

OXIDATIVE METABOLISM OF XENOBIOTICS DURING PREGNANCY:  
SIGNIFICANCE OF MICROSOMAL FLAVIN-CONTAINING MONOOXYGENASE

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**SUMMARY:** Pregnancy related changes in oxidative metabolism of model substrates were examined in CD<sub>1</sub> mice. As compared to nonpregnant females, a significant decrease in the hepatic microsomal aminopyrine-but not in dimethylaniline-N-demethylase activity was observed in pregnant mice. The rates of microsomal flavin-containing monooxygenase-catalyzed N-oxidation of dimethylaniline remained relatively unchanged during pregnancy in the liver, lung, kidney, and uterus. In contrast to this, N-oxidase activity of placental microsomes was increased nearly 5-fold when measured at day 12 and 18 of gestation.

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While most investigations on the oxidative metabolism of xenobiotics have concerned the microsomal heme-containing cytochrome P-450 system, several reports have also established the role of the MFMO in the N- and S-oxidation of xenobiotics including carcinogens, drugs, and pesticides (1-3). Using DMA as a model substrate, MFMO activity has been demonstrated in many animal species (4). Reports indicate that it is present in many tissues including adrenal, bladder mucosa, corpus luteum, liver, lung, kidney, thymus, and thyroid (4). In contrast to the several reports (5-9) indicating a general depression of the microsomal cytochrome P-450-dependent monooxygenase system during pregnancy, there is only one report on the changes in MFMO activity during pregnancy (10). In this report, Devereux and Fouts (10) observed no change in the liver MFMO but detected a 57% increase in lung MFMO activity in 28 day pregnant rabbits. The purpose of our work was to examine MFMO activity in different tissues of mice during pregnancy. We are also

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Abbreviations: MFMO, microsomal flavin-containing monooxygenase (E.C.1.14.13.8); DMA, N, N-dimethylaniline; AP, aminopyrine

reporting for the first time significant amounts of MFMO activity in uterine and placental microsomes. In addition, data on the changes in the rates of hepatic microsomal N-demethylation of DMA and AP during pregnancy in mice are presented.

#### MATERIALS AND METHODS

##### Animals:

CD<sub>1</sub> mice purchased from Charles River Breeding Labs., Wilmington, MA were used. Mice were maintained on a 12 hour day/night cycle and had access to food and water ad libitum. Presence of a vaginal plug marked day 0 of gestation.

##### Tissue Preparation:

Immediately after sacrifice by cervical dislocation, tissues were removed and homogenized in a medium containing ice-cold 0.25 M sucrose, 50 mM Tris, pH 7.4, and 0.1 mM EDTA. The homogenate was centrifuged at 9000 g for 20 minutes and the supernatant decanted and centrifuged at 105,000 g for 60 minutes. The microsomal pellet was suspended in 100 mM glycine, 25 mM phosphate buffer, pH 8.4. Protein estimation was by the biuret method (11).

##### Enzyme Assays:

MFMO activity of microsomes was determined by measuring the rate of DMA N-oxide formation according to the method described by Ziegler and Petit (12). DMA was purchased from Aldrich Chemical Co., Milwaukee, WI, and was redistilled prior to use. Incubation medium (total volume 1.0 ml) consisted of 100 mM glycine, 25 mM phosphate buffer, pH 8.4, 2.4 mM N-octylamine, 2.0 mg microsomal protein, and NADPH generating system consisting of 2.5 mM glucose-6-phosphate, 1.0 mM NADP, and 1 unit of glucose-6-phosphate dehydrogenase. Following preincubation at 37° with shaking for 3 min., reactions were started with the addition of DMA (1.0 mM). Incubations were for 10 min. with shaking at 37°.

DMA- and AP-demethylase activities were estimated in the absence of n-octylamine using incubation media (1.0 ml total volume) containing 100 mM Tris buffer pH 7.4, 1.0 mg microsomal protein, NADPH generating system as described above, and either 1.0 mM DMA or 5.0 mM AP. Following incubation for 10 min. (DMA) or 30 min. (AP) at 37°, reactions were terminated with 1.5 ml ice-cold 12.5% TCA. The amount of formaldehyde produced was measured by the Nash method (13). The non-enzymatic controls consisted of standard incubations with the appropriate buffer in place of microsomal suspensions. Statistical analysis was by one way analysis of variance and Bonferonni comparison of group means (14).

