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ENDOGENOUS INDUCTION OF EPOXIDE HYDROLASE, BENZO(a)PYRENE HYDROXYLASE AND GLUTATHIONE-S-TRANSFERASE IN "RESPONSIVE" C57Bl/6 MICE AND IN "NONRESPONSIVE" DBA/2 MICE DURING PREGNANCY

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SUMMARY : Gestational changes in activity for three enzymes associated with different hepatic and pulmonary drug metabolizing systems were investigated in C57Bl/6 and DBA/2 mice : benzo(a)pyrene hydroxylase, epoxide hydrolase and glutathione-S-transferase. The gestational profiles of hepatic and pulmonary benzo(a)pyrene hydrolase, and epoxide hydrolase were similar in both strains. In addition, we demonstrated higher endogenous stimulation of the three studied enzymic activities in C57Bl/6 mice. During the second moiety of pregnancy, temporal variations were observed : a peak of activity occurred between days 17 and 18 for lung and liver benzo(a)pyrene hydroxylase and at day 20 for epoxide hydrolase in both strains. Hepatic glutathione-S-transferase variations were similar in both strains. However, pulmonary glutathione-S-transferase increased gradually throughout pregnancy in C57Bl/6 mice, while a peak of glutathione-S-transferase activity occurred on day 18 of gestation in DBA/2 mice.

Biotransformations involved in the metabolism of physiological substrates such as cholesterol and bilirubin, in the biosynthesis of steroid hormones and fatty acids, and in the detoxification of xenobiotics, condition the survival of the organism (1, 2). Most of these enzymatic reactions take place in the liver, but may also occur in the kidney, lung, skin, testes, ovary, placenta, etc... The enzyme involved are primarily membrane bound constituents in the endoplasmic reticulum : the cytochrome P-450 system and epoxide hydrolase (3,4,6). They are also found at lower levels in the nuclear membrane (5). Others are cytosolic constituents : the sulfotransferases and the glutathione-S-transferases (7). These enzymes have been also shown to participate in the toxic activation of xenobiotics into mutagens and proximate or ultimate carcinogens. In addition, other physiological pathways can complement these activation reactions, as recently demonstrated for prostaglandin synthetase which can oxidize the proximate carcinogen B(a)P 7,8 diol into one ultimate carcinogen, B(a)P diol epoxide (8,9). The activities of these enzymatic systems

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are induced or regulated either by endogenous factors, such as hormones and the physiological state of the animal, or by numerous exogenous chemicals (10, 11). In a developmental study of these activities in mice, we observed variations in maternal enzymic activities. We report here the variations in these enzymatic systems occurring during pregnancy in two separate strains : C57Bl/6 and DBA/2 mice.

EXPERIMENTAL METHODS

Animals. Pregnant mice of two strains (C57Bl/6, DBA/2) were obtained from the IRSC (CNRS, Villejuif, France). Females and males were mated during the night, the following day being considered as the first day of gestation. Non pregnant females served as controls. The age of females ranged from 10 to 12 weeks (body weight : 20-30 g).

Chemicals. [G-^{3}H] benzo(a)pyrene (37 Ci/mmole) was purchased from the Radiochemical Center, Amersham, U.K. 4,5-^{3}H benzo(a)pyrene 4,5-oxide (10 mCi/mole) and 4,5-dihydrodiol B(a)P were synthesized as described by Jerina *et al.* (12). All other substrates were obtained from Sigma.

