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# Suppression of the Constitutive, Male-Specific Rat Hepatic Cytochrome P-450 2c and Its mRNA by 3,4,5,3′,4′,5′-Hexachlorobiphenyl and 3-Methylcholanthrene

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## SUMMARY

Rat liver cytochrome P-450 2c (P-450 2c) is a constitutive, male-specific enzyme that oxidatively metabolizes both steroid hormones and liphophilic foreign compounds. Exposure of adult male rats to certain xenobiotics can lead to a decrease in the expression of hepatic P-450 2c. The present studies were undertaken to examine the mechanism of this decrease. Treatment of adult male rats with 3-methylcholanthrene (3-MC) or 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB) (two compounds known to induce P-450c and P-450d as a consequence of their interaction with the aromatic hydrocarbon receptor) decreased hepatic content of P-450 2c and its associated steroid hormone 2α- and 16α-hydroxylase activities. Moreover, the present studies demonstrate that decreases in hepatic content of P-450 2c mRNA (determined by electrophoretic analysis of immunoprecipitated translational products) fully account for the effects of 3-MC and HCB on P-450 2c. HCB (50 mg/kg) produced the most striking decrease in P-450 2c and its mRNA, virtually eliminating their expression in hepatic tissue. The time course and dose-response for the decrease in P-450 2c and its mRNA differed markedly from that for induction of P-450c, indicating that the effects of HCB on the two proteins may involve different mechanisms. Additional experiments demonstrated that the sex difference in hepatic expression of P-450 2c is the result of a >5-fold higher content of P-450 2c mRNA in male as compared to female rats. HCB decreased serum testosterone levels by >98% at 5 days in adult male rats. However, the decrease in P-450 2c and serum testosterone levels was not accompanied by an increase in serum estradiol levels or induction of the female-specific enzyme P-450 2d. The present findings clearly establish that the decrease in P-450 2c produced by administration of HCB and 3-MC is the result of a decrease in the hepatic content of P-450 2c mRNA. These xenobiotics may decrease transcription of the mRNA for P-450 2c as a consequence of binding to the aromatic hydrocarbon receptor, or, alternatively, may interfere with the hormonal regulation of the mRNA for this male-specific P-450 enzyme.

P-450, the terminal monooxygenase of the microsomal mixed-function oxidase system, exists as a family of closely related enzymes with various specificities (1). These enzymes catalyze the oxidative metabolism of a wide variety of endogenous substrates such as steroids, fatty acids, and prostaglandins, and exogenous compounds including drugs and environmental chemicals (2, 3). P-450 2c[1] [also termed P-450h (4), P-450 UT-A (5), or P-450 RLM5 (6)] is a male-specific constitutive P-450 enzyme that accounts for most (>85%) of the testosterone and androstenedione 16α-hydroxylase activity catalyzed by liver microsomes isolated from uninduced or polycyclic hydrocarbon-induced adult male rats (7). In addition, P-450 2c catalyzes most of the hepatic microsomal testosterone 2α-hydroxylase activity (7). P-450 2c is also a major catalyst of 17β-estradiol 2- and 4-hydroxylation in liver microsomes isolated from adult male rats (8). Expression of this P-450 enzyme has been shown to be "imprinted" or programmed for induction at puberty in male rats by neonatal exposure to androgens (9–11).

Recent studies have indicated that some constitutive P-450s can be decreased by administration of well-characterized monooxygenase inducers to animals. For example, Aroclor 1254 produced a 65% decrease in P-450 2d-dependent androstanediol disulfate 15β-hydroxylase activity in liver microsomes from

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[1] The nomenclature used for individual forms of P-450 is detailed under Materials and Methods.

**ABBREVIATIONS:** P-450, cytochrome P-450; T, 17β-hydroxyandrost-4-ene-3-one (testosterone); A, androst-4-ene-3,17-dione (androstenedione); HCB, 3,4,5,3′,4′,5′-hexachlorobiphenyl; 3-MC, 3-methylcholanthrene; *Ah*, aromatic hydrocarbon; RIA, radioimmunoassay, IP, intraperitoneal.

adult female rats (12). Significant decreases in liver microsomal P-450 2c (11, 13) and its associated steroid hormone 2$\alpha$- and 16$\alpha$-hydroxylase activities (7) have also been observed in male rats following administration of various classical P-450 inducers, including 3-MC, several polybrominated biphenyl congeners, and phenobarbital. An inverse correlation was noted between the decrease in P-450 2c and the induction of P-450c and P-450d (two 3-MC-inducible P-450 enzymes) (13). The decreases in P-450 2c could result from an increase in degradation of the protein, due, perhaps, to competition between constitutive and inducible apocytochromes for available heme. Alternatively, the decrease in P-450 2c could reflect a decrease in the rate of synthesis of this protein.

The current studies were undertaken to determine whether the suppression of P-450 2c by certain xenobiotics is the result of a decrease in the mRNA for this form of P-450 or whether P-450 2c is decreased by other mechanisms. The present studies examine the effects of 3-MC and HCB on P-450 2c, on P-450 2c-associated microsomal steroid hydroxylase activities, and on the translatable mRNA for P-450 2c in livers of adult male rats. The time course and dose-response for the effects of HCB on P-450 2c and its mRNA are compared to those of HCB on P-450c and its mRNA (14). The present results demonstrate that decreases in hepatic content of P-450 2c mRNA can fully account for the decreases in P-450 2c and its associated steroid hydroxylase activities after administration of HCB and 3-MC.

## Materials and Methods

**Treatment of rats.** Adult male Sprague-Dawley (CD) rats (6–8 weeks of age) (Charles River Laboratories) were given a single IP dose of 3, 10, or 50 mg/kg HCB in corn oil and were killed by decapitation 4, 8, 17, 24 hr or 2, 3, or 7 days later. PB (80 mg/kg) or 3-MC (50 mg/kg) was administered IP as a single dose and rats were sacrificed 17 hr later, or it was given in three daily doses, and rats were sacrificed 24 hr after the last dose. Control rats received corn oil alone. Microsomes were prepared as described previously (15). Blood was collected for serum testosterone assays 2, 5, or 7 days after a single dose of HCB and 1, 3, or 5 days after daily administration of 3-MC or phenobarbital.

