A UNIQUE CHANGE OF STEROID METABOLISM IN RAT LIVER MICROSOMES INDUCED WITH HIGHLY TOXIC POLYCHLORINATED BIPHENYL(PCB) AND POLYCHLORINATED DIBENZOFURAN(PCDF)*

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Pretreatments of rats with the highly toxic compounds such as 3,4,5,3',4'-pentachlorobiphenyl (PenCB), and 2,3,7,8-tetrachloro (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (PenCDF), which are potent 3-methylcholanthrene (MC)-type inducers, increased selectively 7 α -hydroxylation, but strongly suppressed 2 α -, 6 β - and 16 α -hydroxylations as well as 5 α -reduction of progesterone and testosterone in the liver microsomes. This unique change in the metabolic pattern was accompanied by a marked decrease in total metabolism of both steroids and appeared to correlate apparently with their toxic potency. This kind of change was not shown by pretreatments with not only MC itself but also the phenobarbital-type (2,4,5,2',4',5'-hexachlorobiphenyl) and the mixed type PCBs (Kanechlor 400, a PCB mixture with 48% chlorine content), all of which possess only a low acute toxicity. The metabolic change produced by 3,4,5,3',4'-PenCB, 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF might not be due to their stimulatory or inhibitory effects, because when added to the incubation mixture 3,4,5,3',4'-PenCB did not change the metabolic pattern with MC-microsomes to that with 3,4,5,3',4'-PenCB-microsomes. Furthermore, either 2,3,7,8-TCDF or 2,3,4,7,8-PenCDF gave similar metabolic pattern whereas their residual levels in the liver were greatly different from each other at the time of sacrifice. These results suggest that this kind of unique change of the steroid metabolism produced by highly toxic PCBs and PCDFs may be responsible, at least in part, for the endocrine symptoms caused by these compounds *via* disturbance of steroid homeostasis.

Keywords—progesterone; testosterone; microsomal metabolism; selective induction; 3,4,5,3',4'-pentachlorobiphenyl; 2,4,5,2',4',5'-hexachlorobiphenyl; Kanechlor 400; 2,3,7,8-tetrachlorodibenzofuran; 2,3,4,7,8-pentachlorodibenzofuran

INTRODUCTION

In recent years polychlorinated biphenyls (PCBs) and the structurally related compounds such as polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) have become of great concern because not only of their wide spread contamination in global ecosystem but also of the potent toxicity of some members of these compounds. In rodents, PCBs

produced uterine atrophy, ovarian stromal changes, reduced plasma progesterone levels and lengthened estrous cycle.¹⁻³⁾ A severe alteration of menstrual cycles and reproductive dysfunction were observed in adult female monkeys fed low levels of PCB mixture.^{4,5)} PCBs and PCDFs also are known as two of the main causal agents of a subacute poisoning called Yusho in Japan.^{6,7)} The major clinical symptoms of Yusho were acneform

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eruptions, peripheral neuropathy, hepatomegaly with no disturbance of routine liver tests and growth retardation in infants and children. Women with Yusho disease had menstrual cycle irregularities, dysmenorrhea and altered serum levels of ketosteroids.⁶⁾ The mechanism of these endocrine-related alterations produced by PCBs and the related compounds, however, has not been fully understood.

One of the major biologic effects of these polychlorinated aromatic compounds is a potent and long-lasting inductive effect on hepatic microsomal mixed function oxidase (MFO) system,⁸⁻¹⁰⁾ which catalyzes the metabolism of a variety of exogenous compounds, such as drugs, food additives, pesticides and chemical carcinogens. This enzyme system is involved also in the metabolism of certain physiologically important endogenous substances including steroids, fatty acids and vitamins.

Several investigators have shown that steroid metabolism is enhanced by pretreatment with commercial PCB mixtures,¹¹⁻¹³⁾ but only a limited number of reports concerning the effect of individual PCBs on steroid metabolism is available. Dieringer *et al.*¹⁴⁾ demonstrated that the degree and/or position of biphenyl chlorination affect the perinatal response of hepatic steroid metabolizing enzymes to PCBs. Gustafsson and Ingelman-Sundberg¹⁵⁾ reported that the metabolic pattern of steroids was dramatically changed following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic member of the known chlorinated aromatic compounds.