Biochemical methods. After decapitation, livers and lungs were removed. The tissues were weighed, washed with cold phosphate buffer saline, then homogenized in 5 volumes of buffer (0.25 M saccharose, 1 mM EDTA, 1 mM Tris-HCl pH 7.4), filtered on gauze and centrifuged at 10,000 x g for 30 min. The supernatant was filtered on glasswool and centrifuged at 105,000 x g for 1 hour. The 105,000 x g supernatant constituted a cytosolic fraction which served as an enzyme source for determination of glutathione-S-transferase activity with 1-chloro 2,4 dinitrobenzene as the substrate, as described by Habig *et al.* (13). The 105,000 x g pellet constituted the microsomal fractions. Hepatic and pulmonary microsomes were resuspended in 1 ml of 10 mM phosphate buffer pH 7.4 containing 20 % glycerol and stored at - 70°C. Microsomal benzo(a)pyrene hydroxylase activity was determined as described by De Pierre *et al.* (14) using [G-^{3}H] benzo(a)pyrene. Microsomal benzo(a)pyrene 4,5-oxide hydrolase was measured according to Jerina *et al.* (12). Proteins were measured using the method of Lowry modified by Hartree (15), using bovine serum albumin as a standard. For each result presented in the figure and tables, organs were analyzed separately and individually ; the numeric value is expressed as the mean $\pm$ SE of 5 determinations from 5 animals.

RESULTS

a) Benzo(a)pyrene hydrolase activity [B(a)PH]

Figure 1 shows the variations in these enzymic systems from Day 15 to Day 20 of gestation. Both hepatic and pulmonary enzymatic activities were highly stimulated (5 to 10 times) during the gestational period, in the liver of both strains. B(a)PH activity increased until Day 18 in the maternal liver and Day 17 in the lung, then decreased drastically before parturition, returning to a normal level two days later.

b) Epoxide hydrolase activity [EH]

The variations in this enzymatic activity are reported in table I. A difference between the two strains was observed : in C57Bl/6 mouse liver, after a significant decrease as compared to non pregnant fe-

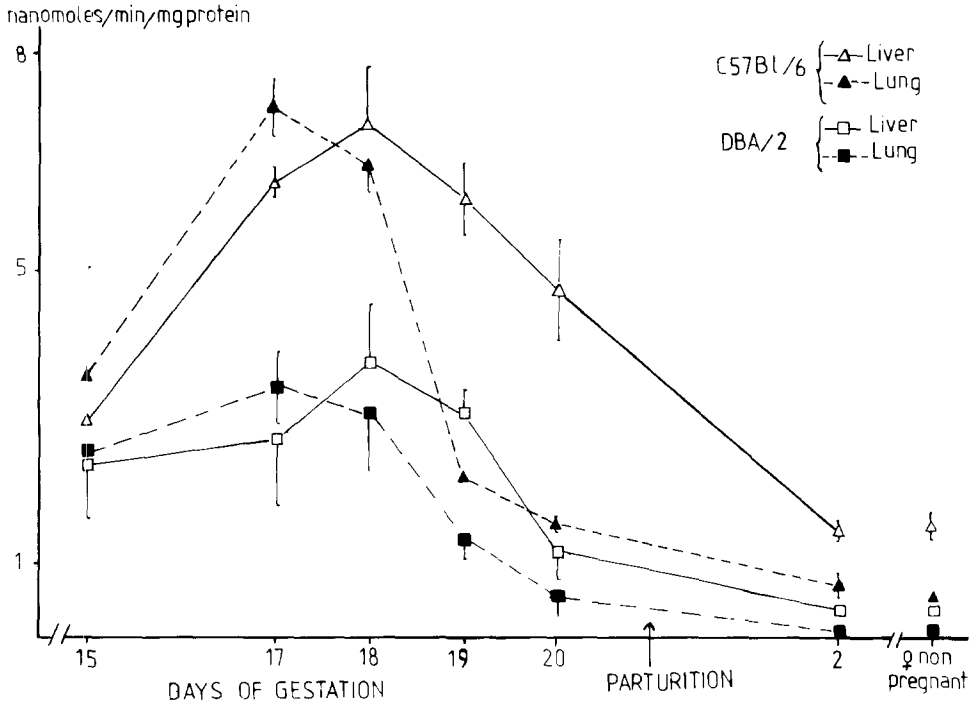

Figure 1. Variations in benzo(a)pyrene hydroxylase activity during pregnancy. Specific activity is expressed as nanomoles of hydroxylated B(a)P products/min/mg protein. Result is a mean ± SE of five individual determinations, with microsomal enzymes prepared from individual organs of 5 animals at each time point.

