Effect of Dietary Methionine and Polychlorinated Biphenyls on Cholesterol Metabolism in Rats Fed a Diet Containing Soy Protein Isolate

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Summary The effects of supplementation of methionine to a 20% soy protein isolate diet on serum level of cholesterol, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, cholesterol 7 α -hydroxylase activity, and biliary and fecal steroids in rats with or without receiving polychlorinated biphenyls (PCB) were investigated. Supplementation of methionine and PCB did not affect the growth. Serum level of cholesterol was higher in rats fed PCB than in controls. In rats fed PCB, addition of methionine elevated serum level of cholesterol synergistically. The activity of HMG-CoA reductase was higher in rats fed the methionine-supplemented diet than in those fed the unsupplemented diet when PCB was included in the diets. Cholesterol 7 α -hydroxylase activity (nmol/h · 100 g body weight) was higher in rats fed PCB than in controls. Biliary secretion of bile acids was higher in rats fed PCB than in controls. On the other hand, fecal excretion of bile acids decreased in PCB-treated rats, but total steroids were not affected by PCB. In rats fed PCB, the addition of methionine did not alter cholesterol 7 α -hydroxylase activity and biliary and fecal steroid output. The data suggest that the increase in serum level of cholesterol due to dietary addition of methionine together with PCB would be mediated through the stimulation of hepatic synthesis of cholesterol.

Key Words methionine, PCB, serum cholesterol, HMG-CoA reductase, fecal steroids, GSH

Administration of PCB (polychlorinated biphenyls), DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane), BHT (2,6-di-*tert*-2,2-butyl-*p*-cresol) or pentobarbital to rats causes an increase in plasma level of cholesterol, urinary excretion of ascorbic

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acid, and the activity of liver microsomal mixed function oxidase system (1, 2). These metabolic responses are greatly influenced by the quantity (3), and the quality (4) of dietary protein. Generally the biological value of protein is positively correlated with serum level of cholesterol, urinary and liver ascorbic acid levels, and hepatic drug-metabolizing enzyme activity (3). Interestingly, in rats fed a diet containing soy protein isolate and PCB, plasma cholesterol and urinary ascorbic acid levels were lower than expected from the biological value of soy protein (5). This finding suggests that soy protein isolate might be deficient in some factors involved in these metabolic responses to PCB. Sulfur amino acids may be involved in such factors, because marked increases in urinary excretion of ascorbic acid and plasma level of cholesterol were observed after supplementation of methionine to the diet containing soy protein isolate and PCB (3, 5). Further studies indicated that a diet containing 0.8% of sulfur amino acids produced a maximum increase in serum level of cholesterol and urinary excretion of ascorbic acid due to PCB-feeding, although a diet containing 0.5% of sulfur amino acids was adequate for maximum growth (6, 7). This observation suggests that more sulfur amino acids might be required for the maximum increase in serum level of cholesterol and urinary excretion of ascorbic acid than for maximum body weight gain and nitrogen balance.

Dietary sulfur amino acids influence the serum level of cholesterol level in many species (7-15). Recently we demonstrated that serum cholesterol and urinary ascorbic acid levels of rats fed PCB had a significant positive correlation with dietary sulfur amino acids (16). However, the mechanism of effect of sulfur amino acids on cholesterol metabolism is not known.

It has been suggested that the increase in serum level of cholesterol due to PCB is mainly attributable to a stimulation of liver cholesterol synthesis (17). PCB-feeding increased 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [EC 1.1.1.34] activity (17, 18).

In this paper, we examined the effects of addition of methionine to a diet containing 20% of soy protein isolate on HMG-CoA reductase activity, cholesterol 7 α -hydroxylase [EC 1.14.13.17] activity, and excretion of steroids in bile and feces in rats fed PCB. Our results suggested that hypercholesterolemia due to dietary methionine together with PCB would be mediated through the stimulation of hepatic cholesterol synthesis.

