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INDUCTION OF HEPATIC MONO-OXYGENASE SYSTEMS OF PREGNANT RATS WITH PHENOBARBITAL AND 3-METHYLCHOLANTHRENE*

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Abstract—When sodium phenobarbital was given to pregnant and non-pregnant female rats (40 mg/kg for 4 days), ethylmorphine *N*-demethylase, a cytochrome P-450-dependent system, was induced about 4-fold in non-pregnant females, but only 2-fold in pregnant females. The induction of microsomal cytochrome P-450 was also lower in pregnant animals. This impairment of phenobarbital induction occurred within 3 days of conception and disappeared after parturition within 5 days. 3-Methylcholanthrene induction of hepatic benzo[a]pyrene hydroxylase, a cytochrome P_1-450-dependent mono-oxygenase system not inducible by phenobarbital, was not impaired during pregnancy. The depressed response of the maternal liver to phenobarbital induction can be partially reversed by the coadministration of 3-methylcholanthrene. The administration of a higher dose of sodium phenobarbital (80 mg/kg/day for 4 days) overcame the pregnancy-related lowered response to phenobarbital induction observed with the smaller dose of the barbiturate. The similarity in responses of the maternal and fetal livers to inducing agents suggests that a common regulatory mechanism operates in both the fetus and the pregnant female.

The absence of P-450 hemoptotein-dependent mono-oxygenase systems from fetal livers of many mammalian species is well documented [1–9]. Although these systems are induced by many chemical agents in the adult, fetal systems are induced only by certain polycyclic hydrocarbons, although as was shown in the accompanying paper [9], some phenobarbital induction occurs when phenobarbital and these hydrocarbons are given simultaneously to the pregnant animal. We postulated from these studies that a selective control mechanism prevents the induction of cytochrome P-450‡ systems by either endogenous or exogenous substances without interfering with the induction of cytochrome P_1-450 systems by xenobiotics. This selective depression of induction was thought to be directed at the synthesis of cytochrome P-450.

If mediated hormonally, this selective mechanism might be expected to operate in the liver of the pregnant rat as well as in the fetus. In fact, some support for this possibility already exists; several studies have demonstrated that various mono-oxygenase activities are diminished in the adult liver during pregnancy [10–14]. The current study offers evidence that a selective mechanism governing induction of the hepatic mono-oxygenase system develops in the adult during pregnancy; this mechanism functions similarly to that observed previously in the fetal liver.

MATERIALS AND METHODS

Materials. Ethyl isocyanide was synthesized by Dr. Don Shoeman. Aniline HCl was obtained from Eastmen Organic Chemicals. Sodium hexobarbital was supplied by Winthrop Laboratories. Progesterone was supplied by Sigma Chemical Co. Other materials were obtained from previously mentioned sources [9].

Animals. Specifications and care of the animals have been described in the companion paper [9]; in fact, most of these rats were those used in that study. Animals were bred overnight; the following day was considered day 1 of pregnancy. Pregnant animals were isolated 1 day prior to parturition; mothers and neonates were housed together until the day they were killed. Non-pregnant females (200–300 g) and weanling (45–50 g) male rats were also used. Females which were to be killed on days 1 and 3 of pregnancy were injected with inducing agents for 3 days or 1

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‡ Throughout this paper cytochrome P-450 is considered to be the P-450 hemoprotein found predominantly in livers of untreated or phenobarbital-treated rats; cytochrome P_1-450 (also known as cytochrome P-448) is considered to be the P-450 hemoprotein found predominantly in livers of rats treated with certain polycyclic hydrocarbons, including 3-methylcholanthrene. We recognize that cytochrome P-450 and cytochrome P_1-450 may describe classes of P-450 hemoproteins rather than specific cytochromes. P-450 hemoprotein designates any cytochrome which gives a spectrum with a maximum of about 450 nm when it is reduced and complexed with carbon monoxide; this includes both cytochrome P-450 and cytochrome P_1-450.

day, respectively, bred with proven males overnight in our laboratory, and then given inducing agents for the remainder of the 4-day injection period. Adult livers were collected on days 1, 3, 5, 7, 10, 13, 16 and on consecutive days from 18 to 25 after conception. Parturition occurred on day 22.

Rats were injected (i.p.) daily for 4 days with sodium phenobarbital (40 mg/kg in saline), 3-methylcholanthrene (16 mg/kg in corn oil) or progesterone (50 mg/kg in corn oil) in a volume of 2 ml/kg. Control rats received i.p. injections of saline (2 mg/kg). Preliminary data showed saline and corn oil controls to be equivalent. The fourth injection was given approximately 15 hr before the animals were killed. In one experiment, rats which had been pregnant for 17 days were injected for 4 days with 80 mg of sodium phenobarbital/kg/day divided into two daily doses.

