$Cs, $^{60}$Co and $^{65}$Zn added separately to 3 tanks containing 40 l of sea water. After a week, the fish were transferred into nonradioactive sea water, and the rearing sea water was frequently replaced to remove the radioactive materials excreted from the fish.

Other groups of the fish were separately contaminated by single administration of 5 g/individual of the soft parts of clam, Gomphina melanaegis, which had been held in high level radioactive sea water for a week and contaminated to the levels of $^{137}$Cs: $1.6 \times 10^6$ cpn/g, $^{60}$Co: $3.7 \times 10^6$ cpn/g and $^{65}$Zn: $1.9 \times 10^6$ cpn/g. During this experiment, three individual fish were taken out of each tank at every sampling time, and the liver and muscle were immediately excised for analysis.

Crude extract was prepared from these samples as shown in Scheme 1. Samples (1–2 g) were

---

*1 The outline of this report was presented at the autumn meeting of the Japanese Society of Scientific Fisheries, Hakodate, Oct., 1979.
*2 Division of Marine Radioecology, National Institute of Radiological Sciences, Nakaminato, Ibaraki 311-12, Japan (鈴木 淑子・中村良一・中原正和・上田正義：放射線医学総合研究施設海洋放射線学研究部)
homogenized with about 10 ml of 0.025 M Tris-acetate buffer solution (pH 8.4) by a high speed homogenizer (20,000 rpm) and centrifuged at 10,000 rpm for 40 minutes. This procedure was repeated twice. A portion of crude extract (5-10 ml) was applied to a Sephadex G-75 gel column (20 mm x 70 cm) equilibrated with above buffer solution. The gel filtration was operated at a flow rate of 40 ml/h using the same buffer solution. The 50 fractions of 5 ml each were taken and 1 ml of each fraction was put into an acrylic counting test tube for the measurement of radioactivity by a well-type gamma ray counter with an automatic sample changer (Aloka auto well gamma system, JDC-752). The radioactivity obtained was standardized in cpm/g of sample. The extraction percent of the radioactivity in this procedure was approximately 82% for liver and 78% for muscle.

After the measurement of radioactivity, the content of protein in each fraction was determined by measurement of absorbance at 280 nm with a Hitachi 124 DB spectrophotometer.

The molecular weight of the protein was estimated on a calibration curve which was obtained using molecular weight markers such as catalase (M=230,000), ribonuclease A(M=13,700), cytochrome C(M=12,400), insulin (M=6,000) and 57Co cyanocobalamin(M=1,370).

Results and Discussion

Fig. 1 shows the uptake-excretion curves of 137Cs in the liver of Lateolabrax japonicus marked through the environmental sea water and food. The shape of these curves was almost the same as those reported so far. Figs. 2 and 3 show the profiles of the gel filtration of the samples corresponding to double circles in Fig. 1. Most of 137Cs in the liver appeared to be bound to a fixed constituent and not to pass into other constituents with time. This tendency was observed in both fish contaminated through sea water and food, and also in the muscle, intestine, kidney and gill. The molecular weight of this constituent was estimated to be 1,100-1,300 from the gel filtration on Sephadex G-25. Thus, this constituent which has a strong affinity for 137Cs in vivo, seems to be a kind
Fig. 2. $^{137}\text{Cs}$ profiles of the gel filtration on Sephadex G-25 for the fractions corresponding to double circles in (A) of Fig. 1.

Fig. 3. $^{137}\text{Cs}$ profiles of the gel filtration on Sephadex G-75 for the fractions corresponding to double circles in (B) of Fig. 1.

Fig. 4. An uptake-excretion curve of $^{60}\text{Co}$ in the liver of Serrula purpurascens marked through sea water.

of peptide which exists in all normal tissues of fish the organisms, and the presence of this substance in the samples was confirmed from the measurement of absorbancy at 280 nm.

Fig. 4 shows the uptake-excretion curve of $^{60}\text{Co}$ through sea water in the liver of Serrula purpurascens and Fig. 5 shows the profiles of the gel filtration on Sephadex G-75 of the samples correspond-
The most remarkable feature in Fig. 5 was the presence of two clear radioactivity peaks. The radioactivity appeared to transfer from a low molecular weight protein to a high one in the uptake and the reverse phenomenon was observed in the excretion. Figs. 6 and 7 show an uptake-excretion curve of $^{60}$Co and the profiles of gel filtration for the liver of Seriola purpurascens marked through food, respectively. The radioactivity in the liver went up to the maximum at 24 h after feeding and dropped to almost half within a day but the rest decreased gradually throughout the period of this experiment. As shown in Fig. 7, the behavior of $^{60}$Co absorbed from the gut wall was found to be practically the same as that from the gill (Fig. 5). Namely, although the total radioactivity of $^{60}$Co in the crude extract of the liver used for the gel filtration was approximately $1 \times 10^6$ cpm on the 1st day after the oral administration, 70\% ($7 \times 10^5$ cpm) was bound to a high molecular weight protein. However, this radioactivity peak fell gradually with time.

The ratio (%) of $^{60}$Co bound to a low molecular

---

**Fig. 5.** $^{60}$Co profiles of the gel filtration on Sephadex G-75 for the fractions corresponding to double circles in Fig. 4.

**Fig. 6.** An uptake-excretion curve of $^{60}$Co in the liver of Seriola purpurascens marked through food.

**Fig. 7.** $^{60}$Co profiles of the gel filtration on Sephadex G-75 for the fractions corresponding to double circles in Fig. 6.
weight substance to the total radioactivity continued to increase and went up to 68% on the 20th day of the excretion. It appears that \(^{60}\text{Co}\) bound to the high molecular weight protein passes into the low one with time in the excretion.

Thus, it was found that \(^{60}\text{Co}\) bound to the two substances did not increase or decrease at the same turnover rate. Therefore, this suggests that these two substances in the liver have each inherent turnover rate for \(^{60}\text{Co}\).

The profiles of the gel filtration of \(^{65}\text{Zn}\) were really complicated. They varied widely with species of fish, tissues or organs even in the same fish and pathways of the uptake. The gel filtration patterns of \(^{65}\text{Zn}\) in the liver of three different species of fish labelled through two different pathways are shown in Fig. 8. The graphs (A) show the patterns on Sephadex G-75 after keeping the three different species of fish for a week in the sea water contaminated by \(^{65}\text{Zn}\), and ones (B) show the patterns in the same species of fish on the 1st day after single administration of the soft parts of \textit{Gomphina melanogaster} labelled by \(^{65}\text{Zn}\) as food.

Most of \(^{65}\text{Zn}\) were bound apparently to more than two kinds of proteins and/or peptides in the liver. The radioactivity peak in the region of 100 ml elution volume was not found in \(^{137}\text{Cs}\) and \(^{60}\text{Co}\), but clearly observed in \(^{65}\text{Zn}\). This protein fraction was estimated to have a molecular weight of approximately 12,000, probably being a metallothioneic one\(^\text{5-7}\).

Thus, it is considered that there exist some rules peculiar to each radionuclide in the binding of radionuclides to proteins in fish, and it is one of the important subjects to elucidate the rules in order to know the mechanism of radioactive contamination of marine fish.

References
\begin{enumerate}
\item J. R. REED: Health Physics, 21, 835–844 (1971).
\end{enumerate}