METHODS

Animals and diets. Male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), averaging about 90 g in weight (250 g in experiment 4), were initially fed a commercial diet (CE-2, Japan Clea Co., Ltd., Tokyo) for 3-7 days. The composition of the basal diet was (in weight percent): soy protein isolate (82% protein, Fuji Seiyu Co., Ltd., Osaka, Japan) 24.3; mineral mixture (7), 5.0; corn oil, 2.0; vitamin mixture (7), 0.5; choline chloride, 0.2; and starch was added to form

100%. Retinyl palmitate, ergocalciferol, and RRR- α -tocopherol acetate were added at the concentrations of 400 μ g, 1.5 μ g, and 10 mg per 100 g of diet, respectively. The basal diet contained 0.56% of sulfur amino acids. The soy protein isolate contained approximately equal amount of methionine and cystine. This amount of sulfur amino acids was enough for the maximum gain in body weight in rats (16). After feeding the basal for 2–6 days, rats were divided into four groups. Animals in groups 1 and 3 were fed the methionine-unsupplemented diet and in groups 2 and 4 were fed the diet supplemented with 0.5% of L-methionine (generously supplied by Ajinomoto Co., Ltd., Tokyo). Diets in groups 3 and 4 were supplemented with PCB (300 mg/kg of diet, Aroclor 1248, Mitsubishi Monsanto Co., Ltd., Tokyo). This amount of PCB produced the marked increase in serum cholesterol without retardation of growth within this experimental period (7 days). Rats were provided these test diets for 7 days, since the addition of methionine and PCB to the basal diet elevated serum cholesterol markedly within 7 days and serum cholesterol remained relatively constant after three-weeks feeding (unpublished data).

Experiment 1. Rats were kept in a room with a 12-h cycle of light (0800–2000) and dark (2000–0800). Tap water was supplied *ad libitum*, and food was withheld between 1000–1800 h. At the end of the experimental period (7 days), rats were killed at 0000 h. Immediately the livers were removed and weighed. Fraction of microsome was obtained for assay of HMG-CoA reductase [EC 1.1.1.34] activity.

Experiment 2. Rats were kept in a room with a 12-h cycle of light (0800–2000) and dark (2000–0800). Tap water was supplied *ad libitum*, and food was withheld between 1000–1800 h. At the end of the experimental period (7 days), rats were killed at 0000 h. Immediately the livers were removed and weighed. Fraction of microsome was obtained for assay for cholesterol 7 α -hydroxylase [EC 1.14.13.17] activity.

Experiment 3. Rats weighing an average of 250 g were kept in a room with a 12-h cycle of light (0800–2000) and dark (2000–0800). Tap water was supplied *ad libitum*, and food was withheld between 1000–1700 h. At the end of experimental period (7 days), rats were anesthetized by intraperitoneal sodium pentobarbital injection during 1900–2000 h, and the bile duct was cannulated with a polyethylene tube (PE-10) to collect bile for 1 h. Feces were collected for two days before rats were killed.

Chemicals. DL-[3-¹⁴C]HMG-anhydride was synthesized essentially as described by Goldfarb and Pitot (19) and then converted to DL-[3-¹⁴C]HMG-CoA by the method of Suzuki *et al.* (20). [5-³H]mevalonolactone was obtained from New England Nuclear. [7(n)-³H]cholesterol was obtained from Amersham. All other chemicals were of analytical grade.

Assay of microsomal HMG-CoA reductase activity. About 1.5 g of liver was homogenized in four volumes of 0.25 M sucrose containing 0.05 M potassium phosphate buffer (pH 7.0), 0.075 M nicotinamide, 10 mM dithiothreitol, and 2.5 mM EDTA. The homogenates were centrifuged at 12,000 $\times g$ for 15 min, and supernatant solution was recentrifuged at 100,000 $\times g$ for 60 min. The microsomal pellet

was suspended in 0.05 M potassium phosphate buffer (pH 7.2) containing 10 mM dithiothreitol and 0.02 M EDTA and used for assay of HMG-CoA reductase (21).

Assay of microsomal cholesterol 7 α -hydroxylase activity (22). About 2 g of liver was homogenized in four volumes of 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 50 mM NaF. The homogenates were centrifuged at $12,000 \times g$ for 15 min, and supernatant solution was recentrifuged at $100,000 \times g$ for 60 min. The microsomal pellet was suspended in the buffer. The incubation mixture contained 0.1 M potassium phosphate (pH 7.4), 0.1 mM EDTA, 50 mM NaF, 2 mM NADPH, 20 mM cysteamine, 200 μ M cholesterol, 1.5 mg/ml Tween 80, 6 μ Ci [7(*n*)-³H]cholesterol, and 1.5–2.0 mg/ml microsomal protein in a final volume of 2 ml. Incubation was carried out at 37°C for 30 min. The reaction was terminated by the addition of 6 ml of 20% trichloroacetic acid. Then the reaction mixture was centrifuged at $1,500 \times g$ for 10 min. Chloroform was added to the supernatant to extract unreacted radiolabeled cholesterol. After the second extraction with chloroform, 1 ml of upper phase was transferred to the counting vial, and the radioactivity was counted in toluene-Triton scintillation fluid [4 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per liter of toluene-Triton X-100 (2:1)].