We previously demonstrated that the individual PCBs fall into two distinct types of MFO inducers, phenobarbital (PB)- and 3-methyl cholanthrene(MC)-type,¹⁶⁻¹⁸⁾ and that the toxic potency of PCB and PCDF congeners is well correlated with their MC-type inducing abilities.¹⁷⁻¹⁹⁾ Therefore, it is of interest to examine the effects of pretreatment with highly toxic PCB and PCDF congeners on the liver microsomal metabolism of progesterone and testosterone in rats.

MATERIALS AND METHODS

Chemicals — Kanechlor(KC-)400, a Japanese PCB preparation with 48% chlorine content, was supplied by the Ministry of Health and Welfare of Japan. 3,4,5,3',4'-Pentachlorobiphenyl (PenCB) was synthesized and purified as reported previously.²⁰⁾ 2,4,5,2',4',5'-Hexachlorobiphenyl (HCB) was purchased from Analab, Inc. (North Haven, Conn., USA). The PCDF congeners used were synthesized from corresponding chlorophenols and chloronitrobenzenes by the method of Kende *et al.*²¹⁾ Their starting materials, mp, and δ of proton magnetic resonances in CDCl₃ (FT NMR, FX 90Q, JEOL, TOKYO) are respectively shown as the followings. 2,8-Dichlorodibenzofuran (DCDF): *p*-chlorophenol and 2,5-dichloronitrobenzene, mp 185–186°C, and 7.88–7.85 m and 7.47–7.45 m. 1,3,6,7-Tetrachlorodibenzofuran (TCDF): 3,5-dichlorophenol and 2,3,4-trichloronitrobenzene, mp 177–178. 5°C, and 8.08 d, 7.54 d, 7.46 d and 7.36 d. 1,3,6,8-TCDF: 2,4-dichlorophenol and 2,4,6-trichloronitrobenzene, mp 177–178°C, and 8.16 d, 7.56 d, 7.52 d and 7.39 d. 2,3,7,8-TCDF: 3,4-dichlorophenol and 2,4,5-trichloronitrobenzene, mp 219–221°C, and 7.97 s and 7.70 s. 1,2,4,6,8-Pentachlorodibenzofuran (PenCDF): 2,4-dichlorophenol and 2,3,5,6-tetrachloronitrobenzene, mp 204–205°C, and 8.25 d, 7.65 s and 7.59 d. 2,3,4,7,8-PenCDF: 2,3,4-trichlorophenol and 2,4,5-trichloronitrobenzene, mp 196–196. 5°C, and 7.97 s, 7.91 s and 7.77 s.

4-¹⁴C-Progesterone (specific radioactivity, 50 mCi/mmol) and 4-¹⁴C-testosterone (specific radioactivity, 50 mCi/mmol) were purchased from New England Nuclear Corp. (Boston, Mass., USA). Unlabelled progesterone, testosterone and androstene-3,17-dione were purchased from Nakarai Chemicals, Ltd. (Tokyo, Japan). 2 α -, 6 β - and 16 α -Hydroxyprogesterone were obtained from Steraroid, Inc. (Wilton, N.H., USA). 2 α -6 β -, 7 α - and 16 α -Hydroxytestosterone were kindly given by Dr. Y. Nakamura, Shionogi Research Laboratory, Shionogi & Co., Ltd., Osaka, Japan. All other chemicals used were of the highest quality commercially available.

Animal Treatments — Male Wistar rats (5 weeks old, weighing about 100 g) were housed in stainless-steel cages without bedding in a temperature- and light-controlled room (23°C, lights on 0600–1900 h) with water and Chow *ad lib*.

All PCBs and PCDFs were dissolved in corn oil and injected into rats *i.p.* at the designated single dose of 300 mg/kg for KC-400, 5 mg/kg for 3,4,5,3',4'-PenCB, 20 mg/kg for 2,4,5,2',4',5'-HCB, 0.1 mg/kg for 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF, 10 mg/kg for 2,8-DCDF and 5 mg/kg for 1,3,6,7-, 1,3,6,8-TCDF and 1,2,4,6,8-PenCDF. In some experiments, 2,3,4,7,8-PenCDF was injected at a single dose of 1 mg/kg. PB in saline and MC in corn oil were injected *i.p.* once a day for 3 consecutive days at the doses of 100 mg/kg and 20 mg/kg, respectively. Control animals were injected with vehicles alone at an equivalent volume (2 ml/kg) to that of the treated animals. Following a fasting for 18 h, the animals (3–4 rats/group) were killed by decapitation 5 d after the injection of PCBs or PCDFs and 24 h after the last injection of PB or MC.

