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Relationship between Chemical Structure and Activity. I. Effects of the Number of Chlorine Atoms in Chlorinated Benzenes on the Components of Drug-Metabolizing System and the Hepatic Constituents[1)]

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To elucidate the relationship between chemical structure and their biological activities, the contents of cytochromes and hepatic constituents in addition to the activities of drug-metabolizing enzymes and δ-aminolevulinic acid (δ-ALA) synthetase were examined in rats treated with various chlorinated benzenes, *i.e.*, monochlorobenzene (MCB), *p*-dichlorobenzene (*p*-DCB), 1,3,5-trichlorobenzene (1,3,5-TRCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TECB), pentachlorobenzene (PECB) and hexachlorobenzene (HCB).

1) The content of cytochrome P-450 and activities of aminopyrine demethylase and aniline hydroxylase were increased by oral administration of all chlorobenzenes except MCB at daily dose of 250 mg/kg, once daily, for 3 days. The contents of microsomal protein and phospholipids also showed a similar tendency to those as described above. The activity of δ-ALA synthetase was increased by treatment with all compounds used.

2) The content of cytochrome P-450 and activity of aminopyrine demethylase were decreased in 24 hr after a single administration of MCB in doses of 125, 250, 500 and 1000 mg/kg, whereas the activity of δ-ALA synthetase was increased markedly by all doses used. The activity of aniline hydroxylase was increased by a dosing of 1000 mg/kg MCB.

3) In the time-course after a single administration of MCB in a dose of 250 mg/kg, the activity of δ-ALA synthetase was decreased in 6 hr after administration, and subsequently restored to normal levels in 12 hr, and then increased markedly in 24 hr. The opposite changes were noted in the content of cytochrome P-450.

Drugs, insecticides and various chemical agents, which possess a different chemical structure and biological activity, have been reported to induce microsomal drug-metabolizing enzymes in the liver when administered to animals.[3)]

These inducing effects are roughly classified into two categories, that is, one is phenobarbital type and the other is 3-methylcholanthrene. But, extent or degree of toxicity may be different according to individual compounds, such as γ-benzene hexachloride (BHC) and β-BHC. Since individual compounds may differ with respect to gastrointestinal absorption, tissue distribution, biotransformation of liver, kidney or the other tissues and excretion in urine and feces, this appears to give rise to a different toxicities.

It is of interest that most of foreign compounds inducing the drug-metabolizing enzyme enhance the activity of δ-aminolevulinic acid (δ-ALA) synthetase which is a rate-limiting

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3) A.H. Conney, *Pharmacol. Rev.*, 19, 317 (1967); R. Kuntzman, *Ann. Rev. Pharmacol.*, 9, 21 (1969).

enzyme for the porphyrine biosynthesis.[4,5] If the synthesized porphyrine could be utilized in heme synthesis, the activity of δ-ALA synthetase would be related with concentration of cytochrome P-450 playing an important role in drug-metabolizing systems.

In a series of this experiment, the authors, therefore, have made an attempt to clarify the relationship between chemical structure and effects on the content of cytochrome P-450 and on the activities of δ-ALA synthetase and drug-metabolizing enzymes by pretreatment with chlorinated benzenes with relatively simple structure. We have also observed the contents of hepatic constituents to estimate for overall evaluation of toxicity.

Materials and Methods

Monochlorobenzene (MCB) and 1,3,5-trichlorobenzene (1,3,5-TRCB) were purchased from Nakarai Chemicals Co. Ltd., Kyoto, Japan. *p*-Dichlorobenzene (*p*-DCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TECB) and hexachlorobenzene (HCB) were purchased from Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan. Pentachlorobenzene (PECB) was purchased from Schuchardt Co. Ltd., Muenchen, Germany. δ-ALA was purchased from Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan. Nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate disodium salt (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PD) were purchased from Boehringer Mannheim GmbH, Mannheim, Germany. Aniline and aminopyrine were obtained from commercial sources and used following purification by redistillation and recrystallization, respectively. The other reagents were obtained from commercial sources and used without further purification.

