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# Glucocorticoid-dependent induction of HMG-CoA reductase and malic enzyme gene expression by polychlorinated biphenyls in rat hepatocytes

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*Administration of xenobiotics to rats results in hypercholesterolemia and in the induction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and malic enzyme. To investigate the mechanism of the induction of the enzymes by xenobiotics, the effects of xenobiotics on gene expressions for HMG-CoA reductase, malic enzyme, and cytochrome P-450 in rat liver and in cultured hepatocyte were investigated. The treatment of rats with polychlorinated biphenyls (PCB) as a xenobiotic induced mRNAs for HMG-CoA reductase and malic enzyme as well as CYP2B1/2 (cytochrome P-450b/e). Other xenobiotics, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), and chloretone, also increased HMG-CoA reductase mRNA. In an investigation of diurnal rhythm of mRNA for HMG-CoA reductase, the induction by PCB was observed in a dark period. Induced expressions of HMG-CoA reductase gene and malic enzyme gene by PCB were observed in primary cultured rat hepatocytes and showed that the action of PCB on the gene expression relating to lipid metabolism was directed on hepatocytes. The induction was observed only in hepatocytes cultured on Engelbreth-Holm-Swarm sarcoma basement membrane gel (EHS-gel), not on type I collagen, which is usually used for monolayer culture of hepatocytes. The induction of CYP2B1/2 gene expression also was observed only in the cells cultured on EHS-gel. The induction of HMG-CoA reductase and malic enzyme by PCB required dexamethasone. However, the addition of dexamethasone per se to medium containing insulin did not show an inductive effect on levels of mRNA for HMG-CoA reductase and malic enzyme. From the data of diurnal variation and hepatocyte culture experiment, HMG-CoA reductase and malic enzyme are considered to be induced by PCB through the so-called "permissive effect" of glucocorticoid.* (J. Nutr. Biochem. 10:644–653, 1999) 

**Keywords:** HMG-CoA reductase; malic enzyme; PCB; glucocorticoid; primary hepatocyte; EHS-gel

## Introduction

It is known that administration of polychlorinated biphenyls (PCB) to rats causes an increase in the serum level of cholesterol, high density lipoprotein cholesterol, tissue and urinary ascorbic acid and liver drug-metabolizing enzymes, as well as an increase in liver weight.[1–4] Some other xenobiotics, including 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), 2,6-di-*tert*-butyl-*p*-cresol, and phenobarbital, also have exhibited a significant increase in serum cholesterol, liver lipids, and urinary ascorbic acid.[1–5] It has also been reported that administration of xenobiotics to rats increased the activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and nicotinamide adenine dinucleotide phosphate (NADPH)-generating enzymes such as malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase.[6–10] The induction of these enzymes by PCB might be responsible for hypercholesterolemia and fatty liver.[6,9,11] However, the mechanism

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by which PCB treatment resulted in the induction of these enzymes remains completely unknown.

The biosynthesis of cholesterol in mammalian liver is regulated principally through microsomal HMG-CoA reductase, which catalyzes the rate-limiting reaction in this pathway.[12] The activity of HMG-CoA reductase is regulated by a mechanism involving (1) negative feedback regulation caused by cholesterol at the transcriptional level,[13] (2) degradation and stability of the enzyme and mRNA for HMG-CoA reductase,[14,15] and (3) modification of the enzyme activity by phosphorylation, thiols, and substrate.[16–18]

Malic enzyme activity is high in lipogenic tissues, where it is regulated by changes in the nutritional or hormonal state.[19,20] Thyroid hormone enhances transcription of malic enzyme gene and induces stabilization of malic enzyme mRNA.[21]

Rat hepatocytes in primary culture have been used to study the mechanism by which growth and differentiation functions are regulated.[22] However, in usual conditions monolayer hepatocytes rapidly lose some liver-specific functions after isolation, for example, induction of some cytochrome P-450s by xenobiotics,[23,24] transcription of albumin gene, α1-antitrypsin gene, and C/EBPα gene.[25] It has been demonstrated that the extracellular matrix plays an important role in maintaining liver-specific gene expression in cultured hepatocytes.[23,24,26] Induction of CYP2B1/2 gene expression by xenobiotics cannot be detected in hepatocytes cultured on type I collagen (TIC). However, its induction was observed in spherical hepatocytes cultured on Engelbreth-Holm-Swarm sarcoma basement membrane gel (EHS-gel).[23,24] Moreover, hepatocytes easily attach to EHS-gel and live longer on it than on TIC without any hormone (Oda et al., unpublished results). Therefore, EHS-gel would appear to be an applicable tool to determine the hormonal effect on gene expression and enzyme activity, especially as they relate to xenobiotic responses.

## Materials and methods

### *Animals and diets*

Male Wistar rats (Japan SLC, Hamamatsu, Japan) aged 5 to 6 weeks and weighing approximately 90 g were used for these experiments. The rats were housed individually and transferred to a semipurified diet (control diet)[3] after feeding a commercial nonpurified diet (CE-2, Japan Clea, Co., Tokyo, Japan) for 3 days. The composition of the control diet was (in percentage): casein, 25.0; mineral mixture,[27] 3.5; corn oil 2.0; vitamin mixture,[27,28] 1.0; choline chloride, 0.2; and a mixture of sucrose and corn starch (1:2) to 100%. In the experimental diet, 200 mg of PCB (Aroclor 1254, Mitsubishi Monsanto Co., Tokyo, Japan), 2 g of DDT, or 5 g of chloretone were added per kilogram of the control diet at the expense of carbohydrate.

All diets and tap water were supplied ad libitum. Room temperature was kept 24°C with a 12-hour light (8:00 AM–8:00 PM)/dark (8:00 PM–8:00 AM) cycle. At the end of the experimental periods, blood from nonfasted rats was collected from 1:00 AM to 3:00 AM by decapitation. Serum was separated by centrifugation at 1,500 × g for 10 minutes. The livers were immediately removed and weighed. A portion of the livers were stored at −80°C for RNA extraction. Microsomes were obtained for HMG-CoA reductase activity.