Biliary and fecal lipids determination. Bile was saponified with alcoholic KOH at 75°C for 30 min. The unsaponifiable fraction was extracted two times with *n*-hexane. Triton X-100 in acetone (150 mg/ml) was added to the pooled extract (23). After the extracts were evaporated, biliary cholesterol was measured by using a commercially available kit (Monotest-cholesterol, Boehringer Mannheim, West Germany). Biliary bile acids were determined enzymatically using 3 α -hydroxysteroid dehydrogenase [EC 1.1.1.50] (24). Biliary phospholipids were determined with a commercially available kit (Phospholipids C-Test, Wako Pure Chemical Industry, Ltd., Japan).

Feces were dried and pulverized. A portion of the pulverized feces, usually 100 mg, was extracted with 10 ml ethanol at 75°C for 1 h three times, and then the extracts were combined and evaporated. To the residue was added 4 ml of 1.25 N sodium hydroxide and the solution was hydrolyzed at 120°C for 6 h. Sterols were extracted with diethyl ether from the reaction mixture, and then bile acids were extracted with diethyl ether after acidifying with 2 N HCl. Each sterol and bile acids fraction was subjected to gas-liquid chromatography, using Silicon OV-17 and Silicon AN-600 (25), respectively. A Shimadzu GC-9A gas chromatograph equipped with a flame ionization detector was employed. All quantitative works were performed by comparison of area responses to peak of known additions of hydrocholic acid and 5 α -cholestane as an internal standard.

Serum and liver cholesterol determination. Serum was separated by centrifugation at $1,500 \times g$ for 10 min after the blood had stood for at least 30 min at room temperature. High-density lipoprotein (HDL) cholesterol was quantitated by the heparin-MnCl₂ method (1). Liver cholesterol was extracted by the method of Folch *et al.* (26). Cholesterol levels were determined by using a commercially available kit.

Other methods. The content of hepatic reduced glutathione (GSH) was determined by the method of Sedlak and Lindsay (27). Microsomal protein was measured using the Lowry assay with bovine serum albumin as a standard.

Statistical analysis. Statistical significance of differences among values was analyzed by two-way analysis of variance (ANOVA). When interaction was significant, Student's *t*-test was performed. The criterion for significance was a *p*-value of less than 0.05 or 0.01.

RESULTS

Data on body weight gain, liver weight, and the content of hepatic GSH are presented in Table 1. As described previously (16), supplementation of PCB and methionine did not affect the growth. Since the basal diet contained 0.56% of sulfur amino acids, which was enough for the maximum gain in body weight in rats (28), the addition of methionine to 20% soy protein isolate did not influence the growth. Liver weight, expressed as the percent of body weight, was higher in rats fed PCB than in controls, and it increased with methionine supplementation. The content of hepatic GSH was higher in rats fed the methionine-supplemented diet than in those fed the unsupplemented diet ($p < 0.01$). The interaction of PCB $\times$ methionine was significant ($p < 0.01$). Increase in hepatic content of GSH due to the addition of methionine was larger in rats fed PCB than in controls ($p < 0.01$).

Table 2 shows serum and liver cholesterol levels. Serum total cholesterol and HDL cholesterol levels were higher in rats fed PCB than in controls ($p < 0.01$). The

Table 1. Effects of supplementation of methionine and PCB to a 20% soy protein isolate diet on body weight gain, liver weight, and the content of GSH in rats (Experiment 1).^a

Measure	Diet	Met PCB					Pooled SE	ANOVA ^b		
			—	+	—	+		PCB	Met	PCB $\times$ Met
Body weight gain (g/7 days)			27.9	30.1	27.3	28.4	1.7	NS	NS	NS
Liver weight (g/100 g body weight)			3.70	3.91	5.08	5.51	0.05	0.01	0.01	NS
Liver GSH (μ mol/g)			2.51	3.54 ^{c,##}	2.66	5.00 ^{d,**}	0.20	0.01	0.01	0.01