Female Wistar rats weighing 100—120 g were used in all experiments and fed commercial rat chow, F-II, Funahashi Nojyo Co. Ltd., Chiba, Japan, for one week prior to the experiments.

Chlorinated benzenes were suspended in 2% tragacanth gum solution and were given orally at a dose of 250 mg/5 ml/kg once daily for 3 days. For a dose-response experiment, MCB was administered orally at a single dose of 125, 250, 500 and 1000 mg/5 ml/kg, respectively. For a time-course experiment, MCB was given at a single dose of 250 mg/5 ml/kg. The control animals received orally an equal volume of the vehicle.

Animals were sacrificed by decapitation 24 hr after the last administration except the time-course experiment, and the livers were perfused with 0.9% NaCl solution *in situ* to remove blood. The livers were removed and immediately the portions of liver were weighed and used for the direct determination of glycogen,[6] triglyceride[7] and also used for the extraction of total lipid with chloroform–methanol (2 : 1, v/v). The rest of liver was immediately placed in ice-cold 0.9% NaCl solution. All subsequent procedures were performed below 4°. The livers were weighed and minced with scissors. The liver-mince was homogenized in 3 volumes of 0.25M sucrose solution containing 1 mM ethylenediaminetetraacetic acid (EDTA) in a motor-driven Potter homogenizer with a Teflon pestle. Preparation of microsomes was carried out by the procedures as described previously[8] except microsomal pellets were suspended in the 1.15% KCl–25 mM Tris-HCl, pH 7.4, to give a concentration of 4.50 mg of microsomal protein per ml. Microsomal protein was estimated by the method of Lowry, *et al.*,[9] using bovine serum albumin as a standard. Incubation mixtures consisted of 4.50 mg of microsomal protein, 5 mM G-6-P, 0.5 mM NADP, 1.75 units G-6-PD, 5 mM $MgCl_2$, substrate (2 mM aminopyrine or 1 mM aniline), and 50 mM Tris-buffer, pH 7.4, in a final volume of 3.0 ml. Incubations were carried out at 37° for 10 min aerobically. Activity of aminopyrine demethylase was measured the amount of formaldehyde produced by the Nash reaction.[10] Activity of aniline hydroxylase was determined by measuring the amount of *p*-aminophenol formed by the method of Imai and Sato.[11] Cytochrome P-450 and b_5 were estimated as described by Omura and Sato.[12] Aniline-induced difference spectra was obtained by the method of Schenkman, *et al.*[13] The extracts of total lipids were washed according to Folch, *et al.*,[14] concentrated in an atmosphere of nitrogen and stored in chloroform under nitrogen. Phospholipids were

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separated from the lipid mixture by silicic acid chromatography.[15] Inorganic phosphorus (Pi) analysis was performed on aliquot of methanol fraction by the method of Fiske and Subbarow.[16] The fatty acid of phospholipid was methylated by a modification of the method of Stoffel, *et al.*,[17] and analyzed by gaschromatography on a 10% diethyleneglycol succinate on a Shimalite W, 60—80 mesh, column using a Shimazu GC-3BF Chromatograph equipped with a hydrogen flame ionization detector. The fatty acids were identified by comparison of retention times with those of known standards. The peaks were evaluated by triangulation. The activity of δ-ALA synthetase was measured in liver homogenates by the method of Marver, *et al.*[5a] The rest of liver was homogenized in 3 volumes of 0.9% NaCl solution containing 0.5 mM EDTA and 10 mM Tris, pH 7.4. The incubation mixture contained a total volume of 2.0 ml; 1.0 ml of homogentate, 20 mM of glycine, 2 mM of EDTA and 15 mM of Tris-HCl buffer, at a final pH 7.2. After incubation for 90 min at 37°, the reaction was stopped with 0.5 ml of 25% (w/w) trichloroacetic acid. The protein-free supernatant was used for the determination of δ-ALA by using acetylacetone and modified Ehrlich reagent in the method of Urata and Granick.[18]

TABLE I. Effects of Treatment with Chlorinated Benzenes for 3 Days on the Hepatic Constituents and the Fatty Acid Composition on Microsomal Phospholipids

