マダイにおける放射性エイコサペンタエン酸の組織への取込み

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Tissue Uptake of Radioactive Eicosapentaenoic Acid in the Red Sea Bream

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The present paper deals with the uptake of radioactivity into the organs and tissues in the red sea bream *Chrysophrys major*, 24 h after injection of [^{14}C] eicosapentaenoic acid (20: 5ω3). [^{14}C] 20: 5ω3 was biologically prepared by incubating a marine *Chlorella* with [1-^{14}C] linolenic acid. The incorporation of radioactivity into the organs and tissues was determined by radioactive measurements with a liquid scintillation counter after combustion of each tissue or organ and also by autoradiography of the total body of the red sea bream.

The results of the present study showed that radioactivity from [^{14}C] 20: 5ω3 was mainly incorporated into the gall bladder, swim bladder, liver, and pyloric caecum, suggesting that dietary 20: 5ω3 may take part in some physiologically important metabolism in these organs.

Fish[1,2] and crustaceans[3] require the ω3 series of fatty acids such as linolenic (18: 3ω3), eicosapentaenoic (20: 5ω3), and docosahexaenoic (22: 6ω3) acids as essential fatty acids (EFA) for their growth in contrast with mammals which require the ω6 series of fatty acids, linoleic (18: 2ω6) and arachidonic (20: 4ω6) acids. Especially, the marine fish such as the red sea bream etc.[2,4–7] necessitate strictly the highly unsaturated fatty acids (ω3 HUFA), 20: 5ω3 and 22: 6ω3, for optimum growth. Although many reports have shown the high nutritive values of ω3 HUFA for various fish by the feeding trials, little is known on the physiological and metabolical roles of dietary ω3 HUFA in the marine fish. As a part of investigating the possible role of dietary 20: 5ω3, we examined the uptake of radioactive 20: 5ω3 into the organs and tissues of the red sea bream *Chrysophrys major*. The present paper deals with these results and discussion.

Materials and Methods

Preparation of Radioactive 20: 5ω3

Radioactive 20: 5ω3 was biologically prepared by incubating a marine type of *Chlorella* (100 g, wet weight) with 20 μCi of [1-^{14}C] 18: 3ω3 dissolved in small amounts of 3% aqueous solution of egg albumin at 20°C for 24 h. Lipids were extracted with chloroform-methanol-water[3] and saponified with 10% potassium hydroxide solution in the usual manner. The fatty acids obtained from the saponifiable matters were methylated with 5% hydrogen chloride in methanol to give the mixture of fatty acid methylesters. The fatty acid methylesters (3.0 g) were chromatographed on 5% (w/w) silver nitrate-impregnated Kieselgel G as described previously.[9] The radioactive 20: 5ω3 (specific activity=50,100 dpm/100 mg) so obtained gave a single peak in gas-liquid chromatography on 10% DEGS (3 m×4 mm i. d., column temperature 190°C)[10] and also a sole radioactive spot in thin-layer chromatography on silver nitrate-impregnated Kieselgel[11] followed by autoradiography.

Incorporation of Radioactive 20: 5ω3 into the Organs and Tissues

The red sea bream, 23.5 g in body weight, was received the peritoneal injection of [^{14}C] 20: 5ω3 (100,000 dpm) dissolved in small amounts of ethanol and maintained in an aquarium at 23–24°C. Twenty-four h after injection of [^{14}C] 20: 5ω3, the red sea bream was taken out from the aquarium and dissected to examine the incorporation of radioactivity into the organs and tissues. Radioactivity was measured with a Beckman liquid scintillation counter LS-230 using a toluene solution of PPO (0.6%) and POPOP (0.04%) as a scintillator after combustion of samples with the automatic

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sample combustion system, Aloka ASC-113.

Autoradiography of the Whole Body of Red Sea Bream

The another specimens of red sea bream were injected with (100,000 dpm) in the same manners and conditions as mentioned above, and then the autoradiogram was made according to the usual procedures.[12] The red sea bream was cut into the slices (40 μ in thickness), lyophilized, put in a pile on a X-ray film (Sakura, Type N), and then exposed at 4°C for 87 days. The organs and tissues on the autoradiograms were identified in comparison with the non-autoradiographed slices of red sea bream.

Results and Discussion

Table 1 shows the incorporation of radioactivity into the organs and tissues of red sea bream 24 h after injection of [^{14}C] 20: 5ω3. As expressed in terms of total radioactivity recovered and distribution (%), radioactivity was highly incorporated into the gill, alimentary tract, swim bladder, and gall bladder. The liver, pyloric caecum, skin, and muscle gave considerably high radioactivity, but only low radioactivity was associated with the heart, adipose tissue, brain, and eye. The specific activity (dpm/g of organ or tissue) was also high in the gall bladder, swim bladder, and pyloric caecum as compared with those of other organs and tissues. The above mentioned profile on the incorporation of radioactivity from [^{14}C] 20: 5ω3 was further confirmed by the autoradiogram (Fig. 1) of the whole body of red sea bream. The results of the present study indicated that the injected [^{14}C] 20: 5ω3 was mainly transported to the gall bladder, swim bladder, liver, and pyloric caecum, with suggestion that dietary 20: 5ω3 may take part in some physiologically important metabolism in these organs.

