Elevated concentrations of α-tocopherol, ascorbic acid, and serum lipids in rats fed polychlorinated biphenyls, chlorobutanol, or phenobarbital

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Experiments were conducted with growing rats to investigate the effects of dietary 0.05% polychlorinated biphenyls (PCB), 0.3% chlorobutanol (Chloretone), and 0.2% phenobarbital sodium on intestinal absorption of α-tocopherol and serum and tissue lipids, and the influence of dietary 5% pectin and 0.2% ethyl p-chlorophenoxyisobutyrate (clofibrate) on the changes in these lipids due to dietary 0.4% Chloretone. Dietary addition of PCB increased serum concentrations of α-tocopherol, cholesterol, and phospholipids. These were mainly attributed to the increments in the fraction of high-density lipoproteins. PCB intake increased tissue α-tocopherol and apparent absorption of α-tocopherol from intestine. Similar changes in serum and tissue lipids and in intestinal absorption of α-tocopherol were observed with dietary Chloretone and phenobarbital. Dietary clofibrate and pectin depressed the increase in serum concentrations of these lipids by Chloretone. Changes in serum levels of α-tocopherol, cholesterol, and phospholipids correlated with those in apparent absorption of α-tocopherol. These results suggest that the increase in serum cholesterol and phospholipids due to xenobiotics relates to the increase in serum and tissue α-tocopherol.

Keywords: α-tocopherol; cholesterol; xenobiotics; clofibrate; pectin; intestinal absorption of α-tocopherol

Introduction

Administration of xenobiotics (including polychlorinated biophenyls (PCB), chlorobutanol (Chloretone), and pentobarbital) to rats causes not only an induction of liver microsomal drug-metabolizing enzymes, but also an increase in serum cholesterol or HDL-cholesterol and urinary ascorbic acid.[1,2] Recently, we demonstrated an increased serum α-tocopherol in rats fed some xenobiotics.[3] Further, we showed that dietary PCB increased concentrations of α-tocopherol in several organs, including kidney, spleen, lung, muscle, etc.[4] These increments in serum and tissue vitamin E seem to be a protective response against lipid peroxidation induced by the chemicals, since PCB feeding increases liver lipid peroxidation and vitamin E requirement.[5] In the present study, to clarify the mechanism of these effects on tissue vitamin E, we studied the effects of dietary addition of PCB, Chloretone, and phenobarbital on intestinal absorption of α-tocopherol.

We previously reported that serum cholesterol generally correlated with serum α-tocopherol in rats fed xenobiotics.[3] Kato et al. reported that the increase in serum cholesterol and α-tocopherol by PCB was potentiated with high protein diets and with copper deficient diets.[4,6,7] In view of these facts, we speculate that the changes in the metabolism of cholesterol due to xenobiotics might relate to the increase in serum α-tocopherol. Feeding of pectin and ethyl p-chlorophenoxyisobutylate (clofibrate) has been reported to reduce serum cholesterol in rats fed PCB.[8,9] The present study further examines the relationship between the changes in serum cholesterol and those in serum α-tocopherol of rats fed xenobiotics, as well as the influence of dietary addition of pectin or clofibrate on the changes in serum α-tocopherol due to xenobiotics, with special reference to serum cholesterol. The distri-

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bution of α-tocopherol in serum lipoproteins of rats exposed to xenobiotics was also studied and compared with serum lipoprotein cholesterol.

Materials and methods

Animals and diets

Male rats of the Wistar strain weighing 36–56 g (Hiroshima Laboratory Animal Center, Hiroshima, Japan) were fed a stock diet (MF, Oriental Yeast Co. Ltd., Tokyo) for 3 days until day 0 when the rats were transferred to the experimental diets. Room temperature was kept at 24°C with a 12-hour cycle of light (8:00 a.m.–8:00 p.m.) and dark.

In experiment 1, 0.05% PCB (PCB-48, Tokyo Kasei Kogyo Co., Ltd., Tokyo) was added to the basal diet as shown in *Table 1*. In experiment 2, 0.3% Chloretone (Wako Pure Chemical Industries Ltd., Tokyo) and 0.2% phenobarbital sodium (Wako Pure Chemical Industries Ltd., Tokyo) were added to the basal diet. In experiment 3, 0.2% clofibrate (Sigma Chemical Co., Ltd., USA) and 5% pectin (from citrus, Wako Pure Chemical Industries Ltd., Tokyo) were added to the basal diet with or without the addition of 0.4% Chloretone. Pectin was added at the expense of 4% cellulose powder plus carbohydrate. The experimental diets and tap water were supplied ad libitum throughout these studies. After feeding the test diets for 14 to 15 days, the diets were removed from the cages at 8:00 a.m., and the animals were lightly anesthetized with ether and killed between 1:00 p.m. and 3:00 p.m. Blood was collected by heart puncture. Aliquots of blood were allowed to clot at 4°C and serum samples were isolated by centrifugation (10 min, 1,500*g*). Liver, kidney, and spleen were immediately excised and weighed. In experiments 1 and 2, feces of each rat were collected daily over a 10-day period from day 3 to day 13 and then combined. In experiment 3, feces were collected over a 13-day period from day 1 to day 14.