^a Each value is the mean of 7 observations. ^b Statistical significance of differences among values was analyzed by two-way analysis of variance (ANOVA). When the interaction (PCB $\times$ Met) was significant, Student's *t*-test was performed. NS, not significant ($p > 0.05$). ^{c,##} This value differed significant ($p < 0.01$) from the value of the basal group (without PCB and methionine) using Student's *t*-test. ^{d,**} This value differed significantly ($p < 0.01$) from the value of the PCB-treated and methionine-unsupplemented group using Student's *t*-test.

Table 2. Effect of supplementation of methionine and PCB to a 20% soy protein isolate diet on serum and liver cholesterol in rats.^a

Measure	Diet		Met	PCB	+	-	+	+	Pooled SE	ANOVA ^b		
										PCB	Met	PCB × Met
Serum cholesterol (mg/dl)												
Experiment 1 (7)	60	61						120 ^{d**}	3	0.01	0.01	0.01
Experiment 2 (6)	59	69 [#]						120 ^{**}	3	0.01	0.01	0.01
Experiment 3 (9)	74	83						122 [*]	4	0.01	0.01	0.05
Experiment 1 (7)												
Serum HDL cholesterol (mg/dl)	26	30						64 [*]	2	0.01	0.01	0.01
Liver cholesterol (mg/g)	2.06	1.99						1.89	0.04	NS	NS	NS
(mg/100 g body weight)	7.60	7.77						10.41	0.21	0.01	NS	NS
Liver microsomal cholesterol (μ g/mg protein)	48.5	47.5						49.9	2.1	NS	NS	NS

^a Each value is the mean. Values in parentheses represent number of rats in each group. ^b Statistical significance of differences among values was analyzed by two-way analysis of variance (ANOVA). When the interaction (PCB × Met) was significant, Student's *t*-test was performed. NS, not significant ($p > 0.05$). ^c ^{*} ^{*} These values differed significantly ($p < 0.05$, $p < 0.01$, respectively) from the values of the basal group (without PCB and methionine) using Student's *t*-test. ^d ^{*} ^{*} ^{*} These values differed significantly ($p < 0.05$, $p < 0.01$, respectively) from the values of the PCB-treated and methionine-unsupplemented group using Student's *t*-test.

effect of dietary methionine was significant ($p < 0.01$). The interaction of PCB $\times$ methionine was significant ($p < 0.01$). In rats fed PCB, dietary addition of methionine increased serum total and HDL cholesterol levels synergistically. In control groups (without PCB), the supplementation of methionine produced significant increase in serum total cholesterol level in experiment 2, in agreement with our previous observation (7, 16). However, in experiments 1 and 3 methionine did not increase serum level of cholesterol in rats fed the diet without PCB. This difference may be due to the short experiment period (7 days) and to the small effect of methionine in rats fed a diet without PCB. It is likely that dietary addition of methionine tends to increase the serum level of cholesterol (7, 13, 14, 16).

Hepatic levels of total cholesterol (mg per g of liver) and microsomal cholesterol (μg per mg of microsomal protein) were not affected by dietary addition of PCB and methionine. And free and esterified cholesterol levels in liver (mg per g of liver) were not changed by the addition of dietary PCB and methionine (data not shown). Liver cholesterol expressed as mg per 100 g body weight was higher for rats fed PCB than in controls ($p < 0.01$).

The activity of liver microsomal HMG-CoA reductase is shown in Table 3. The addition of PCB alone did not influence HMG-CoA reductase activity. It has been demonstrated that PCB induced hepatic HMG-CoA reductase activity in rats fed a diet containing casein (17) or rice protein (Okumura and Yoshida, unpublished data). Okumura and Yoshida also showed PCB did not elevate HMG-CoA reductase activity in rats fed a diet containing soy protein isolate as a protein source. The interaction of PCB $\times$ methionine was significant. In rats fed PCB, the activity of HMG-CoA reductase was higher for rats fed the methionine-supplemented diet than in those fed the unsupplemented diet.

