STUDIES ON THE RADIOLOGICAL CONTAMINATION OF FISHES

III. RADIOCHEMICAL STUDIES OF CONTAMINATED FISH

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(Received: August 25th, 1955)

1. INTRODUCTION

The distribution of radioactivity in fish bodies had been made clearly by our observations on "Shunkotsu Maru". It was also observed that the radioactivity in one organ of a fish was quite different from that of another. However, the radioactive elements contained in fish bodies were left un-identified.

The present work was undertaken to meet the urgent necessity of identification of the radioactive elements contained, thereby the results could guarantee the safety on the edibility of radiologically contaminated fishes from the food sanitation's view points and, further, to obtaining the fundamental knowledge of the influence of radioactivity upon pelagic fishes.

2. DECAY RATES AND ABSORPTION CURVES OF THE SAMPLES

a. Materials and methods

Chemical separation of the radioactive elements was not conducted in the experiments carried out on the expedition ship "Shunkotsu Maru". However, decay rates and absorption curves gave some indication of radioactive elements existed in various fish bodies.

The samples used for measuring the decay of radioisotopes contained in fish bodies were the dried material from 1 g of wet minced tissue in stainless steel dishes (25×5 mm). No correction was made for self absorption.

As for the absorption curves, the samples were prepared from an albacore caught on June 12, at the station 17, about 80 miles distant from Bikini Atoll to the west. Ashing of samples was commenced at the National Institute of Health in early October, 1954, and was continued until the end of the same month. The organs or tissues were dried in an electric oven maintained at 100°C. The dried material was then ashed in an electric muffle thermostatically controled at 500°C until it became ash completely with occasional addition of fuming HNO_3 in order to promote ignition. The ash thickness of the samples was 2 mg/cm² (in the case of white muscle, the thickness was 20 mg/cm², and those for dark muscle and the contents of pyloric coeca were 6 mg/cm²).

Table 1. Radioactivity detected in various organs and tissues

Fish species	Station	Date of catch	Body length (cm)	Body wt. (kg)	Sex	Gross count (cpm) at distance of 10cm			Skin (including scales)	Dorsal muscle	Ventral muscle	Dark muscle
						Surface	Gill	Viscera				
Yellow fin tuna	17	June12	135.3	44.6	♀	500	1500	3500	40 4.2	10 -0.7	15	45 7.3
	"	"	133.2	48.8	♀	1300	3600	8000	130 23.3	18 1.3	0	110 14.9
	"	"	135.4	45.4	♀	270	840	2000	25 2.8	15 0.9	0	54 1.6
	"	"	122.5	36.7	♂	620	1900	2000	120 16.2	11 -0.7	14	100 21.2
	"	"	138.8	52.5	♂	380	1200	3000	27 5.6	12 -1.5	0	10 5.2
Big eye tuna	"	"	136.4	57.7	♂	210	500	3000	0 4.9	0 6.3	0	10 8.2
Albacore	"	"	102.5	24.7	♀	600	3600	7000	50/0.8g 13.6	10 3.5	10	120 49.5
Skip-jack	20	June19	70.0	7.9	♂	3500	10000	24000	730 418.5	160 43.2	180 59.8	580 156
Black mar-line	17	June12	162.3	54.8	♂	500	1000	1800	50 8.2	12 -1.9	13 -0.4	25 2.9

Numericals are shown in cpm per dried sample from 1 g of wet material.

Figures in Roman are those measured on the ship and in Gothic at the

For measuring decay and absorption, the samples were placed on the first shelf of a sample holder and were counted with a 3.9 mg/cm² end window GM tube and Radiation Counter of Model 32 Type (Scientific Research Institute Ltd.).

b. Experimental results

The results on the decay rate with the samples prepared on the ship as well as those on the pattern of the absorption curves with each ashed sample are shown in Table 1 and Fig. 1, in which marked differences in the amount and kinds of the radioactive elements in various organs and tissues are observed.