**Purified cytochromes and antibodies.** Cytochromes P-450b, c, and d were purified as previously described (15). Antibody to P-450c was obtained from rabbits and immunopurified as described previously (16). Cytochromes P-450 2c, 2d, and PB-1 were purified as described, and antibody to P-450 2c was prepared in rabbits (7, 17). Cytochrome P-450g was purified by the method described by Ryan *et al.* (4). P-450 UT-H, purified in this laboratory,[2] was identical on Western blots to authentic P-450 UT-H generously provided by Dr. F. P. Guengerich, Vanderbilt University (18).

The nomenclature for equivalent forms of rat hepatic P-450 purified by various investigators has been detailed previously (19) and can be summarized as follows: P-450 PB-1 (17) = P-450 PB-C (5); P-450 2c (7) = P-450h (4), P-450 male (20), P-450 UT-A (5), and P-450 RLM5 (6); P-450 2d (7) = P-450i (12), P-450 female (21), and P-450 UT-I (11). In this study, P-450b [P-450 PB-4; (22)], P-450c, and P-450d are classified according to the terminology of Ryan *et al.* (23).

**Western blots.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.6% acrylamide) was performed by the method described by Laemmli (24). The proteins were transferred to nitrocellulose sheets by the method of Towbin *et al.* (25). The sheets were immunostained for P-450 2c as outlined previously for P-450s b, c, and d (26).

**Cell-free translation of polysomes.** Hepatic content of the mRNA for P-450 2c was estimated by translation of polysomes followed by immunoprecipitation with antibody to P-450 2c and electrophoresis of the translational products. Total liver polysomes were isolated (14) as previously described and translated in the presence of [$^{35}$S]methionine using a cell-free reticulocyte lysate system by a procedure similar to that described previously for P-450c mRNA (26). Translational products were precipitated with antibody to P-450 2c followed by 100 $\mu$l of *Staphylococcus aureus* cells (10% suspension) and the immunoprecipitates analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Incorporation of radioactivity into a band equivalent in electrophoretic mobility to purified P-450 2c was quantified following electrophoresis and autoradiography of the immunoprecipitates. The bands were excised, incubated with 95% $H_2O_2$ (30%) and 5% $NH_4OH$ overnight, neutralized with glacial acetic acid, and counted. A duplicate area was cut from samples subjected to electrophoresis to which preimmune immunoglobulin G had been added instead of antibody to P-450 2c. The radioactivity in these slices was subtracted from that of the actual samples. Values were expressed as percent of total translation (disintegrations per minute in the corrected specific polypeptide band divided by total trichloroacetic acid-precipitable counts × 100). The amount of antibody required to precipitate the maximum radioactivity in a polypeptide equivalent in electrophoretic mobility to purified P-

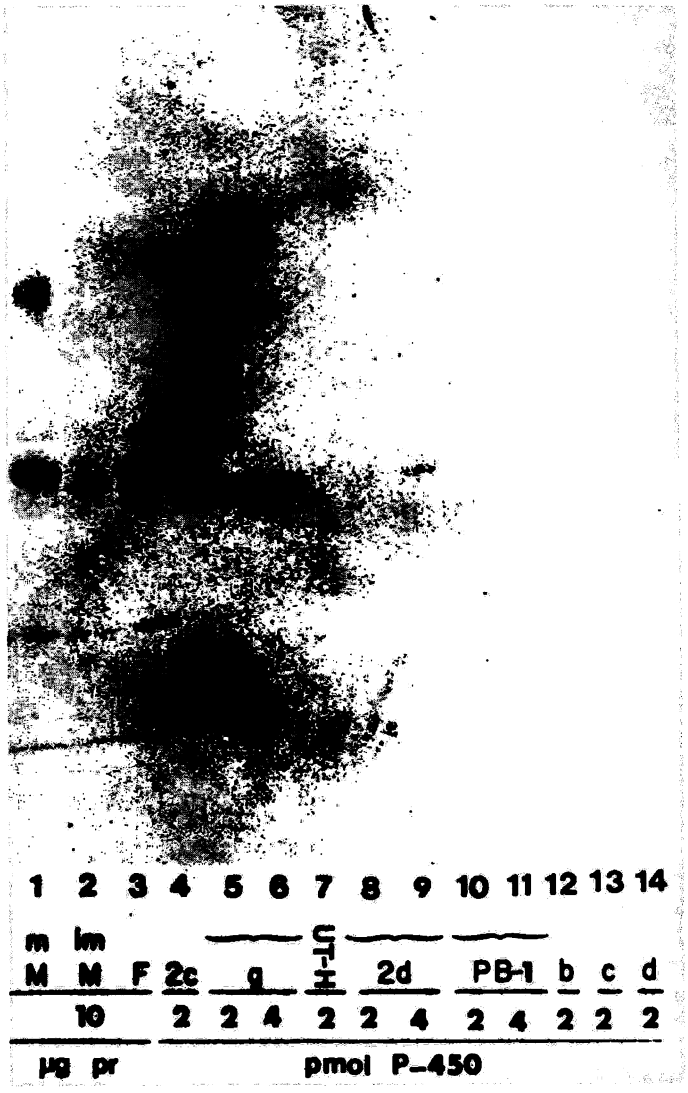


**Fig. 1.** A Western blot demonstrating the specificity of antibody to P-450 2c. The following lanes represent: hepatic microsomes isolated from mature and immature male (*lanes 1 and 2*) and mature female rats (*lane 3*); purified P-450 2c (*lane 4*) and other purified P-450s as indicated (*lanes 5–14*). The *numbers below each lane* indicate the amount of microsomal protein or pmol purified P-450 applied to each well.