Table 4 shows liver microsomal cholesterol 7α -hydroxylase activity. Cholesterol 7α -hydroxylase activity was not affected by PCB when expressed per h $\cdot$ g of liver, although the enzyme activity, expressed as nmol/h $\cdot$ mg of microsomal protein, was lower in rats fed PCB than in controls ($p < 0.01$). When the enzyme activity was expressed per h $\cdot$ 100 g of body weight, the activity was greater in rats fed PCB than in controls ($p < 0.01$). This increase in cholesterol 7α -hydroxylase activity by PCB is due to the hypertrophy of the liver caused by PCB (Table 1). Dietary addition of methionine did not influence cholesterol 7α -hydroxylase activity in both PCB-treated and PCB-untreated groups.

Biliary secretion of lipids and fecal excretion of steroids are shown in Table 5. The bile flow was higher in rats fed PCB than in controls, but the concentrations of bile acids, cholesterol, and phospholipids were lower in rats fed PCB than in controls. The biliary secretion of bile acids was higher in rats fed PCB than in controls when this was expressed in terms of $\mu\text{mol/h} \cdot \text{rat}$. However, biliary secretion of cholesterol and phospholipids were still lower in rats fed PCB when expressed in terms of nmol/h $\cdot$ rat and $\mu\text{mol/h} \cdot \text{rat}$, respectively ($p < 0.01$ and $p < 0.05$, respectively). In rats not fed PCB, biliary cholesterol and phospholipids were lower in the methionine-supplemented group than in the unsupplemented group when they

Table 3. Effect of supplementation of methionine and PCB to a 20% soy protein isolate diet on liver microsomal HMG-CoA reductase activity in rats (Experiment 1).^a

Measure	Diet		Met		PCB		ANOVA ^b	
	Diet	PCB	+	-	+	-	PCB	Met
Hepatic HMG-CoA reductase activity ^c								
(pmol/min · mg protein)	509	464	410	591 ^{d,*}	50	NS	NS	0.05
(nmol/min · g liver)	7.57	7.31	7.56	11.71 ^{**}	0.86	0.05	0.05	0.05
(nmol/min · 100 g body weight)	27.7	28.7	38.5	64.7 ^{**}	3.9	0.01	0.01	0.01

^a Each value is the mean of 7 observations. ^b Statistical significance of differences among values was analyzed by two-way analysis of variance (ANOVA). When the interaction (PCB × Met) was significant, Student's *t*-test was performed. NS, not significant ($p > 0.05$). ^c Pico- or nanomoles mevalonic acid produced. ^{d,***} These values differed significantly ($p < 0.05$, $p < 0.01$, respectively) from the values of the PCB-treated and methionine-unsupplemented group using Student's *t*-test.

Table 4. Effect of supplementation of methionine and PCB to a 20% soy protein isolate diet on liver microsomal cholesterol 7 α -hydroxylase activity in rats (Experiment 2).^a

Measure	Diet		Met		PCB		ANOVA ^b	
	Diet	PCB	+	-	+	-	PCB	Met
Cholesterol 7 α -hydroxylase activity								
(nmol/h · mg protein)	1.31	1.32	0.90	0.97	0.06	0.01	NS	NS
(nmol/h · g liver)	34.9	36.2	31.1	33.4	2.05	NS	NS	NS
(nmol/h · 100 g body weight)	130	143	166	186	10	0.01	NS	NS

^a Each value is the mean of 6 observations. ^b Statistical significance of differences among values was analyzed by two-way analysis of variance (ANOVA). When the interaction (PCB × Met) was significant, Student's *t*-test was performed. NS, not significant ($p > 0.05$).

were expressed in terms of nmol/h·rat and μ mol/h·rat, respectively.

Fecal excretion of total steroids and sterols (cholesterol and coprostanol) were not affected by dietary PCB and methionine. The fecal excretion of bile acids was lower in rats fed PCB than in controls ($p < 0.01$). The ratio of chenodeoxycholic acid and its related bile acids to cholic acid and its related bile acids (CDCA group/CA group) was higher for rats fed PCB than for controls ($p < 0.01$). The absorption efficiency (the ratio of daily intestinal bile acid absorption to daily biliary bile acid secretion) was increased to a small extent but significantly ($p < 0.01$) by the PCB-feeding.