The most conspicuous decay was detected in gall. After about three months, the cpm of liver and other organs was found decreased to about 1/10, whereas that of spleens, kidneys, and gonads was seen to maintain a comparatively high level, which suggested the presence of some long-lived elements in these organs. The decay rate of pyloric coeca and intestinal contents varied from one to another; the fact may have depended on the food they consumed. Considerably high cpm of γ -ray were detected in spleen, gonad, liver, muscle and many other organs.

of contaminated fishes caught by the crew of "Shunkotsu Maru"

Gill	Blood	Heart	Bile	Pylo- ric coeca	Spleen	Sto- mach wall	Inte- stinal wall	Inte- stinal con- tent	Gonad	Kid- ney	Bone	Liver (tip of left blade)
300	340		210	3400	980		700	7700	300	1600	55	1900
42.2			19.4	564	436			159			10.9	214
490	630	160	1400	1500	3200	250		18000	1000	210	15/0.6g	3000
		38	37.3	344	2407	65		5660	387	114	10	371
110	210	35	3500		3500	270	220	3600	80	830	0	1700
8.5		5.5	46.8		564	141	67.5	644	8.5	143	1.8	50.2
480	480		1300/0.95g	5300	640	240	550	1100	200	1800	30	6000
6.8			12.8			36.2	61.8		32.8		13.5	
240	200	54	1300	2100	460	350	890	3200	50	500	40	1400
51.9		17.5	28.9	407	195	33	160		24.2	141	3.5	221
110	130	70	730	2400	270	140	1300	3700		470	0	1100
21.2		6.2	-2.8	447	162	13.9	224	702		110.5	1.9	117
340	680	130	180	2800		640	600	2600	140	2900	40	2600
97.2		50.9		602			129	334	36.5	848		545
1900		1800	3400	29500	5050/0.7g	5900	4800	38000	Base 1300 402	21000	420	47000
474		475	194.7	6452	1463	2462	1830		Tip 1200 343			7690
320	230/ 0.95g	65	2500	2200	210	90	160	1000	130	430	0	1500
55.2		10.2	30.9	382.5	77.2	13.9		31.5	22.2	116	5.6	

laboratory of the National Institute of Health 3 months after.

3. RADIOCHEMICAL AND PHYSICAL ANALYSES

Separation and identification of radioisotopes in each organ and tissue of contaminated fishes was carried out with an albacore (*Germo germo*) and a big eye tuna (*Parathunnus mebachii*) collected during the expedition by "Shunkotsu Maru".

a. Analyses by the qualitative chemical grouping with carriers

(1) *Methods*: The group separation with carriers was conducted by means of an ordinary qualitative analysis of H₂S method, as illustrated in Fig. 2. The carriers used were 5 mg of each Fe⁺⁺, Cu⁺⁺, Ca⁺⁺, and Sr⁺⁺. On account of high content of phosphate in fish organs, common inorganic procedure was not available¹⁾. Therefore, the following procedures were adopted: The residue insoluble in 2 N HCl was taken off (Group I). The remaining solution was diluted ten-fold, in which the concentration of HCl shifted to 0.2 N, and the elements of the Group II were precipitated in the form of sulfides adding with the carriers before-mentioned. The precipitate was filtered off through a filter

Fig. 1. Absorption curves with each ashed sample of various organs and tissues of an albacore (No. 80)

Fig. 2. Radiochemical group separation with carriers

paper of 1.5 cm dia. which was settled on a glass filter (Group II). After heating the filtrate to expel H_2S , 1 cc of concn. H_2SO_4 was added and evaporated until its volume became smaller. After cooling, 10 cc of water and 30 cc of ethanol were added, then the separation of the precipitate of Group IV was undertaken. The remaining solution was heated in order to evaporate excess ethanol out, thereafter added with 1 cc of 3 M NH_4Cl and made it weak alkaline with 6 N NH_4OH . After gentle boiling for 10 minutes, the precipitate produced (Group III A) was separated. Filtrate was bubbled with H_2S for 3 minutes, and then the precipitate produced was filtered off (Group III B). The remaining filtrate was then evaporated to dryness, and the activity counted was regarded as that of Group V. When necessary, re-precipitations were undertaken adding with hold-back carriers to accomplish the purification with the precipitate of each group separately.