Liver preparations. Animals were stunned, decapitated and exsanguinated; their livers were perfused *in situ* with cold 1.15% KCl, excised, blotted dry and weighed. A 25% liver homogenate was prepared using a Dounce glass–glass homogenizer. Washed microsomes were prepared as described previously [15] and used on the day of preparation.

Determination of P-450 hemoprotein. Microsomal P-450 hemoprotein was measured by the method of Omura and Sato [16] using an Aminco DW2 spectrophotometer in the split beam mode. The same spectrophotometer was used for all other spectral measurements.

Spectral binding determination. Ethyl isocyanide (EIC) and substrate binding spectra were determined as described previously [17, 18].

Enzyme assays. Benzpyrene hydroxylase activities of homogenates were determined by the method of Wattenberg and Leong [19]. Microsomal ethylmorphine *N*-demethylase was measured as described previously [18]. Microsomal cytochroome *c* reductase activity was measured by an adaptation [9] of the method of Williams and Kamin [20].

Assay of hepatic phenobarbital levels. Phenobarbital levels of livers from pregnant and non-pregnant female rats were determined as described previously [9].

Statistical analysis. Statistical analysis utilized one- and two-way analyses of variance programs written for the Control Data Cyber 70 model 72 computer. Statistical differences ($P < 0.05$) between individual treatment groups were detected using Duncan's new multiple range test [21].

Protein determination. Protein contents of liver preparations were determined by the method of Lowry *et al.* [22] using bovine serum albumin as the standard.

RESULTS

Induction of ethylmorphine N-demethylase activity in maternal livers. Figure 1 shows the response of ethylmorphine *N*-demethylase activity to administered 3-methylcholanthrene and phenobarbital. Contrary to results reported elsewhere [12], rates of ethylmorphine *N*-demethylation/g of liver were not different in pregnant and non-pregnant control animals. However, livers from pregnant phenobarbital-treated animals possessed only half the activity of livers from their non-pregnant, phenobarbital-treated counterparts. The diminished response to phenobarbital administration is seen within 3 days of conception, persists throughout pregnancy, and disappears during the 3 days after parturition. The simultaneous administration of phenobarbital and 3-methylcholanthrene

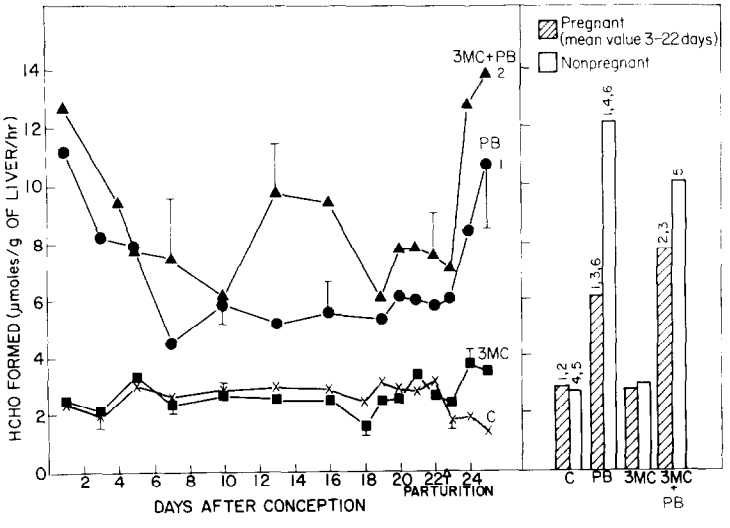

Fig. 1. Ethylmorphine *N*-demethylase activity of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on days −3 through 21 relative to the day they were bred (day 0). Animals were killed 15 hr after the last injection and microsomes from their livers were assayed for ethylmorphine *N*-demethylase activity on the days indicated. C = control (injected with saline only). Bars indicate S. E. N for each point = 4. The graph on the right compares the mean values obtained on days 3–22 of pregnancy (N = 40) with mean values obtained from non-pregnant female rats (N = 4). Values labeled with the same numerals are significantly different at the $P < 0.05$ level.