[2] P. McClellan-Green, manuscript in preparation.

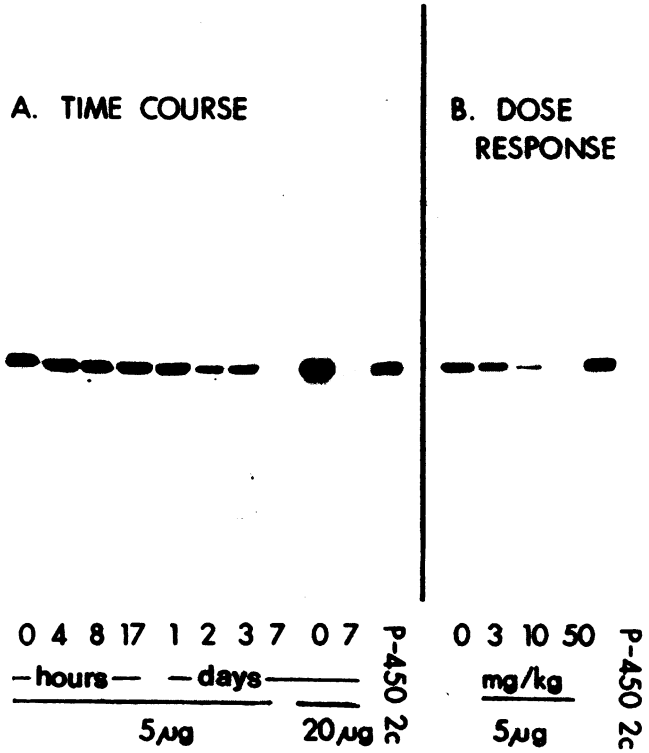


**Fig. 2.** Western blot analysis of P-450 2c suppression upon administration of HCB. (*panel A*) Time-course experiment in which animals were sacrificed at various times following HCB administration and (*panel B*) dose-response experiment at 7 days (3, 10, and 50 mg/kg). Microsomes or purified P-450 2c were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Western blotting. The *numbers below the gel* indicate the amount of microsomal protein in each well.

450 2c was determined in preliminary studies, and this amount of anti-P-450 2c was used in all subsequent experiments.

**Catalytic assays.** Androstenedione and testosterone hydroxylase (7) and 7-ethoxycoumarin-*O*-deethylase activities (17) were measured in liver microsomal samples as described previously.

**RIA for P-450 2c.** Cytochrome P-450c content of microsomes was assayed by RIA (16). P-450 2c in some microsomal samples was analyzed by a $^{125}$I-protein A dot-blot immunoassay similar to that described by Jahn *et al.* (27). Each microsomal sample was analyzed for P-450 2c in duplicate at three concentrations in the linear range and quantified with a standard curve for purified P-450 2c (0.1–0.8 pmol). Cytochrome P-450 2c was radioiodinated using Bolton-Hunter reagent (25 $\mu$Ci/$\mu$g), and microsomal P-450 2c content was also estimated by RIA in a manner similar to that described for P-450c and P-450d (16). Since the RIA for P-450 2c was relatively insensitive (limit of detection ≥3 pmol), requiring excessive utilization of pure antigen for preparation of standard curves, standard curves were routinely prepared with solubilized microsomes from an uninduced male rat, and values were expressed as percent of control. Inhibition of binding was linear when $B/B_0$ was plotted versus the log of the concentration of total microsomal cytochrome P-450 between 10 and 100 pmol. $B/B_0$ represents the amount of radioactivity precipitated in the sample ($B$), divided by that precipitated in tubes containing no nonradioactive P-450 2c ($B_0$). Initial RIA analysis of liver microsomes from adult male rats using a standard curve of purified P-450 2c indicated that P-450 2c comprised approximately 10–20% of the total P-450.

**Specificity of antibody to P-450 2c.** The cross-reactivity of the antibody to P-450 2c used in the present study toward a number of other purified constitutive and xenobiotic-inducible P-450 enzymes was evaluated by Western blotting (Fig. 1). Only faint cross-reactivity to purified P-450g, PB-1, b, and 2d was observed, compared to the reaction with purified P-450 2c. Anti-P-450 2c did not cross-react detectably with the inducible P-450s c and d or with the constitutive P-450 UT-H.

In addition, anti-P-450 2c detected one major band in immunoblots of adult male rat liver microsomes with an electrophoretic mobility identical to that of purified P-450 2c (Fig. 1). Only a faint band with this mobility was observed in liver microsomes from either immature male or adult female rats, consistent with our previous data indicating that P-450 2c is male-specific and developmentally induced at puberty (7). This band could be due to P-450 PB-1 (17) or P-450f (28), both of which have similar mobilities to P-450 2c. A second fainter band was observed in liver microsomes from immature male and adult female rats which co-migrated with purified P-450 2d, consistent with the weak cross-reactivity of the antibody to P-450 2d, an enzyme found in livers of female and immature male rats (11, 29). Although some cross-reactivity of anti-P-450 2c toward pure P-450g was observed in Fig. 1, anti-P-450 2c showed only very weak cross-reactivity toward a polypeptide with the mobility of P-450g (a male-specific enzyme) on immunoblots of liver microsomes from adult male rats known to contain P-450g (Fig. 2). Quantification of P-450 2c by RIA also indicated that liver microsomes from immature male rats contain <10% of that present in adult males (data not shown). All of these data indicate that the cross-reactivity of the preparation of anti-P-450 2c used in the present study with other P-450s present in adult female and immature male rats (P-450f, P-450 UT-H, P-450 PB-1, and P-450 2d), although detectable, is relatively low (<10–20% of the total immunoreactivity).

**Other methods.** Protein was determined by the method of Lowry *et al.* (30). Total cytochrome P-450 was determined by the method of Omura and Sato (31). Serum testosterone and estradiol were measured with commercially available kits (Diagnostic Products Corporation, Los Angeles, CA, and Serono RIA, Braintree, MA).

