Fecal Bile Acids and Neutral Sterols of Rats Fed PCB

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Introduction

Cholesterol is the obligatory precursor of bile acids. Steroid nucleus cannot be broken down in tissues, so liver is the only organ which can eliminate significant amounts of cholesterol from body by producing bile acids and cholesterol present in the bile (1). The rate of synthesis of cholesterol and its degradation to bile acids, and excretion in the feces is of particular importance in the study of cholesterol metabolism. Previously, it was reported that the administration of PCB, an environmental chemicals (xenobiotics), caused a fatty liver, increased serum total cholesterol, HDL-cholesterol, triglyceride and urinary ascorbic acid (2-4). It was found that the activity of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-COA) reductase, the rate limiting enzyme of cholesterol biosynthesis, and cholesterol 7α-hydroxylase, the rate limiting enzyme of cholesterol degradation, were also elevated in the liver of rats exposed to PCB as compared to the control animals (5).

In the present study, fecal bile acids and neutral sterols were assayed in an attempt to elucidate the hypercholesterolemic effect of dietary PCB.

Materials and Methods

Animals and diets : The animals used were female Wistar rats weighing 65-75 g (Shizuoka Agricultural Cooperative Assoc. for Laboratory Animals, Hamamatsu, Japan). They were individually self-fed a commercial stock diet (CE-2, Japan Clea Co. Ltd , Tokyo) for a 4-day adaptation period to the new environment. They were then offered an adequate purified diet (25%

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casein-sucrose diet) for 2 days to adapt them in a new types of purifed diet. The average body weight was 96 g at the time of test diets provided. Test diets (Table-1) were provided for 7 days. There were nine rats per group. In the experimental diet, 300 ppm PCB (Aroclor 1248, Mitsubishi Monsant Co. Ltd., Tokyo) was added to the basal diet at the expense of sucrose. All rats were individually housed; diets and tap water were supplied **ad libitum**. Room temperature was kept 22±2°C with a 12-hour cycle of light (0800-2000 hours) and dark. On the last day of the experimental period, rats were anesthetized with diethyl ether and killed at 1000 hours within a short period. Blood samples were taken from heart puncture for the analysis of serum cholesterol. The liver was removed, weighed and then stored at - 20°C until liver lipids were analyzed. Feces were collected over a 3-day period before killing, with provisions to avoid dietary contamination of feces. The feces were dried at 70°C for 5 hours, weighed and ground to a fine powder. The 3-day samples per rat were pooled and used for the determination of bile acids and neutral sterols.

Table I- Composition of the basal diet *

Component	Amount
	%
Casein	20.00
Sucrose	71.95
Corn oil	2.00
Vitamin mixture [2,3]	0.85
Salt mixture [2]	5.00
Choline chloride	0.20

* Dietary PCB (0.03%) was added to the basal diet at the expense of sucrose. [2]Harper (15). [3]The vitamin mixture did not contain vitamin C. Additional vitamins per 100 g diet: retinyl palmitate, 600 IU; ergocalciferol, 1.5/μg; dl-α-tocopheryl acetate, 10 mg.

Analytical methods : Liver lipids were extracted by the method of Folch et. al. (6) and used for the determination of cholesterol and total lipids. Liver total lipids were determined gravimetrically. Cholesterol levels in the serum and liver were measured according to the method of Pearson et. al. (7). The main four fecal bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid) were determined by the method of Grundy et. al. (8). Fecal lipids were measured according to the method of Leveille and Sanberlich (9) modification of the Searchy and Bergquist (10) method. All the 3-beta-hydroxy sterols were determined by this method.

Results

The effects of dietary PCB (0.03%) on liver weight, serum cholesterol, liver total lipids and cholesterol are shown in Table-2. Serum cholesterol, liver weight, liver total lipids and cholesterol were all significantly increased in the PCB-fed rats than that of control rats ($P < 0.05$). There were no significant differences observed on the body weight gain between the two groups.

Table 2- Effect of dietary PCB on liver weight and tissue cholesterol in Wistar female rats *

Dietary Groups	Body wt. gain (g/7 days)	Liver weight (%of body wt.)	Liver total lipids (mg/g tissue)	Cholesterol Serum (mg/dl)	Cholesterol Liver (mg/g tissue)
A. Control	$16.2 \pm 1.1^{2}{}_{a}$	5.39 ± 0.12^{3a}	72.3 ± 4.2^{3a}	$109.2 \pm 3\ 6^{3a}$	5.1 ± 0.42^{3a}
B. A+0.03% PCB	15.9 ± 0.9^{a}	6.97 ± 0.14^{3b}	112.0 ± 6.1^{3b}	161.5 ± 6.5^{3b}	8.3 ± 0.60^{3b}

* Initial average body weight was 96 g. [2] Mean ± SEM; n=9. [3] Different superscripts indicate significantly different means ($P < 0.05$).

