Studies on Astaxanthin Formation in some Fresh-water Fishes

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Studies on Astaxanthin Formation in some Fresh-water Fishes

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^{14}C labelled β-carotene, lutein and zeaxanthin were administered to rainbow trout (*Salmo gairdneri irideus*). 48 hours after the administration, the lipid fractions of integment, flesh, liver and digestive tract were extracted and their radioactivity was measured.

To confirm the conversion of these carotenoids into astaxanthin, the radioactivity of astacene prepared from astaxanthin extracted from all samples was measured. It was confirmed that rainbow trout was not able to convert these three carotenoids to astaxanthin.

Some discussion was made on the formation of astaxanthin in some fresh-water fish species.

In regard to the origin of astaxanthin in fish, it is generally accepted that fishes are divided to two groops. (1) The fishes of the first group have no ability to convert injested carotenoids into astaxanthin. Therefore they only accumulate the astaxanthin contained in their foods. (2) The fishes belonging to the second have the ability to oxidize injested carotenoids and convert them into astaxanthin. In previous papers (1) (2), the authors demonstrated *de novo* conversion of zeaxanthin to astaxanthin in gold fish. Red carp is also able to use zeaxanthin as the source of astaxanthin. (unpublished result).

It has been thought that trout and salmon belong to the first group, but the experimental evidence showing that these fishes are unable to convert the injested carotenoids which are common in their natural foods, such as β-carotene, lutein and zeaxanthin, is not sufficient. In this experiment, in order to confirm the above mentioned phenomena, ^{14}C labelled β-carotene, lutein and zeaxanthin were administered to rainnow trout (*Salmo gairdneri irideus*),

Experimental Procedure

The rainbow trout used were obtained from a local fish firm. Body lengths were 21 to 22 cm, and body weights were 130 to 180 g.

^{14}C-β-carotene was prepared from *Phycomyces blakesleanus* as reported in a previous paper (1). ^{14}C-lutein and ^{14}C-zeaxanthin were prepared from calyx and fruits of *Physalis alkekengi L. Francheti Hort, forma Bunyardii Makino*, according to the methods reported previously (1).

Carotenoids and tween 80 were dissoved in small quantities of chloroform and the chloroform was removed at a reduced pressure under N_2 gas. Then an adequate volume of water was added and stirred with a grass rod to obtain the carotenoid aquaous solution.

The test fishes were anesthetized with MS–222 (Sandoz, Swiss) and an adequate volume of each carotenoid solutions was administered directly into the stomach with a polyethylene catheter. The amount and its radioactivity of each carotenoids administered in one fish were as follows.

β-Carotene	0.43 mg	145,600 dpm
Lutein	1.23 mg	9,466 dpm
Zeaxanthin	0.26 mg	22,848 dpm

The water temperature was maitained at 25°C.

48 hours after the administeration of carotenoid solutions, the test fishes were killed, the lipid fraction was extracted with acetone and the radioactivity was measured. Samples were discoloured with benzoyl peroxide, suspended with Cab-O-Sil and counted with a liquid scintillation counter.

To confirm the radioactivity of astaxanthin in the samples, a standard astaxanthin ester isolated from shrimps was added. Then astacene was prepared by saponification, purifide twice with Silica Gell thin layer chromatography and the radioactivity was measured.

Results and Discussion

Table one shows the radioactivity distribution among various organs.

β-Carotene accumulated mostly in the digestive tract (85.1%) and among other organs with the liver showing the highest radioactivity. While with the xanthophyls (lutein and zeaxanthin), the integment showed a high radioactivity; the % of

TABLE 1. *Radioactivity Distribution among Various Drgans of Rainbow Trout Administered ^{14}C-Zeaxanthin, ^{14}C-lutein and ^{14}C-β-carotene*

	^{14}C-Zeaxanthin		^{14}C-Lutein		^{14}C-β-Carotene	
	dpm	%	dpm	%	dpm	%
Integment	626	42.82	150	31.3	970	5.6
Flesh	375	25.6	71	14.8	348	2.0
Liver	33	2.3	78	16.2	1280	7.4
Digestive tract	428	29.3	180	37.6	14010	85.1
Total	1462	100	479	100	17408	100
Recovery of radioactivity %	3.3		2.5		6.0	
Radioactivity of astacene	Not detected		Not detected		Not detected	

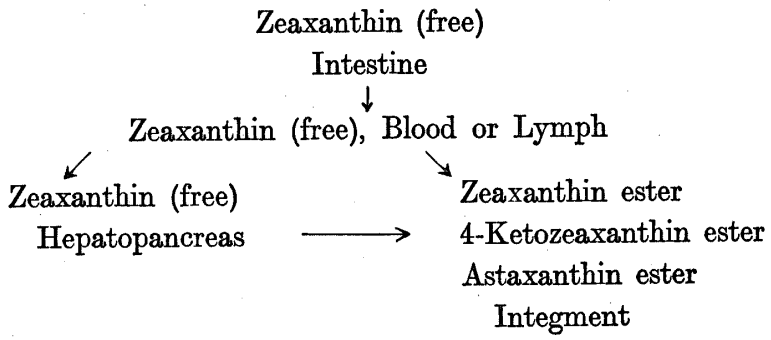
radioactivity in integment to total radioactivity recovered were 40.8% with the zeaxanthin and 31.3% with the lutein.