## Results

**Effects of HCB on liver microsomal steroid hydroxylases.** HCB (50 mg/kg, single dose) decreased P-450 2c-mediated microsomal androstenedione-16$\alpha$-hydroxylase activity by approximately 50% at 2 days, and essentially abolished this catalytic activity by 7 days (Table 1). Administration of a lower dose of HCB (10 mg/kg) resulted in a more modest decrease in 16$\alpha$-hydroxylase activity, but the time course for the decrease was similar to that observed at the higher dose. In contrast, 7-ethoxycoumarin-*O*-deethylase activity [principally

TABLE 1

**Effects of HCB on rat liver microsomal monooxygenase activity: time course and dose response**

Hepatic microsomes were isolated from adult male rats at various time points after administration of a single dose of HCB at either 50 mg/kg (a) or 10 mg/kg (b) and then were assayed for P-450-dependent monooxygenation of androstenedione (A) or 7-ethoxycoumarin, as described in Methods. Values represent mean catalytic activities for four individual rats (±SD) for the indicated metabolites.

| Dose (HCB) | Hydroxylation product | Time after administration of HCB | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 17 hr | 1 day | 2 days | 3 days | 7 days |
| *mg/kg* | | *nmol/mg/10 min* | | | | | |
| (a) 50 | 16 $\alpha$OH-A | 10.6 ± 2.2 | 9.9 ± 3.6 | 9.4 ± 2.2 | 4.7 ± 0.9 | 3.7 ± 1.6 | 0.4 ± 0.1 |
| (b) 10 | 16 $\alpha$OH-A | 11.4 ± 1.8 | 14.1 ± 6.3 | 6.0 ± 2.0 | | 6.3 ± 5.3 | 4.1 ± 0.7 |
| | 7-OH Coumarin | 5.0 ± 0.6 | 38.1 ± 4.3 | 41.0 ± 9.6 | | 61.4 ± 19.9 | 92.5 ± 12.7 |