Analytical procedures

In experiments 1 and 2, 0.8 mL sera of each rat were pooled and subjected to preparative ultracentrifugation. The lipoproteins, including chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), were isolated by flotation.[10]

The concentrations of α-tocopherol in serum, serum lipoproteins, tissues, and feces were measured by HPLC method described previously.[4] Apparent absorption of α-tocopherol was determined as a percentage of intake [100% × (intake-feces)/intake]. Fecal neutral and acid steroids were extracted by the method of Hill and Aries.[11] Neutral steroids were determined by a modification of Liebermann/Burchard reaction, using cholesterol as a standard.[12] Bile acids were determined fluorometrically by the procedure of Levin et al., using cholic acid as a standard.[13] The lipids in serum lipoproteins were extracted by the method of Folch et al.[14] and used for the determination of cholesterol and phospholipids in serum lipoproteins by the procedure of Pearson et al.[12] and by a commercially available kit (Phospholipid-Test, Wako Pure Chemical Industries Ltd., Osaka), respectively. These methods were also used to determine the concentrations of serum cholesterol and phospholipids. Tissues were homogenized with ice-cold 5% metaphosphoric acid and centrifuged for 10 minutes at 1,600*g*. Ascorbic acid in the supernatant was measured by the dinitrophenylhydrazine method.[15]

Table 1 Composition of basal diet

Ingredient	%
Casein	25
α-Corn starch	40
Sucrose	20
Corn oil	5
Cellulose powder	4
Salt mixture[a]	4
Vitamin mixture[b]	2

[a] Harper, A.E. *J. Nutr.* **68,** 405. (1955).
[b] Oriental Yeast Co. Ltd., Tokyo. Provided the following (mg per 100 g diet): thiamine HCl, 2.4; riboflavin, 8; pyridoxine HCl, 1.6; cyanocobalamine, 0.001; DL-α-tocopherol acetate, 10; menadione, 10.4; D-biotin, 0.04; folic acid, 0.4; Ca pantothenate, 10; p-aminobenzoic acid, 10; niacin, 12; inositol, 12; choline chloride, 400; retinyl acetate, (1000 IU); cholecalciferol, (200 IU).

Statistical analysis

Student's *t* test was used to statistically analyze the data in experiments 1 and 2.[16] In experiment 3, two-way analysis of variance[16] and Duncan's multiple-range test[17] were used to analyze the data.

Results

Experiments 1 and 2

Table 2 shows the effects of dietary PCB, Chloretone, and phenobarbital on growth rate, tissue lipids, tissue ascorbic acid, and apparent absorption of α-tocopherol. Growth rate was significantly depressed by dietary PCB and phenobarbital, but not by dietary Chloretone. Dietary PCB caused a 15% reduction in the food intake. Dietary phenobarbital caused a 16% reduction in food intake, but Chloretone intake caused no influence. Dietary PCB and Chloretone significantly increased serum cholesterol (*Table 2*). Dietary phenobarbital caused a trend of increase in serum cholesterol. These chemicals significantly increased serum α-tocopherol and phospholipids. Dietary PCB, Chloretone and phenobarbital caused increases in liver weight (61%, 19%, and 31%, respectively) when expressed as percent of body weight, but caused no influence on the weight of kidney and spleen. Dietary PCB significantly increased the concentrations of α-tocopherol in kidney and spleen. Liver content of α-tocopherol was increased by PCB intake when expressed per 100 g body weight, although dietary PCB caused no effect on liver concentrations of α-tocoph-

Table 2 Effects of some xenobiotics on serum and tissue lipids and intestinal absorption of α-tocopherol in rats