The samples thus separated were counted with the same GM end window tube and scaling circuit used in the previous experiments. In no case, correction of the self-absorption was made.

Table 2. Results of the group separation with various tissues and organs of albacore (No. 80)

	I	II	III		IV	V	Weight of ashed sample	Date of analyses
			A	B				
Liver	233.8 ± 7.5	-4.4 ± 2.7	572.0 ± 16		95.0 ± 3.5	10.8 ± 2.8	mg 30	Oct. 6
Gonad	2469.8 ± 35	8.4 ± 2.6	494.3 ± 16		20.6 ± 3.5	6.0 ± 2.8	30	Oct. 6
Kidney	281.0 ± 13	8.0 ± 2.5	$1,062.0 \pm 23$		404.0 ± 14	7.0 ± 2.5	10	Sept. 21
Intestine	132.0 ± 4.7	3.5 ± 3.2	566.4 ± 14		13.5 ± 2.8	-6.3 ± 2.8	30	Sept. 22
White muscle	227.6 ± 9.6	16.0 ± 3.8	149.9 ± 8.0		1.2 ± 4.1	-2.4 ± 3.7	30	Oct. 15
Dark muscle	174.6 ± 8.6	-4.4 ± 3.7	185.6 ± 8.2		3.2 ± 4.0	-1.4 ± 4.0	30	Oct. 15
Pyloric coeca	86.5 ± 5.6	4.0 ± 3.6	10.0 ± 3.9	435 ± 13	67.0 ± 5.9	0 ± 3.6	30	Oct. 20
Stomach	201.6 ± 8.9	-0.4 ± 3.5	-4.4 ± 3.2	500 ± 4.5	72.0 ± 6.0	1.6 ± 4.1	30	Oct. 20

Carriers used were each 5 mg of Fe^{59} , Cu^{64} , Ca^{45} , Sr^{90} .

(2) *Results*: Results thus obtained are shown in Tables 2 and 3, in which it may be observed that the highest radioactivity was found in Group III. It is important that in the case of fish, Group III B was detected with considerably high counts. Moreover, it was occasionally found with even higher counts than III A. On the other hand, the analytical data revealed that the Group III A (the rare-earth elements) was contained in the largest amount in the case of the ashes collected on "Fukuryu Maru No. 5"²⁾ (so-called "Bikini Ashes"), vegetables or

other agricultural products³⁾ and sea water collected near Bikini⁴⁾. Group IV and II came next and Group III B was found with the lowest count. Group I and II were often detected in an appreciable amount in fish organs especially in the gonad of albacore (see Table 2).

Table 3. Results of the group separation with various tissues and organs of big-eye tuna (No. 16)

	I	II	III		IV	V	Weight of ashed sample mg	Date of analyses
			A	B				
Spleen	5.4±2.9	1.0±2.8	213.0±8.5	180.8±6.8	0.8±2.5	3.0±2.9	100	Nov. 17.
Liver	4.0±2.9	15.8±3.3	243.0±8.4	413.1±12	50.4±4.2	5.0±2.9	100	„
Kidney	3.0±2.9	0.6±2.8	104.4±5.4	478.5±13	8.8±3.1	0.4±2.8	100	„
Pyloric coeca	3.2±2.9	4.0±2.9	48.4±4.2	270.1±8.8	24.2±3.6	-3.6±2.7	100	„

Carriers added were each 5 mg of Fe⁵⁵, Cu⁶⁴, Ca⁴⁵, Sr⁹⁰.

The inconsistent results as described in the above made us reconsider the procedures for separation we adopted. Consequently, it was found that the ashes often became insoluble form in hot HCl even in *aqua regia*, which does not justify to regard the insoluble residual matter as Group I. Moreover, mutual contamination to some extent occurred inevitably in each separation; this tendency was remarkably observed between Ce¹⁴⁴ (Group III A) and Group IV. Therefore, it may be considered that the results obtained from such a brief analytical method will show merely a relative distribution of activities of each group of elements contained in fish organs.