A)		Control	MCB	*p*-DCB	1,3,5-TRCB
Body weight	initial (g)	119±4	117±2	124±2	121±3
	final (g)	129±3	122±4	133±2	129±3
Liver weight (g/100 g b.w.)		5.10± 0.13	6.22± 0.10[a]	5.46± 0.12	5.60±0.28
Glycogen (mg/g)		60.8 ± 2.1	67.5 ± 4.2	68.8 ± 4.3	60.3 ±6.0
Triglyceride (mg/g)		5.8 ± 1.0	6.0 ± 0.8	6.1 ± 0.4	7.8 ±0.9
Microsomal					
protein (mg/g)		16.7 ± 0.5	17.0 ± 0.9	17.5 ± 0.7	19.9 ±0.9[a]
phosphorus of phospholipids (μg/g)		215.0 ±10.7	222.4 ±12.7	244.3 ±14.1	283.7 ±9.3[b]
fatty acid of phospholipids	16:0[c]	17.2 ± 0.4	22.6 ± 0.5[a]	20.1 ± 0.5[b]	17.9 ±0.9
(% to total)	18:0	29.7 ± 0.5	26.1 ± 0.4[a]	28.0 ± 0.5	30.9 ±1.0
	18:2	9.7 ± 0.2	13.7 ± 0.1[a]	10.3 ± 0.4	10.0 ±0.1
	20:4	26.5 ± 0.4	20.3 ± 0.4[a]	25.7 ± 0.3	25.8 ±0.6
	22:6	9.4 ± 0.2	10.3 ± 0.2	9.7 ± 0.2	8.3 ±0.4

B)		Control	1,2,4,5-TECB	PECB	HCB
Body weight	initial (g)	117±2	121±4	122±3	120±4
	final (g)	129±1	132±3	132±3	131±4
Liver weight (g/100 g b.w.)		4.87± 0.12	5.07± 0.13	5.72± 0.09[a]	5.25±0.11[b]
Glycogen (mg/g)		72.6 ± 3.3	66.6 ± 5.5	40.5 ± 4.7[a]	64.9 ±3.1
Triglyceride (mg/g)		7.0 ± 0.7	8.2 ± 0.9	15.5 ± 1.0[a]	8.5 ±0.9
Microsomal					
protein (mg/g)		15.5 ± 0.5	17.9 ± 0.4[a]	20.4 ± 0.2[a]	18.9 ±0.6[a]
phosphorus of phospholipids (μg/g)		221.9 ±19.5	298.8 ±15.1[b]	270.3 ± 9.0[b]	252.5 ±8.6
fatty acid of phospholipids	16:0[c]	16.9 ± 0.5	18.5 ± 0.6	19.0 ± 0.5[b]	19.7 ±0.8[b]
(% to total)	18:0	29.5 ± 0.6	29.6 ± 0.6	31.5 ± 0.4	30.1 ±0.7
	18:2	9.8 ± 0.3	12.0 ± 0.2[b]	12.6 ± 0.4[b]	11.5 ±0.1[b]
	20:4	26.4 ± 0.5	24.9 ± 0.5	24.3 ± 0.5	23.0 ±0.4[a]
	22.6	9.6 ± 0.2	8.7 ± 0.2	7.1 ± 0.2[b]	9.1 ±0.3

Rats were pretreated orally with monochlorobenzene (MCB), *p*-dichlorobenzene (*p*-DCB), 1,3,5-trichlorobenzene (1,3,5-TRCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TECB), pentachlorobenzene (PECB), and hexachlorobenzene (HCB) in a dose of 250 mg/kg daily for 3 days, respectively, and were sacrificed 24 hr after the last administration. All values are mean ± S.E. of 6 rats except phospholipids, and fatty acid values are mean ± S.E. of 3 groups of 2 rats.

a) significantly different from control, $p<0.01$

b) significantly different from control, $p<0.05$

c) carbon number: double bond number

15) D.J. Hanahan, J.G. Dittmer, and E.A. Warashina, *J. Biol. Chem.*, **228**, 685 (1957); R.J. Morin, *Cancer Res.*, **25**, 118 (1965).
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TABLE II. Effects of Treatment with Chlorinated Benzenes for 3 Days on Cytochromes, Drug Metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

