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# Interaction Between Phencyclidine and its Pyrolysis Product, 1-Phenylcyclohexene[1]

ARVIND K. CHATURVEDI[2] AND DAVID J. KUNTZ[3]

*Departments of Pharmaceutical Sciences and Toxicology, College of Pharmacy North Dakota State University, Fargo, ND 58105*

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CHATURVEDI, A. K. AND D. J. KUNTZ. *Interaction between phencyclidine and its pyrolysis product, 1-phenylcyclohexene.* PHARMACOL BIOCHEM BEHAV **30**(4) 1035–1043, 1988.—The interaction between phencyclidine (PCP) and its pyrolysis product, 1-phenylcyclohexene (PC), at metabolic level was evaluated in Swiss male mice (21–24 g). PC (1.1, 2.2 and 4.4 mmol/kg/day for 4 days, IP, in corn oil) treatment to mice induced the in vitro metabolism ($p<0.05$) of amidopyrine (17%), aniline (12%), phenacetin (62–100%), pentobarbital (20–26%), PCP (25–80%) and benzo[*a*]pyrene (81–147%) in the 9000 g liver fraction and the hepatic microsomal contents of cytochrome P-450 (18–42%). The induction of the mixed function oxygenase (MFO) system was consistent with the decreases in the concentrations of IP administered pentobarbital (0.27 mmol/kg, in saline) and PCP (16.4, 32.8 and 65.6 μmol/kg, in saline) in the serum, brain, liver and kidneys of PC pretreated mice. At 1 hr after the above doses of PC, the in vitro metabolism of amidopyrine, aniline, or phenacetin was not inhibited. However, the biotransformation of benzo[*a*]pyrene was inhibited by 33 to 45%. Though PC after a single dose did not alter the tissue concentrations of PCP, it increased the pentobarbital concentrations in the tissues studied ($p<0.05$). These results indicate that PC has a potential to induce the MFO system after the 4-day treatment. This property of PC plays an important role in the reduction of the action of PCP by enhancing its metabolism, thereby decreasing its tissue levels.

Phencyclidine Pyrolysis product 1-Phenylcyclohexene Metabolic interaction
Mixed function oxygenase system Hepatic microsomal cytochrome P-450 contents Pentobarbital
Phenobarbital SKF-525A Gas-liquid chromatography Nitrogen-phosphorus detector

PHENCYCLIDINE (PCP), a psychotomimetic drug of abuse [3,42], is commonly taken by smoking [23,31]. During this process, about 50% of PCP pyrolyzes into 1-phenylcyclohexene (PC) and piperidine [20]. PCP is primarily metabolized by the hydroxylation of the cyclohexane and piperidine rings followed by conjugation [15]. The metabolites of PCP are considered less active pharmacologically/toxicologically than the parent compound [9, 18, 25, 40]. It appears that the cytochrome P-450 system is involved in the biotransformation of PCP [9,27]. Though PC is considerably less behaviourally active in producing motor impairment and has a higher $LD_{50}$ than PCP in mice [24,26], it has a potential to produce chronic toxicity. The irreversible binding of [$^{14}$C]-PC metabolites to tissue protein in rats [7] and the allylic oxidation involving the cyclohexene double bond of the PC molecule in humans [14,15] have been documented.

In both first time and chronic smokers, the possibility for the interaction between PCP and PC exists. The disposition of PC following the inhalation of smoke by mice [35] and humans [14,15] from PCP-loaded cigarettes has been studied. Epidemiological studies have indicated months or years of regular daily use in chronic smokers [3, 6, 31]. Thus, the toxicity resulting from repeated exposure to large amounts of PC and interaction with PCP in chronic situations remains a cause for concern. PC increases the liver/body weight ratio, decreases the pentobarbital-induced sleep and causes the proliferation, along with the dilatation and fragmentation, of the endoplasmic reticulum in the liver of mice [26]. PC after a single or 4-day treatment significantly lowers the PCP- (16.4 μmol/kg, IP) stimulated locomotion [26]. The mechanism by which PC exerts its effects has not been established. Therefore, the interaction between PCP and PC at metabolic level was evaluated by observing the effects of PC after

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[2]Requests for reprints should be addressed to Arvind K. Chaturvedi, Department of Toxicology, College of Pharmacy, North Dakota State University, Fargo, ND 58105.

[3]Present address: Department of Pharmacology and Toxicology, The University of Utah, Salt Lake City, UT.

single and 4-day exposures on the mixed function oxygenase (MFO) system and on the tissue concentrations of pentobarbital and PCP in mice.

## METHOD

### *Materials*

Phencyclidine (PCP) hydrochloride was supplied by the National Institute on Drug Abuse, Rockville, MD. l-Phenylcyclohexene (PC) was obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. Sodium phenobarbital (PB), pentobarbital, 2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride (SKF-525A), glutethimide and meperidine·HCl were gifts of Merck & Co., Inc., Rahway, NJ, Abbott Laboratories, North Chicago, IL, Smith Kline & French Labs., Philadelphia, PA, USV Laboratories, Inc., Manati, PR and Wyeth Laboratories, Philadelphia, PA, respectively. Corn oil (Mazola®, Best Foods, Englewood Cliffs, NJ) was purchased locally. Other chemicals of analytical grade were purchased from commercial sources.

Solutions of chemicals were prepared in deionized water. Fresh drug solutions for animal injection were prepared in physiological saline before use, unless otherwise stated. PC and 3-methylcholantherene (MC) were dissolved, while PB was suspended in corn oil. Animals in the control and experimental groups were administered equivalent amounts of the vehicle.

### *Animals*

Swiss-Webster male mice (21–24 g), purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN, were housed in plastic cages of 29.2×19×12.7 cm dimensions, in groups of 5, in a centralized animal care facility. Softwood shavings were used as bedding in the cages. The facility, maintained at 24 to 25°C with the relative humidity of 40 to 60%, was kept in a 12-hr light (7 a.m. to 7 p.m.)/dark (7 p.m. to 7 a.m.) cycle with fluorescent lights. Animals were allowed mouse chow and tap water ad lib.

### *Experimental Design*

Experiments were designed to evaluate the effects of PC after a single, as well as 4-day, exposure. *For acute experiments*, animals in 4 groups, each consisting of 4 mice, were treated IP with corn oil (10 ml/kg) or with 1.1, 2.2, or 4.4 mmol/kg of PC in corn oil. These doses correspond to 1/20, 1/10 and 1/5 of the $LD_{50}$ of PC in mice [26]. One hr after the treatment, animals were killed using diethyl ether to remove the liver for the in vitro microsomal drug metabolizing enzyme assays. It had been established that the PC accumulation in various organs in rats peaked within 1 hr following its IP administration [7]. The influence of PC, at the above doses, on the tissue concentration of pentobarbital or PCP was studied by the IP injection of pentobarbital (0.27 mmol/kg) or PCP (16.4, 32.8, or 65.6 μmol/kg), in saline, at 1 hr after the PC or corn oil administration to mice in 16 groups each consisting of 10 animals. Four groups of animals were utilized for the pentobarbital study, while the remaining groups of mice were used in the PCP experiments.

