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EISEI KAGAKU
25 (6) 309—314 (1979)

Studies on the Influence of PCB on Aquatic Organisms. VII.¹⁾ The Effect of Oral Administration of PCB on the Lipid Metabolism of the Carp

YASUYUKI ITO,^{2a)} RYOHEI KIMURA, and TOSHIRO MURATA^{2b)}
Women's Junior College Tokai University^{2a)} and Shizuoka College of Pharmacy^{2b)}

(Received June 18, 1979)

The changes of lipid metabolism in carp caused by feeding a PCB diet were as follows.

- 1) In *in vivo* experiments, total lipid and triglyceride in the carp hepatopancreas increased slightly, and glycogen decreased.
- 2) In *in vivo* experiment, the rate of incorporation of 1-¹⁴C-palmitate into the hepatopancreatic lipid fractions did not change, but the incorporation of radioactivity into blood-plasma lipid increased in the triglyceride and free fatty acid fractions, and decreased in the phospholipid and free cholesterol fractions.
- 3) In *in vitro* experiments, the incorporation of 1-¹⁴C-palmitate into lipids of the hepatopancreas increased slightly in the triglyceride fraction, especially in the α, α' -position of the triglyceride.
- 4) The triglyceride synthetase and α -glycerophosphate dehydrogenase activities in hepatopancreas were slightly increased by PCB administration.

The changes of lipid metabolism in the carp hepatopancreas on PCB administration are discussed in comparison with those in mammals.

Keywords—PCB ; carp, *Cyprinus carpio* ; triglyceride ; triglyceride synthetase ; α -glycerophosphate dehydrogenase ; 1-¹⁴C-palmitate ; lipid metabolism ; glycogen ; hepatopancreas

Introduction

Many authors have reported that the administration of polychlorinated biphenyls (PCB) induces abnormal lipid metabolism in mammals.³⁾

In the previous paper⁴⁾ of this series the authors reported that the serum triglyceride (TG) levels in carp fed with PCB were

much higher than those in the control fish, and it appeared that PCB caused hyperglyceridemia in carp as well as in mammals.^{3a, d, i, j)} However, the mechanism of the effect of PCB on the lipid metabolism in mammals has not yet been clarified, though many hypotheses have been proposed.^{3d, f, h, i)}

In the present study, the authors at-

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- 2) Location : a) 101, Miyamae-cho, Shizuoka 420, Japan ; b) 2-2-1, Oshika, Shizuoka 422, Japan.
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tempted to elucidate the effect of PCB on lipid metabolism in the carp hepatopancreas.

Materials and Methods

1. Reagents—NAD, ATP, glutathione (GSH), coenzyme A (CoA), and bovine serum albumin were purchased from Sigma Chemical Co. Pancreatic lipase was obtained from Nutritional Biochemicals Corp. Aroclor 1248 was obtained from Monsanto Chemical Co. $1\text{-}^{14}\text{C}$ -Palmitic acid (53.2 mCi/mmol) was obtained from Daiichi Pure Chemicals Co., Ltd. 2,5-Diphenyloxazole (PPO), 2,2'-*p*-phenylene-bis-(5-phenyloxazole) (POPOP), and α -glycerophosphate were obtained from Nakarai Chemicals Ltd. All other chemicals were of analytical grade, and were used without further purification.

2. Experimental Fish—Carp, *Cyprinus carpio*, weighing 180–200 g, were kindly supplied by Chubu Shiryō Co., Ltd. and were maintained on a commercial diet for at least 10 days, then were divided into two groups of four.

3. Preparation of PCB Diet—The PCB used in this study was Aroclor 1248, which contains 48% chlorine by weight. An ethanol solution of the PCB was added to the commercial diet and mixed mechanically. The mixture was then made into pellets with a mincing machine. After removing ethanol, the pellets (containing 250 ppm of PCB) were used as a PCB diet.