In the present study, hepatic content of GSH was changed by feeding the experimental diets (Table 1). It is known that thiols or disulfides can modulate several enzyme activities (29), including HMG-CoA reductase (30) and cholesterol 7 α -hydroxylase (31). We then performed the additional experiments to compare the effect of glutathione on thiol-deficient microsome-derived HMG-CoA reductase and cholesterol 7 α -hydroxylase *in vitro*. To prepare thiol-deficient microsome, thiols were omitted from the preparation medium. Preincubation of HMG-CoA reductase and cholesterol 7 α -hydroxylase with 5 mM GSH for 15 min at 37°C resulted in 5-fold and 1.5-fold increase of these enzyme activities, respectively (Fig. 1). HMG-CoA reductase activity was stimulated by up to 9.2-fold by the inclusion of 20 mM GSH in the assay mixture (Fig. 1A). This shows that HMG-CoA reductase activity is

Fig. 1. Modulation of HMG-CoA reductase (A) and cholesterol 7 α -hydroxylase (B) activity by GSH or GSSG (inset). Thiol-deficient microsomes were obtained from rats, weighing 250–270 g, fed a commercial diet. Thiol-deficient microsomes were preincubated with cofactors for 15 min at 37°C with the indicated amounts of GSH, or preincubated with cofactors for 15 min at 37°C with 5 mM GSH and the indicated amounts of GSSG (inset). Each point is the mean of duplicate determinations.

Fig. 2. Modulation of HMG-CoA reductase (A) and cholesterol 7 α -hydroxylase (B) activity by [GSH]/[GSSG] ratio. Thiol-deficient microsomes were obtained from rats, weighing 250–270 g, fed a commercial diet. The assays contained 1.25 mM (Δ), 2.5 mM ($\circ$), and 5 mM ($\bullet$) of GSH. The enzymes were preincubated with cofactors for 15 min at 37°C. Each point is the mean of duplicate determinations.

stimulated to the greater extent by GSH than cholesterol 7 α -hydroxylase activity. The inhibitory effect of GSSG (oxidized glutathione) on the activity of these enzymes is shown in Fig. 1 (inset). It is apparent that GSSG can reverse or prevent these enzymes activation promoted by GSH. The activity of cholesterol 7 α -hydroxylase is dependent on the ratio of [GSH] to [GSSG] (Fig. 2B), while HMG-CoA reductase activity is dependent not only on the ratio of [GSH]/[GSSG] but also on the total and reduced glutathione concentrations (Fig. 2A). Therefore HMG-CoA reductase activity is closely dependent on the ratio of $[GSH]^2/[GSSG]$. Recently, Cappel and Gilbert (32) have reported that HMG-CoA reductase is very sensitive to dithiol oxidation both kinetically and thermodynamically. These observations suggest that cholesterol 7 α -hydroxylase may form protein mixed disulfides and HMG-CoA reductase may form protein-protein disulfides during reversible oxidation of glutathione (33, 34).

DISCUSSION

In agreement with our previous report (7, 16), the supplementation of methionine markedly increased serum level of cholesterol in rats fed PCB (Table 2). In the present study, to investigate the mechanism of the increase in serum level of cholesterol by the addition of methionine in rats fed PCB, we determined HMG-CoA reductase activity, cholesterol 7 α -hydroxylase activity, and excretion of steroids in bile and feces.

Kato and Yoshida (17) demonstrated that an increase in the activity of hepatic

HMG-CoA reductase is responsible for hypercholesterolemia induced by PCB. Jenke (18) also reported PCB-feeding resulted in the increase in the activity of the enzyme in accordance with the increase in the amount of mRNA coding for HMG-CoA reductase. In the present study, however, supplementation of PCB alone did not induce HMG-CoA reductase activity significantly (nmol/min · 100 g body weight) (Table 3), in spite of the increase in serum cholesterol by PCB (Table 2). Kato and Yoshida used casein as protein source and Jenke did not mention protein source. Soy protein isolate was used as the protein source in the present study. Our results show that soy protein isolate might be deficient in some factors involved in the induction of HMG-CoA reductase activity by PCB.

Sulfur amino acids, especially methionine (16), would be involved in such factors. Hepatic HMG-CoA reductase activity was higher in rats fed the methionine-supplemented diet than in those fed the unsupplemented diet when PCB was included in the diets. These results suggested that, in rats fed PCB, dietary methionine stimulates hepatic synthesis of cholesterol through the increase in the activity of HMG-CoA reductase. Moreover, serum cholesterol level had a significant positive correlation with hepatic HMG-CoA reductase activity ($r = 0.81$, $p < 0.001$, $n = 28$). From these observations, it is considered that methionine-induced hypercholesterolemia in PCB-treated rats is attributable to the stimulation of liver cholesterol synthesis.