Groups	Experiment 1		Experiment 2		
	Control	0.05% PCB	Control	0.3% Chloretone	0.2% Phenobarbital
Gains in body wt. (g/14 days)	105 ± 3[a]	89 ± 4*	114 ± 4	118 ± 4	93 ± 3*
Serum					
α-Tocopherol (μmol/L)	55.5 ± 2.8	85.0 ± 2.8*	54.1 ± 3.0	89.6 ± 7.4*	86.4 ± 4.4*
Cholesterol (mmol/L)	3.59 ± 0.16	6.18 ± 0.34*	2.95 ± 0.23	4.14 ± 0.31*	3.67 ± 0.41
Phospholipids (mmol/L)	1.76 ± 0.09	3.05 ± 0.26*	2.80 ± 0.08	3.52 ± 0.19*	3.51 ± 0.21*
Liver					
α-Tocopherol (nmol/g tissue)	157 ± 14	150 ± 7	122 ± 6	145 ± 10*	172 ± 18*
Ascorbic acid (μmol/g tissue)	1.25 ± 0.08	3.16 ± 0.08*	1.38 ± 0.06	3.49 ± 0.12*	2.89 ± 0.15*
Kidney					
α-Tocopherol (nmol/g tissue)	99 ± 3	126 ± 8*	110 ± 6	160 ± 19*	209 ± 15*
Ascorbic acid (μmol/g tissue)	0.87 ± 0.05	1.98 ± 0.08*	0.87 ± 0.03	2.02 ± 0.02*	1.65 ± 0.11*
Spleen					
α-Tocopherol (nmol/g tissue)	194 ± 7	228 ± 10*	165 ± 16	229 ± 16*	254 ± 8*
Ascorbic acid (μmol/g tissue)	ND[b]	ND	2.20 ± 0.09	3.15 ± 0.12*	2.88 ± 0.05*
Apparent absorption of α-Tocopherol (%)	61.3 ± 1.7	67.7 ± 1.1*	57.5 ± 1.8	63.9 ± 1.9*	61.0 ± 4.2

[a] Means ± SE ($N = 6$)
[b] Not determined.
* Significantly different from control group ($P < 0.05$).

erol (nmol/g tissue). Chloretone or phenobarbital intake increased the concentrations of α-tocopherol in liver, kidney, and spleen. The concentrations of ascorbic acid in tissues examined here were increased by the three chemicals (*Table 2*).

Apparent absorption of α-tocopherol was significantly increased by PCB and Chloretone. Phenobarbital intake caused a trend of increase in the apparent absorption of α-tocopherol. In experiment 2, apparent absorption of α-tocopherol significantly ($P < 0.05$) correlated with serum α-tocopherol ($r = 0.556$), cholesterol ($r = 0.660$), and phospholipids ($r = 0.496$). Fecal excretion of neutral steroids and bile acids was not significantly affected by the chemicals.

Distributions of α-tocopherol, cholesterol, and phospholipids in serum lipoproteins were illustrated in *Figures 1* and *2*. PCB intake increased these lipids in all of the fractions. In addition, the increases of these lipids in serum due to PCB were mainly due to the increments in the fraction of HDL. As shown in *Figure 2*, dietary Chloretone and phenobarbital also increased the levels of α-tocopherol, cholesterol, and phospholipids in the fractions of chyromicrons, LDL, and HDL, although the lipids in VLDL were slightly decreased by the chemicals.

Experiment 3

Table 3 shows the effects of dietary 5% pectin and 0.2% clofibrate on growth rate, serum lipids, and apparent absorption of α-tocopherol in rats fed 0.4% Chloretone containing diets. Dietary Chloretone significantly depressed growth rate. Dietary pectin slightly depressed growth rate.

Dietary Chloretone remarkably increased serum α-tocopherol, cholesterol, and phospholipids. These effects were significantly depressed by dietary pectin and clofibrate. In the rats not receiving Chloretone, dietary clofibrate also decreased serum cholesterol and phospholipids, and there was a decreasing trend of serum α-tocopherol in rats fed clofibrate (*Table 3*). The concentration of liver α-tocopherol was significantly increased by Chloretone intake, and dietary

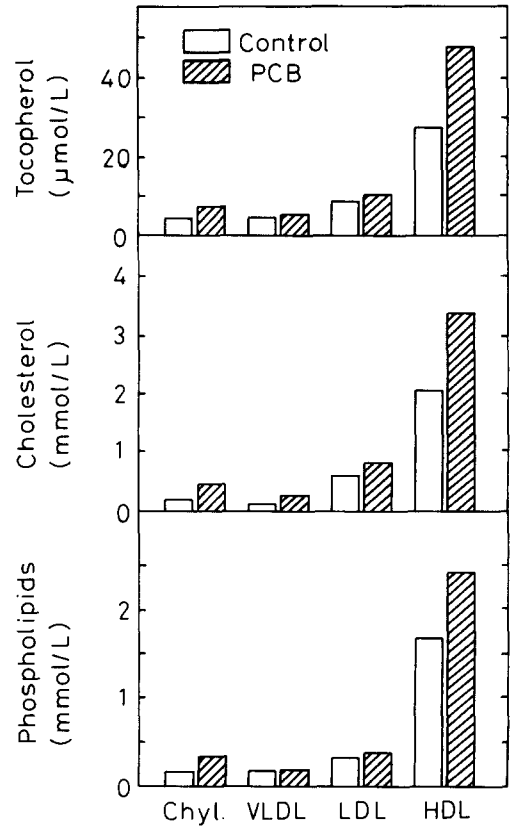

Figure 1 Effects of dietary PCB on the distributions of lipids in serum lipoproteins (Chyl.: chylomicrons.)