The primary bile acids in the feces, cholic acid and chenodeoxycholic acid, were also found significantly higher in the rats exposed to PCB compared with controls. No significant differences were observed on the secondary bile acids, deoxycholic acid and lithocholic acid between the control and that of the PCB-fed rats (Fig. 1). On the basis of total determined bile acids, PCB-fed rats excreted 2-fold higher than that of controls (control diet-fed group 9.24 and PCB-fed group 20.16 mg/day/100g body weight). Fecal sterols were also found significantly increased in the animals fed PCB (control diet-fed group 0.90 and PCB-fed group 2.96 mg/day/100g body weight) (Fig. 2).

Discussion

The increase in serum and liver cholesterol, liver total lipids accompanied by the enlargement of liver due to PCB-feeding confirmed our previous observations (2-4,11). Nearly similar observations have been reported with other xenobiotics, such as phenobarbital, pentobarbital, caffeine, 2,6-di-tert-butyl-p-cresol (BHT) and 1,1,1,-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) (12-14).

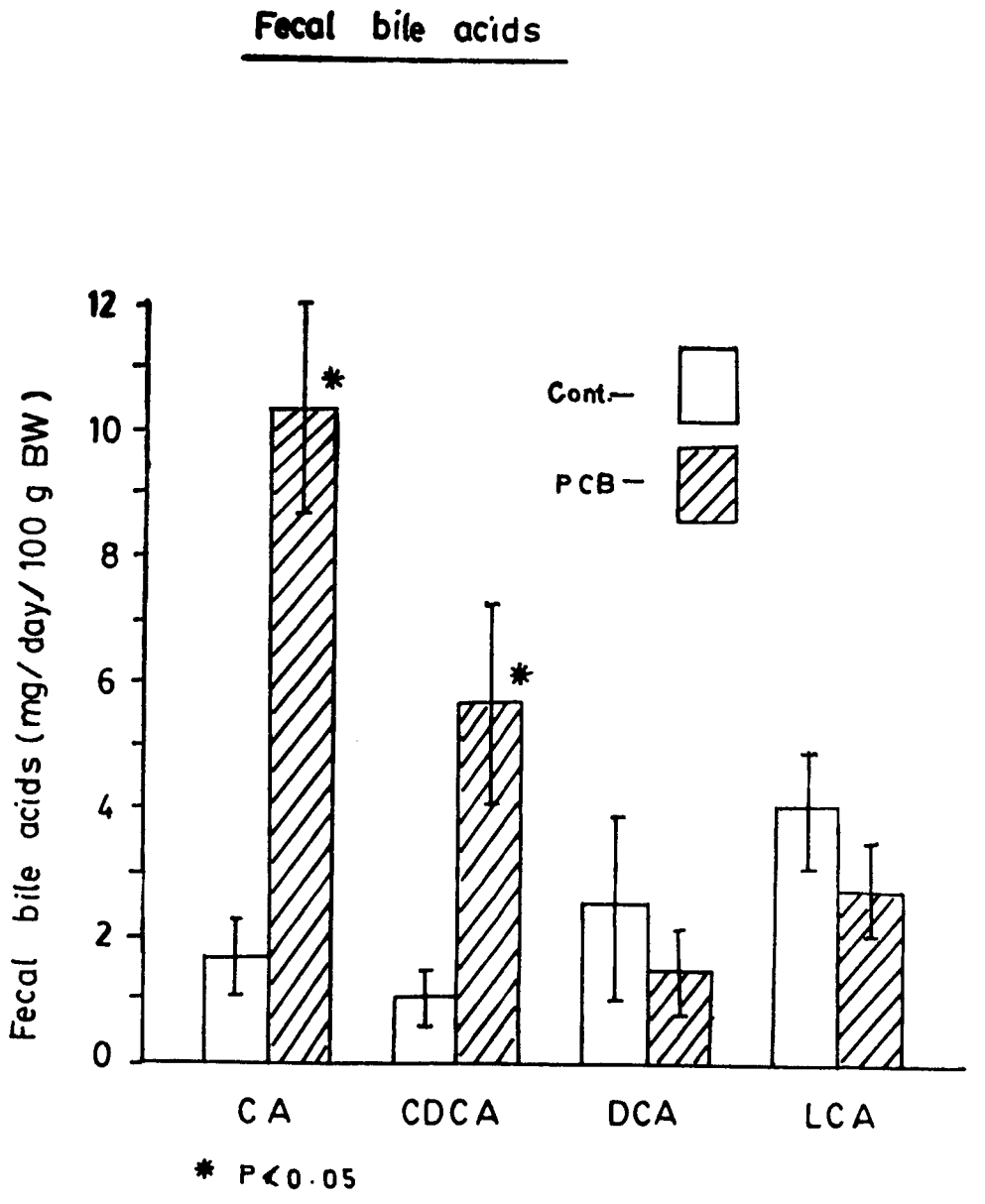

Fig. 1. Fecal bile acids in rats with or without received PCB. Vertical bars indicate standard errors. Abbreviations used: CA, cholic acid; CDCA chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