### *Preparation of cultured adult rat hepatocytes*

Rat parenchymal hepatocytes were isolated by perfusion of liver with collagenase as described previously[29] and cultured in Waymouth's MB 752/1 medium containing insulin ($10^{-8}$M), amphotericin B (0.25 mg/mL), penicillin (5 IU/mL), and streptomycin (5 mg/mL). Hepatocytes were plated at an initial density of $1 \times 10^7$ cells into a collagen-coated dish (100 mm, IWAKI-Corning Co. Ltd., Tokyo, Japan) and EHS-gel coated dish. The medium was changed 4 hours after plating. Twenty-four hours after plating, hepatocytes were treated with PCB (20 μg/mL), insulin ($10^{-8}$ M), and/or dexamethasone ($10^{-6}$ M) for 48 hours. EHS-gel was prepared from EHS tumor passed to C57BL/6J female mice as described by Kleinman et al.[30]

### *Assay of HMG-CoA reductase*

Approximately 1.5 g of liver was homogenized in four volumes of 0.05 M potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose, 0.075 M nicotinamide, 10 mM dithiothreitol, 50 mM NaF, and 2.5 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 12,000 × g for 15 minutes at 4°C to remove mitochondria and nuclei. The supernatant liquid was centrifuged at 100,000 × g for 60 minutes at 4°C. 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. In case of total activity, 10 units of *Escherichia coli* alkaline phosphatase was added to dephosphorylate HMG-CoA reductase, and the mixture was incubated at 37°C for 60 minutes. In case of the activity of the active form of the enzyme, alkaline phosphatase was not added. The incubation mixture contained 10 μmol potassium phosphate (pH 7.2), 1 μmol dithiothreitol, 2 μmol EDTA, 2 μmol NADPH, 0.05 μmol DL-[3-$^{14}$C]HMG-CoA, and 250 to 500 μg protein of microsomes in a final volume of 100 μL. Incubation was carried out in a metabolic shaker at 37°C for 15 minutes. The reaction was terminated by the addition of 15 μL of 10 M HCl, and [5-$^{3}$H]mevalonic acid was added as an internal standard. After lactonization of mevalonic acid, mevalonolactone was separated by thin layer chromatography and scraped into the counting vial.

### *RNA isolation and Northern blot analysis*

RNA was extracted according to the method of Chomczynski and Sacchi.[31] Ten to twenty micrograms of total cellular RNA was subjected to electrophoresis on 1% agarose containing 2.2 M formaldehyde. To ensure that the equal amount of RNA was loaded, RNA was visualized with ethidium bromide. RNA was transferred to Hybond-N+ membrane (Amersham, Pharmacia Biotech, Tokyo, Japan) by overnight capillary blotting. The membranes were baked at 80°C, then prehybridized at 42°C for at least 8 hours. The amount of $^{32}$P-labeled cDNA probe added to the hybridization solution was $6 \times 10^6$ cpm/mL, and hybridization was carried out for 48 or 24 hours for HMG-CoA reductase mRNA or other mRNA, respectively. After washing, filters were exposed to Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY) for 1 to 3 days, and the radioactivity on the filters was quantified by a radioanalytical imaging system (Ambis Systems, San Diego, CA). The cDNA clones of hamster HMG-CoA reductase (ATCC#37365),[32] rat malic enzyme,[19] rat apolipoprotein A-I,[33] mouse apolipoprotein E,[34] rat CYP2B1,[35] and mouse β-actin were labeled with [5′α-$^{32}$P]dCTP using Megaprime DNA labeling system (Amersham, Pharmacia Biotech, Tokyo, Japan). Membranes were repeatedly reprobed with the above mentioned cDNA probes. Because the level of apolipoprotein E mRNA was not affected by PCB in rat liver and hepatocyte, apolipoprotein E mRNA was used as a reference.

**Table 1** Effect of dietary PCB on body weight gain, liver weight, and serum levels of cholesterol and apolipoprotein A-I in rats

| | Body weight gain (g) | Liver weight (g/100 g body weight) | Serum cholesterol (mmol/L) | Serum apolipoprotein A-I (arbitrary units) |
|---|---|---|---|---|
| 1 day | | | | |
| Control | 5 ± 1 | 4.72 ± 0.10 | 2.55 ± 0.14 | 100.0 ± 8.3 |
| PCB | 5 ± 1 | 4.84 ± 0.05 | 2.24 ± 0.07 | 91.3 ± 5.7 |
| 3 days | | | | |
| Control | 17 ± 1 | 4.61 ± 0.04 | 2.51 ± 0.15 | 100.0 ± 5.1 |
| PCB | 17 ± 1 | 5.36 ± 0.04† | 2.81 ± 0.11 | 103.6 ± 5.6 |
| 7 days | | | | |
| Control | 36 ± 1 | 4.44 ± 0.07 | 2.55 ± 0.14 | 100.0 ± 3.4 |
| PCB | 33 ± 1 | 6.10 ± 0.10† | 3.25 ± 0.23* | 163.0 ± 6.0† |

Rats were fed polychlorinated biphenyls (PCB) for 1, 3 or 7 days. Values are means ± SEM for 6 rats.
*,†Significant differences as compared with control on each day ($P < 0.05$, $P < 0.001$, respectively).

### *Other methods*

Cholesterol levels were determined with a commercially available kit (Boehringer Mannheim, Mannheim, Germany). The serum level of apolipoprotein A-I was quantified by densitometric scanning of nonreducing SDS-PAGE patterns of serum proteins.[36] Microsomal protein was measured using the Lowry assay with bovine serum albumin as a standard. The statistical significance of differences among values was analyzed by Student's *t*-test and by one-way analysis of variance plus Duncan's multiple-range test.

## Results

### *Activity and mRNA of HMG-CoA reductase induced in rats fed PCB*

Data on body weight gain, liver weight, and serum levels of cholesterol and apolipoprotein A-I are presented in *Table 1*. The addition of PCB to the diet did not affect growth. Liver weight, expressed as a percentage of body weight, was higher in rats fed PCB for 3 or 7 days. Serum levels of cholesterol and apolipoprotein A-I were higher in rats fed PCB for 7 days than controls.