A)	Control	MCB	*p*-DCB	1,3,5-TRCB
Cytochromes				
P-450 (nmoles/mg protein)	0.68± 0.04	0.56± 0.02[a]	0.73± 0.04	0.73± 0.03
b_5 (nmoles/mg protein)	0.35± 0.01	0.41± 0.03	0.40± 0.02	0.43± 0.03[a]
Aniline hydroxylase				
formed *p*-aminophenol				
(nmoles/mg protein/min)	0.56± 0.08	0.45± 0.03	0.65± 0.03	0.67± 0.09
(nmoles/g liver/min)	9.29± 1.20	7.60± 0.37	11.29± 1.15	12.92± 1.62
(nmoles/100 g b.w./min)	47.12± 5.76	47.26± 2.29	61.47± 6.11	70.30± 7.20
formed *p*-aminophenol/P-450				
(nmoles formed/nmoles P-450/min)	0.83± 0.10	0.81± 0.05	0.89± 0.05	0.92± 0.09
Aminopyrine demethylase				
formed formaldehyde				
(nmoles/mg protein/min)	3.70± 0.33	2.22± 0.55	4.98± 0.53	5.52± 0.29[a]
(nmoles/g liver/min)	62.18± 6.45	42.45± 7.75	88.03±10.58	109.64± 6.69[a]
(nmoles/100 g b.w./min)	317.71±35.26	266.79±48.74	476.33±54.05	612.62±41.36[a]
formed formaldehyde/P-450				
(nmoles formed/nmoles P-450/min)	5.45± 0.47	3.98± 0.98	6.82± 0.84	7.60± 0.35[a]
Spectral change				
aniline–cytochrome P-450				
($E_{430-480}$/mg protein) × 10^3	16.3 ± 1.4	14.4 ± 0.5	15.0 ± 1.7	18.2 ± 0.7
δ-ALA synthetase				
(nmoles/g liver/hr)	29.5 ± 2.0	49.2 ±10.6	31.9 ± 2.6	30.2 ± 2.4

B)	Control	1,2,4,5-TECB	PECB	HCB
Cytochromes				
P-450 (nmoles/mg protein)	0.66± 0.03	0.99± 0.04[b]	1.16± 0.06[b]	0.97± 0.08[b]
b_5 (nmoles/mg protein)	0.36± 0.01	0.42± 0.02	0.42± 0.01	0.41± 0.01
Aniline hydroxylase				
formed *p*-aminophenol				
(nmoles/mg protein/min)	0.65± 0.04	0.89± 0.04[b]	0.82± 0.03[b]	0.59± 0.04
(nmoles/g liver/min)	10.02± 0.70	15.82± 0.78[b]	16.63± 0.70[b]	11.11± 0.76
(nmoles/100 g b.w./min)	48.52± 2.77	80.37± 5.13[b]	95.11± 3.98[b]	58.63± 4.84
formed *p*-aminophenol/P-450				
(nmoles formed/nmoles P-450/min)	0.98± 0.07	0.90± 0.03	0.71± 0.03[a]	0.61± 0.07[a]
Aminopyrine demethylase				
formed formaldehyde				
(nmoles/mg protein/min)	3.47± 0.34	5.71± 0.62[a]	6.67± 0.64[b]	4.69± 0.35[a]
(nmoles/g liver/min)	53.45± 4.73	102.23±11.64[b]	135.73±12.88[b]	88.36± 6.97[a]
(nmoles/100 g b.w./min)	262.39±28.16	520.71±65.74[a]	781.50±84.25[b]	469.50±43.89[a]
formed formaldehyde/P-450				
(nmoles formed/nmoles P-450/min)	5.25± 0.30	5.78± 0.32	5.75± 0.54	4.85± 0.29
Spectral change				
aniline–cytochrome P-450				
($E_{430-480}$/mg protein) × 10^3	15.7 ± 0.80	27.1± 1.8[b]	29.6± 1.4[b]	25.3± 1.5[b]
δ-ALA synthetase				
(nmoles/g liver/hr)	34.9 ± 3.2	56.0± 4.8[a]	98.7± 6.4[b]	51.8± 5.5[a]

Conditions were as described in Table I. All values are mean ± S.E. of 6 rats.
a) significantly different from control, $p<0.05$
b) significanty different from control, $p<0.01$

Results

Influence of the Number of Chlorine Atoms

The effects of chlorine-number in the chlorinated benzenes on the hepatic constituents and on the fatty acid composition in microsomal phospholipid fraction were shown in Table I.