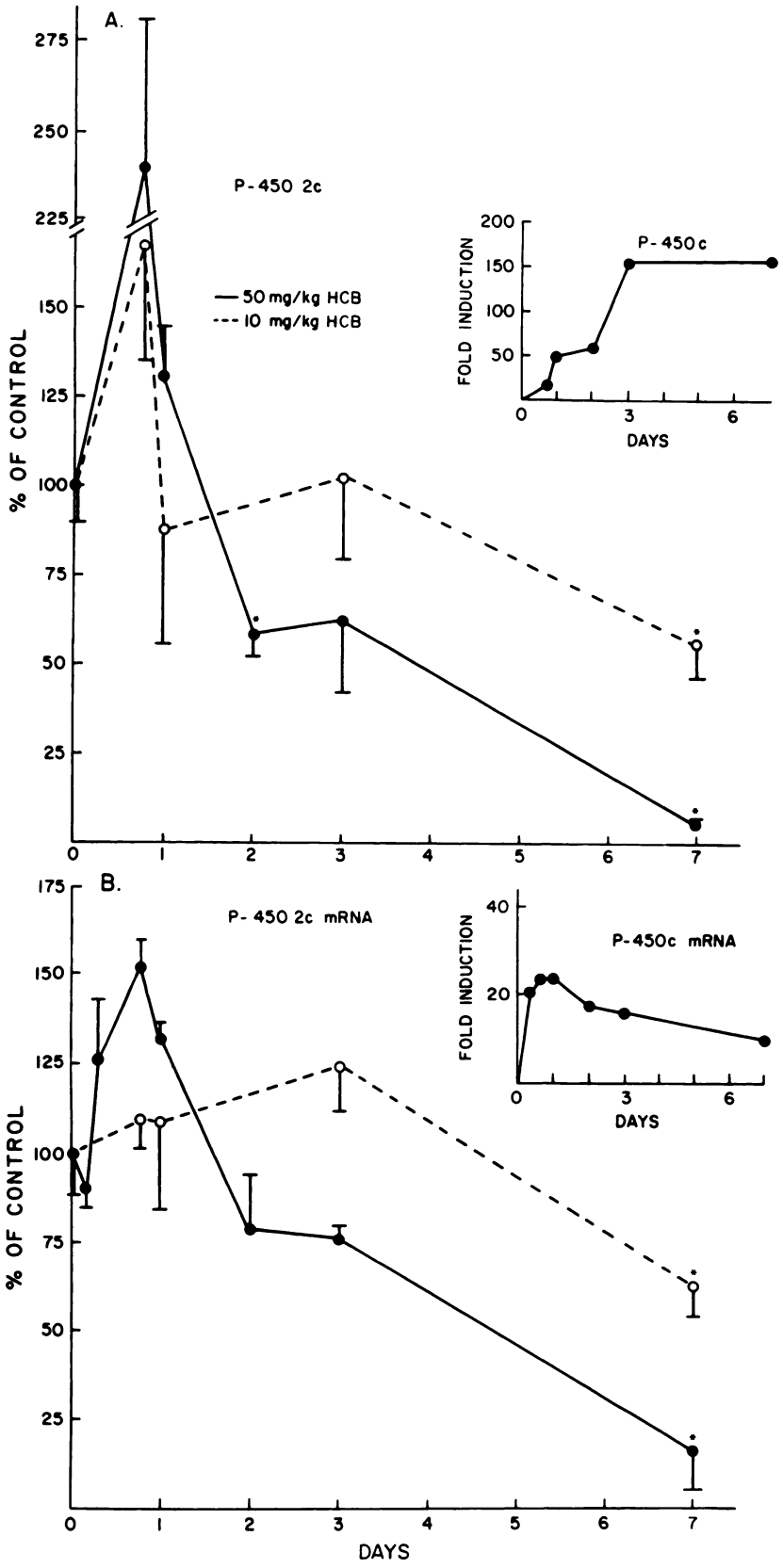


**Fig. 3.** Levels of P-450 2c (*panel A*) and P-450 2c mRNA (*panel B*) at various times after administration of HCB at 50 mg/kg and 10 mg/kg to adult male rats. *Panel A.* Suppression of P-450 2c in hepatic microsomes and (*inset*) the contrasting induction of P-450c as determined by RIA. Values represent means (± SE) of four individual rats (plotted as percent of control). The apparent increase in P-450 2c at 17 hr (note break in scale) was not statistically significant, due to large interanimal variability at this time point. *Panel B.* P-450 2c mRNA and P-450c mRNA were measured by translation in a reticulocyte lysate system followed by immunoprecipitation, electrophoresis of the immunoprecipitates, and determination of the radioactivity incorporated into a polypeptide band corresponding in $M_r$ to that of the isozyme. Percentage translation represents the dpm in the specific polypeptide band minus background radioactivity divided by the total trichloroacetic acid-precipitable counts. Results are expressed as percent of control (average value of control is 0.06 ± 0.01% of total translation for 2c mRNA) and represent the mean ± SD of duplicate determinations on three individual animals. *Significance $p < 0.05$.

mediated by P-450c in HCB-induced microsomes (32)] was increased maximally (~20-fold) 3–7 days after administration of 10 mg/kg HCB.

**Effects of HCB on P-450 2c.** Western blot analysis of hepatic microsomes isolated from male rats at various times after administration of HCB (50 mg/kg) verified a gradual decrease in P-450 2c (Fig. 2A). The polypeptide band with a mobility identical to this constitutive cytochrome was virtually abolished by 7 days. Smaller decreases in P-450 2c were seen at doses of 3 and 10 mg/kg at 7 days (Fig. 2B). Quantification

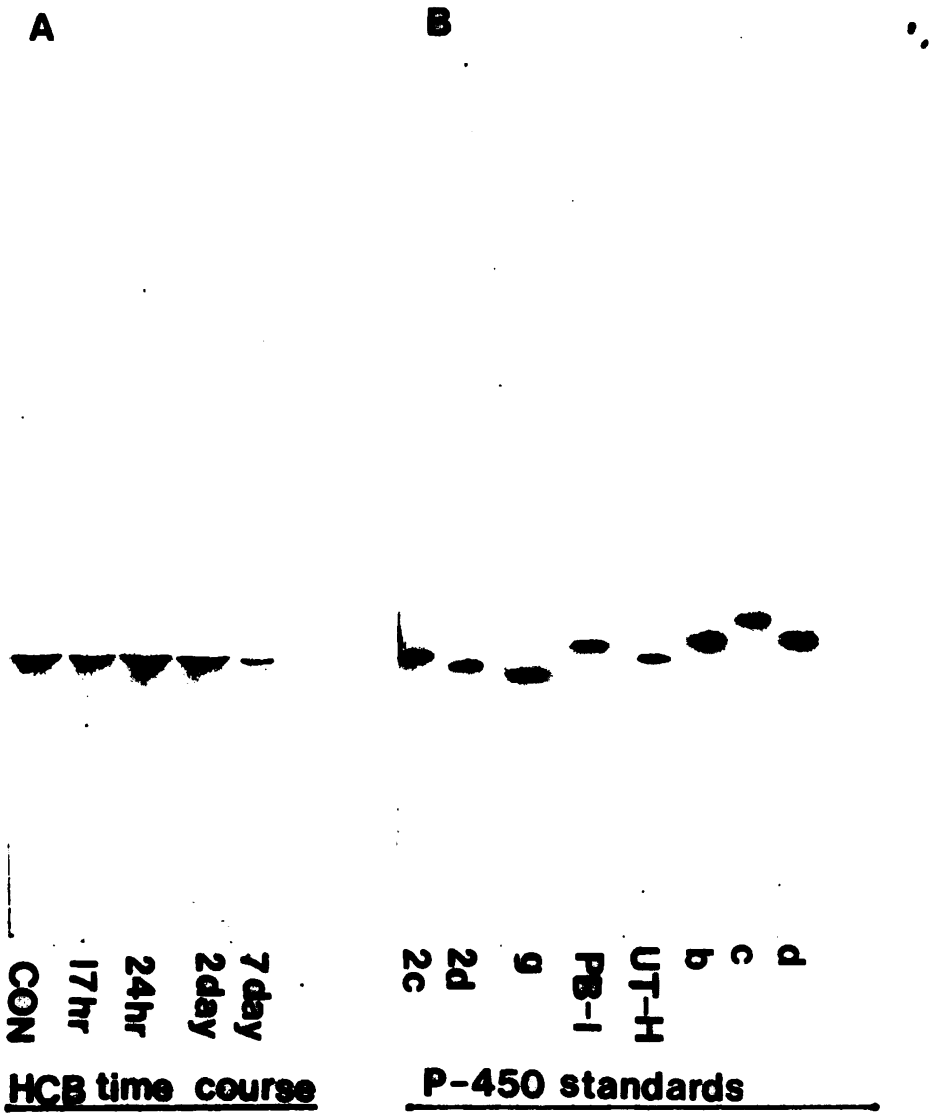


**Fig. 4.** *A.* Autoradiograph of sodium dodecyl sulfate gel of immunoprecipitates of translation products of liver polysomes from male rats treated with 50 mg/kg HCB at representative time points (17, 24 hr, and 2, 7 days). *B.* a sodium dodecyl sulfate gel showing the mobilities of purified P-450s. *A.* aliquots of approximately $2 \times 10^6$ dpm from each translation were immunoprecipitated with anti-P-450 2c, and a similar volume of each sample was subjected to electrophoresis and autoradiographed. *B.* purified P-450 standards (2 pmol/well), electrophoresed on the same gel shown in *panel A*, then stained with Coomassie blue, demonstrating the similarity of the electrophoretic mobilities of purified P-450 2c and the major protein immunoprecipitated by anti-P-450 2c.

of liver microsomal P-450 2c by RIA (Fig. 3A) indicated a moderate increase in P-450 2c 17 hr after administration of 50 mg/kg HCB, which was reproducible but not statistically significant, followed by a 40% decrease in this enzyme at 2 days, and a 90% decrease at 7 days. In contrast, HCB induced P-450c 150-fold by 3 days, and this P-450 enzyme remained elevated for at least 7 days (Fig. 3A, *inset*).