*Figure 1* shows the activity of HMG-CoA reductase in liver microsomes of rats fed PCB. As demonstrated previously,[7,8] the total enzyme activity was increased by 1 day of feeding of PCB, and the activity increased with PCB feeding time. The activity of the active form of HMG-CoA reductase, the dephosphorylated form, increased in rats fed PCB on days 3 and 7. Northern blot analysis of liver mRNA for HMG-CoA reductase, malic enzyme, apolipoprotein A-I, apolipoprotein E, and CYP2B1/2 is shown in *Figure 2*. The level of apolipoprotein E mRNA was not affected by PCB treatment. Therefore, we used apolipoprotein E mRNA as a reference, and normalized other mRNA with the mRNA. The level of HMG-CoA reductase mRNA and malic enzyme mRNA were increased 1.8- to 2.8-fold and 2.6- to 4.4-fold by PCB feeding, respectively. CYP2B1/2 mRNA was also induced by PCB. Apolipoprotein A-I mRNA was increased slightly but significantly by PCB on days 3 and 7 (1.4-fold and 1.3-fold, respectively; $P < 0.01$).

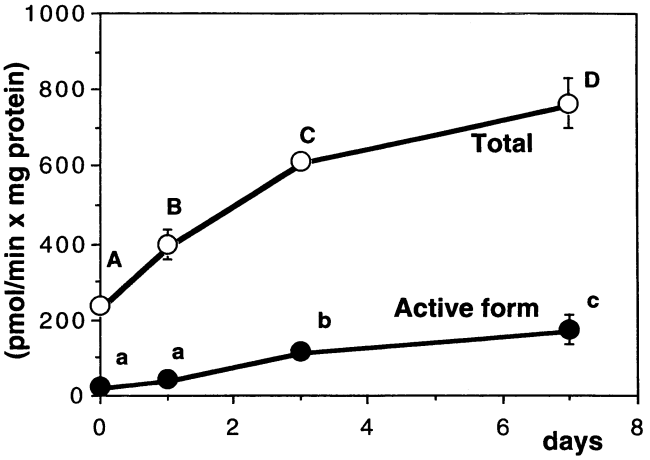


**Figure 1** Effect of dietary polychlorinated biphenyls on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in rats. Rats were fed PCB for 1, 3, or 7 days and were sacrificed by decapitation between 1:00 and 3:00 AM. Values are means ± SEM for 4 rats in each group. Points without vertical bars indicate that the bars fall within the size of the points. Statistical significance of differences among values was analyzed by one-way analysis of variance. Because the treatment was significant, Duncan's multiple-range test was performed. Points not followed by the same letter in the line are significantly different ($P < 0.05$, capital letter for total activity, small letter for the activity of the active form).

### *Induction of HMG-CoA reductase mRNA by xenobiotics*

Because several xenobiotics that cause hypercholesterolemia in rats induced liver HMG-CoA reductase,[5] we should determine if the other xenobiotics that result in hypercholesterolemia induce HMG-CoA reductase gene expression in rat liver. DDT and chloretone as well as PCB were chosen for this study because of the relatively high incidence with which they induce hypercholesterolemia in rats.[5] The xenobiotics elevated liver weight and serum cholesterol without changing body weight gain (*Table 2*). The level of HMG-CoA reductase mRNA was significantly increased by feeding PCB, DDT, and chloretone (*Figure 3*).

### *Diurnal variation of hepatic HMG-CoA reductase mRNA in rats fed PCB*

The activity and mRNA of HMG-CoA reductase show a diurnal rhythm.[37] In both control and PCB-treated rats, the highest mRNA for HMG-CoA reductase was found in the mid-dark period (*Figure 4*). The induction of the reductase by PCB was observed in the mid-dark period (1:00 AM) and just before the dark period (7:00 PM). Insulin has been implicated in governing the diurnal rhythm of HMG-CoA reductase activity and in mediating the postprandial rise in the reductase activity of fasted rats.[38] In adrenalectomy experiments, glucocorticoid hormone also played an important role in diurnal rhythm of the reductase.[39,40] Although the rat liver was continuously exposed to PCB in the present study, reductase induction was seen only in the mid-dark period. Therefore, it was expected that its induction by PCB was mediated by hormones such as insulin and/or glucocorticoid hormone. However, it is not easy to determine the