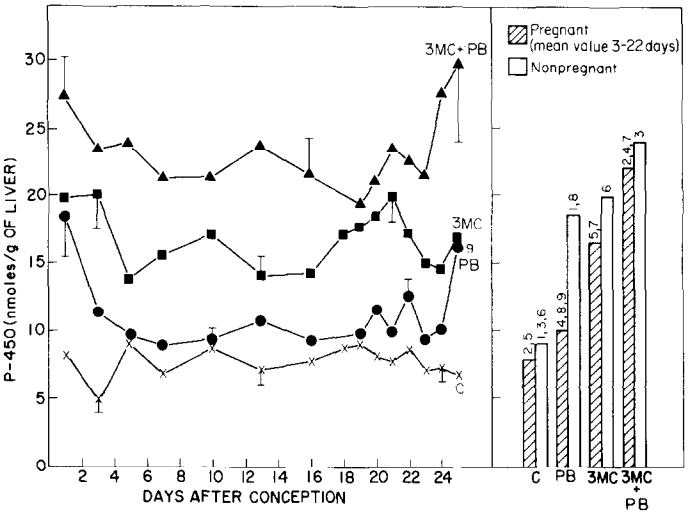

Fig. 2. P-450 hemoprotein content of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on days −3 through 21 relative to the day they were bred (day 0). Animals were killed 15 hr after the last injection and microsomes from their livers were assayed for P-450 hemoprotein content on the days indicated. C = control (injected with saline only). Bars indicate S. E. N for each point = 4. The graph on the right compares the mean values obtained on days 3–22 of pregnancy (N = 40) with the mean values obtained from non-pregnant female rats (N = 4). Values labeled with the same numerals are significantly different at the $P < 0.05$ level.

to pregnant animals increased activity significantly above that observed with phenobarbital alone, but a significant difference was not seen in non-pregnant animals. 3-Methylcholanthrene given alone produced no increase in ethylmorphine metabolism.

Induction of P-450 hemoprotein in maternal livers. P-450 hemoprotein levels of livers from untreated pregnant rats were not significantly different from those of their non-pregnant counterparts (Fig. 2). Phenobarbital caused increases in hepatic P-450 hemoprotein levels which were only about half those produced in non-pregnant animals. Values obtained from pregnant and non-pregnant rats treated with 3-methylcholanthrene were not significantly different. Levels produced by treatment with 3-methylcholanthrene + phenobarbital were significantly higher than those produced by phenobarbital alone in pregnant rats, but not in non-pregnant rats.

During the 3 days after parturition, P-450 hemoprotein levels and *N*-demethylase activities in phenobarbital-treated pregnant rats rose to those of non-pregnant phenobarbital-treated rats. Poor correlation is seen between P-450 hemoprotein levels and ethylmorphine *N*-demethylase activities in 3-methylcholanthrene-induced animals. This is readily explained by earlier studies which showed 3-methylcholanthrene-induced hemoprotein (cytochrome P_1-450) to be metabolically unreactive with ethylmorphine, or nearly so [18, 23, 24].

Induction of benzpyrene hydroxylase activity in maternal livers. Hepatic benzo[a]pyrene hydroxylase activity was unaltered by pregnancy in either treated or untreated rats (Fig. 3). Thus, the maternal liver resembles the fetal liver in its ability to respond to the induction of benzo[a]pyrene hydroxylase by 3-methylcholanthrene [9].

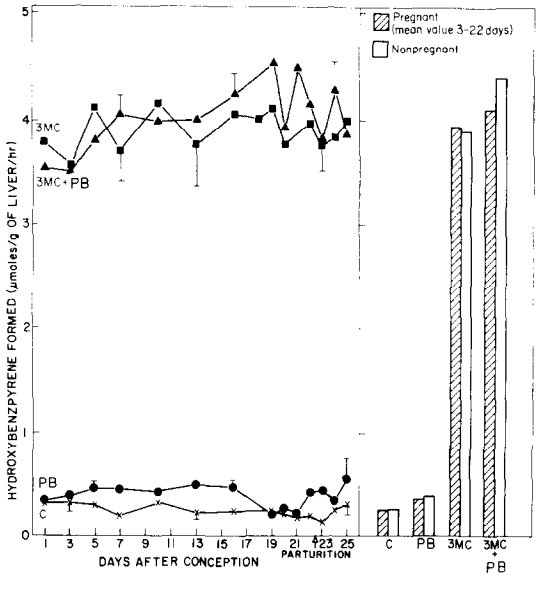

Fig. 3. Benzpyrene hydroxylase activity of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on days −3 through 21 relative to the day they were bred (day 0). Animals were killed 15 hr after the last injection and microsomes from their livers were assayed for benzo[a]pyrene hydroxylase activity on the days indicated. C = control (injected with saline only). Bars indicate S. E. N for each point = 4. The graph on the right compares the mean values obtained on days 3–22 of pregnancy (N = 40) with mean values obtained from non-pregnant female rats (N = 4).