**Effects of HCB on P-450 2c mRNA.** Only one radiolabeled electrophoretic band was observed in autoradiographs of translational products of polysomes from adult male rats precipitated with anti-P-450 2c (Fig. 4A). This band had an electrophoretic mobility identical to that of authentic P-450 2c (Fig. 4B). The amount of translatable mRNA for P-450 2c was estimated to be at least 5 times higher in livers of adult male rats (0.06 ± 0.01% of the total translational products) than in adult female rats (0.01 ± 0.002%), indicating that the male specificity of this enzyme reflects sex differences in hepatic mRNA content.

HCB produced a 50% increase in P-450 2c mRNA at 17 hr, followed by a 20% decrease by 2 days and a 90% decrease at 7 days (Fig. 3B). These changes paralleled the decreases in the concentration of the isozyme (Fig. 3A). The decrease in P-450 2c mRNA was relatively specific, insofar as HCB did not decrease total translatable mRNA at any time point. Moreover, HCB increased P-450c mRNA maximally by 24 hr (>20-fold), and this mRNA remained elevated (10-fold) at 7 days (Fig. 3B). Doses of 3, 10, and 50 mg/kg HCB produced parallel decreases in P-450 2c, its associated steroid hydroxylase activities, and P-450 2c mRNA (Fig. 5). The dose of HCB (50 mg/kg) required to produce maximum suppression of P-450 2c was somewhat higher than the dose required to produce maximum induction of P-450c (10 mg/kg) (data not shown).

**Response of P-450 2c and its mRNA to 3-MC and phenobarbital.** 3-MC, another compound known to induce synthesis of P-450c, also decreased liver microsomal P-450 2c content and 16$\alpha$-steroid hydroxylase activity significantly, with a 55–60% decrease observed following daily administration of 3-MC for 3 days (Fig. 6). These decreases were preceded by a 65% decrease in P-450 2c mRNA 17 hr after a single dose of 3-MC. The induction of P-450c mRNA also precedes the induction of the protein in a similar manner (14). In contrast to 3-MC, phenobarbital (an inducer of P-450b and P-450e) de-

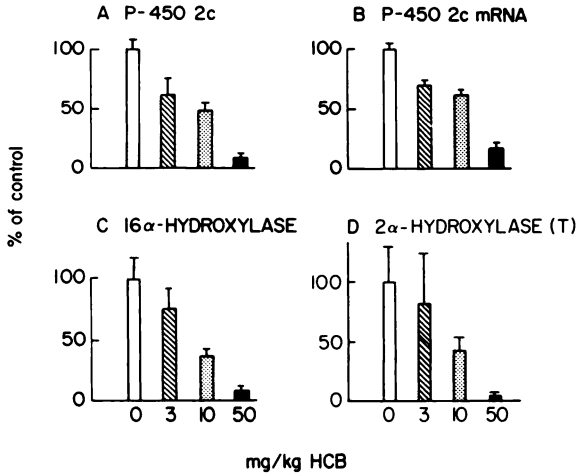


**Fig. 5.** Dose-response for levels of P-450 2c, its mRNA, and its associated steroid hydroxylase activities in livers isolated from adult male rats 7 days after administration of HCB at 3, 10, and 50 mg/kg. *Panel A.* P-450 2c levels were determined by RIA. Results are plotted as percent of control. Values represent the mean ± SD of duplicate determinations on four individual animals. Panel B. P-450 2c mRNA was measured as described in the legend to Fig. 3. Results are plotted as percent of control. Data at each point represent the mean ± SD of duplicate determinations on three individual animals. The catalytic activities toward androstenedione (A)-16α-OH (*C*), and testosterone (T)-2α-OH (*D*) (see Methods) are plotted as percent of control (control levels for A-16α- and T-2α-hydroxylase were 1.1 ± 0.2 and 1.1 ± 0.3 nmol/min/mg microsomal protein, respectively).

creased P-450 2c mRNA by ~40% 17 hr after a single dose, but it had less effect on the microsomal P-450 2c content (8–14% decrease 17 hr after a single dose or after three daily doses) (data not shown).

**Effects of HCB on P-450 2d and serum testosterone levels.** P-450 2c is a male-specific cytochrome that is believed to be regulated primarily by the pattern of growth hormone secretion (33). Therefore, we attempted to determine whether the suppression of P-450 2c by HCB might reflect a general demasculinization of the hepatic enzymes of steroid metabolism. Western blot analysis of hepatic microsomes isolated from male rats 7 days after administration of 3, 10, or 50 mg/kg HCB using antibody to P-450 2d (a female-specific P-450 enzyme) demonstrated that P-450 2d was not induced by HCB (data not shown). Moreover, HCB did not markedly increase the activity of microsomal androstenedione 5α-reductase (control activity 1.3 ± 0.6 nmol/min/mg versus 2.2 ± 0.9 nmol/min/mg 7 days after HCB (50 mg/kg)), an enzyme expressed at approximately 10- to 20-fold higher levels in liver microsomes of female rats than in those of male rats (9, 11). Serum estradiol levels in adult male rats (26.6 ± 3.7 pg/ml) were not increased by HCB (50 mg/kg) at 7 days (17.1 ± 3.1 pg/ml). However, HCB at 50 mg/kg dramatically decreased serum testosterone levels (>98%) (Fig. 7). This decrease was evident by 5 days. Lower doses of HCB, although effective in suppressing P-450 2c (Fig. 5), had no significant effect on serum testosterone levels. Administration of 3-MC produced a more modest but significant decrease in serum testosterone levels after 5 days continuous dosing (Fig. 7C), but phenobarbital had no effect (not shown).