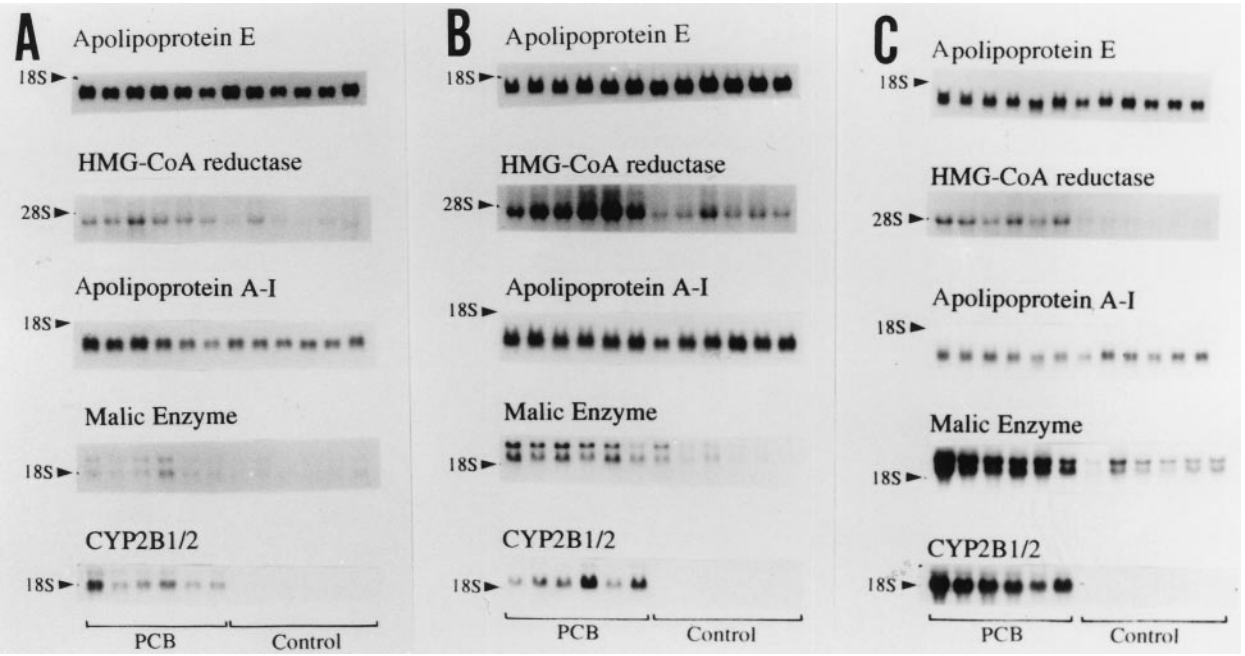


**Figure 2** Northern blot analysis of liver mRNA for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, malic enzyme, CYP2B1/2, apolipoprotein A-I, and apolipoprotein E in rats fed polychlorinated biphenyls (PCB) for *(A)* 1 day, *(B)* 3 days, and *(C)* 7 days. Rats were sacrificed between 1:00 and 3:00 AM by decapitation. Each group had 6 rats. Total RNA from each rat was loaded in each lane.

factors involved in the induction of the reductase in vivo. To obtain more information about the induction, a hepatocyte culture was performed.

### *Induction of HMG-CoA reductase by PCB in rat hepatocytes*

Hepatocytes cultured on TIC showed a flat monolayer (*Figure 5A*), whereas those cultured on the EHS-gel exhibited a spherical configuration (*Figure 5B*). The morphologic changes might be important for the function of hepatocytes. However, the components of extracellular matrix would play a more important role in liver-specific function.[24,41]

We did not observe any induction of HMG-CoA reductase activity in monolayer hepatocyte culture,[29] although dietary xenobiotics induced hepatic HMG-CoA reductase and malic enzyme in rats (*Figures 1 and 2*). By using the EHS-gel as an extracellular matrix, the induction of HMG-CoA reductase and malic enzyme mRNA could be observed in rat hepatocyte culture (*Figure 6*).[42] The induction of HMG-CoA reductase, malic enzyme, and CYP2B1/2 mRNA by PCB was not observed in hepatocytes cultured on

**Table 2** Effect of xenobiotics on body weight gain, liver weight, and serum level of cholesterol in rats fed the experimental diets for 7 days

| | Body weight gain (g) | Liver weight (g/100 g body weight) | Serum cholesterol (mmol/L) |
|---|---|---|---|
| Control | 33 ± 1 | 4.46 ± 0.10[a] | 2.31 ± 0.09[a] |
| PCB | 33 ± 2 | 6.50 ± 0.05[b] | 3.18 ± 0.07[c] |
| DDT | 29 ± 3 | 6.18 ± 0.21[b] | 2.69 ± 0.16[b] |
| Chloretone | 32 ± 3 | 6.03 ± 0.20[b] | 3.36 ± 0.07[c] |

Values are means ± SEM for 4 rats. Statistical significance of differences among values was analyzed by one-way anlysis of variance. When the treatment was significant, Duncan's multiple-range test was performed. Means within a column not followed by the same letter are significantly different ($P < 0.05$).
PCB, polychlorinated biphenyls. DDT, 1,1,1-trichloro-2-2-bis(*p*-chlorophenyl)ethane.

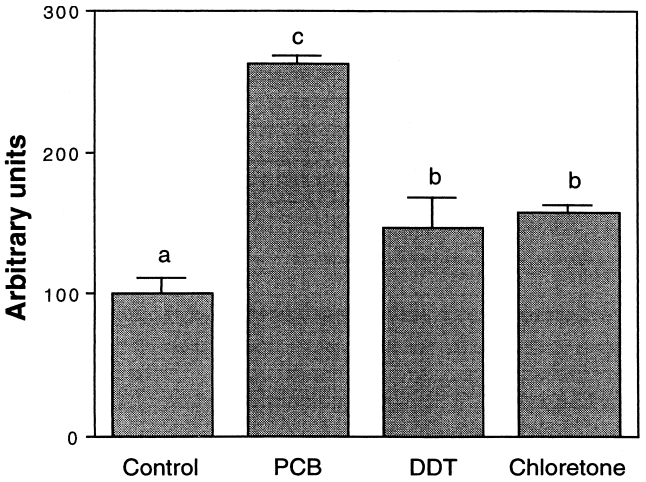


**Figure 3** Effect of dietary polychlorinated biphenyls (PCB), 1,1,1-trichloro-2.2-bis(*p*-chlorophenyl)ethane (DDT), or chloretone on liver 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA in rats. Rats were fed PCB, DDT, or chloretone for 7 days and were sacrificed by decapitation between 1:00 and 3:00 AM. Values are means ± SEM for 4 rats. Values were normalized by apolipoprotein E mRNA and expressed as a percentage relative to the control group. Statistical significance of differences among values was analyzed by one-way analysis of variance. Because the treatment was significant, Duncan's multiple-range test was performed. Bars not followed by the same letter are significantly different ($P < 0.05$).

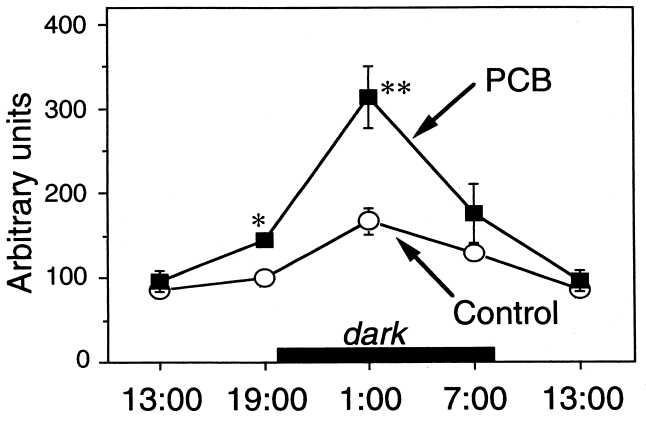


**Figure 4** Diurnal variation of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase mRNA in rats with (closed square) or without (open circle) polychlorinated biphenyls. After rats were fed a semipurified diet for 7 days, the experimental diets were given to animals at 7:00 PM. Twenty-four hours after starting the experimental diets, rats were sacrificed at intervals of 6 hours. Values are means ± SEM for 6 rats. Values were normalized by apolipoprotein E mRNA and expressed as percentage relative to control group at 7:00 PM. Points without vertical bars indicate that the bars fall within the size of the points. * and ** indicate significant difference as compared with control in each time ($P < 0.05$ and $P < 0.01$, respectively).