It is a noticeable fact that the activity detected in various samples of Group IV, in which such harmful elements as Sr⁹⁰, Sr⁸⁹ and so forth are included, was slight or even negligible.

b. Group separation with ion exchange resin

In order to find out the kinds and amounts of radioactive elements in contaminated fish organs, ion exchange method, using Dowex-50, was applied for the separation. Prior to the present analyses by the author, Okano, a research worker of the Scientific Research Institute, who participated in the similar study, had presumed the existence of Zn⁶⁵ in the material using a scintillation counter⁵⁾. His presumption was based on the energy value of γ -ray observed in the spectrum of the muscle of skipjack (*Katsuwonus pelamis*), which was caught by the crew of "Shunkotsu Maru". Therefore, in the first step, the author's main effort was concentrated to the separation and identification of Zn⁶⁵, the presence of which had never been reported in the "Bikini Ashes".

(1) *Samples and method*: Ashed samples of various organs and tissues of an albacore and a big eye tuna caught by the crew of "Shunkotsu Maru" on June 12, 1954, near Bikini Atoll, were used for the present study. The ion exchange resin employed was Dowex-50, a cationic type resin. In order to separate radioactive elements contained in the samples, 0.2 N HCl, 0.5% oxalic acid and 5% ammonium citrate buffer (pH 3.5, 4.1, 4.6, 5.1, 5.6 and 6.4) were used as the eluants²). The flow rate was 0.5–1.0 cc/min., and each effluent was collected separately in the amount of 5–20 cc by means of a fraction collector. Each fraction thus collected was poured into a crucible, was dried in an electric oven, and then ashed in an electric muffle at 500°C until it became complete ashes, which was transferred into a stainless steel dish with a small amount of water and was dried. The radioactivity of the sample was determined by an end window GM tube and scaler. (Equipment and condition were the same as written in Chapter 1,a).

(2) *Results*: Among the elution curves thus figured with those samples, the representative curves of the muscle and liver of albacore are shown in Figs. 3 and 4. The data of the appreciable activities appeared in various portions of effluent are summarized in Table 4.

In the case of muscle tissues, cationic radioactivity was found in the fractions of 0.5% oxalic acid and of 5% ammonium citrate buffer at pH 4.1; the former was far less than the latter. On the other hand, with viscera, higher activity was counted compared with that with the muscle, especially with kidney, liver and pyloric coeca, in both fractions above-described. Also, considerable activity appeared in the effluents at pH 4.6. Activity was hardly detectable in the fraction, so-called anionic one, eluted with 0.2 N HCl. Further analyses will form the following chapter.

c. Identification of the radioisotope eluted with 5% ammonium citrate at pH 4.1

(1) *Chemical confirmation*: Being encouraged by Okano's finding, further analyses were made, with an intention to identify the radioactive element contained in the pH 4.1 fraction, which showed the highest count in the muscle of albacore. Group separation of radioisotope of this fraction was undertaken according to the scheme cited in Fig. 5. The figures in Fig. 5 were obtained with the sample of kidney (albacore), in which the most active portion in the pH 4.1 fraction was separated in groups (I to V) by means of the qualitative analysis with carriers. The carriers added were 1 mg of Ag⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Sr²⁺. As shown in Fig. 5, the element obtained was verified to belong to Group III B. Further analysis with the intention to identify the portion thus obtained proceeded using carriers of 1 mg of Cu²⁺, Ni²⁺, Mn²⁺, Co²⁺, Fe³⁺ and Zn²⁺. The highest activity was found in the portion of Zn, this fact seemed to be indicative that the radioisotope contained in the pH 4.1 fraction of kidney was Zn⁶⁵. Identical experiments were conducted on every portion of the effluents at pH 4.1 of various fish organs, and just the same results as that with kidney were obtained.