Enzyme Preparation and Assays — The liver dissected was perfused with ice-cold saline and homogenized with three vol. of 0.25 M sucrose containing 0.1 mM EDTA and 10 mM Tris-HCl (pH 7.4) by use of a glass-teflon homogenizer. The $9000\times g$ supernatant prepared by the method described previously¹⁹⁾ was centrifuged at $105000\times g$ for 60 min. The pellet was washed once with isotonic KCl and resuspended in the Tris-sucrose buffer.

The metabolic study on steroids was performed as follows; the incubation mixture consisted of microsomes (about 3 mg protein), 200 μ g of 14 C-steroid (0.4 μ Ci), 3.6 mM $MgCl_2$, 0.5 mM NADP, 6 mM glucose-6-phosphate, 5 units of glucose-6-phosphate dehydrogenase and 50 mM Tris-HCl buffer (pH 7.4) to make a final volume of 5 ml. Incubation was carried out at 37°C for 10 min following a preincubation (1 min) without an NADPH-generating system. In the experiment with the lower toxic PCDFs, incubation was continued for 30 min. The metabolites were extracted with 20 ml of

ethylacetate by use of a mechanical shaker and the organic phase was evaporated to dryness *in vacuo*. The residue was redissolved in small amounts of methanol and filtered through a membrane filter with 0.45 μ pore size. An aliquot (50–100 μ l) of the filtrate (0.2 ml) was applied to a high performance liquid chromatograph (Waters Assoc., Inc., Milford, Mass., USA, Model ALC 202/401) equipped with 6000 A pump, a solvent programmer type 660 (Waters Assoc.) and a radioactivity monitor, BL 503 (Berthold, Wildbad, Germany).

Samples were chromatographed on a Radial-PAK A column (Waters Assoc., reversed phase) by stepwise elution with 30% and 45% tetrahydrofuran (THF) in water (v/v, changed at 22 min after injection) for progesterone metabolites or 25% and 35% THF in water (v/v, changed at 18 min after injection) for testosterone metabolites at a flow rate of 3 ml/min. The metabolites were identified by their retention volumes on high performance liquid chromatography (HPLC) relative to reference standards or by comparisons of *R_f* value on thin layer chromatography and GC/MS fragmentation pattern of the isolated metabolites with those reported by others. A quantitative determination of the metabolite was carried out by measuring an absorbance at 254 nm or a radioactivity (Packard, Model 3375 liquid scintillation counter) of the corresponding peaks on HPLC.

Experiment to assess *in vitro* effects of PenCB on the steroid metabolism was carried out by use of the liver microsomes obtained from MC-treated rats according to the method of Schmoldt *et al.*²²⁾

Protein was determined according to the method of Lowry *et al.*²³⁾ using bovine serum albumin as a standard.

Toxicological Study — Upon sacrifice, the thymus was dissected out, blotted and weighed.

Statistical Method — Statistical analysis of significance of the various parameters measured was assessed by two-sided Mann-Whitney U tests.²⁴⁾

RESULTS

Effects of PCBs on the Steroid Metabolism by Rat

Liver Microsomes

In vitro metabolism of ^{14}C -progesterone and ^{14}C -testosterone with the liver microsomes of rats pretreated with various inducers was followed by

HPLC. The chromatograms and quantitative determination of the metabolites were summarized in Figs. 1 and 2, respectively. PB, 2,4,5,2',4',5'-HCB and KC-400 increased 1.5 to 2-fold

FIG. 1. High Performance Liquid Chromatograms of Progesterone Metabolites(A) and Testosterone Metabolites(B) formed by Incubation with Liver Microsomes from Rats pretreated with PB, MC and PCBs