TIC. This result showed that PCB exerted the effect on hepatocytes directly.

We next determined the effect of media on the inducible expression of HMG-CoA reductase and malic enzyme mRNAs. Induction of HMG-CoA reductase and malic enzyme mRNAs was observed with similar magnitude in all media used in the present study, although the basal expression of the genes, except for apolipoprotein E, was highest in Waymouth's medium (*Figure 7*). Interestingly, no induction of CYP2B1/2 was detected in hepatocytes cultured in minimum essential medium (MEM). The only nutrients lacking in MEM are aspartic acid, glutamic acid, glycine, proline, and vitamin $B_{12}$. These nutrients might play a significant role in the induction of CYP2B1/2 gene expression. Because hepatocytes in Waymouth's medium showed higher gene expression of HMG-CoA reductase and malic enzyme, the medium and EHS-gel were used for additional experiments.

Hepatocytes cultured even on EHS-gel in a medium containing insulin as the sole hormone did not show the induction of HMG-CoA reductase and malic enzyme gene expression by PCB (*Figure 6*). In a medium containing both insulin and dexamethasone, PCB treatment resulted in increased mRNA for HMG-CoA reductase and malic enzyme (*Figure 6*). This observation suggested that the PCB induction of HMG-CoA reductase and malic enzyme required dexamethasone alone or both dexamethasone and insulin. To clarify the role of insulin and dexamethasone in the induction, hepatocytes were cultured on EHS-gel in media containing dexamethasone and/or insulin. HMG-CoA reductase and malic enzyme were induced by PCB either in media containing dexamethasone alone or both dexamethasone and insulin (*Figure 8*). As shown in *Figures 6* and *8*, there was no induction by PCB when insulin was

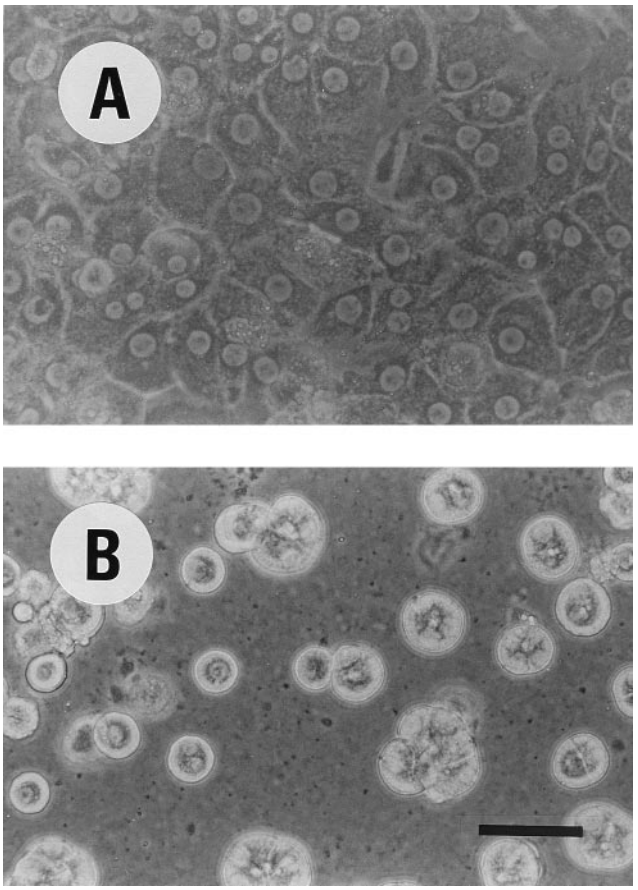


**Figure 5** Phase-contrast micrographs of rat hepatocytes cultured on *(A)* type I collagen or *(B)* Engelbreth-Holm-Swarm sarcoma basement membrane gel for 24 hours after isolation. Scale bar indicates 100 μm.

the sole hormone. These results demonstrate that glucocorticoid hormone, not insulin, is necessary for PCB to induce HMG-CoA reductase and malic enzyme mRNA. The treatment of dexamethasone itself did not increase HMG-CoA reductase mRNA level. With malic enzyme, dexamethasone influenced gene expression negatively. The effect of dexamethasone resembles the "permissive effect" seen in cAMP-dependent induction of phosphoenolpyruvate carboxykinase.[43] As reported previously,[44] glucocorticoid hormone served to induce CYP2B1/2 gene expression by PCB (*Figure 8*). Treatment with insulin, however, negatively affected CYP2B1/2 gene expression.[45]

## Discussion

Treatment with xenobiotics such as PCB results in hypercholesterolemia. The endogenous hypercholesterolemia is characterized by high levels of high density lipoproteins and apolipoprotein A-I,[3,6] and cholesterol-rich very low density lipoproteins.[11] Xenobiotics that result in hypercholesterolemia induced the activity[5] and mRNA of HMG-CoA reductase (*Figures 2 and 3*).[8] The induction of cholesterol synthesis is considered one of the etiologic factors in hypercholesterolemia. Moreover, we recently found that apolipoprotein A-I gene expression was induced by PCB feeding (*Figure 2*, unpublished results). Now we suppose