A ratio of liver weight to 100 g body weight was increased by treatment of animals with MCB, PECB, or HCB. No effects were observed in contents of glycogen and triglyceride by treatment with these compounds, except that PECB decreased markedly glycogen content and increased triglyceride. In microsomal protein, significant increase was noted in 1,3,5-TRCB, 1,2,4,5-TECB, PECB and HCB treatment, respectively. A similar result was observed for the Pi content in microsomal phospholipids by pretreatment with the compounds possessing more than chlorine-number of three except HCB. The proportion of arachidonic acid to total fatty acids was decreased markedly and that of palmitic and linoleic acid in microsomal phospholipids were increased by treatment with MCB or PECB.

The influences on the contents of cytochromes, the magnitude of aniline-induced spectral change and both activities of drug-metabolizing enzymes and δ-ALA syntherase were summarized in Table II. The content of cytochrome P-450, the magnitude of aniline-cytochrome P-450 binding spectrum and the activity of aminopyrine demethylase were increased by treatment with 1,2,4,5-TECB, PECB and HCB, respectively. On the contrary, MCB treatment decreased significantly the activity of aminopyrine demethylase and slightly the activity of aniline hydroxylase in proportion to the decrease of cytochrome P-450 content, whereas the cytochrome b_5 content showed the tendency to be increased by each treatment. Activity of δ-ALA synthetase was more markedly increased by PECB treatment than 1,2,4,5-TECB or HCB treatment. MCB treatment also enhanced the activity of δ-ALA synthetase, but not statistically significant.

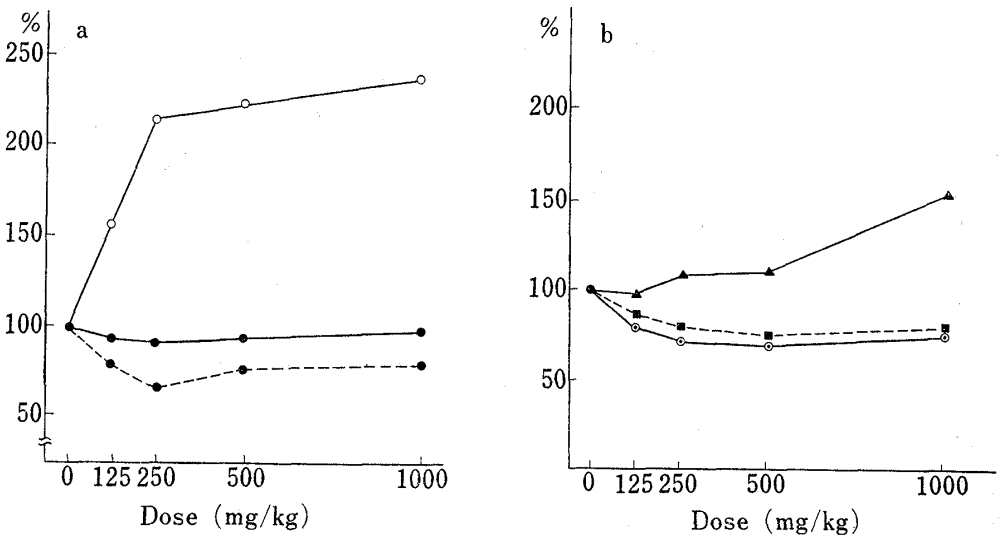

Fig. 1. a, b). Dose-Response Relationship for Monochlorobenzene on the Cytochromes, δ-ALA Synthetase and Drug-Metabolizing Enzymes