The liver microsomes were obtained from the rats pretreated with PB, MC or PCBs at each dose indicated. Either ^{14}C -progesterone or ^{14}C -testosterone ($0.4 \mu\text{Ci} / 200 \mu\text{g}$) was incubated with about 3 mg protein of the microsomes in the presence of an NADPH-generating system for 10 min at 37°C . Following extraction with 20 ml of ethylacetate, the extract was redissolved into methanol (0.2 ml) and filtered through a membrane filter. An aliquot (50–100 μl) of the filtrate was injected onto a Radial-PAK A column (reverse phase). Stepwise elution was adopted as follows; (A) 30% followed by 45% THF in water (v/v) at 22 min after injection, (B) 25% followed by 35% THF in water (v/v) at 18 min after injection.

(A) Progesterone metabolism

(1): $16\alpha\text{-OH}$, (2): $7\alpha\text{-OH}$, (3): $6\alpha\text{-OH}$, (4): $6\beta\text{-OH}$, (5): $2\alpha\text{-OH}$, (6): $5\alpha\text{-pregnane-3}\alpha\text{-ol-20-one}$ (7): progesterone, (8): $5\alpha\text{-pregnane-3, 20-dione}$, (I.S.): internal standard (testosterone).

(B) Testosterone metabolism

(1): $7\alpha\text{-OH}$, (2): $16\alpha\text{-OH}$, (3): $6\beta\text{-OH}$, (4): $2\alpha\text{-OH}$, (5): $5\alpha\text{-androstane-3}\alpha, 17\beta\text{-diol}$ (6): androstene-3, 17-dione, (7): testosterone, (8): $5\alpha\text{-androstane-17}\beta\text{-ol-3-one}$.

the 16 α -, 7 α - and 6 β -hydroxylations of progesterone. A similar tendency was also observed in the metabolites of testosterone. MC enhanced the 7 α - and 16 α -hydroxylations of progesterone and the 7 α -hydroxylation of testosterone, while the 6 β -hydroxylation of both steroids was not affected by this treatment.

A most unique change of the metabolic profile of progesterone and testosterone was demonstrated by the pretreatment with the highly toxic 3,4,5,3',4'-PenCB. This PCB enhanced more than 3-fold the 7 α -hydroxylation of both steroids, but

unlike MC treatment the hydroxylations of 6 β - and 16 α -positions of the steroids were strongly suppressed to less than 20% of the control. In addition, the 2 α -hydroxylation of progesterone (peak 5 in Fig. 1(A)) and the formation of androstenedione (peak 6 in Fig. 1(B)) from testosterone were also decreased only by the PenCB treatment. The 5 α -reductions of progesterone to 5 α -pregnane-3,20-dione and testosterone to 5 α -androstane-17 β -ol-3-one were decreased more intensively by 3,4,5,3',4'-PenCB than the other inducers. And this unique

FIG. 2. Effects of Pretreatment with PB, MC and PCBs on Metabolism of Progesterone (A) and Testosterone (B) with Rat Liver Microsomes

The liver microsomes were obtained from the rats pretreated with PB, MC or PCBs at each dose indicated. The incubation and analysis of the metabolites were performed as described in the legend of Fig. 1. The activity was expressed as a relative value to that of the control group indicated. The vertical line represents the standard deviation of the mean of four rats per group. †† nd; not detectable

a) Significantly different from the control, $p < 0.05$

(A) Progesterone metabolism
control activity

16 α -OH	6.93 $\pm$ 1.52
7 α -OH	3.34 $\pm$ 1.16
6 β -OH	14.81 $\pm$ 1.03
5 α -Reduction	21.13 $\pm$ 9.91

(B) Testosterone metabolism
control activity

16 α -OH	8.06 $\pm$ 1.79
7 α -OH	6.70 $\pm$ 0.86
6 β -OH	19.12 $\pm$ 1.19
5 α -Reduction	17.77 $\pm$ 8.25

effect of PenCB on the metabolism of progesterone and testosterone resulted in a marked depression of total microsomal metabolism of both steroids.

