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Conversion of β-Carotene and Retinol$_1$ to Retinol$_2$ in Freshwater Fish

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Summary

In vivo and *in vitro* conversion of β-carotene to retinol$_1$ and retinol$_2$, and retinol$_1$ to retinol$_2$ was demonstrated with two fresh-water fish species, "Funa" *Carassius auratus*) and "Raigyo" (*Channa argus* CANTOR), using the radioactive isotope tracer technique.

The ratio of retinol$_1$ to retinol$_2$, that were biologically synthesized from β-carotene, was different between the two fish species. "Raigyo," naturally containing retinol$_1$ almost exclusively, showed the higher retinol$_1$ formation than "Funa", naturally containing considerable amount of both retinols.

Retinol$_1$, (3-dehydroretinol) with a Carr-Price maximum at 693 nm, was recognized in 1931 as a vitamin A congener in the unsaponifiable fraction of sea-fish liver oils. In 1937, it was prepared in a purer state from the unsaponifiable fractions of the liver oils or eye lipids of fresh-water fish and was designated by partial shynthesis from vitamin A$_1$ aldehyde (retinal$_1$) and from vitamin A$_1$ acid (retinoic acid$_1$). Retinol$_1$ naturally occurs very often in fresh-water fish. Many kinds of fresh-water fishes contain more retinol$_2$ series than that of retinol$_1$, and sometimes almost all of retinol series are retinol$_2$ members. Although in some fresh-water fishes it has been reported that the interconversion of retinol$_1$ and retinol$_2$ can take place, we demonstrated the *in vitro* and *in vivo* conversion of β-carotene and retinol$_1$ to retinol$_2$ by radioisotope tracer techniques using two kinds of fresh-water fishes. One (crucian carp, "Funa", Carassius auratus), contains both retinols at considerable amount and the other ("Raigyo", *Channa argus* CANTOR) contains almost only retinol$_2$ as retinol component.

Experimental Procedure

Two kinds of fresh-water fishes, crucian carp ("Funa", *Carassius auratus*), and "Raigyo" (*Channa argus* CANTOR) were used as experimental fish. All of the fish used were collected from local ponds and stored in the experimental

aqualium for a long time. Water temperature was kept at 20°C. Two to four fishes were used in each experiment.

Carbinol-^{14}C-retinol$_1$ was obtained from the Radiochemical Center in England.

^{14}C-β-Carotene was prepared biosynthetically using *Phycomyces blakesleanus* as the organism and $CH_3\,{}^{14}COOH$ as the source of ^{14}C. Existing position of ^{14}C in β-carotene molecule prepared were 5, 5′, a, a′, 13, 13′.

β-Carotene and retinol$_1$ were disolved in Tween 80 and mixed with distilled water into adequate concentration.

In *in vivo* experimentation, fishes were anesthetised by putting them into 1% urethan solution, and β-carotene or retinol$_1$ solution prepared by the above mentioned method was administered directly into the stomach (in "Raigyo") or the digestive tract(in "Funa") through a polyethylene catheter which was fixed to the injection syringe. Fifty hours after administration, the fishes were killed and various organs were taken as samples.

In *in vitro* experimentation, hepatopancleas (3.55 g) and digestive tract of "Funa" and liver of "Raigyo" were homogenized with 10 ml of physiological salt solution for fresh-water fish, mixed with 20 ml of physiological salt solution and an adequate volume of β-carotene solution and incubated for 90 min. at 30°C. The digestive tract of the "Raigyo" was taken from the anesthetized fish, 1/6 of total length was cut off from the upper part and 1/3 from the end. β-Carotene solution was administered inside of it. It was tied at both ends, put into 15 ml of physiological salt solution and incubated for 90 min. at 30°C.

All the samples were saponified with NaOH-ethanol solution at the NaOH concentration of 11N for two hours, then an equal volume of distilled water was added. It was moved into a separating funnel and the unsaponifiable fraction was extracted with ethyl ether. After distilling the ethyl ether, the residue was redisolved in petrolium ether.

β-Carotene was separated by almina column chromatography in the usual way.