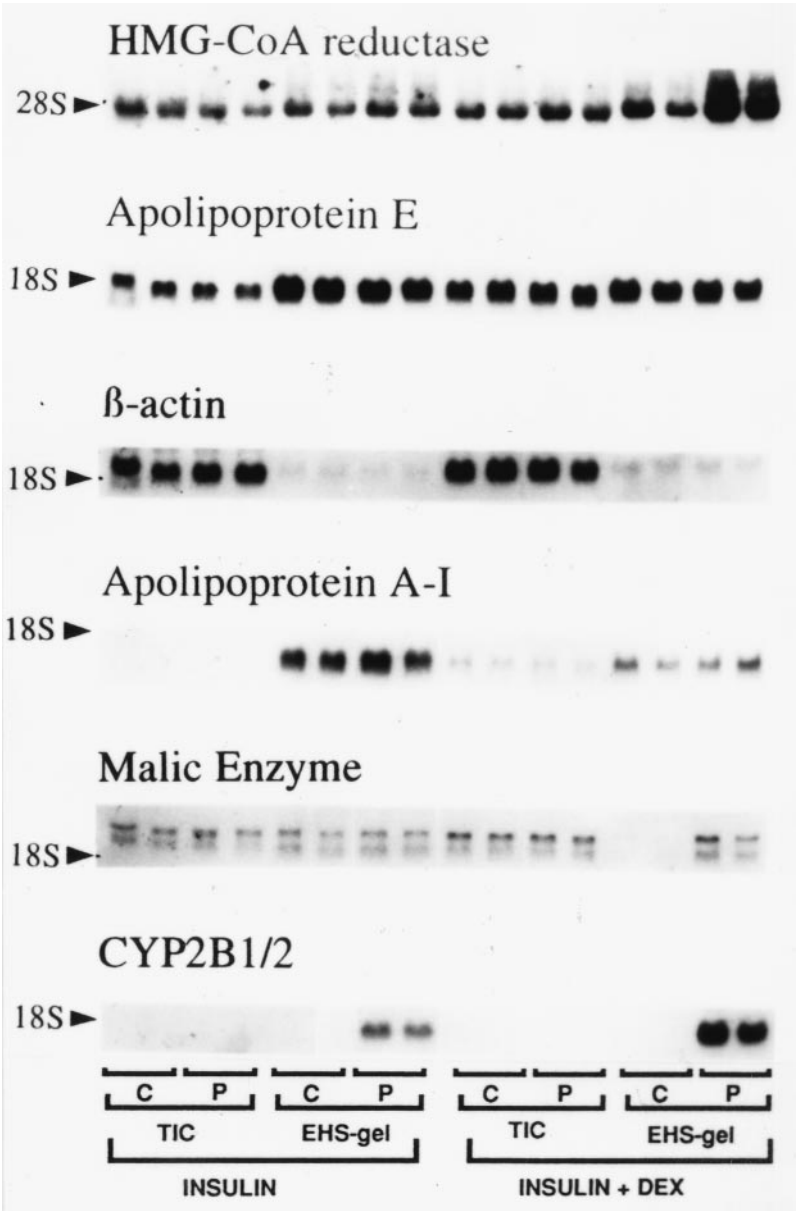


**Figure 6** Northern blot analysis of mRNA for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, malic enzyme, CYP2B1/2, apolipoprotein A-I, apolipoprotein E, and β-actin in rat hepatocytes cultured on type I collagen (TIC) or Engelbreth-Holm-Swarm sarcoma basement membrane gel (EHS-gel). Parenchymal hepatocytes were isolated by collagenase-perfusion method and cultured in serum-free medium with insulin ($10^{-8}$ M). Hepatocytes were plated at an initial density of $1 \times 10^7$ cells into 100 mm plastic dishes coated with TIC or EHS-gel. The medium was changed 4 hours after plating. Twenty-four hours after plating, hepatocytes were exposed to polychlorinated biphenyls (PCB; 20 μg/mL) and dexamethasone (DEX; $10^{-6}$ M) for 48 hours. A duplicate sample was loaded in each group. C, control group without PCB; P, PCB group (20 μg/mL).

that the enhancement of both cholesterol synthesis and apolipoprotein A-I synthesis is responsible for xenobiotic-induced hypercholesterolemia. However, the mechanism by which xenobiotics induce the activity and gene expression of HMG-CoA reductase in the liver is not known. Although we previously measured an endocrine system involved in the cholesterol metabolism, such as insulin, glucagon, corticosterone, and triiodothyronine, there were no significant changes in the serum levels of these hormones in rats fed PCB[43] (Nagaoka and Yoshida, unpublished results). This result demonstrated that those hormones per se were not primary inducers of HMG-CoA reductase gene expression in rats fed PCB. In the present study, to elucidate the mechanism of HMG-CoA reductase induction by xenobiotics, we investigated HMG-CoA reductase gene expression in vivo and in hepatocyte culture.

We first studied the diurnal rhythm of HMG-CoA reductase mRNA in rats fed PCB to obtain information on factors involved in the induction of the reductase by PCB. Its induction was observed only in the dark period (*Figure 4*), suggesting that some hormonal factors involved in diurnal rhythm played an important role in the induction of HMG-CoA reductase by PCB, and that an interaction between hormones and PCB enhanced the induction of HMG-CoA reductase gene expression. It has been demonstrated that insulin governed the diurnal rhythm of HMG-CoA reductase.[38] Glucocorticoid hormone also played an important role in diurnal variation of the reductase.[39,40]

We next determined whether these same hormones are involved in the induction of HMG-CoA reductase in vitro by using primary rat hepatocyte culture. PCB did not induce HMG-CoA reductase activity in monolayer hepatocytes cultured on TIC.[29] HMG-CoA reductase mRNA was induced only in hepatocytes cultured on EHS-gel (*Figure*