In Vitro Effects of 3, 4, 5, 3', 4'-PenCB on the Steroid Metabolism by MC-microsomes

Since 3,4,5,3',4'-PenCB is highly accumulated in the liver of rats and mice,^{25,26)} it seemed that the unique change of the steroid metabolism produced by 3,4,5,3',4'-PenCB pretreatment might be due to stimulatory or inhibitory effects of the residual 3,4,5,3',4'-PenCB on the microsomal enzymes *in vitro*. Therefore, to assess the *in vitro* effects of 3,4,5,3',4'-PenCB on the microsomal metabolism of progesterone and testosterone, 3,4,5,3',4'-PenCB was added to the incubation

mixture containing the microsomes obtained from the MC-pretreated rat liver.

As shown in Table I, 3,4,5,3',4'-PenCB added partially inhibited not only the 16 α - and 6 β -hydroxylations but also the 7 α -hydroxylation of both steroids mediated by MC-microsomes, whereas the 5 α -reduction was not affected any more. Consequently, the existence of 3,4,5,3',4'-PenCB in the incubation mixture was unable to change the metabolic pattern by the MC-microsomes to that by the 3,4,5,3',4'-PenCB-microsomes.

These results strongly suggest that the unique metabolic profile produced by 3,4,5,3',4'-PenCB pretreatment is not due to the *in vitro* effects of the residual 3,4,5,3',4'-PenCB.

TABLE I. Inhibitory Effect of 3,4,5,3',4'-PenCB on Metabolism of Progesterone and Testosterone with Liver Microsomes from MC-treated Rats^{a)}

Substrate	16 α -OH	7 α -OH (Percent inhibition)	6 β -OH	5 α -Reduction
Progesterone	32 $\pm$ 6	24 $\pm$ 10	21 $\pm$ 4	0
Testosterone	16 $\pm$ 2	15 $\pm$ 2	17 $\pm$ 6	0

a) The microsomes were obtained from the rats pretreated with MC at a dose of 20 mg/kg for 3 consecutive days. 3,4,5,3',4'-PenCB was added to the incubation mixture at a concentration of 30 μ M and the incubation was performed as described in the legend of Fig. 1.

TABLE II. Effects of Pretreatment with 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF on Metabolism of Progesterone with Rat Liver Microsomes^{a)}

Metabolite	Pretreatment	Control	TCDF (0.1 mg/kg) (nmol/mg protein/10 min)	PenCDF (0.1 mg/kg)
16 α -OH		6.93 $\pm$ 1.52	3.09 $\pm$ 1.28 ^{b)}	3.10 $\pm$ 0.83 ^{b)}
7 α -OH		3.34 $\pm$ 1.16	8.35 $\pm$ 3.07 ^{b)}	8.60 $\pm$ 4.00 ^{b)}
6 β -OH		14.81 $\pm$ 1.03	8.71 $\pm$ 1.59 ^{b)}	8.81 $\pm$ 2.90 ^{b)}
2 α -OH		7.31 $\pm$ 4.01	1.01 $\pm$ 0.61 ^{b)}	0.93 $\pm$ 0.38 ^{b)}
5 α -Pregnane-3,20-dione		21.13 $\pm$ 9.91	11.93 $\pm$ 4.63	13.13 $\pm$ 5.15
Total metabolism		68.11 $\pm$ 8.23	37.18 $\pm$ 6.75 ^{b)}	37.84 $\pm$ 11.48 ^{b)}

a) The liver microsomes were obtained from the rats pretreated with TCDF or PenCDF at a single dose indicated. ¹⁴C-Progesterone (0.4 μ Ci/200 μ g) was incubated with about 3 mg protein of the microsomes as described in the legend of Fig. 1. The total metabolism was calculated from the amount of disappeared substrate. The value represents the mean $\pm$ S.D. of four rats.

b) Significantly different from the control, $p < 0.05$.

Effects of PCDF Congeners on the Progesterone Metabolism by Rat Liver Microsomes

Although the accumulation of 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF in the liver was greatly different from each other, both PCDFs exhibited almost equipotent toxicity and inducibility, which are comparable with those of 3,4,5,3',4'-PenCB.¹⁹⁾ Table II shows their effects on the progesterone metabolism by the microsomes obtained from the rats pretreated with 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF at a single dose of 0.1 mg/kg. Either 2,3,7,8-TCDF or 2,3,4,7,8-PenCDF exhibited the similar effect on the progesterone metabolism to that with 3,4,5,3',4'-PenCB. The 7 α -hydroxylation was selectively increased, but the other hydroxylations and the 5 α -reduction were strongly suppressed. On the contrary, none of the lower toxic PCDF congeners such as 2, 8-DCDF, 1,3,6,7- and 1,3,6,8-TCDFs

and 1,2,4,6,8-PenCDF produced such a unique change of the progesterone metabolism (Fig. 3).