Separation and isolation of retinol$_1$ and retinol$_2$ in the retinol fraction obtained from almina column was fairly accomplished by the following procedure. Samples were treated with HCl-ethanol at the concentration of 1/30 N, for 40 min. at 20°C. By this treatment, retinol$_1$ and retinol$_2$ were changed to their anhydroderivatives and were easily separated by a silica gel thin layer chromatography, using petrolium ether-ethyl ether (90:10) solution as a developing solvent.

Absorption spectra of isolated β-carotene, anhydroretinol$_1$ and anhydroretinol$_2$ from the fish smaple by the above procedure are shown in Fig. 1.

The total analysis procedure is shown in Fig. 2.

Each band on the thin layer plate was scraped off, suspended with Cab-O-Sil and counted with the scintilation counter.

All of the organic solvents used were purified and redistilled in the usual manner.

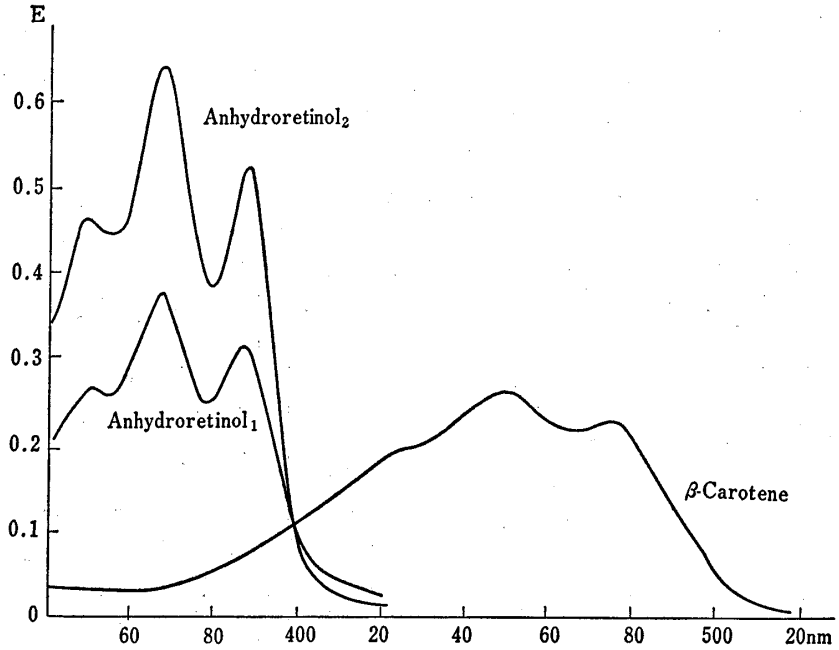

Fig. 1. Absorption spectra of isolated β-carotene, anhydroretinol$_1$ and anhydroretinol$_2$.

Results and Discussion

Table 1 shows the radioactivity of β-carotene, anhydroretinol$_1$ and anhydroretinol$_2$ fractions isolated from hepatopancleas and digestive tract of "Funa" administered ^{14}C-β-carotene. Table 2 is the result of the same experiments obtained with "Raigyo".

TABLE 1. *Radioactivity in c.p.m. β-Carotene, Anhydroretinol$_1$ and Anhydroretinol$_2$ fractions Isolated from Hepatopancleas and Digestive Tract of "Funa" Administered ^{14}C-β-Carotene*

	Fractions	c.p.m.
Hepatopancreas	β-Carotene	131
	Anhydroretinol$_1$	814
	Anhydroretinol$_2$	135
Pyloric caecum	β-Carotene	6, 334
	Anhydroretinol$_1$	278
	Anhydroretinol$_2$	172

Although only in a small amount, β-carotene was converted to retinol$_1$ and retinol$_2$. The ratio of retinol$_1$ to retinol$_2$ biologically synthesized from β-carotene was different between the two fish species; in "Raigyo" the amount of retinol$_2$ was larger than retinol$_1$ while in "Funa" the result was reverse.

Table 3 and table 4 show the results of in vitro experiments designed to demonstrate the conversion of β-carotene to retinol$_1$ and retinol$_2$.