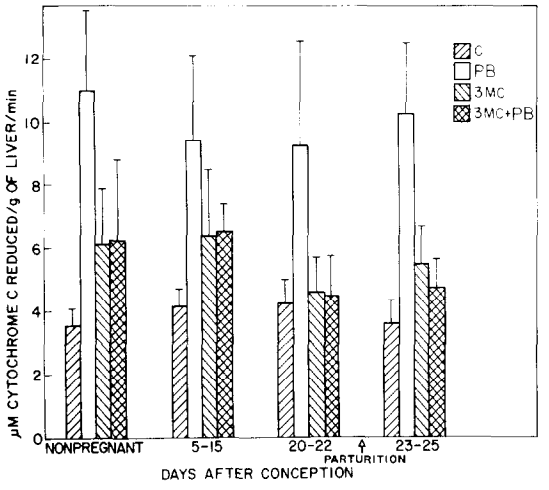

Fig. 4. NADPH–cytochrome *c* reductase activity of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on days 1–21 relative to the day they were bred (day 0). Animals were killed 15 hr after the last injection and microsomes from their livers were assayed for NADPH–cytochrome *c* reductase activity on the days indicated. C = control (injected with saline only). Bars indicate S. E. N for each point = 4.

Induction of NADPH-cytochrome c *reductase activity in maternal livers.* NADPH-cytochrome *c* reductase activity was not different, and was induced by phenobarbital to the same degree, in both pregnant and non-pregnant rats (Fig. 4). Not only did 3-methylcholanthrene fail to induce reductase activity in livers of either pregnant or non-pregnant rats, but when 3-methylcholanthrene was coadministered with phenobarbital, phenobarbital no longer induced reductase activity.

Spectral binding of ethyl isocyanide, hexobarbital and aniline by microsomes from maternal livers. Alteration of the ratio of the heights of the 455 and 430 nm maxima of the spectrum produced when EIC is added to hepatic microsomes has been used to distinguish between cytochrome P-450 and P_1-450 [18, 23]. Microsomes from control and phenobarbital-treated pregnant and non-pregnant rats exhibited the same 455/430 nm peak height ratios (Fig. 5). 3-Methylcholanthrene produced the much higher ratios characteristic of cytochrome P_1-450 in both pregnant and non-pregnant animals. The mean ratio obtained with microsomes from 3-methylcholanthrene + phenobarbital-treated rats was intermediate between those obtained with each of the inducing agents given singly, as would be expected if both cytochrome P-450 and cytochrome P_1-450 had been induced. Pregnancy had no significant effect on any of the EIC measurements.

Pretreatment of animals with phenobarbital increases the capacity of microsomes to bind both hexobarbital and aniline; 3-methylcholanthrene enhances type II binding only [17]. Figures 6 and 7 show the effects of inducing agents on the substrate binding spectra of microsomes from pregnant and non-pregnant animals. In non-pregnant animals, significantly less aniline binding was observed after 3-methylcholanthrene + phenobarbital treatment than with 3-methylcholanthrene alone (Fig. 6). Pregnant animals demonstrated no such difference. This may be viewed as a diminished response to phenobarbital induction during pregnancy.

Figure 7 shows type I difference spectra produced from pregnant, non-pregnant and 3-day postpartum maternal rats. In all cases, typical type I spectra (λ_{max}

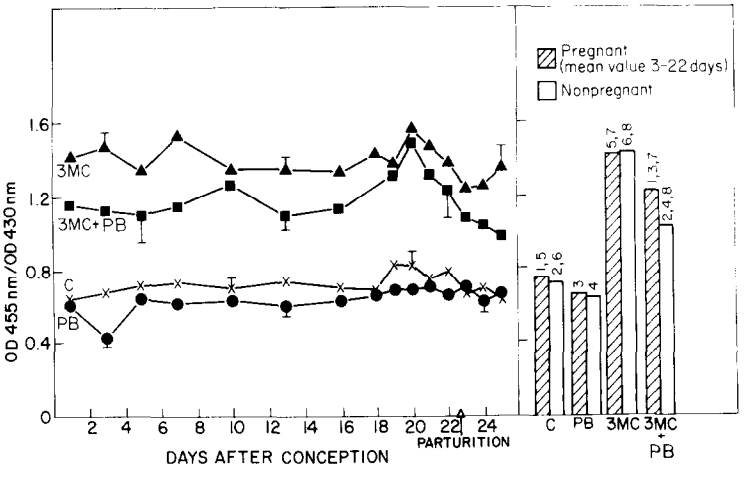

Fig. 5. Ethyl isocyanide binding spectra of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on days −3 through 21 relative to the day they were bred (day 0). Animals were killed 15 hr after the last injection and microsomes from their livers were assayed for ethyl isocyanide binding spectra on the days indicated. C = control (injected with saline only). Bars indicate S. E. N for each point = 4. The graph on the right compares the mean values obtained on days 3 22 of pregnancy (N = 40) with mean values obtained from non-pregnant female rats (N = 4). Measurement of 455 and 430 nm maxima of the ethyl isocyanide spectrum produced with microsomes was made at pH 7.4.