4. Administration of PCB and $1\text{-}^{14}\text{C}$ -Palmitate—1) *In Vivo* Experiment: Carp were maintained on the PCB diet (*ca.* 1.5 mg PCB/body/day) in circulating water tanks (75 (L) $\times$ 40 (W) $\times$ 45 (D) cm) at 21–23° for 14 days. Two hours after the last feeding, 0.3 ml of 5.0 μCi sodium $1\text{-}^{14}\text{C}$ -palmitate (6 μmol) in 0.75% NaCl solution was injected intraperitoneally. Six hours after the injection, the carp were killed and the radioactivities in lipids of the hepatopancreas and plasma were measured.

2) *In Vitro* Experiment: Each group of carp was fed the control or test diet for 14 days, then

Fig. 1. Incorporation of $1\text{-}^{14}\text{C}$ -Palmitate as a Function of Incubation Period

Carp hepatopancreas $500\times g$ supernatant (equivalent to 0.75 g of hepatopancreas) was incubated at 25° for the indicated periods. To each flask, 0.5 μCi of sodium $1\text{-}^{14}\text{C}$ -palmitate was added.

the hepatopancreas of each carp was removed and homogenized with 3 volumes of cold 0.25 M sucrose. The homogenates obtained were centrifuged at $500\times g$ at 0° for 15 min. The albumin-bound sodium $1\text{-}^{14}\text{C}$ -palmitate was prepared according to Sgoutas and Kummerow.⁵⁾ A reaction mixture consisting of 0.2 ml of 0.5 μCi 10% albumin-bound sodium $1\text{-}^{14}\text{C}$ -palmitate (3 μmol), 3 ml of the $500\times g$ supernatant (equivalent to 0.75 g of hepatopancreas), 4 mM α -glycerophosphate, 4 mM ATP, 8 mM phosphate buffer (pH 7.5), 4 mM MgCl_2 , 1 mM β -mercaptoethanol, 60 μM CoA, and 45 mM KCl–6 mM Tris HCl buffer (pH 7.5) in a total volume of 7.5 ml was incubated at 25° for 40 min. The incubation was stopped by the addition of 20 volumes of chloroform-methanol (2:1, by vol.).⁶⁾ In a preliminary experiment, incorporation of $1\text{-}^{14}\text{C}$ -palmitate into hepatopancreas lipid as a function of time was measured using $500\times g$ supernatant of hepatopancreas of carp fed a normal diet. Based on the results shown in Fig. 1, an incubation period of 40 min was selected.

5. Extraction and Separation of Lipid—

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Total lipid was extracted according to the method of Folch *et al.*⁷⁾ The lipids were separated by thin-layer chromatography (TLC) using plates with 1 mm thick Silica Gel G and developing with petroleum ether-diethyl ether-acetic acid (82 : 18 : 1, by vol.).⁸⁾ Spots were visualized with iodine vapor.

6. Determination of Radioactivity—The lipid bands on the TLC plate were scraped off and dissolved in 10 ml of a scintillator solution composed of 2 g of PPO, 50 mg of POPOP and 40 g of Cab-O-Sil per liter of toluene.⁹⁾ Radioactivities were measured in an Aloka LSC-602 liquid scintillation counter.

7. Determination of Lipid—Total lipid was determined gravimetrically. The content of TG was determined by the method of van Handel and Zilversmit.¹⁰⁾ The TG fraction was then hydrolyzed with pancreatic lipase.¹¹⁾ The β -monoglycerides and free fatty acids thus obtained were separated by TLC using a mixture of petroleum ether-diethyl ether-acetic acid (80 : 30 : 1, by vol.) as a developing solvent.¹²⁾

8. Enzyme Assays—TG synthetase activity was determined as follows; a mixture consisting of 0.2 ml of 10% hepatopancreas homogenate, 16.6 mM of Sørensen's phosphate buffer (pH 7.4), 6.66 mM ATP, 83 μ M CoA, 3.3 mM α -glycerophosphate, 10 mM $MgCl_2$, 13.3 mM KF, 2.66 mM GSH, and 1.2 mM potassium 1-¹⁴C-palmitate (1 μ Ci) in a total volume of 2.0 ml was incubated at 25° for 40 min.¹³⁾ The mixture was extracted with isopropanol-*n*-heptane-1N H_2SO_4 (40 : 10 : 1, by vol.). One ml of *n*-heptane layer was added to 12 ml of the scintillator solution (2 g of PPO and 50 mg of POPOP per