As shown in the Tables, of the added radioactivity as ^{14}C-β-carotene only 33.9–

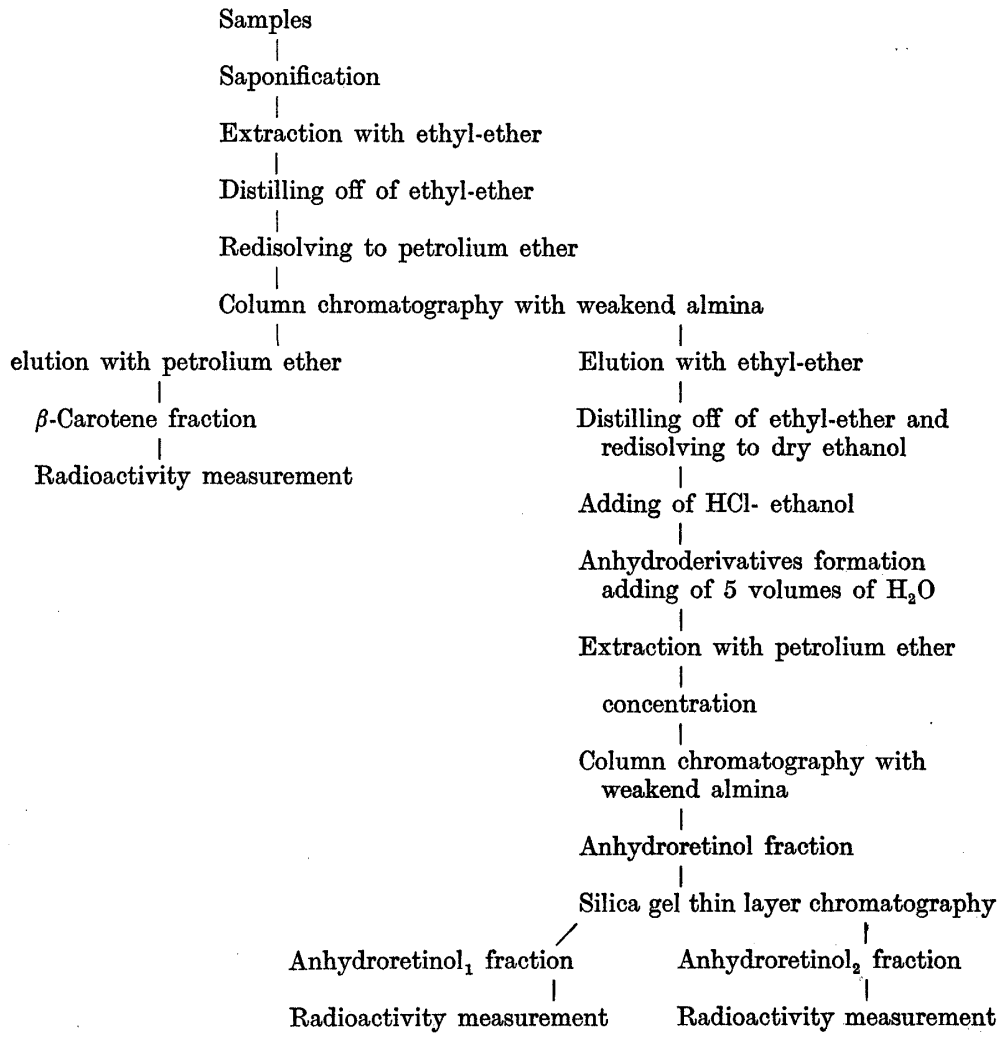

FIG 2 Total analyzation procedure

TABLE 2. *Radioactivity in c.p.m. of β-Carotene, Anhydroretinol$_1$ and Anhydroretinol$_2$ Fractions Isolated from Liver, Pyloric Caecum and of "Raigyo" Administered ^{14}C-β-Carotene*

	Fractions	c.p.m.
Liver	β-Carotene	223
	Anhydroretinol$_1$	—
	Anhydroretinol$_2$	297
Pyloric caecum	β-Carotene	2, 636
	Anhydroretinol$_1$	10
	Anhydroretinol$_2$	25

62.8% was recovered in the β-carotene, retinol$_1$ and retinol$_2$ fraction. This result suggests that the considerable amount of β-carotene added as a substrate was converted to another component. As in the *in vivo* experiments, both radioactive retinols occured in both fish species, and again in "Raigyo" more retinol$_2$ was synthesized than retinol$_1$.