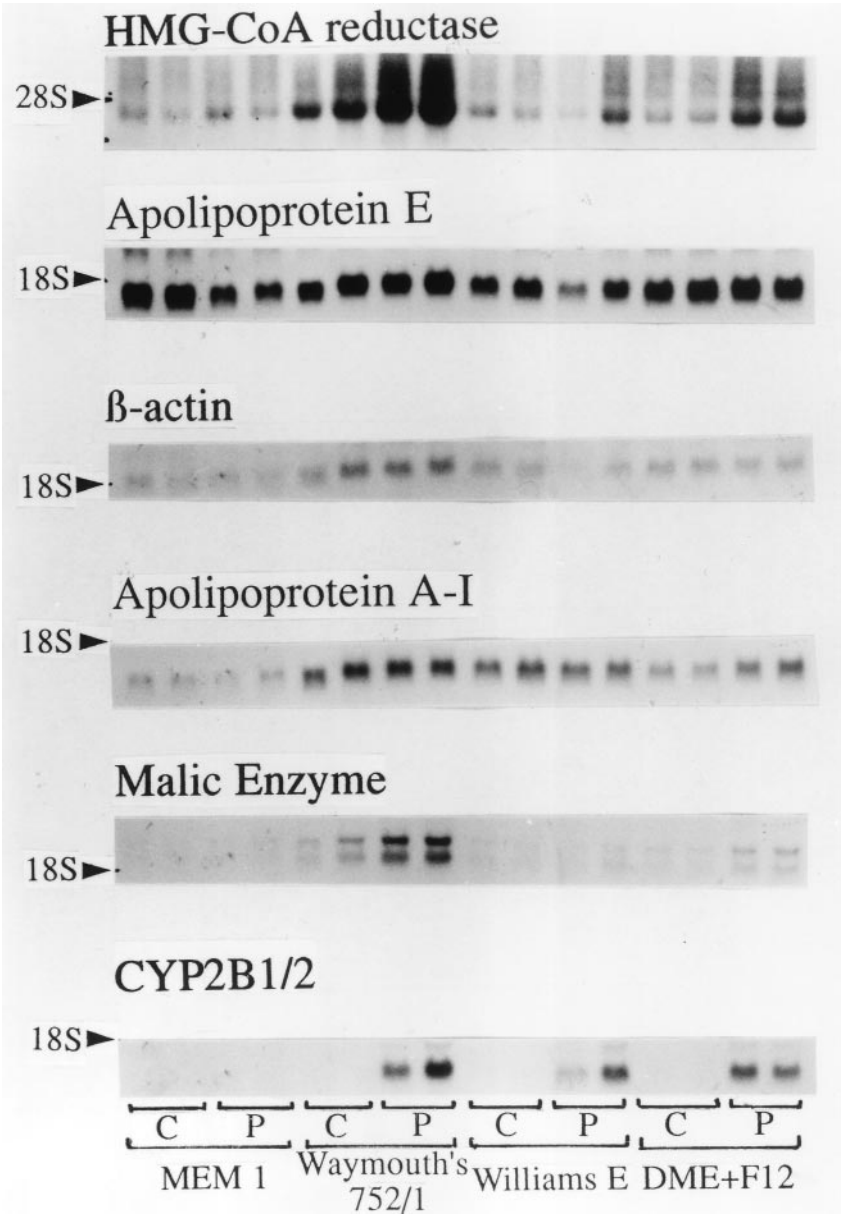


**Figure 7** Effect of culture media on the induction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and malic enzyme gene expression in rat hepatocytes cultured on Engelbreth-Holm-Swarm sarcoma basement membrane gel (EHS-gel). Parenchymal hepatocytes were isolated by collagenase-perfusion method and cultured in serum-free medium with insulin ($10^{-8}$ M) and dexamethasone ($10^{-6}$ M). Hepatocytes were plated at an initial density of $1 \times 10^7$ cells into 100 mm plastic dishes coated with EHS-gel. The medium was changed 4 hours after plating. Twenty-four hours after plating, hepatocytes were cultured in different culture media with polychlorinated biphenyls (PCB; 20 μg/mL) for 48 hours. C, control group without PCB; P, PCB group; MEM, minimum essential medium; DME, Dulbecco's modified Eagle medium.

*6*).[42] Some unknown regulatory factors must be changed by the extracellular matrix. Interestingly, the alteration of the lipid metabolism by xenobiotics is directly mediated to hepatocytes by xenobiotics per se. It has been shown that treatment with insulin resulted in increased HMG-CoA reductase activity.[39] Although its addition to the dexamethasone-containing medium in fact increased HMG-CoA reductase mRNA (*Figure 8*), the induction of the reductase mRNA by PCB in hepatocytes required dexamethasone, not insulin. In our previous report,[46] the serum corticosterone level was not changed by dietary PCB in rats. Moreover, in the present report, the addition of dexamethasone to insulin-containing medium did not show an inductive effect on HMG-CoA reductase mRNA in hepatocytes (*Figure 8*). Therefore, we conclude that PCB induced HMG-CoA reductase mRNA through the so-called "permissive effect" of dexamethasone, which has been seen in cAMP-dependent induction of phosphoenolpyruvate carboxykinase.[43]

Although we found that PCB induced HMG-CoA reductase gene expression in a glucocorticoid-dependent manner, the mechanism for the induction is still unclear. The activity of HMG-CoA reductase is regulated at several steps. The mRNA level of HMG-CoA reductase is known to be regulated by transcription of the gene[13] and by the stability of the mRNA.[14,15] It remains to be determined which steps are involved in the induction of HMG-CoA reductase gene expression by PCB. The activity of HMG-CoA reductase is also regulated at the protein level such as phosphorylation. The ratio of the activity of the active form of the enzyme to the total activity was relatively constant (*Figure 1*). Because the total activity of HMG-CoA reductase might refer to the amount of the enzyme, the amount of the enzyme would be increased by PCB as it is in the mRNA level.