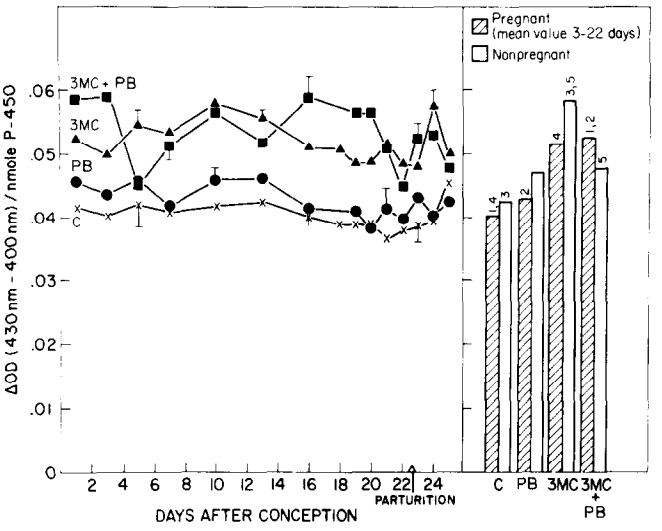

Fig. 6. Aniline binding spectra of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on days −3 through 21 relative to the day they were bred (day 0). Animals were killed 15 hr after the last injection and microsomes from their livers were assayed for aniline binding spectra on the days indicated. C = control (injected with saline only). Bars indicate S. E. N for each point = 4. The graph on the right compares the mean values obtained on days 3–22 of pregnancy (N = 40) with mean values obtained from non-pregnant female rats (N = 4).

395 nm; λ_{min}, 419 nm) were observed with microsomes from control and phenobarbital-treated rats. Microsomes from 3-methylcholanthrene-treated rats gave the expected reverse type I spectrum [17, 25]. The binding spectra obtained after phenobarbital + 3-methylcholanthrene treatment appear to be the sum of the type I and reverse type I components. By inference, the relative presence of cytochromes P-450 and P_1-450 are such that the O.D. values at 395 and 419 nm are proportional and inversely proportional, respectively, to the cytochrome P-450 content of the microsomes, whereas the reverse is the case for the content of cytochrome P_1-450. By these criteria, it can be seen that the non-pregnant rats responded to 3-methylcholanthrene + phenobarbital with increases in both cytochromes P-450 and P_1-450 (Fig. 7b), but

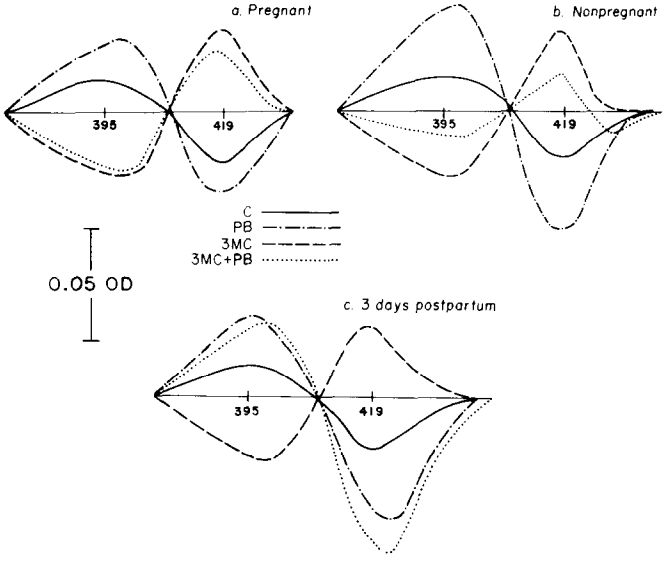

Fig. 7. Hexobarbital binding spectra of microsomes from livers of pregnant and non-pregnant rats given phenobarbital (PB), 3-methylcholanthrene (3MC) or both agents. Rats were injected daily with NaPB (40 mg/kg), 3MC (16 mg/kg) or the same doses of NaPB + 3MC for 4 days beginning on day 15 or 16 relative to the day they were bred (day 0). Hepatic microsomes were harvested 15 hr after the last injection. The individual spectral tracings shown in the figure were selected to represent typical spectra.

Table 1. Correlation of hepatic phenobarbital levels with ethylmorphine *N*-demethylase activities and P-450 hemoprotein levels in livers of pregnant and non-pregnant rats*

Source of microsomes	Treatment (mg/kg/day)	Hepatic PB level (g PB/g liver)	Ethylmorphine *N*-demethylation (moles HCHO/g liver/hr)	P-450 Hemoprotein (nmoles/g liver)
Pregnant females	PB (40)	114 ± 18	5.12 ± 0.7	6.50 ± 0.7
Pregnant females	PB (80)	218 ± 62	11.32 ± 1.2	14.49 ± 2.0
Pregnant females	PB (40)	138 ± 21	7.60 ± 1.0	24.50 ± 2.5
Non-pregnant females	PB (40)	105 ± 21	11.33 ± 1.4	14.36 ± 2.1
Non-pregnant females	PB (80)	237 ± 58	13.50 ± 1.6	17.68 ± 0.9
Non-pregnant females	PB (40)	144 ± 30	9.80 ± 1.3	22.20 ± 3.0