TABLE 3. *Radioactivity of Each Fractions Isolated from the Organs of "Funa" after Incubation with ^{14}C-β-Carotene*

		Radioactivity cpm	Recovery* %
Hepatopancreas	β-Carotene	314, 900	62. 8
	Anhydroretinol$_1$	128	
	Anhydroretinol$_2$	60	
Digestive tract	β-Carotene	75, 200	44. 9
	Anhydroretinol$_1$	118	
	Anhydroretinol$_2$	35	

* % of added radioactivity

TABLE 4. *Radioactivity of Each Fractions Isolated from the Organs of "Raigyo" after Incubation with ^{14}C-β-Carotene*

		Radioactivity cpm	Recovery* %
Liver	β-Carotene	115, 600	33. 9
	Anhydrotetinol$_1$	146	
	Anhydroretinol$_2$	274	
Digestive tract	β-Carotene	20. 100	39. 3
	Anhydroretinol$_1$	79	
	Anhydroretinol$_3$	96	

* % of added radioactivity

TABLE 5. *Radioactivity Distributions among Various Oragns of "Funa" Administered Carbinol-^{14}C-Retinol$_1$ Acetate*

Organs	Weight g	Lipid mg	Radioactivity cpm (%)	Relative radioactivity cpm/g
Hepatopancreas	1. 90	64. 0	145, 000 (40. 6)	76, 500
Kidney	0. 83	12. 3	37, 300 (10. 5)	45. 200
Spleen	0. 36	3. 9	21, 300 (5. 9)	58, 900
Digestive tract	3. 22	51. 1	119, 000 (33. 4)	37, 100
Ovary	14. 66	515. 5	30, 400 (8. 5)	2, 070
Eye	3. 65	22. 2	2, 610 (0. 7)	714
Total			355, 610 (100)	

Radioactivity distributions among various organs of fishes administered with retinol$_1$ acetate labeled with ^{14}C as the carbonyl carbon atom of retinol$_1$ are shown in Table 5 and in Table 6 for "Funa" and "Raigyo". Of the administered radioactivity as ^{14}C-retinol$_1$ acetate, 16.7% in "Funa" and 35.8% in "Raigyo" was recovered in all the organs examined.

The radioactivity was found in all of the organs examined and the hepatopancleas and the digestive tract in "Funa" and the liver in "Raigyo'' were the main places of accumulation.

Table 7 and Table 8 represent the radioactivity of retinol$_1$ and retinol$_1$ fractions,

TABLE 6. *Radioactivity Distributions among Various Organs of "Raigyo" Administered Carbinol-^{14}C-retinol$_1$ acetate*

Organs	Weight g	Lipid mg	Radioactivity cpm (%)	Relative radioactivity cpm/g
Liver	7.72	911.0	456,000(68.4)	59,100
Kidney	2.28	40.4	12,300(1.8)	5,400
Spleen	1.01	20.1	2,780(0.4)	2,770
Stomach	4.09	48.3	42,300(6.4)	10,300
Digestive tract	2.35	55.4	59,400(8.9)	25,200
Pylolic caecum	1.90	30.2	81,500(12.2)	42,600
Ovary	3.72	115.0	11,500(1.7)	3,090
Eye	1.56	18.7	987(0.15)	618
Total			666,767(100)	

TABLE 7. *Radioactivity in Anhydroretinol$_1$ and Anhydroretinol$_2$ Fractions Isolated from Hepatopancreas, Digestive Tract and Eye of "Funa" Administered Carbinol-^{14}C-retinol$_1$ acetate*

Organs	Fractions	Radioactivity cpm
Hepatopancreas	Anhydroretinol$_1$	755,400
	Anhydroretinol$_2$	7,970
Digsetive tract	Anhydroretinol$_1$	24,500
	Anhydroretinol$_2$	77,700
Eye	Anhydroretinol$_1$	2,270
	Anhydroretinol$_2$	4,060

TABLE 8. *Radioactivity in Anhydroretinol$_1$ and Anhydroretinol$_2$ Fractions Isolated from Liver, Digestive Tract and Pylolic Caecum of "Raigyo" Administered Carbinol-^{14}C-retinol$_1$ acetate*

Organs	Fractions	Radioactivity cpm
Liver	Anhydroretinol$_1$	7,880
	Anhydroretinol$_2$	12,300
Digestive tract	Anhydroretinol$_1$	1,150
	Anhydroretinol$_2$	2,320
Pylolic caecum	Anhydroretinol$_1$	473
	Anhydroretinol$_2$	721

isolated from various organs as their anhydroderivatives.