The recoveries of radioactivity were 6.0% with the β-carotene, 2.5% with the lutein and 2.3% with the zeaxanthin.

No radioactivity was found in the astacene prepared from the astaxanthin in any sample. From these results it is concluded that rainbow trout were not able to convert the three carotenoids used to astaxanthin.

In the previous experiment (3), it was demonstrated in goldfish that cynthiaxanthin (7,8,7′,8′-tetradehydrozeaxanthin) was metabolized to 7,8,7′,8′-tetradehydroastaxanthin, and later (1, 2) the conversion of zeaxanthin to astaxanthin in goldfish was directly confirmed. In regard to the possible precurssor of astaxanthin in goldfish, Katayama et al (4) emphasized that lutein is the main source in nature, according their experimental results. From our experimental results, however, it must be concluded that lutein is oxidized to doradexanthin (4-ketolutein) but it is not further oxidized to astaxanthin. The integment of goldfish administered lutein, becomes yellowish in the early stage and then gradually increases its colour intensity, but its colour is redish orange and not so lucid red as in the case of the administration of astaxanthin or zeaxanthin. The shape of the absorption spectra of main carotenoid is similar to that of 4-keto lutein. No radioactivity was found in the astacene prepared from the astaxanthin fraction which was isolated from the test fish administered ^{14}C-lutein. This indicate that the lutein is not metabolized to astaxanthin in goldfish. While the integment of goldfish administered zeaxanthin, increases its colour intensity and the colour is lucid red. The existence ratio of astaxanthin to total carotenoids in the integment twice as much as in ordinary cases. If lutein were converted to astaxanthin, then the α-structure of lutein must be converted to β-structure of astaxanthin at some step of its pathway. This conversion in nature has not yet been clearly confirmed. In regard to this conversion in fish, Crozier et al. (5) suggested the possibility but it has not been sufficiently confirmed. At least in goldfish and red carp, this coversion seems to be impossible, and the main source of astaxanthin been accumulated in their integment in nature is not lutein but zeaxanthin.

A possible metabolic pathway from zeaxanthin to astaxanthin is as follows:



Goldfish ponds are generally eutrophic and the dominant species of flora are *Scenedesmus* and *cyanophyceae Microcystis*, *Merismopedia*, *Aphanocapsa*, and *Lyngbya*. (6–12). Green algae contains lutein and zeaxanthin as the xanthophyll members; for instance *Scenedesmus* contains lutein 2.5 mg/g dry matter and zeaxanthin 0.5 mg/g dry matter. (13) *Cyanophyceae*, however, contains only astaxanthin. The amount of zeaxanthin in *Microcystis* is 0.3 mg/g drymatter and that in *Merismopedia* is 0.5 mg/g dry matter. (14) Green algae contains astaxanthin, also. It is reported that the astaxanthin found in green algae, such as *Chlamidomonas*, *Haematococcus*, *Ankistrodesmus*, *Chlorella*, *Chlorococcum* and *Scenedesmus*, is synthesized as a secondary carotenoid under certain physiological conditions. (15) The amounts of synthesized astaxanthin are 1.39 mg/g dry matter in *Ankistrodesmus braunii*, 0.66 mg/g dry matterin *Chlorella fusca*, 1.42 mg/g dry matter in *Chlorella zofimgiensis*, 0.52 mg/g dry matter in *Scenedesmus* sp. and 1.29 mg/g dry matter in *Haematococcus pluvialis*. These values are equal to or larger than the lutein content.

Goldfish is omnivorous and feeds on these phyto plankton freely. Therefore the flora of phytoplankton and their carotenoid compositions suggest that zeaxanthin, which is the precursor of astaxanthin, and astaxanthin itself are supplied to goldfish sufficiently as the source of the astaxanthin accumulated in the integment.

In the case of rainbow trout, the carotenoid metabolism seems to be different from that of goldfish. It belongs to the first group, that have no ability to oxidize the injested xanthophylls, lutein and zeaxanthin, and β-carotene. They only accumulate the injested astaxanthin as the colour component of its flesh, egg and integment. Although in regard to the formation of astaxanthin from other carotenoids *de novo* (such as in Crustacea), following process has been demonstrated, such a pathway has not yet been found in any kind of fish.

β-carotene→echinenone→canthaxanthin→phenicoxanthin→astaxanthin

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