Fig. 3. Elution curve of the muscle of albacore

Date of analyses Nov. 18-27, 1954. Weight of ashed sample examined; 3.0 g
Column used; cationic exchange resin, Dowex-50
110 g (1.6×60 cm)

Fig. 4. Elution curve of the liver of albacore

Date of analyses; Dec. 3-8, 1954. Weight of ashed sample examined; 0.3 g
Column; Dowex-50 25 g (0.9×50 cm)

Table 4. Maximum radioactivity detected in each fraction of the effluents by complex elution of ion exchange resin

Tissues and organs	Ash wt. analysed	Column Dowex-50	Maximum radioactivity detected in each fraction								Date for analyses
			0.2 N HCl	0.5% Oxalic acid	pH 3.5 NH ₄ -citr.	pH 4.1	pH 4.6	pH 5.1	pH 5.6	pH 6.4	
Kidney (albacore)	0.3 g	25	cpm 38.6	cpm 76.6	cpm 20.0	cpm 3070	cpm 582.9	—	—	—	Oct. 16~25 (1954)
Pyloric coeca (")	0.3	25	58.9	32.1	—	362.0	175.4	—	—	—	Nov. 2~9
Dark muscle (")	3.0	110	—	41.6	—	582.2	16.6	—	—	—	Nov. 9~15
White muscle (")	3.0	110	4.4	55.1	—	217.9	20.0	—	—	—	Nov. 19~27
Liver (")	0.3	25	78.9	1134.5	—	4929.4	3979.0	—	—	—	Dec. 3~8
Gonad (")	0.3	25	10.5	78.6	—	3189.8	14.6	—	—	—	Dec. 11~17
Skin & scales (")	3.0	100	—	14.8	—	49.2	—	—	—	—	Feb. 12~17 (1955)
White muscle (Big-eye tuna)	3.0	110	—	18.6	—	32.6	10.6	—	12.6	—	Dec. 7~13 (1954)
Spleen (")	0.14	25	9.5	59.5	—	341.7	—	—	—	—	Feb. 12~17 (1955)
Pyloric coeca (")	1.0	25	12.7	30.3	—	177.3	20.8	—	—	—	Feb. 22~ Mar. 2
Liver (")	1.0	25	13.1	257.1	—	289.2	17.5	—	—	—	Mar. 5~12
Addendum			anionic ion fraction			Zn ⁶⁵ was detected					

*—means almost same as the natural background or negligible count detected

Fig. 5. Group separation and identification of radioisotopes contained in the effluent when eluted with ammonium citrate at pH 4.1

Sample analysed: most active portion among the pH 4.1 effluents obtained from the sample of kidney (albacore, No. 80)

Ashed sample (one portion of pH 4.1 fraction)

(2) Absorption curve:

As for the absorption curves, ashed samples of various organs, a portion of the pH 4.1 fraction, were placed on the first shelf of a sample holder and counted with a 3.9 mg/cm² end window GM tube and Radiation Counter. When the activity was found low, measurement was carried out with a 2.8 mg/cm² end window GM tube and Tracer Lab's Autoscaler. Absorbers used were tin foils and aluminium plates. Since Zn⁶⁵ is one of the k-capture elements, the $E_{\max}$ of β ray would not be obtainable from the absorption curve with aluminium absorbers. In Figs. 6 and 7, absorption curves are plotted for the pH 4.1 fraction in various samples. The general patterns of these curves are similar to each other

Fig. 6. Absorption curves of samples prepared from the pH 4.1 fraction of various organs with aluminium absorbers

and seems to agree with that of Zn⁶⁵. Further, the pattern of the absorption curves of samples prepared from the pH 4.1 fraction of various organs with Sn absorbers coincided with that of Zn⁶⁵ (see Fig. 7).

(3) *Results:*

The results described in the above indicated that the radioisotope existed in the fraction of pH 4.1 eluted with 5% ammonium citrate was identical with Zn⁶⁵.