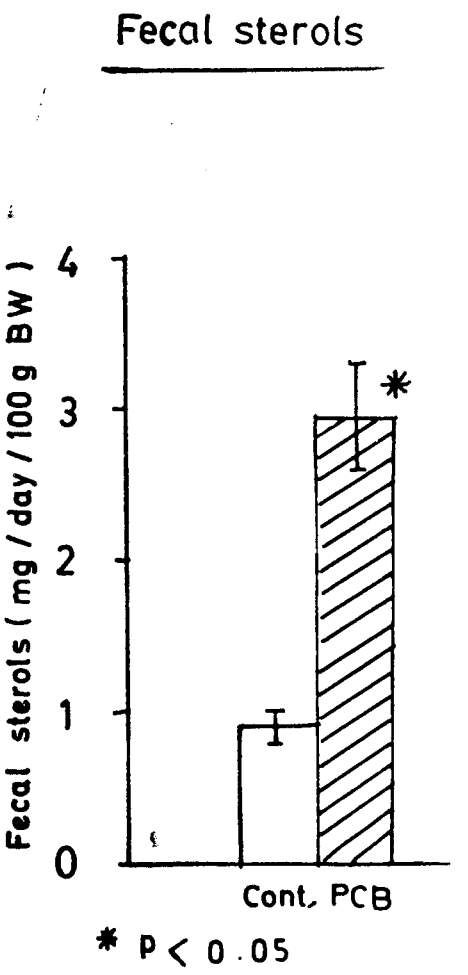

Fig. 2. Fecal neutral sterols in rats with or without received PCB. Vertical bars indicate standard errors.

Liver is the main site of cholesterol synthesis in the body. We found both total (active plus inactive) and expressed (active) activities of the HMG-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis, were significantly higher ($P < 0.05$) in the rats receiving PCB (5). We concluded that dietary PCB stimulates the microsomal cholesterol synthesizing system and as a result serum and liver cholesterol levels were elevated in the PCB-fed rats. It was also observed that the activity of cholesterol 7α-hydroxylase, the first rate limiting enzyme of cholesterol degradation to bile acids, was also elevated in rats exposed to PCB as compared to the control animals (5). The increase in HMG-CoA reductase and cholesterol 7α-hydroxylase activities can be expressed as being due to increased synthesis of the enzyme proteins in the PCB-fed rats. Previously, we reported higher levels of serum albumin and protein in rats exposed to PCB

(2). In the present experiment, we found excess excretion of primary bile acids (cholic acid and chenodeoxy-cholic acid) in the PCB administered rats. Primary bile acids change to secondary bile acids by bacterial degradation in the intestine. It seems that bacterial degradation of primary bile acids to secondary bile acids was similar in both control and PCB-fed animals. The increase excretion of primary bile acids reflected the increase activity of hepatic cholesterol 7α-hydroxylase observed previously in the PCB-fed rats (5). During enterohepatic ciculation, bile acids help to digest and absrob dietary fats. Bile acids may also help the bile excretion of xenobiotics like PCB. So the excess bile acids excretion in rats exposed to xenobiotics may be physiologically significant. Also neutral sterols in the feces of experimental rats were higher. Since PCB feeding drains out significant amounts of bile acids and neutral sterols through the feces, it follows that such constant removal of bile acids and bile cholesterol from the liver should lead to the activation of cholesterol biosynthesis in such rats. The rate of cholesterogenesis in the liver of rats fed PCB may be higher than the rate of cholesterol degradation and excretion, to account for the higher accumulation of tissue cholesterol.

Summary

Effect of 0.03% polychlorinated biphenyls (PCB) in the rat diet on serum and liver cholesterol, and facal bile acids and neutral sterols were investigated. Liver weight, total lipids, cholesterol, and serum cholesterol, were found significantly increased in PCB-fed rats. The primary bile acids in the feces, cholic acid and chenodeoxycholic acid, were also significantly higher in the experimental rats. There were no significant differences in the secondary bile acids, deoxycholic acid and lithocholic acid, between the control and PCB fed rats. Fecal sterols excretion were increased in the animals fed PCB. These results indicated that not only serum and liver cholesterol levels were higher, but also the excretion of cholesterol as primary bile acids and neutral sterols were increased in PCB-fed animals.

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