*For 4-day experiments*, animals were divided into 4 groups, each comprising 4 mice. Animals of each group were challenged IP with 1.1, 2.2, or 4.4 mmol/kg of PC in corn oil or with corn oil (10 ml/kg) daily for 4 days. Twenty-four hr after the last injection, mice were killed to obtain the liver samples for the in vitro microsomal enzyme assays. It appeared that the daily PC treatment for 4 days would provide a sufficient time for the de novo protein synthesis of the MFO system [26]. In the in vivo study, animals were also injected daily for 4 days, IP, the doses of vehicle, PC, PB (0.3 mmol/kg) and SKF-525A. For SKF-525A administration, animals received only the vehicle (10 ml/kg) daily for 4 days, and twenty-four hr after the last corn oil administration, mice were treated IP with 0.10 mmol/kg of SKF-525A in saline. Twenty-four hr after the last treatment or 1 hr following the SKF-525A administration, animals were challenged with pentobarbital or PCP. Animals were divided into 24 groups. Each of the groups contained 10 mice. Six groups of mice were used in the pentobarbital experiment, while the remaining groups of animals were utilized for the PCP treatments.

The pentobarbital-challenged animals were killed using diethyl ether to collect the brain, liver, kidney and blood samples at 45 min postinjection, while the PCP-treated animals were killed at 30 min following its administration. Blood samples from animals of the same group were pooled into one group to obtain sufficient amounts of serum for the quantitative analysis of pentobarbital or PCP, while brain, liver, or kidney samples of the same group were divided into three pools. Samples were frozen at −70°C until analyzed.

At the 0.27 mmol/kg dose of pentobarbital, the average sleep time in the control mice was 47.0±4.0 min (n=17). Based on this, it was determined that the 45-min termination time would be appropriate to study the effects of PC on the tissue concentrations of pentobarbital [11]. The 30-min termination time for animals treated with PCP was consistent with the disposition of $^3$H-PCP in mice, where the peak tissue concentration of the radioactivity occurred at about 15–30 min after the IP administration of PCP [33]. The 16.4 μmol/kg dose of PCP had been determined to produce locomotion in mice with the effect lasting 60 min [10,13]. The 32.8 and 65.6 μmol/kg doses of PCP were able to produce other neurobehavioural effects, e.g., circular movements, side-to-side head movements, convulsions, etc., during a period of 30 min [10]. Furthermore, the concentrations of PCP, which were not detectable in the 1 ml of 20% brain homogenate at the lower PCP doses, were easily determined at the higher PCP dose by a gas-liquid chromatographic method.

### *Assays of Hepatic Microsomal Enzymes*

The activities of amidopyrine N-demethylase [1,2], aniline hydroxylase [28], phenacetin O-dealkylase [22,29] and benzo[*a*]pyrene hydroxylase [17,29] in the hepatic 9000 g supernatant fractions were determined by the methods reported earlier.

### *In Vitro Hepatic Metabolism of Pentobarbital or PCP*

The in vitro metabolism of pentobarbital or PCP was investigated in the hepatic 9000 g supernatant fractions prepared from mice (3 per group) treated IP daily for 4 days with corn oil (10 ml/kg) or PC (1.1, 2.2, or 4.4 mmol/kg, in corn oil). Twenty-four hr after the last injection, animals were killed and the 9000 g liver fractions were obtained. The components of the metabolic reaction mixture and the conditions for the assay were the same as in the metabolism of amidopyrine, aniline, or phenacetin, except the volume of the solution of each component of the reaction mixture was doubled to compensate the final volume of the reaction mixture to 6.0 ml, instead of 3.0 ml [1, 2, 22, 28, 29]. After preincubating the reaction mixture for 5 min, the metabolism

TABLE 1

ACUTE EFFECTS OF PC ON HEPATIC MICROSOMAL ENZYMES

| Enzyme[a] | Control (Corn oil) | PC (mmol/kg, IP) 1.1 | 2.2 | 4.4 |
|---|---|---|---|---|
| Amidopyrine N-Demethylase[b] | 67.2 ± 1.7 | 64.7 ± 6.4 | 67.1 ± 2.4 | 80.9 ± 0.86† |
| Aniline Hydroxylase[c] | 8.5 ± 0.2 | 8.3 ± 0.7 | 7.6 ± 0.4 | 9.0 ± 0.3 |
| Phenacetin O-Dealkylase[d] | 2.4 ± 0.2 | 2.5 ± 0.1 | 2.8 ± 0.2 | 3.2 ± 0.3 |
| Benzo[*a*]pyrene Hydroxylase[e] | 18.5 ± 0.9 | 12.4 ± 0.9* | 10.8 ± 0.8† | 10.1 ± 0.6† |

[a]Assay procedures are described in the text. Each of the values is the mean ± S.E. of the separate enzyme preparations obtained from 4 mice. Experiments were conducted in duplicate.

[b]nmol of HCHO formed/mg protein/30 min.

[c]nmol of p-aminophenol formed/mg protein/30 min.

[d]nmol of acetaminophen formed/mg protein/30 min.

[e]Δ in fluorescence/mg protein/15 min.

*$p<0.01$ different from the control; †$p<0.001$ different from the control.

was initiated by the simultaneous additions of nicotinamide adenine dinucleotide phosphate (NADP) and pentobarbital (530 nmol) or PCP (493 nmol). The disappearance of pentobarbital or PCP from the reaction mixture as a function of incubation time was determined by gas-liquid chromatography using a flame-ionization detector. A 750 μl aliquot of the reaction mixture taken out at 5, 30, 60, or 120 min interval for pentobarbital or at 5, 15, 30, or 45 min interval for PCP was quickly transferred to a 5 ml centrifuge tube which was placed in a boiling water bath for 2 min to stop the reaction. After cooling the aliquot at room temperature, it was centrifuged in a clinical centrifuge for 5 to 10 min at maximum speed, and 500 μl of the supernatant was transferred to another 5 ml centrifuge tube. To that, 100 μl of $CHCl_3$ containing the internal standard, glutethimide (50 mg/l), was added. After vortexing the mixture for 30 sec, it was centrifuged and 2 to 3 μl of the organic layer was injected onto a gas-liquid chromatograph. For the analysis of PCP, 100 μl of 1 N NaOH was added to the 500 μl supernatant prior to the $CHCl_3$ extraction.