Rats were given orally monochlorobenzene at the single doses of 125, 250, 500 or 1000 mg/kg, and sacrificed 24 hr after the administration. The values of each point are mean of 4 rats and represented as percentage of control. ○——○, δ-ALA synthetase activity (control value: 31.8 nmoles/g liver/hr); ●·····●, cytochrome P-450 content (control value: 0.82 nmoles/mg protein); ●——●, cytochrome b_5 content (control value: 0.38 nmoles/mg protein); ▲——▲, aniline hydroxylase activity (control value: 0.52 nmoles/mg protein/min); ■·····■, aminopyrine demethylase activity (control value: 4.78 nmoles/mg protein/min); ◎——◎, aniline-cytochrome P-450 (control value: $E_{430-480}$ 17.3/mg protein $\times 10^3$)

Influence of MCB

As shown in Figure 1a, cytochrome P-450 content was decreased at 24 hr after MCB treatment at single doses of 125, 250, 500 and 1000 mg/kg, respectively, whereas activity of δ-ALA synthetase was increased at 24 hr after dosing. The changes in the activity of aminopyrine demethylase and the magnitude of aniline-cytochrome P-450 binding spectrum were paralleled approximately with changes in the cytochrome P-450 content, while activity of aniline hydroxylase was enhanced by MCB treatment at 1000 mg/kg dosing (Figure 1b).

The results of a single administration of MCB in a dose of 250 mg/kg were shown in Figure 2a, cytochrome P-450 content was increased slightly in 6 hr after dosing, and subsequently decreased within 24 hr, then gradually restored to normal level after 360 hr. On the contrary, activity of δ-ALA synthetase was significantly decreased in 6 hr after dosing, restored after 12 hr, and markedly increased after 24 hr, followed by a gradual decrease to the control level. Activity of aniline hydroxylase was increased in 6 and 12 hr after dosing, but decreased in 48 and 120 hr, whereas, activity of aminopyrine demethylase behaved as did cytochrome P-450 (Figure 2b). Cytochrome b_5 content, however, was not affected under these experimental conditions.

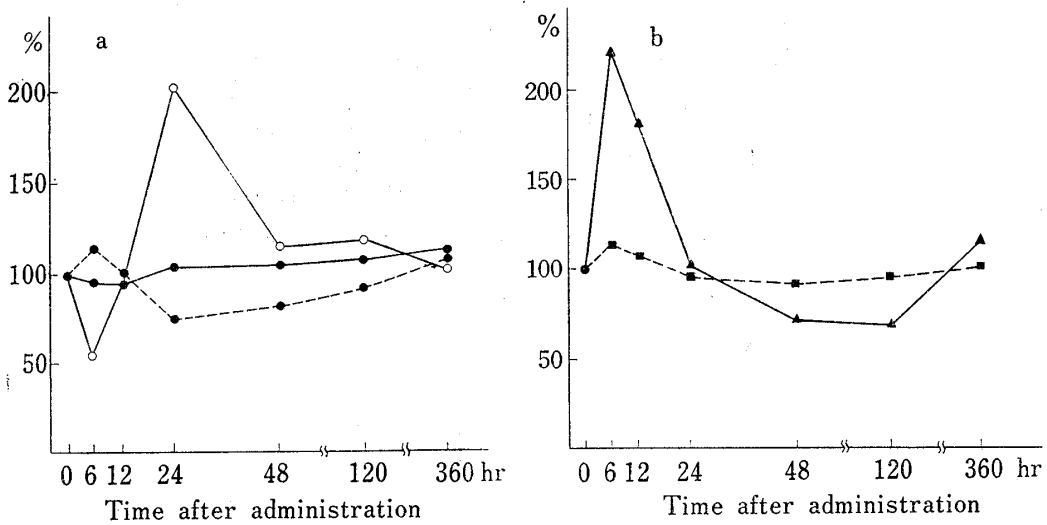

Fig. 2. a, b). Time-Course after a Single Administration of Monochlorobenzene

Rats were given orally monochlorobenzene at a single dose of 250 mg/kg, and sacrificed at the indicated time after the administration. The values of each point are mean of 4 rats and represented as percentage of control. ○——○, δ-ALA synthetase activity (control value: 33. 8nmoles/g liver/hr); ●·····●, cytochrome P-450 content (control values: 0.74 nmoles/mg protein); ●——●, cytochrome b_5 content (control value: 0.35 nmoles/mg protein); ▲——▲, aniline hydroxylase activity (control value: 0.41 nmoles/mg protein/min); ■·····■, aminopyrine demethylase activity (control value: 4.55 nmoles/mg protein/min).