Cholesterol 7 α -hydroxylase activity, which is the rate-limiting enzyme of bile acid biosynthesis from cholesterol, was higher in rats fed PCB when the enzyme activity was expressed in terms of nmol/h · 100 g body weight. Biliary secretion of bile acids increased by PCB-feeding. However, fecal excretion of bile acids was reduced by PCB. This difference might be caused by the increase in the absorption efficiency (the ratio of daily intestinal bile acid absorption to daily biliary bile acid secretion). Several dietary manipulations change the ratio of CDCA group/CA group ratio (35, 36). In rats fed PCB, a higher ratio of CDCA group/CA group was observed (Table 5). The increase in the ratio of CDCA group/CA group ratio may relate to the increase in the absorption efficiency and the reduction of fecal bile acids in PCB-fed rats (36). Although fecal bile acids decreased by PCB-feeding, fecal excretion of total steroids was not influenced by dietary addition of PCB and methionine (Table 5). This suggested that the elevation of serum level of cholesterol by PCB and methionine is not attributable to change in catabolism of cholesterol. As for cholesterol balance, the data suggest that the increase in serum level of cholesterol due to dietary methionine under the presence of PCB in the diet would be mediated through the stimulation of hepatic cholesterol synthesis.

It is well known that HMG-CoA reductase activity is reversibly regulated by the phosphorylation/dephosphorylation mechanism (37). Hepatic cholesterol 7 α -hydroxylase also exists in a phosphorylated (active) and dephosphorylated (inactive) form (38). When microsomes were prepared in the absence of fluoride, 70% of total HMG-CoA reductase was dephosphorylated (active) (39). Brown *et al.* (39) demonstrated that long-term alterations in cholesterol synthesis in rat liver are due

not to changes in the state of phosphorylation of the reductase, but rather to changes in the total amount of the enzyme protein. PCB-feeding resulted in an increase of liver HMG-CoA reductase activity, but the ratio of expressed (dephosphorylated)/total activity was constant for 42 days (18). Accordingly the activity of HMG-CoA reductase shown here would represent the amount of the enzyme, although the enzyme content was not measured in the present study.

Both HMG-CoA reductase and cholesterol 7 α -hydroxylase are activated by GSH and inactivated by GSSG *in vitro* (Fig. 1). GSH activated HMG-CoA reductase to a greater extent than cholesterol 7 α -hydroxylase. Moreover, HMG-CoA reductase activity was dependent not only on the ratio of [GSH]/[GSSG] but also on the total and reduced glutathione concentrations, although cholesterol 7 α -hydroxylase activity was dependent on only the ratio of [GSH]/[GSSG] (Fig. 2). Hepatic content of GSH depended on the dietary level of sulfur amino acids. In the present study, hepatic content of GSH was increased by addition of methionine, and it was the highest in rats fed methionine and PCB concurrently (Table 1). These facts raise a possibility that higher content of GSH due to dietary addition of methionine activates HMG-CoA reductase *in vivo*, and biosynthesis of cholesterol increases. However, in the control group (without PCB), the increasing effect of supplementation of methionine on serum level of cholesterol was small, in spite of the increase in hepatic content of GSH (Table 1). Moreover, the dietary addition of cystine, which also increases in hepatic content of GSH, had less serum cholesterol-elevating effect compared with that of methionine (7, 16). Alteration of the hepatic content of GSH by dietary manipulation continues chronically (7, 16). Therefore the possibility that glutathione plays a role for cholesterol metabolism cannot be excluded.

It has been reported that single amino acids (tyrosine, histidine, or cystine) which induce hypercholesterolemia stimulate hepatic cholesterol synthesis (21, 40, 41). Although these amino acids were contained excessively in the diet (5–12%), it is interesting that the amount of methionine necessary to increase serum cholesterol and hepatic cholesterol synthesis was only 0.5% and less than one tenth of these amounts. These findings suggest that dietary methionine plays a significant role in cholesterol metabolism, especially in the presence of xenobiotics.

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