A Packard Model 417 Becker gas chromatograph (Downers Grove, IL) equipped with a silanized glass column (1.83 m × 2 mm i.d., packed with 3% OV-17 by weight on 100–120 mesh Chromosorb W-HP, Applied Science, Deerfield, IL) and a Hewlett-Packard 3390 A integrator (Avondale, PA) were used in the analysis. The flows of carrier gas ($N_2$), compressed air and $H_2$ were 33, 300 and 30 ml/min, respectively. The injector port, column and detector temperatures for the pentobarbital determinations were 250, 190 and 300°C, while they were correspondingly 195, 190 and 300°C for the PCP analysis. The injector port and oven temperature during PCP determinations were kept below 200°C to minimize the thermal conversion of PCP to PC [19]. The retention times for pentobarbital, PCP and the internal standard (glutethimide) at 190°C oven temperature were correspondingly 2.9, 4.2 and 5.5 min. The other common components and products present in the metabolic reaction mixture did not appear to interfere with the above retention times.

The standard curves using concentrations of 2.5, 5, 10 and 20 mg/l of pentobarbital or PCP in the metabolic reaction mixture containing the boiled 9000 g liver supernatant were obtained on every day of analysis. Plots of peak area ratio of pentobarbital/glutethimide vs. pentobarbital (mg/l) and PCP/glutethimide vs. PCP (mg/l) were determined to be linear in the above concentration range. The value for coefficient of variation for pentobarbital analysis was 3%, while it was 13% for PCP. A level of as low as 1.5 mg/l of pentobarbital or PCP could be easily determined from the plots.

*Cytochrome P-450 Assay Procedure*

Twenty-four hr after the last injection, mice in 6 groups, each consisting of 3 animals, treated IP daily for 4 days with corn oil (10 ml/kg), PC (1.1, 2.2, or 4.4 mmol/kg), PB (0.30 mmol/kg), or MC (0.26 mmol/kg), were killed using diethyl ether to obtain the liver samples. Hepatic microsomes were obtained, and the cytochrome P-450 activity in the microsomal suspensions was determined by its dithionite difference spectrum as described by Omura and Sato [41], using an extinction coefficient (ΔOD 450–490) of 91.1 $mM^{-1} \cdot cm^{-1}$.

*Determination of Pentobarbital in Tissues*

A modification of the method of Chaturvedi and Rao [11] was used for the analysis of pentobarbital in tissues. The tissues, except serum, were mixed with ice-cold deionized water in a ratio of 1:4 (w/v), homogenized in a Polytron PT 10/35 homogenizer (Brinkman Instruments, Westbury, NY) at a submaximal speed for 20 sec and centrifuged in a clinical centrifuge at maximum speed for 10 min. The tissue homogenate supernatants and serum samples were analyzed along with the appropriate standards to determine pentobarbital concentrations. The chromatrographic operating parameters were the same as described in the In Vitro Hepatic Metabolism of Pentobarbital section. The plot of the peak area ratio of pentobarbital/internal standard vs. pentobarbital (mg/l) for each tissue homogenate supernatant or serum was obtained on every day of analysis, and each of them was found to be linear in the range of 2.5 to 20 mg/l.

TABLE 2

EFFECTS OF 4-DAY PC PRETREATMENT ON HEPATIC MICROSOMAL ENZYMES

| Enzyme[a] | Control (Corn oil) | PC (mmol/kg, IP) 1.1 | 2.2 | 4.4 |
|---|---|---|---|---|
| Amidopyrine N-Demethylase[b] | 67.6 ± 1.5 | 63.2 ± 1.7 | 70.5 ± 2.1 | 79.1 ± 3.7* |
| Aniline Hydroxylase[c] | 8.3 ± 0.3 | 7.9 ± 0.1 | 7.7 ± 0.4 | 9.3 ± 0.1* |
| Phenacetin O-Dealkylase[d] | 2.6 ± 0.1 | 2.7 ± 0.1 | 4.2 ± 0.6* | 5.2 ± 0.2† |
| Benzo[*a*]pyrene Hydroxylase[e] | 13.3 ± 1.2 | 16.9 ± 1.1 | 24.1 ± 1.1† | 32.9 ± 1.9† |

[a]Every value is the mean ± S.E. of the separate enzyme preparations obtained from 4 mice. Enzyme assays were conducted in duplicate. Details of the procedures of these assays are given in the text.
[b]nmol of HCHO formed/mg protein/30 min.
[c]nmol of p-aminophenol formed/mg protein/30 min.
[d]nmol of acetaminophen formed/mg protein/30 min.
[e]Δ in fluorescence/mg protein/15 min.
*$p<0.05$ vs. controls; †$p<0.001$ different from the controls.

The lower detection limit for pentobarbital was about 1.5 mg/l. The coefficient of variation was <15%.

*PCP Determination in Tissues*

A method, similar to reported previously [5], for the determination of PCP in various tissue homogenates or serum was developed by gas-liquid chromatography using a nitrogen-phosphorus detector. The tissue homogenate supernatants were prepared in ice-cold water as described in the previous section. Standard curves for each of the tissue homogenate supernatants and serum were obtained by spiking drug free mice tissue homogenates or serum with a stock PCP solution to achieve the PCP concentrations of 0.2, 0.4, 0.8, 1, 2 and 4 mg/l. A set of new respective standards was analyzed every day.

To 1 ml of tissue homogenate supernatant, serum, standard, or blank in a 15 ml screw cap centrifuge tube, 100 μl of 25 mg/l aqueous solution of meperidine (internal standard) and 100 μl of 40% NaOH were added. The mixture was extracted with 5 ml of diethyl ether by vortexing for 30 sec. It was then centrifuged in a clinical centrifuge for 5 min, and the organic layer was completely transferred to another 15 ml centrifuge tube containing 1 ml of 1 N $H_2SO_4$. After vortexing the mixture for 30 sec and centrifugation, the organic layer was removed by aspiration. The aqueous layer was basified by the addition of 200 μl of 40% NaOH and brought to room temperature prior to the addition of 50 μl of $CHCl_3$. The mixture was then vortexed and centrifuged, and 2 to 5 μl of organic layer was injected onto a gas-liquid chromatograph.