* Sixteen-day-pregnant rats were given PB, 3-methylcholanthrene (3MC) or both agents for 4 days (the 80 mg dose of NaPB was divided into two 40 mg doses/day). Livers were assayed 15 hr after the last injection. Values are the mean ± S. E. of three experiments.

that the response in pregnant rats was an increase in cytochrome P_1-450 with little or no increase in cytochrome P-450 (Fig. 7a). By the same criteria, it may be seen in Fig. 7c that 3 days after parturition the relative presence of cytochromes P-450 and P_1-450 after 3-methylcholanthrene + phenobarbital treatment appears to be the reverse of that observed in livers from pregnant rats; 3-methylcholanthrene appears to have lost its ability to induce cytochrome P_1-450.

Levels of phenobarbital in livers from maternal and non-pregnant rats treated with inducing agents. The possibility was considered that observed differences in the degrees of phenobarbital induction between livers from pregnant and non-pregnant rats might be due to differences in accumulation of phenobarbital in their livers. Twenty-one-day pregnant rats and adult non-pregnant female rats were given one or two 40 mg/kg doses of phenobarbital/day for 4 days. Their livers were removed 15 hr after the last injection and analyzed for phenobarbital content. Results summarized in Table 1 permit the following conclusions: (a) the lowered inductive response caused by pregnancy when the dose of phenobarbital was 40 mg/kg/day (Figs. 1 and 2) is not attributable to a lowered accumulation of phenobarbital in the liver; for a given dose of phenobarbital, whether 40 or 80 mg/day, levels of phenobarbital were essentially the same in livers of pregnant and non-pregnant animals; (b) the coadministration of 3-methylcholanthrene does not increase the accumulation of phenobarbital in maternal livers.

Effects of an increased dose of phenobarbital on the induction of ethylmorphine N*-demethylase activity and P-450 hemoprotein level in maternal livers.* In Table 1, it can be seen that the depression of phenobarbital induction of demethylase activity and P-450 hemoprotein level in pregnant rats receiving 40 mg phenobarbital/kg, observed in preceding studies, was overcome when the dose was increased to 80 mg/kg. Thus, while the pregnant rat is less responsive to induction than the non-pregnant rat, it is not refractive.

Attempts to depress response of non-pregnant female rats to phenobarbital and weanling male rats with pro-

Table 2. Effect of progesterone on phenobarbital (PB) induction of livers from weanling male and adult female rats*

Source of microsomes	Treatment	Ethylmorphine *N*-demethylation (μmoles/g liver/hr)	P-450 Hemoprotein (nmoles/g liver)
Weanling	None	3.39 ± 0.26	5.95 ± 0.31
Weanling	Progesterone	5.92 ± 0.71	7.29 ± 0.82
Weanling	PB	23.36 ± 2.48	27.23 ± 3.32
Weanling	Progesterone + PB	23.49 ± 0.71	24.66 ± 1.63
Adult	None	1.42 ± 0.20	4.86 ± 0.30
Adult	Progesterone	2.20 ± 0.21	4.19 ± 0.42
Adult	PB	6.57 ± 0.73	10.95 ± 0.87†
Adult	Progesterone + PB	6.84 ± 1.59	7.75 ± 1.27†

* Progesterone (50 mg/kg) and NaPB (40 mg/kg) were given (i.p.) daily for 4 days; the animals were killed 15 hr after the last injection. Values represent mean ± S. E. of four experiments.

† Significantly different from each other ($P < 0.05$).

gesterone treatment. The coincidental development of the diminished hepatic response to phenobarbital with the onset of pregnancy suggests that some hormonal factor associated with pregnancy might cause the liver to be less responsive to phenobarbital induction. To investigate this possibility, we gave progesterone with phenobarbital to male weanlings and non-pregnant adult females and evaluated their hepatic mono-oxygenase systems. As shown in Table 2, progesterone did not affect the response of P-450 hemoprotein or ethylmorphine *N*-demethylase to phenobarbital in weanlings. However, in non-pregnant adult females, progesterone decreased phenobarbital induction of P-450 hemoprotein significantly, but not the induction of ethylmorphine *N*-demethylase activity.