DISCUSSION

Polyhalogenated aromatic compounds such as PCBs, PCDFs, PCDDs and polybrominated biphenyls (PBBs) are the closely related family of compounds for their similarity not only in the structures but also in the biologic and toxic natures.²⁷⁾ Most of these compounds can induce the hepatic MFO and resulted in accelerated metabolism of a variety of xenobiotics and endogenous substrates. The type and the potency of induction by these compounds are associated with their structural features in terms of the number and the position of halogen atoms substituted on the parent ring structures.^{16,27,28)}

Our previous data¹⁷⁻¹⁹⁾ and others^{27,29)} have shown that the MC-type inducing ability of PCBs, PCDFs and PCDDs well correlated with their toxic potency inducing atrophies of thymus and spleen, hypertrophy of liver in rats or mice and edema in chickens. Many investigators demonstrated that the halogenated aromatic compounds also caused the endocrine-related alterations and reproductive dysfunction.¹⁻⁵⁾ Since steroid are one of the major endogenous substrates for MFO, it is easy to consider that some of the endocrine-related alterations might be produced through modification of steroid metabolism. Indeed, PCBs and PBBs markedly enhanced the metabolism of sex hormones *in vitro*^{11,12,30)} and *in vivo*¹³⁾ In all of these studies, however, a commercial preparation of PCBs or PBBs, which consists of PB-type and MC-type congeners and thereby shows much lower toxicity than 3,4,5,3',4'-PenCB, 2,3,7,8-TCDF or 2,3,4,7,8-PenCDF, was used. Considering from the fact that the toxic potency is varied with the individual congeners, it might be more important to evaluate the effects of pure individual congeners in steroid metabolism. Recently Dieringer *et al.*¹⁴⁾ have demonstrated the different effects of 4-monochlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl and 2,4,5,2',4',5'-HCB on the 16 α -hydroxylation of androstenedione and the 5 α -

FIG. 3. Effects of Pretreatment with PCDF Congeners on 7 α - and 2 α -Hydroxylation of Progesterone with Rat Liver Microsomes

The microsomes were obtained from the pooled liver of 4 rats per group pretreated with the respective PCDF congener at a single dose indicated. ¹⁴C-Progesterone (0.4 μ Ci/200 μ g) was incubated with about 3 mg protein of the microsomes in the presence of an NADPH-generating system for 30 min at 37°C.

▨ 7 α -OH,
□ 2 α -OH.

reduction of testosterone in rats.

In the present study, the effects of pretreatments with highly toxic PCB and PCDF congeners on the microsomal metabolism of progesterone and testosterone in the rat liver were examined comparing with those of lower toxic congeners and the known MFO inducers, PB and MC. Lower toxic 2,4,5,2',4',5'-HCB and KC-400, which are classified as a PB-type and a mixed type, respectively, as well as PB itself significantly enhanced the 16 α -, 7 α - and 6 β -hydroxylations of progesterone and testosterone.

On the contrary, the highly toxic 3,4,5,3',4'-PenCB, showing extremely potent MC-type induction,¹⁷⁾ selectively enhanced the 7 α -hydroxylation of both steroids, but strongly suppressed the 16 α - and 6 β -hydroxylations of both steroids, the 2 α -hydroxylation of progesterone, and the formation of androstenedione from testosterone. Almost similar effects were also demonstrated only by the toxic PCDF congeners, 2,3,7,8-TCDF and 2,3,4,7,8-PenCDF, both of which exhibit a potent MC-type inducing ability.¹⁹⁾

These characteristic effects by the highly toxic compounds differed from that of MC itself, which increased the 7 α -hydroxylation of testosterone, but did not affect the 6 β -hydroxylation of both steroids. The 5 α -reduction, which is a rate-limiting metabolic reaction of catabolism of Δ^4 -steroids, was also decreased most intensively by the toxic congeners. By these effects, the highly toxic PCBs and PCDFs resulted in a marked depression of total metabolism of both steroids rather than enhancement.