A Hewlett-Packard, Model 5880A, gas chromatograph and a Hewlett-Packard 5880A gas chromatograph terminal were used in the analysis. The operating parameters were as follows:

Column: 1.83 m × 4 mm i.d. silanized glass column, packed with 3% OV-17 by weight on 80–100 mesh Gas-Chrom Q.
Gas flow: Carrier gas (helium): 50 ml/min.
Hydrogen: 3 ml/min.
Compressed air: 70 ml/min.
Injector port and column temperature: 195°C.
Detector temperature: 290°C.

Plots of peak area ratio of PCP/meperidine vs. PCP (mg/l) for each tissue homogenate or serum were found to be linear in range of 0.2 to 4 mg/l. This method could detect as little as 0.1 mg/l of PCP. The coefficient of variation for the analysis in the various tissues was <15%. The retention time for PCP was 6.0 min, while for meperidine it was 4.0 min. The extracts of tissue homogenates and serum did not exhibit peaks which interfered with the retention times of PCP and meperidine.

*Protein Determination*

Protein concentrations in the hepatic 9000 g supernatants and microsomal fractions were measured as described by Lowry *et al.* [32]. Bovine serum albumin was used as a standard.

*Statistical Analysis*

Values are presented as mean±S.E. of minimum of 3 determinations. Significance of differences between means of all observations was checked by Student's *t*-test [21]. A difference between 2 means was considered significant with $p<0.05$. Correlation coefficient, slope and Y-intercept of a straight line were calculated by linear regression analysis.

## RESULTS

*Effects of PC on Hepatic Microsomal Enzymes*

PC after the single exposure did not inhibit the activities of amidopyrine N-demethylase, aniline hydroxylase and phenacetin O-dealkylase. However, it was able to inhibit the biotransformation of benzo[*a*]pyrene by aromatic hydrocarbon hydroxylase (AHH) in a dose dependent fashion (Table 1). The inhibition of the enzyme activity in comparison to the control was, respectively, 33, 42 and 45% at the 1.1, 2.2 and 4.4 mmol/kg PC doses ($p<0.05$).

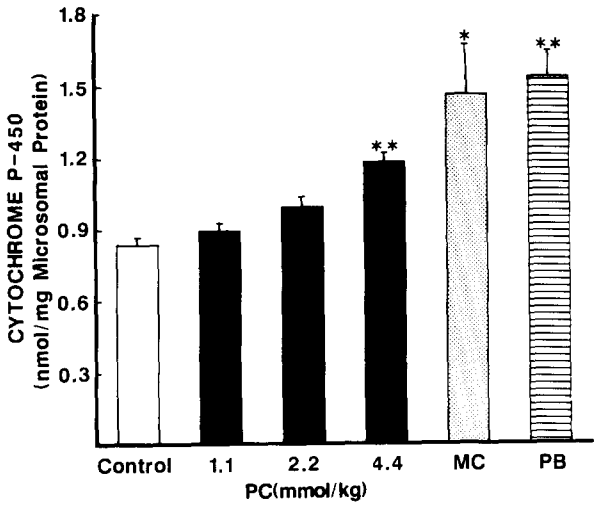


FIG. 1. Effects of the 4-day PC (1.1, 2.2, or 4.4 mmol/kg), PB (0.30 mmol/kg), or MC (0.26 mmol/kg) pretreatment on the hepatic microsomal cytochrome P-450 contents in mice. The control group mice were given corn oil (10 ml/kg). Each bar represents the mean±S.E. (n=3). A significant change, $p<0.05$, in the cytochrome P-450 contents than the control is designated by *, while at $p<0.01$ it is by **. The $p$ value for the difference in the mean cytochrome P-450 contents between the control group and 2.2 mmol/kg PC group was in the range of 0.1 to 0.05.

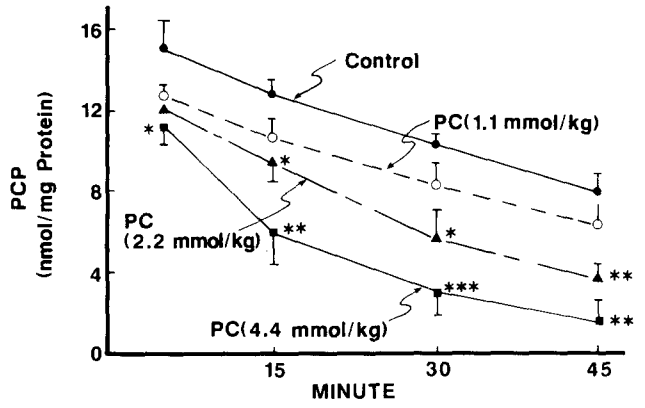


FIG. 3. In vitro disappearance of PCP from the mouse 9000 g liver fractions as a function of time. Each of the points is the mean±S.E. of the observations from 3 animals pretreated with corn oil (control; 10 ml/kg) or PC (1.1, 2.2, or 4.4 mmol/kg) daily for 4 days. The difference between 2 means at $p$ values less than 0.05, 0.01 and 0.001 are depicted by *, ** and ***, respectively.

Contrary to this, the 4-day PC treatment induced the in vitro metabolism of aniline, phenacetin and benzo[$a$]pyrene (Table 2). The enhancements were the most prominent at the higher dose of PC (4.4 mmol/kg), where the activities of aniline hydroxylase, phenacetin O-dealkylase and benzo[$a$]pyrene hydroxylase, in relation to the control, increased by 12, 100 and 147%, respectively ($p<0.05$). The statistically significant increase (17%) seen in the amidopyrine N-demethylase activity at the 4.4 mmol/kg dose of PC does not necessarily represent an induction of the enzyme as a similar percentage increase was noted in the acute experiment (Table 1).

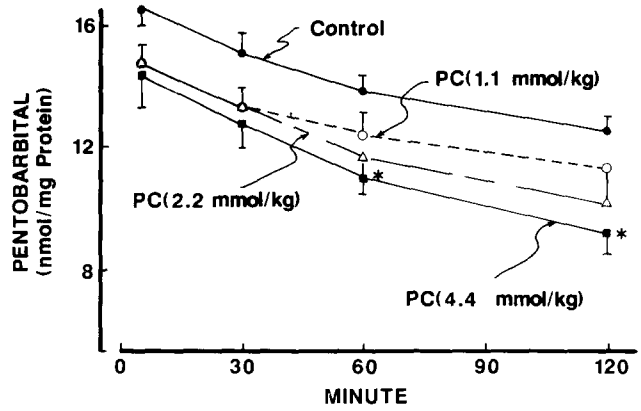


FIG. 2. In vitro metabolism of pentobarbital by the mouse 9000 g liver fractions obtained from 4 groups of animals, 3 in each group, pretreated with corn oil (10 ml/kg), or with PC (1.1, 2.2, or 4.4 mmol/kg, in corn oil) daily for 4 days. As described in the text, the pentobarbital metabolism was studied by its disappearance from the metabolic reaction mixture. Each of the points is the mean±S.E. of the observations from 3 animals. A significant difference ($p<0.05$) at a particular time point from the control is denoted by *.