## Discussion

The present studies examine the effects of 3-MC and HCB, two well-known inducers of cytochromes P-450c and P-450d (16), on P-450 2c, a constitutive, male-specific rat liver P-450 enzyme which is the major catalyst of steroid hormone 2α- and 16α-hydroxylase activities in liver microsomes from uninduced adult male rats (7). HCB produced a sustained decrease in both hepatic microsomal content of P-450 2c and its associated androstenedione 16α-hydroxylase activity, virtually abolishing expression of this enzyme by 7 days. This sustained depression of P-450 2c probably reflects the half-life of HCB in the rat. P-450 2c and its associated steroid hydroxylase activities were also decreased by 3-MC, although the decreases were not as extensive as those produced by HCB. The decreases in P-450 2c content after administration of either 3-MC or HCB could be attributed to parallel decreases in the hepatic content of the translatable mRNA for P-450 2c. Thus, the suppression of this isozyme by these and presumably other P-450 inducers (7, 11, 13) is the result of a decrease in synthesis of this isozyme, rather than competition between apocytochrome P-450 2c and inducible P-450 apoenzymes for available heme. Since hepatic P-450 2c mRNA content was estimated by translation of liver polysomes in an *in vitro* system, the present data do not rule out the possibility that the decrease in translatable message for P-450 2c reflects changes in the translational efficiency of the mRNA for P-450 2c. However, HCB does not affect the total translational efficiency of liver polysomes (14), and a decrease in the level of P-450 2c mRNA seems the most likely explanation for the virtual elimination of the translatable message for this isozyme.

Certain 3-MC type inducers have been shown to increase transcription of the mRNAs for P-450c (34) and $P_1$-450 (35) as a consequence of their interaction with the *Ah* receptor. The

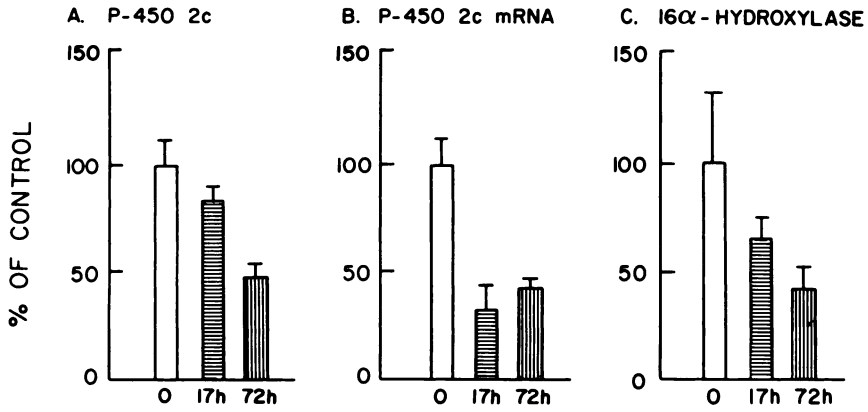


**Fig. 6.** Levels of P-450 2c (*A*), its mRNA (*B*), and androstenedione 16α-hydroxylase (*C*) in hepatic microsomes isolated from adult male rats treated with either a single dose (17 hr) or 3 daily doses (72 hr) of 3-MC (50 mg/kg). P-450 2c was measured by $^{125}$I-protein A dot-blot immunoassay. The mRNA levels and the catalytic activities were measured as described in Figs. 3 and 5. Values represent mean of four individual rats ± SD, except for the mRNA values (*B*), which represent the mean ± SD of three rats. Results are plotted as percent of control. (A-16α-hydroxylase activity in untreated rats was 1.6 ± 0.6 nmol/min/mg protein.)

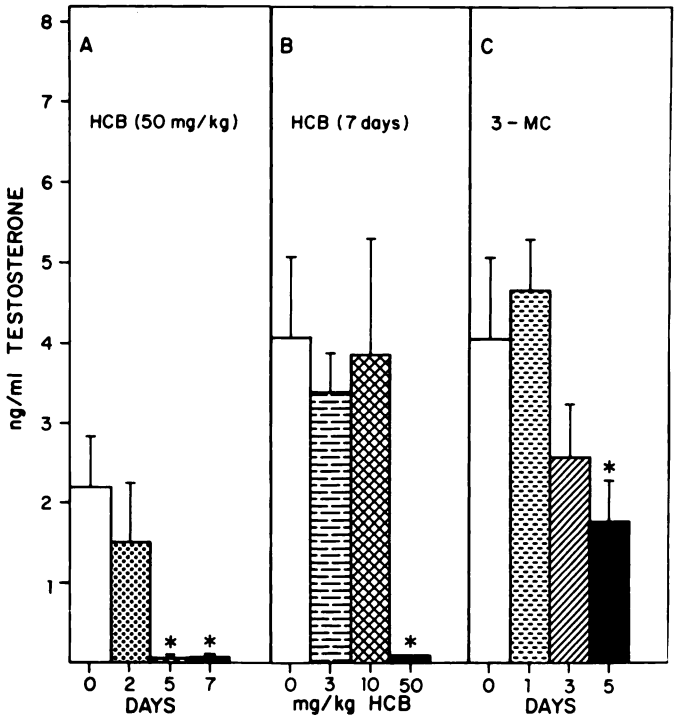


**Fig. 7.** Effects of HCB and 3-MC on serum testosterone levels. Serum testosterone levels were measured in groups of four or five rats at the following times and doses: *A*, at 0, 2, 5, and 7 days in rats treated with a single dose of HCB (50 mg/kg). *B*. 7 days after a single dose of HCB at either 3, 10, or 50 mg/kg. *C*. 0, 1, 3, and 5 days after daily doses of 3-MC (50 mg/kg). *Significance $p < 0.05$ compared with control.