DISCUSSION

That P-450 hemoprotein-dependent mono-oxygenase systems of the liver are deficient or absent in fetuses of several laboratory animals is well established [1–9]. That certain mono-oxygenase activities can be induced in fetal liver by polycyclic hydrocarbons, but not by phenobarbital, is also well documented [4, 8, 9, 26]. In the companion paper [9], we developed the concept that a suppressive mechanism exists in the fetus which prevents the induction of cytochrome P-450-dependent mono-oxygenases without preventing the induction of cytochrome P_1-450-dependent mono-oxygenases. The current paper provides evidence that a similar selective mechanism exists in the adult female rats during pregnancy. The following parallelisms are presented to illustrate this hypothesis:

(a) Phenobarbital does not induce aminopyrine *N*-demethylase, a cytochrome P-450-dependent mono-oxygenase system, in the fetal liver [9]; phenobarbital induces less ethylmorphine *N*-demethylase, a cytochrome P-450-dependent mono-oxygenase system, in the pregnant rat than in the non-pregnant rat (see Fig. 1).

(b) Cytochrome P-450 is not induced in the fetal liver when phenobarbital is given to the mother [9]; phenobarbital induces less cytochrome P-450 in the pregnant rat than in the non-pregnant female rat (see Fig. 2).

(c) Rate limitation of the cytochrome P-450 mono-oxygenase systems in both fetal [9] and maternal livers is imposed by a suppressed synthesis of cytochrome P-450, not a deficiency of NADPH-cytochrome *c* reductase (see Fig. 4).

(d) 3-Methylcholanthrene induces benzo[a]pyrene hydroxylase, a cytochrome P_1-450-dependent mono-oxygenase system, in the fetal liver [9]; pregnancy does not impair 3-methylcholanthrene induction of benzpyrene hydroxylase in the maternal liver (see Fig. 3).

(e) 3-Methylcholanthrene induces cytochrome P_1-450 in the fetal liver [9]; pregnancy does not impair 3-methylcholanthrene induction of cytochrome P_1-450 in the maternal liver (see Fig. 2).

(f) When fetuses are exposed to 3-methylcholanthrene and phenobarbital simultaneously, their resistance to phenobarbital induction is partially reversed [9]. A similar effect is observed in maternal livers. Thus, when 3-methylcholanthrene + phenobarbital was given to pregnant rats, ethylmorphine *N*-demethylase activity was increased above that seen after the administration of phenobarbital alone; this was not the case in non-pregnant females (see Fig. 1). Since 3-methylcholanthrene induces cytochrome P_1-450, and phenobarbital induces cytochrome P-450, and because ethylmorphine *N*-demethylase is a cytochrome P-450-dependent mono-oxygenase, not a cytochrome P_1-450-dependent mono-oxygenase, it is likely that the coadministration of 3-methylcholanthrene partially negated the apparent block of phenobarbital induction associated with pregnancy. This interpretation was strengthened by the observed effects of coadministered 3-methylcholanthrene and phenobarbital on microsomal P-450 hemoprotein and spectral binding of hexobarbital (see Figs. 2 and 7). The cytochrome P-450 level in microsomes from pregnant rats after the administration of phenobarbital alone was only about half that observed with non-pregnant rats. After 3-methylcholanthrene + phenobarbital treatment, P-450 hemoprotein levels in pregnant and non-pregnant rats were the same. Since the induction of cytochrome P_1-450 in pregnant rats after the administration of 3-methylcholanthrene alone was about the same as that in non-pregnant rats, the increase in the P-450 hemoprotein level in pregnant rats after coadministration of 3-methylcholanthrene and phenobarbital, relative to that seen after phenobarbital alone, could be due to an increase in cytochrome P_1-450, in cytochrome P-450 or both. That ethylmorphine *N*-demethylase, a mono-oxygenase induced by phenobarbital, but not by 3-methylcholanthrene [17, 22], is found in a higher concentration in maternal livers from rats injected with 3-methylcholanthrene + phenobarbital than in rats injected with phenobarbital alone (see Fig. 1) indicates that the increase in P-450 hemoprotein was due to an increase in cytochrome P-450 as well as cytochrome P_1-450. In passing, it is worth noting that the inductive effects or 3-methylcholanthrene and phenobarbital on P-450 hemoprotein levels are not additive in either pregnant or non-pregnant female rats when the two agents are coadministered. In male rats the effects are additive [27, 28]. This may represent a sexual difference, but because young males were used in the one case and much older females in the current studies, the difference could be a matter of age rather than of sex. It is of interest in this connection that Gillette [29] observed only a small induction of acetanilide metabolism in microsomes from immature female rats, a large induction with benzo[a]pyrene, but an induction almost three times greater than the sum of the two individual inductions when the two inducing agents were coadministered.

Depression of phenobarbital induction was observed 3 days after conception. The chronology of development of resistance to phenobarbital induction in the maternal liver, therefore, coincides roughly with the development of the corpus luteum, not with the much later appearance of the placenta and the fetus.