*Influence of 4-Day PC Treatment on Cytochrome P-450*

PC, at doses ranging from 1.1 to 4.4 mmol/kg, was tested for its ability to induce the hepatic microsomal cytochrome P-450 contents (Fig. 1). Though PC was not effective at 1.1 mmol/kg, it was able to induce the cytochrome P-450 system at the higher doses. This was reflected by 18 ($p=0.1$–$0.05$) and 42% ($p<0.01$) increases in the cytochrome P-450 contents at the 2.2 and 4.4 mmol/kg doses of PC, respectively (n=3). Under the identical experimental conditions, MC and PB pretreatments correspondingly increased the cytochrome P-450 contents by 76 and 85%.

*In Vitro Metabolism of Pentobarbital and PCP*

Effect of the 4-day PC treatment on the metabolism of pentobarbital and PCP by the mouse 9000 g liver fraction was studied. The disappearances of these substrates from the incubation medium as a function of time are depicted in Figs. 2 and 3.

Five min after the initiation of the metabolism, 16.5 nmol of pentobarbital/mg protein were present in the control incubation medium. During the following 115 min, there was a decrement of 4 nmol of pentobarbital/mg protein in the control group, while in the treated groups, decreases of 3.4 nmol with 1.1 mmol/kg PC, 4.5 nmol with 2.2 mmol/kg PC and 5.2 nmol with 4.4 mmol/kg PC were observed. This was in agreement with the statistically significant increases noted in the metabolism of pentobarbital of 20% at 60 min and 26% at 120 min with respect to the corresponding control points in the 4.4 mmol/kg PC group.

As is evident from Fig. 3, PC enhanced the metabolism of PCP in a dose-related manner as a function of time. Though such an increase was not seen at the lower dose of PC, it was clearly visible with the higher doses of PC. With the 2.2 mmol/kg PC group, the increases in the metabolism with respect to the control at 5, 15, 30 and 45 min were correspondingly 17, 26, 44 and 52%, while these increases were 25, 53, 71 and 80% with the 4.4 mmol/kg PC dose. During the

TABLE 3

INFLUENCE OF PC ON TISSUE CONCENTRATIONS OF PENTOBARBITAL IN MICE

| Pretreatment | Pentobarbital (nmol/ml or g)[a] | | | |
|---|---|---|---|---|
| | Serum | Brain | Liver | Kidney |
| | Single Exposure | | | |
| Control | 63.2 ± 0.3 | 121.7 ± 2.1 | 234.1 ± 22.5 | 183.2 ± 9.6 |
| PC (mmol/kg) | | | | |
| 1.1 | 83.0 ± 0.9‡ | 144.7 ± 4.5‡ | 320.3 ± 23.4* | 225.7 ± 4.1† |
| 2.2 | 78.0 ± 2.0‡ | 144.3 ± 5.0† | 310.8 ± 11.3* | 227.1 ± 5.6† |
| 4.4 | 83.2 ± 0.9‡ | 178.3 ± 4.6‡ | 434.1 ± 43.6† | 262.7 ± 6.7‡ |
| | 4-Day Exposure | | | |
| Control | 35.1 ± 2.2 | 97.1 ± 2.7 | 238.3 ± 13.0 | 162.6 ± 10.1 |
| PC (mmol/kg) | | | | |
| 1.1 | 43.3 ± 3.1 | 110.8 ± 4.4† | 244.2 ± 11.4 | 164.5 ± 4.2 |
| 2.2 | 41.9 ± 3.6 | 93.4 ± 2.9 | 208.4 ± 6.3 | 143.3 ± 4.9 |
| 4.4 | 27.3 ± 2.5* | 83.0 ± 3.0† | 182.8 ± 7.1† | 118.5 ± 2.2‡ |
| PB[b] | 2.9 ± 1.0‡ | ND[c] | 26.3 ± 11.3‡ | ND |
| SKF-525A[d] | 71.3 ± 5.8‡ | 186.4 ± 8.8‡ | 445.0 ± 22.1‡ | 267.3 ± 9.2‡ |

[a]Details of the experimental design and the analysis in various tissues are described in the text. Blood samples from the animals of the same group were pooled into one group to obtain sufficient amount of serum for the analysis, while each of the other tissues of the same group were divided into 3 pools. Serum was analyzed for a minimum of 3 determinations in duplicate. Similarly, each of the three pools of brain, liver, or kidney was analyzed at least once in duplicate. Values are means ± S.E.

[b]0.30 mmol/kg daily for 4 days.

[c]Not detected in the 1 ml of 20% homogenate.

[d]Animals were pretreated with corn oil (10 ml/kg) daily for 4 days. Twenty-four hr after the last injection, they were challenged with the 0.10 mmol/kg dose of SKF-525A.

*Significantly different from control values, $p<0.05$; †significantly different from control values, $p<0.01$; ‡significantly different from control values, $p<0.001$.

40-min period, i.e., between the 5 and 45 min incubation time, 7.2 nmol of PCP/mg protein were metabolized in the control and 6.5, 8.8 and 9.7 nmol of PCP/mg protein were biotransformed in the 1.1, 2.2 and 4.4 mmol/kg PC groups, respectively.

*Influence of PC on Tissue Levels of Pentobarbital*

Single exposures of mice to PC, ranging from 1.1 to 4.4 mmol/kg, increased the concentrations of pentobarbital in all the tissues analyzed (Table 3). In relation to the control group, the increases in the pentobarbital concentrations in the 4.4 mmol/ kg PC group were 32% in the serum, 47% in the brain, 85% in the liver and 43% in the kidneys, whereas these increases in various tissues ranged from 19 to 37% in the lower PC dose groups.

In contrast to the single exposure, PC (4.4 mmol/kg/day for 4 days) lowered the pentobarbital concentrations with statistical significance in the serum (22%), brain (15%), liver (23%) and kidneys (27%). At the lower doses of PC, the decrease was not significantly evident. However, an increase of 14% ($p<0.01$) in the brain pentobarbital concentration was noted with the 1.1 mmol/kg PC group. The PB pretreatment lowered the pentobarbital concentrations in the four tissues, while they were significantly higher in the SKF-525A pretreated group. Depending upon the tissue, the decrease ranged from 89 to 100% in the PB group, and the increase ranged from 64 to 104% in the SKF-525A group.