liter of toluene) to determine the radioactivity.¹⁴⁾

α -Glycerophosphate dehydrogenase activity was determined according to Shibata *et al.*¹⁵⁾ as follows; a mixture of 20 mM Tris-HCl buffer (pH 8.6), 50 mM $MgCl_2$, 15 mM NAD, 100 mM α -glycerophosphate and 0.4 ml of 2% hepatopancreas homogenate in a total volume of 2.0 ml was incubated at 37° for 30 min. On completion of the incubation, 0.4 ml of Na_2WO_4 and 2.8 ml of acetone were added to the reaction mixture. After centrifugation the optical density of the supernatant was measured at 340 nm.

9. Determination of Protein and Glycogen—Protein was determined by the method of Lowry *et al.*¹⁶⁾ with bovine serum albumin as a standard. The content of glycogen was measured by the method of Seifter *et al.*¹⁷⁾

Results

Changes in the Amounts of Hepatopancreatic Components of Carp after Oral Administration of PCB Diet

As shown in Table I, the contents of total lipid and TG (expressed per g of tissue) in the hepatopancreas of carp fed the PCB diet were increased, but the increments were not significant when expressed per mg of protein of the hepatopancreas. No change in the protein content was observed, but the hepatopancreatic glycogen level decreased significantly.

Incorporation of 1-¹⁴C-Palmitate into the Hepatopancreatic and Plasma Lipid Fractions in Carp

1) *In Vivo* Experiment—Tables II and

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TABLE I. Hepatopancreatic Composition of Carp Fed a PCB Diet

Components	mg/g tissue		mg/mg protein	
	Control	PCB ^{a)}	Control	PCB ^{a)}
Total lipid	62.1±3.4	68.7±1.2	0.91±0.04	0.92±0.07
Triglyceride	34.3±5.4	50.3±8.7	0.51±0.09	0.67±0.11
Protein	68.0±3.2	74.9±6.3	—	—
Glycogen	111.8±4.2	83.8±7.1	1.64±0.14	1.12±0.02

a) The carp were fed a diet containing 250 ppm of PCB (Aroclor 1248) per day for 14 days. Each value is the mean ± S.E. for 4 carp.

TABLE II. Incorporation of 1-¹⁴C-Palmitate into Hepatopancreatic Lipids of Carp Fed a PCB Diet

Lipid fractions	Radioactivity (dpm/mg tissue)			
	Control	(%) ^{a)}	PCB	(%) ^{a)}
Triglyceride	39.2±6.5	68.5	38.6 ±8.5	69.4
Phospholipid	16.4±2.7	28.7	15.0 ±3.1	27.0
Free cholesterol	1.1±0.1	1.9	1.2 ±0.2	2.2
Cholesterol ester	0.12±0.02	0.2	0.18±0.05	0.3
Free fatty acid	0.41±0.06	0.7	0.67±0.40	1.2

Each value is the mean ± S.E. for 4 carp.
a) % of total radioactivity incorporated.

III show the effects of PCB on the incorporation of 1-¹⁴C-palmitate into the lipids of the hepatopancreas and plasma in carp, respectively. The incorporation of 1-¹⁴C-palmitate into each of the hepatopancreas lipid fractions was not affected by feeding the PCB diet. On the other hand, the radioactivities of plasma TG and free fatty acid fractions increased, but those of the phospholipid and free cholesterol fractions decreased.

2) *In Vitro* Experiment—The effect of PCB administration on the incorporation of 1-¹⁴C-palmitate into the 500×g supernatant of hepatopancreas homogenate is shown in Table IV. The incorporation of 1-¹⁴C-palmitate into the TG fraction of the homogenate was increased by PCB administration. However, no difference between the control and PCB-treated groups was observed in the distribution of the radioactivity into each lipid fraction.