Progesterone was employed in an attempt to diminish the response of weanlings and non-pregnant adult females to phenobarbital (see Table 2). Results were equivocal; induction of cytochrome P-450 was lowered, but induction of mono-oxygenase was not.

This experiment does not exclude progesterone as a mediator of suppressed phenobarbital induction in maternal livers; the metabolic clearance rate of progesterone after a single dose in the rat is very high [30], and the steady state concentration of progesterone existing during pregnancy may not be achieved with four daily intraperitoneal doses of progesterone.

Our data are compatible with the hypothesis that a hormonal substance, accessible to both fetal and maternal livers, acts to suppress phenobarbital induction. Validity of this hypothesis awaits identification of the hormonal substance. We have viewed the failure of development of the mono-oxygenase systems in the fetal liver and the lowered inducibility of these systems in the maternal liver as the result of a suppression of the mechanism controlling the synthesis of these systems, more specifically, the synthesis of cytochrome P-450. Alternatively, the deficiency of mono-oxygenase systems in fetal liver might be viewed as a failure to develop the necessary cellular components for the synthesis of these systems. In this case, parturition would trigger a rapid synthesis of the required components rather than remove the liver from a repressor substance contained in the maternal environment. The loss of the ability of the maternal liver to respond to the inductive effects of phenobarbital within 3 days of conception would be more difficult to explain by this alternative hypothesis. Apart from speculation concerning the precise mechanisms involved, the phenomenological similarities between the control of the fetal systems and that of the maternal systems suggest that the same mechanism may regulate mono-oxygenase induction in both fetal and maternal livers.

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REFERENCES

1. J. R. Fouts and R. H. Adamson, *Science, N.Y.* **129**, 897 (1959).
2. L. G. Hart, R. H. Adamson, R. L. Dixon and J. R. Fouts, *J. Pharmac. exp. Ther.* **137**, 103 (1962).
3. E. Bresnick and J. G. Stevenson, *Biochem. Pharmac.* **17**, 1815 (1968).
4. R. M. Welch, B. Gommi, A. P. Alvares and A. H. Conney, *Cancer Res.* **32**, 973 (1972).
5. R. L. Dixon and V. J. Willson, *Archs int. Pharmacodyn. Thér.* **172**, 453 (1968).
6. G. Feuer and A. Liscio, *Int. J. clin. Pharmac.* **3**, 30 (1970).
7. W. R. Jondorf, R. P. Maickel and B. B. Brodie, *Biochem. Pharmac.* **1**, 352 (1959).
8. E. Schlede and H. J. Merker, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **272**, 89 (1972).
9. T. M. Guenthner and G. J. Mannering, *Biochem. Pharmac.* **26**, 567 (1977).
10. G. Feuer and A. Liscio, *Nature, Lond.* **223**, 68 (1971).
11. M. G. Neale and D. V. Parke, *Biochem. Pharmac.* **22**, 1451 (1973).
12. A. M. Guarino, T. E. Gram, D. H. Schroeder, J. B. Call and J. R. Gillette, *J. Pharmac. exp. Ther.* **168**, 224 (1969).
13. M. E. Dean and B. H. Stock, *Drug Metab. Dispos.* **3**, 325 (1975).
14. E. Schlede, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **281**, 341 (1974).
15. M. W. Anders and G. J. Mannering, *Molec. Pharmac.* **2**, 319 (1966).
16. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
17. D. W. Shoeman, M. D. Chaplin and G. J. Mannering, *Molec. Pharmac.* **5**, 412 (1969).
18. N. E. Sladek and G. J. Mannering, *Molec. Pharmac.* **5**, 186 (1969).
19. L. W. Wattenburg and J. L. Leong, *Cancer Res.* **25**, 365 (1962).
20. C. H. Williams and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
21. C. I. Bliss, in *Statistics in Biology* (Ed. C. I. Bliss), pp. 253–7. McGraw-Hill, New York (1967).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
23. N. E. Sladek and G. J. Mannering, *Biochem. biophys. Res. Commun.* **24**, 688 (1966).
24. G. J. Mannering, *Metabolism* **20**, 228 (1971).
25. J. B. Schenkman, D. L. Cinti, P. W. Moldeus and S. Orrenius, *Drug. Metab. Dispos.* **1**, 111 (1973).
26. D. P. Bogdan and M. R. Juchau, *Eur. J. Pharmac.* **10**, 119 (1970).
27. N. E. Sladek and G. J. Mannering, *Molec. Pharmac.* **5**, 174 (1969).
28. K. Bidleman and G. J. Mannering, *Molec. Pharmac.* **6**, 697 (1970).
29. J. Gillette, *Adv. Enzyme Regulat.* **1**, 215 (1963).
30. G. J. Pepe and J. Rothchild, *Endocrinology* **93**, 1200 (1973).