*Effects of PC on Tissue Concentration of PCP*

Tissue concentrations of PCP after the single and 4-day PC pretreatments in mice are given in Tables 4 and 5. Though PCP could not be detected in 1 ml of the brain homogenate by the analytical technique utilized at its 16.4 or 32.8 μmol/kg IP dose, it was easily detected at a higher dose (65.6 μmol/kg). The concentrations of PCP in tissues increased proportionally to its dose. PC after a single dose did not significantly alter the concentrations of PCP in the serum, brain, liver and kidneys. However, the multiple PC treatments lowered the PCP concentrations in those tissues. Such a decrease was more distinct at the higher doses of PCP. When PC (2.2 and 4.4 mmol/kg) pretreated animals were challenged with PCP (32.8 μmol/kg), a statistically significant reduction in the PCP levels, ranging from 36 to 75%, in the liver and/or kidneys was observed. At the 65.6 μmol/kg PCP dose, the decrease in the PCP concentration was noted in most of the tissues studied. There was a 78%

TABLE 4

ACUTE EFFECTS OF PC ON TISSUE CONCENTRATIONS OF PCP IN MICE

| Pretreatment | PCP (nmol/ml or g)* Serum | Brain | Liver | Kidney |
|---|---|---|---|---|
| | PCP (16.4 μmol/kg, IP) | | | |
| Control | 1.7 ± 0.08 | ND† | 9.4 ± 0.4 | 10.5 ± 1.2 |
| PC (mmol/kg) | | | | |
| 1.1 | 1.4 ± 0.05 | ND | 11.0 ± 0.4 | 9.1 ± 1.7 |
| 2.2 | 1.5 ± 0.05 | ND | 10.3 ± 2.5 | 11.8 ± 2.5 |
| 4.4 | 2.3 ± 0.09 | ND | 12.9 ± 1.3 | 15.1 ± 1.8 |
| | PCP (32.8 μmol/kg, IP) | | | |
| Control | 2.1 ± 0.15 | ND | 20.3 ± 0.6 | 17.2 ± 1.7 |
| PC (mmol/kg) | | | | |
| 1.1 | 2.4 ± 0.2 | ND | 20.3 ± 1.0 | 20.1 ± 0.4 |
| 2.2 | 2.1 ± 0.1 | ND | 20.1 ± 0.4 | 24.4 ± 1.7 |
| 4.4 | 1.9 ± 0.1 | ND | 25.6 ± 1.4 | 23.7 ± 1.6 |
| | PCP (65.6 μmol/kg, IP) | | | |
| Control | 4.4 ± 0.2 | 5.7 ± 0.9 | 38.8 ± 6.5 | 39.6 ± 2.8 |
| PC (mmol/kg) | | | | |
| 1.1 | 3.2 ± 0.2 | 8.3 ± 2.3 | 38.0 ± 2.4 | 48.1 ± 2.8 |
| 2.2 | 3.9 ± 0.3 | 6.3 ± 1.5 | 40.0 ± 2.5 | 41.1 ± 2.6 |
| 4.4 | 3.8 ± 0.2 | 5.8 ± 1.2 | 47.0 ± 4.9 | 57.3 ± 4.8 |

*Same as in Table 3.
†Not detected in the 1 ml of 20% homogenate.

reduction in the brain PCP concentration in 2.2 mmol PC group, while it was not detectable in the 4.4 mmol PC group. Under similar experimental conditions, the PB pretreatments decreased the concentrations of PCP in these tissues at all the three doses of PCP, whereas the SKF-525A pretreatment increased the tissue concentrations of PCP.

## DISCUSSION

Though the PC doses used in the present study were much higher than that a PCP addict might be exposed to during PCP smoking at one sitting [3, 6, 31], the duration of PC exposure in chronic smokers is generally longer, i.e., months or years of regular daily use [3, 6, 31], than 4 days. Consequently, the high doses of PC were used to demonstrate its potential to induce the MFO system, thereby the PCP metabolism, after a relatively short exposure period. PC was determined to induce the hepatic MFO system in mice, as it increased the metabolism of various agents including PCP and pentobarbital and the contents of microsomal cytochrome P-450. These observations were consistent with the previous findings where PC treatments reduced the pentobarbital-induced sleep, increased the liver/body weight ratio and produced the proliferation, along with the dilatation and fragmentation, of the hepatic endoplasmic reticulum in mice [26] and induced the rat hepatic microsomal cytochrome P-450 contents [8]. The induction of AHH by PC, as with MC [37,38], reflected by the biotransformation of benzo[*a*]pyrene via epoxidation, can be eventually linked to a correlation between susceptibility to the carcinogens and AHH inducibility [36, 44–46]. PC is biotransformed into reactive metabolites including the generation of PC-epoxides not only in mice [34,35] and rats [8,16], but also in humans [14,15]. Similar metabolites are capable of covalent binding to tissue macromolecules [12, 39, 47]. Therefore, it can be speculated that in the heavy chronic PCP smokers there is a potential for the induction of the MFO system, including AHH, thereby leading to the generation of reactive metabolites of PC or polynuclear hydrocarbons present in the environment, i.e., during tobacco smoking [4] which is not uncommon in PCP smokers [23,43].

The observed PB-induced decrease and the SKF-525-caused increase in the tissue levels of PCP and pentobarbital further supported the involvement of cytochrome P-450 system in the metabolism of these agents [9, 27, 30]. Though less potent than PB, PC was also effective in decreasing the tissue levels of PCP and pentobarbital. The PC-induced decrease in the tissue levels and increase in the in vitro metabolism of these agents explain the ability of the 4-day PC treatment to lower the PCP-stimulated locomotion and pentobarbital-induced sleep [26], since PCP metabolites, as is also true with pentobarbital metabolites, are considered weaker in action [9, 18, 25, 40]. The potential of PC to induce PCP metabolism and, therefore, to decrease its tissue levels might contribute, at least in part, to the development of tolerance observed in chronic smokers [3,6]. However, the possible interaction between PCP and PC at levels other than metabolic cannot be completely ruled out.