We hypothesized that PCB and dexamethasone induce reductase gene expression either directly or indirectly. Do PCB and dexamethasone per se directly transactivate the

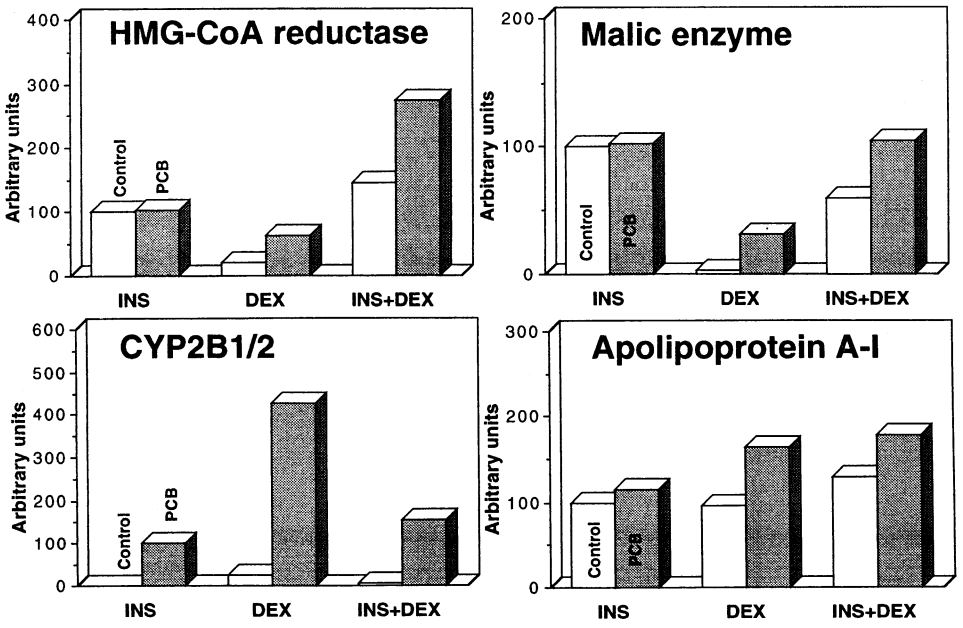


**Figure 8** Effect of dexamethasone (DEX) on mRNA levels for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, malic enzyme, CYP2B1/2, and apolipoprotein A-I in rat hepatocytes cultured on Engelbreth-Holm-Swarm sarcoma basement membrane gel (EHS-gel). Parenchymal hepatocytes were isolated by collagenase-perfusion method and cultured in serum-free medium with insulin (INS; $10^{-8}$ M). Hepatocytes were plated at an initial density of $1 \times 10^{7}$ cells into 100 mm plastic dishes coated with EHS-gel. The medium was changed 4 hours after plating. Twenty-four hours after plating, hepatocytes were exposed to polychlorinated biphenyls (PCB; 20 μg/mL), DEX ($10^{-6}$ M), and insulin ($10^{-8}$ M) for 48 hours. Values are means for 2 samples. Values were expressed as percentage relative to control (INS) group except for the case of CYP2B1/2. Values of CYP2B1/2 were expressed as percentage relative to the PCB (INS) group.

HMG-CoA reductase gene and stabilize its mRNA? Some genes induced by aromatic hydrocarbon have a xenobiotic responsive element in the promoter region of those genes.[47] It is well known that glucocorticoid hormone induces transcription through the binding of the receptor homodimer to the glucocorticoid responsive element. However, there are no reports that HMG-CoA reductase gene has a xenobiotic responsive element and/or glucocorticoid responsive element. It also has been reported that dexamethasone decreased hepatic HMG-CoA reductase mRNA by stimulating its degradation.[15] Therefore, we speculated that PCB and dexamethasone do not induce HMG-CoA reductase directly by themselves in the light of current knowledge. Still, the possibility of direct reductase induction by PCB and dexamethasone cannot be excluded, because, as discussed below, even when there was no detectable induction of CYP2B1/2 in the cells cultured on EHS-gel with MEM medium, the induction of HMG-CoA reductase was observed (*Figure 7*).

How do PCB and dexamethasone indirectly induce HMG-CoA reductase gene expression? CYP2B1/2 was induced in hepatocytes cultured on EHS-gel, but not on TIC (*Figure 6*).[24] We would speculate that the induction of cytochrome P-450 indirectly leads to the induction of HMG-CoA reductase gene expression. Proliferation of endoplasmic reticulum is reportedly accompanied by the induction of drug-metabolizing enzymes by xenobiotics.[48] Proliferation of endoplasmic reticulum should result in insufficiency of membrane components such as cholesterol and fatty acids. As seen in *Figures 6* and *8*, dexamethasone enhanced induction of CYP2B1/2 mRNA, suggesting an enhanced proliferation of the endoplasmic reticulum. Treatment with dexamethasone might cause an even greater shortage of membrane cholesterol. Therefore, the enhanced CYP2B1/2 gene expression by dexamethasone is postulated to lead to glucocorticoid-dependent induction of HMG-CoA reductase gene expression by PCB. Presently unknown signals indicating the insufficiency of cholesterol in endoplasmic reticulum might be transmitted to the nucleus. Recently, novel types of transcription factors, SREBP-1 and SREBP-2, which are involved in the cholesterol metabolism, were cloned.[49,50] The transcription factor SREBP-1 acts as a sensor for cholesterol mainly in endoplasmic reticulum, and the proteolytic 68kD form translocates to the nucleus and transactivates the low density lipoprotein receptor gene.[51] Although SREBP-1 and SREBP-2 regulated transcription of low density lipoprotein receptor and HMG-CoA synthase gene, no transactivation of HMG-CoA reductase gene (−277 to +231) by SREBPs was observed.[49,50] However, a mechanism similar to that for SREBPs should exist in the regulation of HMG-CoA reductase gene expression.

Although no reports to date have detailed the induction of malic enzyme by PCB, we would speculate that malic enzyme gene expression was induced due to PCB in a glucocorticoid-dependent manner by a mechanism similar to the case of HMG-CoA reductase discussed above. It was demonstrated that fatty acids suppressed malic enzyme gene expression in hepatocytes.[52] Binding of thyroid hormone to its receptor was inhibited by fatty acyl-CoA in vitro.[53] Moreover, fatty acids regulate gene expression mediated by peroxisome proliferator-activated receptor, a member of the steroid receptor superfamily.[54] The peroxisome proliferator-activated receptor recently has been found to be a new heterodimerization partner for thyroid hormone receptor,[55] which is important for malic enzyme gene expression.[21] These steroid receptor family proteins or unknown proteins may sense the status of triglyceride, phospholipid, or fatty acids and transactivate genes of lipogenic enzymes. Further research is required to examine how malic enzyme is induced by xenobiotics.

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