KITAJIMA *et al.*[13] have pointed out that the curvature of verteral column was correlative to the undeveloped swim bladder in the hatchery-reared red sea bream. Later, the occurrence of undeveloped swim bladder has been speculated to be closely related to the nutritive values, especially ω3 HUFA content[14,15] of the rotifers used as a diet.[16] Further nutritional studies on the larval red sea bream have shown that the ω3 HUFA such as 20: 5ω3 and 22: 6ω3 probably play an important role in the development and subsequent opening of swim bladder,[*1-3] although the aeration rate of sea water also seemed to be responsible for the occurrence of undeveloped swim bladder to some extent.[*3]

In mammals, phospholipids rich in polyunsaturated fatty acids are known to be indispensable constituents for the formation of biomembrane

Table 1. Incorporation of radioactivity into the organs and tissues of the red sea bream 24 h after injection of [^{14}C] 20: 5ω3

Organ or tissue	Weight (mg)	Radioactivity		Specific activity (dpm/g of tissue or organ)
		Total activity recovered (dpm)	Distribution (%)*	
Gill	639	2,070	22.3	3,240
Alimentary tract	433	1,770	19.1	4,090
Swim bladder	104	1,020	11.1	9,900
Gall bladder	86	980	10.5	11,500
Muscle	14,280	890	9.6	60
Liver	216	870	9.4	4,010
Pyloric caecum	98	800	8.6	8,190
Skin	1,506	590	6.4	400
Adipose tissue	79	180	1.9	2,240
Heart	21	50	0.5	2,400
Eye	632	40	0.4	60
Brain	116	30	0.3	270

* Percentage of all tissues and organs examined.

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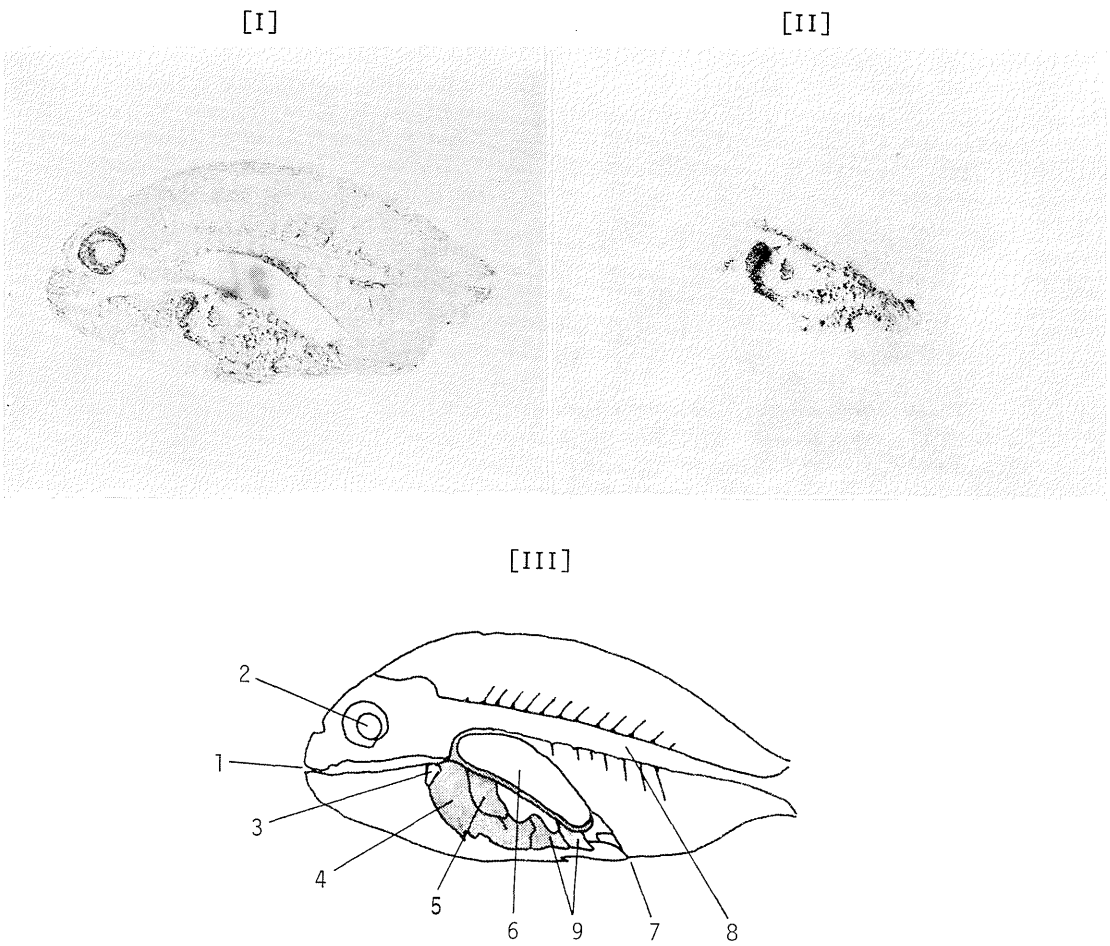

Fig. 1. Autoradiograms of the vertical sections of the red sea bream 24 h after injection of [^{14}C] 20: 5ω3. I, section close to median axis; II, section slightly far from median axis; III, diagrammatic display of the incorporation of radioactivity into the organs. 1, mouth; 2, eye; 3, heart; 4, liver; 5, stomach; 6, swim bladder; 7, anus; 8, spinal column; 9, intestine.

structures and for the manifestation of biomembrane function.[17] The present study showed the remarkable incorporation of radioactivity into the swim bladder in the red sea bream injected with [^{14}C] 20: 5ω3. In consideration of the data on the occurrence of undeveloped swim bladder in the red sea bream, we suspect that dietary 20: 5ω3 is possible important not only as EFA but also as the constituent fatty acids of biomembrane phospholipids; especially in the larval red sea bream, 20: 5ω3 may take part in the development of swim bladder.

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