TABLE 5

EFFECTS OF 4-DAY PC PRETREATMENT ON TISSUE CONCENTRATIONS OF PCP IN MICE

| Pretreatment | PCP (nmol ml or g)[a] | | | |
|---|---|---|---|---|
| | Serum | Brain | Liver | Kidney |
| | PCP (16.4 μmol/kg, IP) | | | |
| Control | 0.94 ± 0.01 | ND[b] | 5.5 ± 0.7 | 10.4 ± 1.5 |
| PC (mmol/kg) | | | | |
| 1.1 | 0.92 ± 0.01 | ND | 5.6 ± 1.0 | 12.2 ± 1.0 |
| 2.2 | 0.76 ± 0.02 | ND | 5.4 ± 0.8 | 9.7 ± 1.0 |
| 4.4 | 0.74 ± 0.02 | ND | 3.5 ± 0.6 | 10.6 ± 0.2 |
| PB[c] | 0.53 ± 0.04‡ | ND | 2.0 ± 0.9† | 8.1 ± 0.8 |
| SKF-525A[d] | 2.8 ± 0.2‡ | 4.4 ± 0.1‡ | 13.5 ± 3.2‡ | 13.6 ± 2.5 |
| | PCP (32.8 μmol/kg, IP) | | | |
| Control | 1.6 ± 0.1 | ND | 10.7 ± 0.9 | 27.5 ± 3.8 |
| PC (mmol/kg) | | | | |
| 1.1 | 0.92 ± 0.01 | ND | 8.3 ± 1.2 | 18.9 ± 5.2 |
| 2.2 | 1.6 ± 0.01 | ND | 12.9 ± 1.2 | 17.7 ± 0.7* |
| 4.4 | 1.4 ± 0.04 | ND | 5.0 ± 0.7* | 7.0 ± 7.0‡ |
| PB | 1.9 ± 0.1 | ND | 3.5 ± 0.9‡ | 13.0 ± 2.2† |
| SKF-525A | 2.8 ± 0.1* | 11.3 ± 2.7‡ | 19.1 ± 5.1* | 39.3 ± 3.8* |
| | PCP (65.6 μmol/kg, IP) | | | |
| Control | 3.2 ± 0.1 | 4.1 ± 0.7 | 23.0 ± 1.1 | 43.0 ± 2.7 |
| PC (mmol/kg) | | | | |
| 1.1 | 2.7 ± 0.2 | 3.9 ± 1.9 | 20.4 ± 2.0 | 37.6 ± 2.1 |
| 2.2 | 1.9 ± 0.1‡ | 0.9 ± 0.5† | 21.8 ± 1.9 | 32.3 ± 1.5* |
| 4.4 | 1.3 ± 0.1‡ | ND | 8.7 ± 2.1‡ | 30.9 ± 2.0* |
| PB | 2.7 ± 0.2* | 1.5 ± 1.5* | 6.6 ± 0.9‡ | 34.0 ± 1.5* |
| SKF-525A | 6.3 ± 0.6‡ | 12.1 ± 3.3‡ | 37.4 ± 3.4* | 47.7 ± 2.9 |

[a]Same as in Table 3.

[b]Not detected in the 1 ml of 20% homogenate.

[c]0.30 mmol/kg daily for 4 days.

[d]Mice were pretreated with corn oil (10 ml/kg) daily for 4 days. Twenty-four hr after the last injection, they were challenged with SKF-525A (0.10 mmol/kg).

*$p<0.05$ vs. respective controls; †$p<0.01$ different from controls; ‡$p<0.001$ vs. controls.

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## REFERENCES

1. Ali, B.; Spencer, H. W.; Auyong, T. K.; Parmar, S. S. Selective induction of hepatic drug metabolizing enzymes by lithium treatment in dogs. J. Pharm. Pharmacol. 2:131–132; 1975.
2. Ali, B.; Kaur, S.; Kumar, A.; Bhargava, K. P. Comparative evaluation of stimulatory effects of oral tobacco and nicotine consumption on hepatic microsomal N-demethylations. Biochem. Pharmacol. 29:3087–3092; 1980.
3. Aniline, O.; Pitts, F. N. Phencyclidine (PCP): A review and perspectives. CRC Crit. Rev. Toxicol. 10:145–177; 1982.
4. Badger, G. M.; Kimber, R. W. L.; Novotny, J. The formation of aromatic hydrocarbons at high temperatures. Aust. J. Chem. 15:616–625; 1962.
5. Bailey, D. N.; Shaw, R. F.; Guba, J. J. Phencyclidine abuse: Plasma levels of clinical findings in casual users and in phencyclidine-related deaths. J. Anal. Toxicol. 2:233–237; 1978.