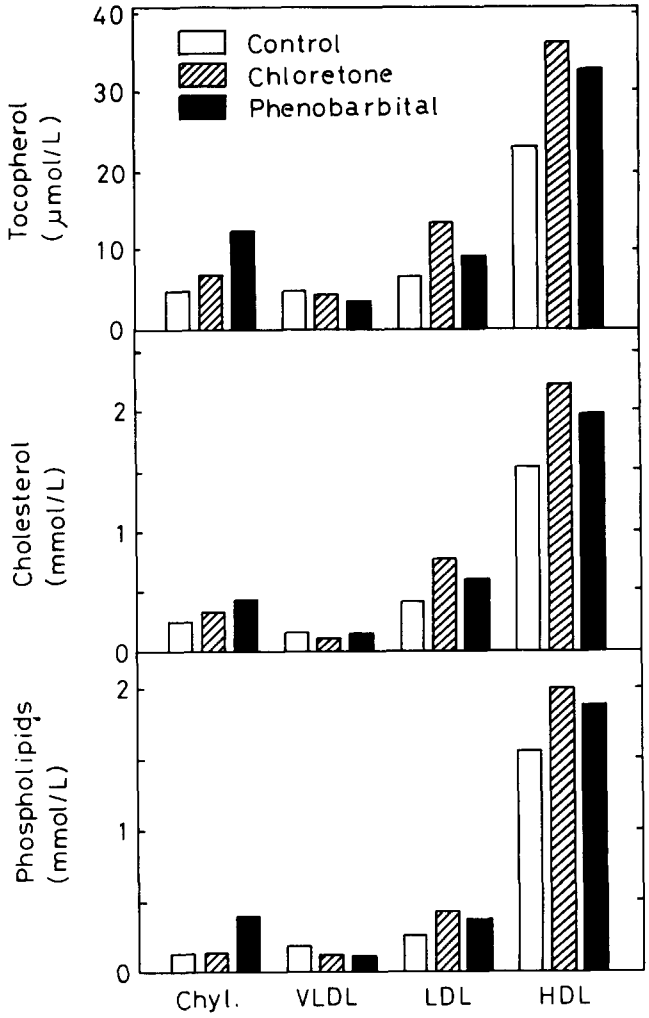

Figure 2 Effects of dietary Chloretone and phenobarbital on the distributions of lipids in serum lipoproteins. (Chyl.: chylomicrons.)

pectin and clofibrate significantly decreased liver α-tocopherol, regardless of Chloretone intake.

In this experiment, dietary Chloretone, pectin, and clofibrate caused a significant effect on apparent absorption of α-tocopherol. Apparent absorption of α-tocopherol was significantly increased by Chloretone intake, which was depressed by dietary clofibrate. In the rats not receiving Chloretone, dietary pectin significantly decreased apparent absorption of α-tocopherol. Pectin intake caused a trend of depression in the increment of apparent absorption of α-tocopherol by Chloretone. Apparent absorption of α-tocopherol significantly ($P < 0.05$) correlated with serum concentrations of α-tocopherol ($r = 0.669$), cholesterol ($r = 0.723$), and phospholipids ($r = 0.680$) in this experiment. Dietary 0.4% Chloretone caused a 26 to 35% reduction in fecal neutral steroids ($P < 0.05$) except for pectin treated groups. However, dietary addition of pectin and clofibrate caused no significant influence on fecal neutral steroids. Fecal bile acids were not significantly affected by these dietary manipulations.

Discussion

The present study demonstrated an increase in apparent absorption of α-tocopherol from intestine caused by dietary PCB and Chloretone (*Tables 2* and *3*). A similar trend was observed with dietary phenobarbital (*Table 2*) The results indicate that the increase in serum and tissue α-tocopherol due to xenobiotics is at least in part attributed to the increase in intestinal absorption of α-tocopherol.