males, EH activity gradually increased to 7 times the basal value until the end of gestation and returned to a normal level after parturition. On

Table I. EFFECTS OF PREGNANCY ON EPOXIDE HYDROLASE ACTIVITY

		Epoxide hydrolase activity (pmole/min/mg protein)			
		Liver		Lung	
Strains		C57Bl/6	DBA/2	C57Bl/6	DBA/2
non pregnant		310 ± 90	100 ± 50	160 ± 70	80 ± 20
Gestational Day	15	130 ± 40	100 ± 20	100 ± 50	100 ± 20
	17	280 ± 50	120 ± 30	110 ± 55	100 ± 30
	18	560 ± 170	150 ± 25	140 ± 60	120 ± 40
	19	1200 ± 420	100 ± 40	170 ± 50	160 ± 40
	20	2060 ± 520	120 ± 40	260 ± 100	120 ± 50
Post-Partum Day	2	180 ± 30	100 ± 30	130 ± 20	95 ± 40

Specific activity of epoxide hydrolase is expressed as pmoles of 4,5 diol benzo(a)pyrene formed/min/mg protein. Each value represents the mean ± SE of the activity for five determinations with enzyme source prepared from individual organs of five animals at each time point.

Table II. EFFECTS OF PREGNANCY ON GLUTATHIONE-S-TRANSFERASE ACTIVITY

		Glutathione-S-transferase activity (μmole/min/mg protein)			
		Liver		Lung	
Mouse strain		C57Bl/6	DBA/2	C57Bl/6	DBA/2
non pregnant		10.1 ± 1.6	10.1 ± 2.3	0.59 ± 0.25	0.61 ± 0.25
Gestational Day	15	7.5 ± 1.9	5.8 ± 2.8	0.47 ± 0.16	0.57 ± 0.28
	17	12.5 ± 5.9	10.1 ± 3.1	0.57 ± 0.25	0.42 ± 0.26
	18	16.1 ± 1.4	9.2 ± 2.5	0.69 ± 0.10	0.92 ± 0.28
	19	28.5 ± 6.3	21.7 ± 5.6	0.73 ± 0.21	0.48 ± 0.29
	20	12.2 ± 1.1	9.5 ± 0.9	0.81 ± 0.29	0.61 ± 0.24
Post-partum Day	2	12.2 ± 3.2	9.6 ± 2.2	0.87 ± 0.16	0.57 ± 0.34

Activity measured at 37°C and pH 6.5 using 1 mM 1 chloro-2,4-dinitrobenzene and 1 mM glutathione as substrates. Specific activity is expressed as μmole of conjugated product/min/mg protein. Each value represents mean ± SE of five determinations performed individually with microsomal preparation from mouse non pooled organs.

the other hand, in DBA/2 mice, the hepatic EH activity remained close to the control level. The pulmonary EH activity did not significantly change in either strain.

c) Glutathione-S-transferase [GST]

Table II shows the variations in liver, and lung GST activities. In both strains, hepatic GST activity showed a parallel pattern with a burst on gestational Day 19, reaching twice the control level, returning to normal at Day 20. In the lung of C57Bl/6 mice, this activity increased gradually throughout the second moiety of gestation, whereas in DBA/2 mice, this enzymic system was not stimulated except for one possible peak at Day 18 of pregnancy.

DISCUSSION

These results indicate that mouse maternal liver and lung drug metabolizing activities increase during pregnancy. For this study, we chose two strains of mice for which numerous genetic data are available, in particular, on the induction pattern by polycyclic aromatic hydrocarbons. The first,C57Bl/6, is highly responsive to inducibility of aryl-

hydrocarbon hydroxylase activity by these chemicals, while the second, DBA/2, shows a lack of inducibility (16). In both strains, hepatic mono-oxygenase activity such as B(a)PH was highly stimulated several days before parturition while stimulation of EH activity occurred during the final days of gestation. The gestational enzymic patterns suggest that "stimulation" of oxidative pathways could be correlated with hormonal variations. It is well known that induction of hepatic cytochrome P-450 is regulated by natural and synthetic steroids and the role of corticosteroids in the maintenance and induction of cytochromes P-450 in cell culture has recently been shown (17,18,19). Unfortunately, to our knowledge the regulation by other endocrine products has not yet been studied.