same *Ah*-receptor-ligand complexes that presumably induce transcription of P-450c and P-450d might concomitantly repress transcription of P-450 2c and other constitutive mRNAs. Alternatively, the decrease in P-450 2c mRNA may be a secondary response or be unrelated to the effects of the *Ah* receptor. Phenobarbital, an inducer of cytochromes P-450b and P-450e, also produced a moderate decrease in the mRNA for P-450 2c, but had less effect on the immunoreactive protein. It is not clear whether the induction of P-450b and e by phenobarbital is receptor-mediated, but induction by this compound does not involve the *Ah* receptor (36). Our results agree with those of Dannan *et al.* (13) who suggest that, although other types of P-450 inducers can decrease P-450 2c, compounds that interact with the *Ah* receptor produce the most striking effect. However, the dose of HCB required to decrease P-450 2c and its mRNA was somewhat higher than the dose required to induce P-450c. Moreover, the induction of P-450c mRNA by HCB clearly preceded the decrease in P-450 2c mRNA, suggesting that HCB may affect these isozymes via different mechanisms. Alternatively, the differences in the time course for the effects of HCB on the mRNAs for these two enzymes may reflect differences in the half-lives of the mRNAs.

Three lines of evidence indicate that the effects of HCB on P-450 2c and its mRNA do not reflect general hepatotoxicity. First, histological examination of the livers showed only occasional single cell necrosis 7 days after 50 mg/kg HCB and no single cell necrosis after 10 mg/kg HCB (data not shown). Second, HCB did not produce a general decrease of P-450-dependent monooxygenase activities. P-450c-dependent 7-ethoxycoumarin-*O*-deethylation was induced maximally by HCB at 7 days in the present study, at a time when P-450 2c was maximally suppressed. Finally, total translational products of hepatic polysomes were not decreased by HCB, indicating that the suppression of P-450 2c mRNA was selective for this P-450. In addition, the translatable mRNA for P-450c was increased markedly by 24 hr (20-fold), and remained elevated for at least 7 days. Thus, P-450 2c and its mRNA are selectively suppressed by HCB, under conditions in which other P-450s are induced.

The present study shows that P-450 2c mRNA was at least 5-fold higher in livers of male rats than those of female rats, indicating that the previously reported sex specificity of this P-450 (7, 21, 33) is due to sex differences in hepatic content of P-450 2c mRNA. Immunoreactive P-450 2c has been reported to be ~20- to 30-fold higher in livers of mature males than in those of mature females (11, 21, 33). Since a recent report indicates some homology between a cDNA probe for P-450 2c and mRNA(s) in livers of female rats,[3] and the existence of common antigenic sites between P-450 2c and several forms of P-450 present in female rats (2d and f) has been documented (7, 28), the present studies probably underestimate the magnitude of the sex differences in the mRNA for P-450 2c due to the presence of some cross-reactivity between anti-P-450 2c and P-450 forms present in the female rat.

Since P-450 2c is a male-specific enzyme, one explanation for the suppression of this P-450 by HCB might be feminization of the male rat by this polychlorinated biphenyl isomer. However, androstenedione 5$\alpha$-reductase activity, which is $\geq$10-fold higher in liver microsomes of female rats than male rats (9, 11), was not significantly increased by HCB. In addition, HCB did not increase either the hepatic microsomal content of P-450 2d, a female-specific enzyme, or serum estradiol levels. A recent report (37) shows a similar decline in P-450 2c in the livers of diabetic male rats, and this decrease does not appear to reflect feminization of the male rats. HCB decreased serum testosterone dramatically 5–7 days after a dose of 50 mg/kg. However, HCB did not decrease testosterone at lower doses which decreased P-450 2c moderately. Interestingly, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, another compound known to bind the *Ah* receptor, recently has been reported to decrease serum testosterone levels (36, 38). A number of compounds known to interact with the *Ah* receptor, suppress 2$\alpha$-, 6$\beta$-, and 16$\alpha$-hydroxylation of steroid hormones in hepatic microsomes from male rats (39). Therefore, we cannot rule out the possibility that HCB and structurally related compounds act, at least in part, by interrupting the hormonal control of the synthesis of P-450 2c at the level of the pituitary or testes. However, the decrease in serum testosterone seen in the present study probably does not fully account for the observed ~90% loss of P-450 2c, insofar as only a 40% decrease in P-450 2c has been reported after castration of adult male rats (11). Therefore, HCB and 3-MC may decrease synthesis of P-450 2c by a more direct mechanism, such as interaction of the *Ah* receptor-ligand complex with a regulatory portion of the gene for P-450 2c.

In conclusion, the sex specificity of cytochrome P-450 2c, a constitutive, male-specific enzyme, reflects the hepatic content of its mRNA in male rats. Expression of P-450 2c is almost

[3] After completion of this study, Yoshioka *et al.* [*J. Biol. Chem.* **262**:2787–2793 (1987)] reported the isolation of cDNA clones for P-450 M-1 (P-450 2c) mRNA. Under stringent conditions, the cDNA probe hybridized to poly A$^+$ mRNA isolated from the livers of both male and female rats, presumably due to high homology between the male-specific P-450 2c mRNA and a female P-450 form.

totally abolished in male rats following administration of a polychlorinated biphenyl isomer known to interact with the *Ah* receptor. The decrease in P-450 2c is accompanied by a marked decrease in the ability of liver microsomes to hydroxylate steroid hormones such as androstenedione and testosterone at the 16$\alpha$-position and testosterone at the 2$\alpha$-position. These decreases have been shown to be the consequence of a decrease in the synthesis of P-450 2c, since the hepatic levels of the translatable mRNA for this isozyme are virtually eliminated by HCB administration. A similar though less striking decrease in P-450 2c and its mRNA is observed after administration of 3-MC, another compound which interacts with the *Ah* receptor. The effects of large doses of HCB were accompanied by dramatic decreases in serum testosterone levels, and thus may reflect, at least in part, disruption of the hormonal control of the expression of P-450 2c.

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