TABLE III. Incorporation of 1-¹⁴C-Palmitate into Plasma Lipid of Carp Fed a PCB Diet

Lipid fractions	Radioactivity (dpm/ml plasma)			
	Control	(%) ^{a)}	PCB	(%) ^{a)}
Triglyceride	920	41.3	1483	54.7
Phospholipid	623	28.0	333	12.3
Free cholesterol	110	4.9	90	3.3
Cholesterol ester	70	3.1	78	2.9
Free fatty acid	503	22.6	728	26.8

Values are those for pooled blood collected from 4 carp.
a) % of total radioactivity incorporated.

Incorporation of 1-¹⁴C-Palmitate into the α , α' - and β -Positions of the Hepatopancreas TG *in Vitro*

The incorporation of 1-¹⁴C-palmitate was increased in the α , α' -position of TG of the hepatopancreas homogenate by the PCB treatment. The isotope distributions, however, were similar in the control and PCB-treated groups (Table V).

Effect of the PCB Diet on the TG Synthetase and α -Glycerophosphate Dehydrogenase

TABLE IV. *In Vitro* Incorporation of 1-¹⁴C-Palmitate into Hepatopancreatic Lipid of Carp Fed a PCB Diet

Lipid fractions	Radioactivity (dpm/mg lipid)			
	Control	(%) ^{a)}	PCB	(%) ^{a)}
Total lipid	19650±840		24750±3340	
Triglyceride	1061±123	23.1	1252±74	23.9
Phospholipid	2568±289	55.9	2956±295	56.5
Free cholesterol	684±59	14.9	836±214	16.0
Cholesterol ester	21±2	0.5	20±4	0.4
Free fatty acid	259±55	5.6	166±48	3.2

Each value is the mean ± S.E. for 4 carp.

a) % of total radioactivity incorporated.

TABLE V. Incorporation of 1-¹⁴C-Palmitate into Hepatopancreatic Triglyceride of Carp Fed a PCB Diet

	Radioactivity (dpm/mg triglyceride)			
	Monoglyceride (β-position)	(%) ^{a)}	Free fatty acid (α, α'-positions)	(%) ^{a)}
Control	1004±117	25.4	2948±268	75.6
PCB	968±37	21.7	3503±388	78.3

Each value is the mean ± S.E. for 4 carp.

a) % of total radioactivity incorporated.

TABLE VI. Changes in Triglyceride Synthetase and α-Glycerophosphate Dehydrogenase Activities in the Hepatopancreas of Carp Fed a PCB Diet

Enzymes	Control	PCB
Triglyceride synthetase (dpm/mg protein)	10233±107	10686±93
α-Glycerophosphate dehydrogenase (OD ₃₄₀ /mg protein)	0.123±0.011	0.148±0.010

Each value is the mean ± S.E. for 4 carp.

Activities in Carp Hepatopancreas

The TG synthetase and α-glycerophosphate dehydrogenase activities were slightly increased in carp hepatopancreas by PCB treatment (Table VI).

Discussion

In this study, the increases in the contents of total lipid or TG in the carp hepatopancreas after oral administration of PCB were less marked than those reported in mammals.^{3c)} PCB had little effect on the

incorporation of 1-¹⁴C-palmitate into the carp hepatopancreas either *in vivo* or *in vitro*. On the other hand, there was a marked decrease in the hepatopancreatic glycogen of carp fed with PCB, together with slight elevations of both α-glycerophosphate dehydrogenase and TG synthetase activities in the hepatopancreas. The increases in both enzyme activities suggest possible stimulations of the supply of α-glycerophosphate and of the synthesis of TG, followed by glycogenolysis.

In conclusion, the fluctuations of lipid metabolism in carp hepatopancreas caused by PCB administration seem to be similar in tendency, but smaller in magnitude, compared with those in mammals.

The reason for the discrepancy between carp and mammals may be a consequence of the experimental conditions used in this study. Even though a fresh-water fish was used as an experimental animal, the study was car-

ried out under the same conditions used in the experiments with mammals. For example, the activities of drug-metabolizing enzymes from carp hepatopancreas are about one-third of those from rat liver under the same experimental conditions.¹⁸⁾

Acknowledgement The authors are grateful to Mr H. Takano of Chubu Shiryō Co., Ltd. for a generous supply of carp and diet.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged.

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