6. Burns, R. S.; Lerner, S. E. Phencyclidine deaths. J. Am. Coll. Emerg. Physicians 7:135–141; 1978.
7. Chakrabarti, S.; Song, Y. Y.; Law, F. C. P. Metabolic disposition of l-phenylcyclohexene in rats. Fed. Proc. 41:1714; 1982.
8. Chakrabarti, S.; Law, F. C. P. Effects of N-ethyl-l-phenylcyclohexylamine (PCE) and l-phenylcyclohexene (PC) on hepatic drug metabolism in rats. IRCS Med. Sci. 10:572; 1982.
9. Chaturvedi, A. K.; Rao, N. G. S.; Berg, I. E. The role of hepatic microsomal enzymes in the modulation of phencyclidine-induced toxicity. Toxicology 22:245–254; 1981.
10. Chaturvedi, A. K. Effects of mecamylamine, nicotine, atropine and physostigmine on the phencyclidine-induced behavioral toxicity. Pharmacol. Biochem. Behav. 20:559–566; 1984.
11. Chaturvedi, A. K.; Rao, N. G. S. Influence of phencyclidine on the translocation of pentobarbital in mice. Toxicology 30:149–155; 1984.
12. Chaturvedi, A. K. Metabolism, metabolites and toxicity of poisons: "Metabolites." *A Prime Lecture in the International Conference on Forensic Sciences*, Madras, India, December 12–16, 1985.
13. Chen, G.; Ensor, C. R.; Russell, D.; Bohner, B. The pharmacology of l-(l-phenylcyclohexyl)-piperidine·HCl. J. Pharmacol. Exp. Ther. 127:241–250; 1959.
14. Cook, C. E.; Brine, D. R.; Quin, G. D.; Perez-Reyes, M.; Di Guiseppi, S. R. Phencyclidine and phenylcyclohexene disposition after smoking phencyclidine. Clin. Pharmacol. Ther. 31:635–641; 1982.
15. Cook, C. E.; Perez-Reyes, M.; Jeffcoat, A. R.; Brine, D. R. Phencyclidine disposition in humans after small doses of radiolabeled drug. Fed. Proc. 42:2566–2569; 1983.
16. Cook, C. E.; Brine, D. R.; Tallent, C. R. Identification of *in vitro* rat metabolites of l-phenylcyclohexene. Drug Metab. Dis. 12:186–192; 1984.
17. Dehnen, W.; Tomingas, R.; Roos, J. A modified method for the assay of benzo[*a*]pyrene hydroxylase. Anal. Biochem. 53:373–383; 1973.
18. Domino, E. F. Neurobiology of phencyclidine—An update. In: Petersen, R. C.; Stillman, R. C., eds. Phencyclidine (PCP) abuse: An appraisal. Rockville, MD: National Institute on Drug Abuse, Research Monograph 21; 1978:18–43.
19. Foltz, R. L.; Fentiman, A. F.; Foltz, R. B. GC/MS assays for abused drugs in body fluids. Rockville, MD: National Institute on Drug Abuse, Research Monograph 32; 1980:25–38.
20. Freeman, A. S.; Martin, B. R. Quantification of phencyclidine in mainstream smoke and identification of phenylcyclohex-l-ene as pyrolysis product. J. Pharm. Sci. 70:1002–1004; 1981.
21. Goldstein, A. Biostatistics. New York: The Macmillan Company; 1964.
22. Gwilt, J. R.; Roberton, A.; McChesney, E. W. Determination of blood and other tissue concentrations of paracetamol in dog and man. J. Pharm. Pharmacol. 15:440–444; 1963.
23. Henderson, G. Phencyclidine, a widely abused but little understood psychotomimetic agent. Trends Pharmacol. Sci. 3:248–250; 1982.
24. Holsapple, M. P.; Munson, A. E.; Freeman, A. S.; Martin, B. R. Pharmacological activity and toxicity of phencyclidine (PCP) and phenylcyclohexene (PC), a pyrolysis product. Life Sci. 3:803–813; 1982.
25. Holsztynska, E. J.; Domino, E. F. Overview of phencyclidine biotransformation. In: Kamenka, J. M.; Domino, E. F.; Geneste, P., eds. Phencyclidine and related arylcyclohexylamines: Present and future applications. Ann Arbor, MI: NPP Books; 1983:157–194.
26. Hu, C. Y.; Choudhuri, M. S. K.; Berg, I. E.; Rao, N. G. S.; Chaturvedi, A. K. Toxicity of l-phenylcyclohexene and its interaction with phencyclidine. Toxicol. Appl. Pharmacol. 76:403–413; 1984.
27. Kammerer, R. C.; Schmitz, D. A.; Hwa, J. J.; Cho, A. K. Induction of phencyclidine metabolism by phencyclidine, ketamine, ethanol, phenobarbital and isosafrole. Biochem. Pharmacol. 33:599–604; 1984.
28. Kato, R.; Gillette, J. R. Effect of starvation of NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. Exp. Ther. 150:279–284; 1965.
29. Kaur, S.; Ali, B. Stimulation of drug and carcinogen metabolism by prolonged oral tobacco consumption. Biochem. Pharmacol. 31:1595–1597; 1982
30. LaDu, B. N.; Mandal, H. G.; Way, E. L. Fundamentals of drug metabolism and drug disposition. Baltimore, MD: The Williams and Wilkins Co.; 1971:3–143.
31. Lerner, S. E.; Burns, R. S. Phencyclidine use among youth: History, epidemiology, and acute and chronic intoxication. In: Petersen, R. C.; Stillman, R. C., eds. Phencyclidine (PCP) abuse: An appraisal. Rockville, MD: National Institute on Drug Abuse, Research Mongraph 21; 1978:66–118.
32. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
33. Martin, B. R.; Vincek, W. C.; Balster, R. L. Studies on the disposition of phencyclidine in mice. Drug Metab. Dis. 8:49–54; 1980.
34. Martin, B. R.; Bailey, B. B.; Awaya, H.; May, E. L.; Narasimhachari, N. *In vitro* metabolism of l-phenyl-l-cyclohexene, a pyrolysis product of phencyclidine. Drug Metab. Dis. 10:685–689; 1982.
35. Martin, B. R.; Freeman, A. S. Disposition of phencyclidine and its pyrolytic products in mice exposed to smoke. Fed. Proc. 42:2561–2565; 1983.
36. Nebert, D. W.; Atlas, S. A.; Guenthner, T. M.; Kouri, R. E. The *Ah* locus: Genetic regulaton of the enzymes which metabolize polycyclic hydrocarbons and the risk for cancer. In: Gelboin, H. V.; Ts'o, P. O. P., eds. Polycyclic hydrocarbons and cancer: Molecular and cell biology. vol. 2. New York: Academic Press; 1978:345–390.
37. Nebert, D. W.; Eisen, H. J.; Negishi, M.; Lang, M. A.; Hjelmeland, L. M. Genetic mechanisms controlling the induction of polysubstrate monoxygenase (P-450) activities. Annu. Rev. Pharmacol. Toxicol. 21:431–462; 1981.
38. Negishi, M.; Nebert, D. W. Structural gene products of the *Ah* locus: Genetic and immunochemical evidence for two forms of mouse liver cytochrome P-450 induced by 3-methylcholanthrene. J. Biol. Chem. 254:11015–11023; 1979.
39. Nelson, S. D. Metabolic activation and drug toxicity. J. Med. Chem. 25:753–765; 1982.
40. Ober, R. E.; Gwynn, G. W.; Chang, T.; McCarthy, D. A.; Glazko, A. J. Metabolism of l-(l-phenylcyclohexyl)piperidine (Sernyl®). Fed. Proc. 22:539; 1963.
41. Omura, T.; Sato, R. The carbon monoxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. J. Biol. Chem. 239:2370–2378; 1964.
42. Petersen, R. C.; Stillman, R. C. Phencyclidine: An overview. In: Petersen, R. C.; Stillman, R. C., eds. Phencyclidine (PCP) abuse: An appraisal. Rockville, MD: National Institute on Drug Abuse, Research Monograph 21; 1978:1–17.
43. Vadlamani, N. L.; Pontani, R. B.; Misra, A. L. Effect of chronic nicotine pretreatment on phencyclidine (PCP) disposition in the rat. Arch. Int. Pharmacodyn. 265:4–12; 1983.
44. Watanabe, M.; Watanabe, K.; Konno, K.; Sato, H. Genetic differences in the induction of aryl hydrocarbon hydroxylase and benzo[*a*]pyrene carcinogenesis of C3H/He and DBA/2 strains of mice. Jpn. J. Cancer Res. 66:217–226; 1975.
45. Watanabe, M.; Konno, K.; Sato, H. Properties of aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase in lung microsomes in mice. Jpn. J. Cancer Res. 69:1–8; 1978.
46. Watanabe, M.; Watanabe, K.; Sato, H. Effect of trichloropropene oxide and benzoflavone on polycyclic hydrocarbon carcinogenesis in C3H/He and DBA/2 mice. Jpn. J. Cancer Res. 70:83–87; 1979.
47. Wills, E. D. The role of glutathione in drug metabolism and protection of the liver against toxic metabolites. In: Garrod, J. W., ed. Testing for toxicity. London, U.K.: Taylor and Francis Ltd.; 1981:95–109.