In both fishes, the results clearly showed the *in vivo* conversion of retinol$_1$ to retinol$_2$. As seen in the experiments with ^{14}C-β-carotene, in "Raigyo" radioactivity found in retinol$_2$ was larger than that of retinol$_2$. The eye of "Funa" showed a higher radioactivity in retinol$_2$ than in retinol$_1$. Naito et al (1) reported retinol$_1$ to retinol$_2$ conversion in the eye of sunfish *Lepamis* species.

There are two possible ways to explain the roll of the existence of the two

retinols in these species of animals. (1) The existing ratio of the retinols in certain animals is only a reflection of the ratio of the two retinols contained in its food. It cannot interconvert two retinols, but only can accumulate them as they are. (2) The animal has some specific enzyme system and converts one retinol to another at anywhere in its body. Existence of retinol$_2$ in some kinds of birds, mammals and amphibias has been explained by (1). (4, 5). In the case of fish there are many examples that cannot be explained in that way. For instance, among the two species used in this experiment, "Funa", naturally containing considerable amounts of both retinols (Table 9) in various organs, is omnivorse and its foods in nature do not seem to contain a sufficiant amount of retinol$_2$ to account for the amount in its body. In addition, the other species, "Raigyo" ,naturally contains retinol$_1$ almost exclusively as a retinol component. (Table 9) It is carnivorous and its foods in nature consist of small fishes, adults and larva of amphibia and many other small aquatic animals. So this fish has taken considerable amounts of retinol$_1$ from its foods in nature.

TABLE 9 *Contents of Retinol$_1$ and retinol$_2$ in Various Organs in "Funa" and "Raigyo"*

Organs	"Funa"		"Raigyo"	
	Retinol$_1$ mg%	Retinol$_2$ mg%	Retinol$_1$ mg%	Retinol$_2$ mg%
Liver			0.61	41.4
Hepatopancreas	12.6	13.3		
Eye	9.1	47.7	—	0.50
Gonad	0.1	0.2	—	0.29
Digestive tract	0.2	0.2	—	0.50
Stomach			—	0.12
Flesh	0.1	0.3	—	0.03
Heart	1.4	0.9	—	0.20
Spleen	0.7	0.5	—	0.46
Kidney	0.7	0.6	—	0.12

As in other animals, fish cannot synthesize retinol from lower molecular materials. They can only hydrolize certain carotenoids and convert them to retinol. Conversion of such carotenoids that have a retinol structure at one or both ends, like β-carotene or cryptoxanthin, has been demonstrated in many animal species. As shown in this experiment, fish can also use β-carotene as the source of retinol$_1$ so that fish obviously possess an enzyme system that catalyzes the cleaving of carotenoids into the coresponding half size components. If there are some carotenoids having the characteristic structure of retinol$_2$, 3,4,didehydro-, in one or both sides of their molecule, in nature and in the foods of certain fish, the fish that takes this food can prepare and accumulate the retinol$_2$. But in the fact this kind of carotenoids has not yet been found in nature. Therefore, in the case of these fishes, it seems reasonable to recognize that the species' specific existing ratio of two retinols found in nature must be attributed to the enzyme systems

that each fish species genetically possesses. In this experiment the conversions of β-carotene to retinol$_1$ and retinol$_2$, and retinol$_1$ to retinol$_2$ were demonstrated *in vivo* and *in vitro*, with two fresh-water fish species, using the radioactive isotope tracer technique. These results clearly indicate the existence of enzyme systems that catalyze the conversion of retinol$_1$ to retinol$_2$. It is interesting that "Raigyo", naturally possessing retinol$_2$ almost exclusively as its retinol component, showed the higher retinol$_2$ formation in every experiment. These results suggest the existence of genetical species' specificity in the enzyme systems that are controling the balance of two retinols.

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