Our results fit an induction pattern where the endogenous factors would act as regulators or suppressors of drug metabolizing enzymes. The abrupt decrease observed after the peak of activity for B(a)PH could be due to the well known rapid turnover of cytochrome P-450 forms (20), the half life of which may vary from a few hours to a few days. The induction of heme biosynthesis and δ-aminolevulinate synthetase by progesterone is well known *in vivo* or in cultured chick embryo liver cells (21) and may similarly occur in mice. B(a)PH is readily induced by many xenobiotics such as polycyclic aromatic hydrocarbons and 5,6-benzoflavone in C57Bl/6 mice ; on the other hand, these compounds do not induce B(a)PH in DBA/2 (22,23), a more active inducer such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin, for instance being necessary. In the present work, no great difference in induction between the two strains during pregnancy was seen, thus involving another mechanism of induction by endogenous factors. Soyka and Long (24) have shown that pregnenolone and related metabolites of progesterone inhibit drug demethylation and hydroxylation *in vitro*. Soyka and Deckert (25) have found that progesterone and its metabolites, as well as structurally similar steroids such as 18 pregnene or pregnane, were potent inhibitors of a model microsomal drug metabolizing system : para nitroanisole demethylase ; they proposed that inhibition of hepatic mono-oxygenase by pregnane steroids may play a physiological role in intact organs. Such an inhibition by progesterone or its metabolites could explain the decrease in hepatic mono-oxygenase activity occurring a few days before parturition, despite the fact that these activities are measured *in vitro*. Destruction of cytochrome P-450, as observed with oral contraceptive agents having a 17 α-ethinyl substituant, is less likely, since this kind of substrate acts as a suicide inhibitor for the heme of cytochrome P-450 (26, 27, 28), which is not the case for natural steroids. Inhibition of mono-oxygenase activities by insaturated phospholipids had also been previously emphasized to account for the low activity found in

pregnant and lactating rats as compared to male rats (29). The lipogenesis in females may be enhanced as a result of their high level of estrogenic hormones. Accordingly, such an explanation could also be valid for mice.

Oesch (30) has shown that the onset of inducibility of benzo(a) pyrene hydroxylase and epoxide hydrolase activities occurred at different times during pregnancy. But no systematic study of EH activity in pregnant animals has been published. The observation that EH activity is highly stimulated only in C57Bl/6 mice is intriguing ; it is known that several endogenous steroid 16α and 17α-epoxides are excellent substrates for EH (31, 32, 33), and Oesch has suggested that certain steps in the biosynthesis and biodegradation of steroid hormones were catalyzed by EH. But the fact that DBA/2 mice have an unmodified EH level shows that this phenomenon is not general. This suggests that endogenous inducibility of EH activity is crucial in pregnant C57Bl/6 mice, but not in pregnant DBA/2 mice.

For glutathione-S-transferase activity, the report by Schoemaker *et al.* (34) that this enzyme reaches maturity after 9 weeks dictated the choice of females aged 10-12 weeks, and the stimulation observed during pregnancy was presumably due to endogenous gestational factors, since no variation was observed in control mice of the same age. Our results are similar to those obtained for rat liver GST during pregnancy (35). Thus, these modifications in drug metabolizing enzymes should be accounted for when administering drugs during gestation, and may be of importance in the teratogenic effect of certain chemicals. Our experimental results irrefutably confirm that drug metabolizing enzymes are controlled by hormonal status and that endogenous compounds involved in the developmental stage may interfere with enzymic activities in mammals.

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