Discussion

Recently, Litterst, *et al.*[19] has demonstrated that there is a close relationship between the chlorine content and the enzyme inducing effect of the Aroclors.

In the present studies, the content of hepatic constituents, such as microsomal protein and phospholipids including the composition of fatty acid, appeared to be increased with an increase of chlorine-number of the benzene ring. In addition, the remarkable enhancement of δ-ALA synthetase activity and increase of cytochrome P-450 content accompanying significant changes in activity of aminopyrine demethylase and in magnitude of aniline-cytochrome P-450 binding spectrum were observed in 24 hr after administration of 1,2,4,5-TECB, PECB

19) C.L. Litterst, T.M. Farber, A.M. Baker, and E.J. Van Loon, *Toxicol. Appl. Pharmacol.*, 23, 112 (1972).

and HCB respectively. These findings would also suggest to have a relationship between the number of chlorine atoms and their biological effects. However, the effects of HCB were less than that of PECB in the doses used. Similar results was obtained by Chen and DuBois[20] who found that polychlorinated biphenyls of 54% chlorination produced a greater stimulation of O-demethylation than did polychlorinated biphenyls of 60% chlorine content. In general, higher chlorinated benzenes are not readily absorbed, so that the metabolites accounted for in the urine tend to become less as the chlorine content increases. For example, it was shown[21] that 1,3,5-TRCB, 1,2,4,5-TECB and PECB were converted to small extent into corresponding phenols, 2,4,6-trichlorophenol (5%), 2,3,5,6-tetrachlorophenol (2%) and pentachlorophenol (less than 1%), respectively. Furthermore, HCB seemed to be metabolically inert,[22] therefore, it tends to remain in the tissue for a considerable time in the unchanged state. On the contrary, MCB was principally metabolized to catechol (27%), and mercapturic acid (25%), whereas *p*-DCB formed mostly phenols, *i.e.*, 2,5-dichlorophenol (35%).[21] These facts may partly be associated with the differences in the biological effects inducing by each treatment of chlorinated benzenes.

In the dose-response experiment of MCB 24 hr after administration, MCB decreased significantly cytochrome P-450 content, but enhanced markedly activity of δ-ALA synthetase. In the time-course study, cytochrome P-450 content was initially increased, and subsequently decreased throughout 2 or 3 days, and then recovered to the control level. δ-ALA synthetase, on the other hand, displayed the opposite tendency with the elapse of time.

From these findings, it seemed to speculate that MCB would increase the degradation of cytochrome P-450 or heme protein, and lower the utilization of porphyrine which might be accumulated by enhancing the activity of δ-ALA synthetase. Wada, *et al.*[23] observed that the increase of activity of δ-ALA synthetase by administration of porphyria-induced drugs, such as phenobarbital, tolbutamide and HCB, was in considerable agreement with the increase of content of cytochrome P-450. Therefore, when the content of cytochrome P-450, as one of the components of drug-metabolizing system, was insufficient, it seems likely that the marked increase of activity of δ-ALA synthetase may occur to supply cytochrome P-450.

Furthermore, the changes in cytochrome b_5 content was approximately paralleled in each treatment with the number of chlorine atoms. It is doubtful that there is a distinct effect on the cytochrome P-450 and b_5, both of which would probably exist in the same microsomal membrane, when chlorinated compounds used were given to animals. This may be due to the difference in turn-over rate or the difference of heme utilization of both cytochrome P-450 and b_5.

Further investigation should be required for the relationship between activity of δ-ALA synthetase and contents of cytochrome P-450 and b_5. This seems useful for elucidating the etiology of porphyria induced by organic chlorinated compounds, such as polychlorinated biphenyls, dichlorodiphenyltrichloroethane (DDT), BHC and so on.

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