We have reported increased cholesterol and α-tocopherol in serum HDL due to PCB intake.[2,4] In accordance with these studies, the present study showed that the increment in serum cholesterol, phospholipids, and tocopherol were mainly attributable to

Table 3 Influence of dietary pectin and clofibrate on serum and tissue lipids and intestinal absorption of α-tocopherol in rats fed 0.4% Chloretone

Groups	None	Normal pectin	Clofibrate	None	Chloretone pectin	Clofibrate	Main effects[2]
Gains in body wt. (g/14 days)	116 ± 3[a,1]	104 ± 2[b]	111 ± 3[a,b]	89 ± 4[c]	87 ± 4[c]	95 ± 3[c]	CH, PE
Serum							
α-Tocopherol (μmol/L)	39.5 ± 4.6[a]	37.6 ± 3.5[a]	30.6 ± 2.1[a]	95.7 ± 5.3[b]	63.2 ± 4.4[c]	61.5 ± 3.0[c]	CH, CL, PE
Cholesterol (mmol/L)	3.52 ± 0.21[a]	3.08 ± 0.13[a]	2.48 ± 0.08[b]	5.77 ± 0.31[c]	4.97 ± 0.18[d]	4.47 ± 0.23[d]	CH, CL, PE
Phospholipids (mmol/L)	2.40 ± 0.08[a,b]	2.19 ± 0.10[b,c]	1.86 ± 0.09[c]	3.47 ± 0.18[d]	3.05 ± 0.18[e]	2.71 ± 0.10[a,e]	CH, CL, PE
Liver							
α-Tocopherol (nmol/g tissue)	125 ± 8[a]	110 ± 4[b]	95 ± 4[c]	163 ± 6[d]	110 ± 5[a,b]	113 ± 5[b]	CH, CL, PE
Apparent absorption of α-tocopherol (%)	58.0 ± 2.3[a,b]	45.1 ± 3.8[c]	50.4 ± 2.6[a,c]	71.0 ± 1.7[d]	64.3 ±2.9 [d,e]	62.5 ± 4.9[a,e]	CH, CL, PE

[1] Means ± SE ($N = 6$). Data were analyzed by two-way analysis of variance and Duncan's multiple range test. Means not followed by the same letter are significantly different ($P < 0.05$).
[2] CH: Significantly affected by dietary Chloretone ($P < 0.05$). CL: Significantly affected by dietary clofibrate ($P < 0.05$). PE: Significantly affected by dietary pectin ($P < 0.05$).

the increase in the fraction of HDL. Similar effects of Chloretone and phenobarbital were also observed. Furthermore, an increased α-tocopherol in the fraction of chylomicrons by xenobiotics was found (*Figures 1* and *2*). α-Tocopherol is absorbed in the small intestine and transported in lymph associated with chylomicrons.[18] The increased α-tocopherol in chylomicrons by xenobiotics might be due to the increase in intestinal absorption of α-tocopherol.

Previously, we reported that serum cholesterol generally correlated with serum vitamin E in rats fed xenobiotics.[3] Further, in the present study, serum cholesterol and phospholipids correlated well with serum α-tocopherol and apparent absorption of α-tocopherol. In addition, there were similar trends in the changes of α-tocopherol, cholesterol, and phospholipids in the fractions of serum lipoproteins by xenobiotics (*Figures 1* and *2*). Some workers also reported general correlation between serum cholesterol and α-tocopherol in rats or humans under several dietary conditions.[19,20] These facts stress the importance of the metabolic interrelationship among these lipids.

Quazi et al.[8] and Nakagawa et al.[9] showed that the increase in cholesterol due to PCB intake was depressed by pectin and clofibrate intake. Schaus et al. showed a depressed availability of vitamin E by dietary pectin.[21] The present study reported here demonstrated that dietary pectin and clofibrate depressed the increase in serum α-tocopherol, cholesterol, and phospholipids and in liver α-tocopherol by Chloretone (*Table 3*). In view of these facts, we speculate that the increase in serum cholesterol and phospholipids due to xenobiotics may be necessary for the increase in serum and tissue α-tocopherol. Obviously, until more direct experimental evidence is obtained, this idea remains speculative.

On the other hand, in agreement with previous reports,[1,22,23] dietary PCB, Chloretone, and phenobarbital increased tissue ascorbic acid (*Table 2*). It has been reported that the increment in tissue and urinary ascorbic acid by xenobiotics is attributable to the increased ascorbic acid synthesis.[22,23] Vitamin C has been shown to be important for the regeneration of vitamin E from the tocopherol radical in in vitro systems.[24,25] Thus, the increase in tissue ascorbic acid may also contribute to the increase in tissue α-tocopherol by xenobiotics. Further studies are in progress to examine this possibility.

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