Similar depressive effects on certain hydroxylations^{15,31)} and 5 α -reduction¹⁵⁾ of steroids have been demonstrated by the pretreatment with the most highly toxic TCDD. Nienstedt *et al.*³²⁾ also reported TCDD-treatment decreased the total metabolism of testosterone by rat liver.

This unique change of the steroid metabolism produced by the highly toxic PCB and PCDFs might be responsible for qualitative and quantitative changes of the microsomal cytochrome P-450 (448) rather than the *in vitro* effects of the resided

compounds in the liver (Table I and II).

It is believed that different forms of cytochrome P-450 (448) are involved in the hydroxylations at different positions of testosterone and the content of such P-450 (448) in microsomes are changed by pretreatment with various MFO inducers.

Recently Levin and his coworkers^{33,34)} purified three forms of cytochrome P-450 (448) which showed a different spectrum for substrate specificity from each other, from the rats pretreated with PB, MC and PCBs. These investigators have shown that the 7 α -hydroxylation of testosterone is preferentially catalyzed by cytochrome P-450a showing mol. wt 48000 and the CO-complex maximum at 452 nm, which is a minor P-450 in either untreated or treated microsomes. Although 3,4,5,3',4'-PenCB markedly induced the cytochrome P-448 (mol. wt 54000), which is similar to cytochrome P-448 mainly induced with MC,^{25,35)} it is possible that a species like cytochrome P-450a may also be induced by the highly toxic PCBs and PCDFs judging from the increased size of protein band at mol. wt 48000 on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (unpublished data).

To explain a selective enhancement of 7 α -hydroxylation there might be another possibility. Shiverick and Neims³⁶⁾ reported that the 7 α -hydroxylation of testosterone was enhanced 2-fold by the addition of microsomal lipids to the reconstituted system consisting of partially purified cytochrome P-450 from untreated rats, whereas the 16 α - and 6 β -hydroxylations were affected a little or none. Therefore, the increased liver lipids may also participate in a selective enhancement of the 7 α -hydroxylation of progesterone and testosterone because only the highly toxic PCBs and PCDFs caused a severe fatty liver.^{17,19)}

Thymus atrophy is known to be mediated by corticoids.³⁷⁾ However, on the TCDD studies adrenalectomy did not prevent the thymus atrophy.^{38,39)} In the present study, as shown in Fig. 4, the extents of suppression of total metabolism and

FIG. 4. Correlation between Thymus Atrophy and depressed Total Metabolism(A), and increased 7 α -Hydroxylation(B) of Testosterone induced by Pretreatment with PB, MC and PCBs in Rats

A) ● phenobarbital 100 mg/kg $\times$ 3; Δ 3-methylcholanthrene 20mg/kg $\times$ 3; $\square$ KC-400 300 mg/kg $\times$ 1; $\circ$ 2,4,5,2',4',5'-HCB 20 mg/kg $\times$ 1; $\blacksquare$ 3,4,5,3',4'-PenCB 5 mg/kg $\times$ 1; $r = 0.817$ ($\alpha < 0.001$)
 B) ● phenobarbital 100 mg/kg $\times$ 3; Δ 3-methylcholanthrene 20mg/kg $\times$ 3; $\square$ KC-400 300 mg/kg $\times$ 1; $\circ$ 2,4,5,2',4',5'-HCB 20 mg/kg $\times$ 1; $\blacksquare$ 3,4,5,3',4'-PenCB 5 mg/kg $\times$ 1; $r = -0.686$ ($\alpha < 0.01$)

induction of 7 α -hydroxylation of testosterone were apparently correlated with the thymus atrophy.

Although we do not have a direct evidence, the results obtained here suggest that a selective induction of steroid 7 α -hydroxylase and suppressions of 2 α -, 6 β - and 16 α -hydroxylases as well as 5 α -reductase by the highly toxic PCBs and PCDFs may be responsible, at least in part, for the endocrine symptoms caused by these compounds *via* disturbance of steroid homeostasis.

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