Fig. 7. Absorption curves of samples prepared from the pH 4.1 fraction of various organs with tin-foil absorbers

4. DISCUSSION

Concurrently with our experiment, Yamada et al⁶⁾ of the Tokai Regional Fisheries Research Laboratory, who participated in the similar study, independently made analyses employing cation exchange resin (Dowex-50) on the ashed sample of the muscle of skipjack caught at the station 20. They also confirmed the presence of Zn^{65} in the fraction separated with 5% ammonium citrate buffer of pH 4.18. The findings were further confirmed by Saeki et al⁷⁾ of the Tokyo University and Nagasawa et al of the National Hygienic Laboratory. These findings indicate the wide distribution of Zn^{65} in various fishes. The origin of Zn^{65} has not yet been clearly known but it is most likely that it has been formed from certain metal parts induced by the effect of neutron emitted at the explosion of hydrogen bomb.

It is a matter of importance as well as of interest that such radioactive element as Zn^{65} which is scarcely detected in sea water, was condensed and

accumulated in marine organisms especially in fish bodies.

If such harmful element as Sr^{90} is contained in the edible part of fish, especially in the muscle of tuna, it would form a serious problem from the view points of food sanitation. However, according to Knobf's report⁸⁾ concerning the contamination of fresh-water fishes in White Oak Lake and in Clinch River, high activity was detected in bones or scales, whereas comparatively low in muscles, of which 97% was proved to be Cs^{137} and the remaining minute amount was found to be Sr^{89} , Sr^{90} and Y^{90} . The report⁹⁾ on the radiological survey of Bikini and other atolls following the test explosion of atomic bomb in 1949 stated that Ce^{144} and its daughter Pr^{144} occupied the greater part of radioactive elements in fish tissues and in other materials. On the other hand, quick transferring without appreciable accumulation was observed following the experimental inoculation of Sr^{90} intramuscularly carried out by Hiyama et al¹⁰⁾ of the Tokyo University. Our investigations along the same line also indicated the low activity of Group IV in the muscles.

In our experiments, Zn^{65} was detected from various parts of contaminated fishes, though the fact does not necessarily deny the presence of other radioactive elements, probably comparatively long-lived isotopes. Separation and identification of these elements are now in progress, especially of the elements detected in the fraction of 0.5% oxalic acid and in the 5% ammonium citrate effluent at pH 4.6.

5. SUMMARY AND CONCLUSION

1. From the decay rates and absorption curves of the samples prepared from various organs and tissues of contaminated fishes caught the crew of "Shunkotsu Maru", marked differences among the radioactive elements contained were presumed both in the kinds and in the amounts.

2. Highest activities in the Group III A (rare earth group) had been reported making experiments with "Bikini Ashes" and other contaminated materials, whereas, radiochemical group separation with carriers of radioactive elements contained in each organ and tissue of contaminated fishes elucidated that the most active element belongs to Group III B.

A small amount of elements belonging to Group IV, including harmful element, was detected in various organs.

3. In the separation of the samples from muscle with cationic ion exchange resin, appreciable activity was found in the fraction eluted with 0.5% oxalic acid and 5% ammonium citrate at pH 4.1, and considerable activity was also observed in the fraction of pH 4.6 from various samples of viscera.

4. The principal constituent of the radioisotopes contained in the muscle of albacore was identified to be Zn^{65} , which was found to be widely distributed various organs and tissues.

5. The presence of some other radioisotopes than Zn^{65} in contaminated fishes is recognized and their identifications are now in progress. Further findings will be presented in the later paper.

6. ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. Y. Tohyama of the National Institute of Health, Dr. K. Amano of the Tokai Regional Fisheries Research Laboratory, Prof. T. Mori of the Tokyo University and Dr. F. Yamazaki of the Scientific Research Institute for their kind guidance. The author is also indebted to Messrs. K. Yamada, H. Tozawa, S. Okano, S. Saeki and A. Takase for their kind advice on the radiochemical analyses. The present work was supported by the special research grant for "Shunkotsu Maru" expedition from the Fisheries Agency and the special grant from the Ministry of Health and Welfare.

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