#### RESULTS AND DISCUSSION

Table 1 shows MFMO activity in various extrahepatic tissues of pregnant and nonpregnant mice. Pregnancy had no statistically significant effect on MFMO in either the lung, kidney, or uterus, although some decline in activity was observed in the latter two tissues. The fact that we did not find an increase in MFMO activity in lung microsomes from pregnant mice as reported for rabbits (10) suggests a species difference in either the MFMO enzyme molecule itself and/or in the endogenous modifying factors affecting lung

**Table 1.** Changes in MFMO activity of the mouse extrahepatic tissues during pregnancy. Assays were carried out as described under Materials and Methods. MFMO activity is expressed as nmoles of DMA N-oxide formed/min/mg microsomal protein. Values represent mean  $\pm$  S.E. (n). Relative MFMO activities are given in parentheses below means.

Tissue	MFMO Activity		
	Non-pregnant	Pregnant	
		Day 12	Day 18
Uterus	0.59 $\pm$ 0.13 (5) (100)	0.26 $\pm$ 0.04 (5) (44)	0.43 $\pm$ 0.18 (5) (73)
Kidney	1.44 $\pm$ 0.08 (5) (100)	0.77 $\pm$ 0.18 (5) (53)	1.26 $\pm$ 0.26 (5) (88)
Lung	1.00 $\pm$ 0.03 (4) (100)	1.15 $\pm$ 0.11 (4) (115)	0.96 $\pm$ 0.02 (4) (96)
Placenta	--	0.29 $\pm$ 0.03 (8) (100)	1.40 $\pm$ 0.02*(7) (483)

\*Significantly different,  $P < 0.05$

MFMO activity during pregnancy. In addition to these tissues, we have detected significant N-oxidase activity in the mouse placenta. The activity was low at day 12 of gestation, but increased nearly five-fold reaching a level that was about 50% of maternal liver MFMO (Table 2) by day 18 of gestation.

In vitro studies of placental xenobiotic oxidation are complicated by the hemoglobin contamination of microsomes. Available information, however, suggests that hemoglobin-mediated oxidation is not a general phenomenon applicable to all the xenobiotics undergoing oxidation; but is rather substrate specific (15). Furthermore, due to poor affinity, very high substrate concentrations are required to observe low measurable activity. Thus, in contrast to 1.0 mM DMA employed in this study, Blisard and Mielal (16) reported a need to use 40 mM aniline to measure p-hydroxylase activity of hemoglobin. We have determined that in standard tissue-free DMA incubations hemoglobin could catalyze the formation of 0.29 nmoles of DMA N-oxide/min/mg hemoglobin. Despite expected substantial variation in the degree of

Table 2. Changes in the mouse hepatic microsomal monooxygenase activities during pregnancy. Assays were carried out as described under Materials and Methods. MFMO activity is expressed as nmoles of DMA N-oxide formed/min/mg microsomal protein while N-demethylase activities are expressed as nmoles of formaldehyde produced/min/mg microsomal protein. Values represent mean  $\pm$  S.E. (n). Relative activities are given in parentheses below means.

Status	MFMO	DMA N-demethylase	AP N-demethylase
Non-pregnant	2.77 $\pm$ 0.15 (13) (100)	1.79 $\pm$ 0.11 (5) (100)	6.81 $\pm$ 0.28 (11) (100)
Pregnant (Day 12)	3.14 $\pm$ 0.08 (11) (113)	1.70 $\pm$ 0.08 (9) (95)	5.73 $\pm$ 0.32*(13) (84)
Pregnant (Day 18)	2.66 $\pm$ 0.15 (12) (96)	1.98 $\pm$ 0.20 (5) (111)	5.44 $\pm$ 0.28* (9) (80)

\* Significantly different from non-pregnant,  $P \leq 0.01$ .

hemoglobin contamination, fairly uniform MFMO activities were found in the different preparations of placental microsomes (Table 1). In view of these points and the fact that MFMO specific activity increased markedly toward the end of gestation strongly suggest that the observed placental DMA N-oxide formation is chiefly catalyzed by the constitutive microsomal MFMO and not by the contaminating hemoglobin. These observations are noteworthy since, to our knowledge, MFMO activity has not been reported in placental microsomes of any animal species before. More importantly, placental MFMO activity near term appears to be quite high compared to the barely detectable levels reported for microsomal cytochrome P-450 dependent xenobiotic metabolizing activities (17). This suggests that MFMO represents a major oxidative pathway in the placenta during pregnancy and may perform an important role in xenobiotic metabolism in an otherwise oxidative deficient fetal-placental unit. These results are important since DMA-N-oxide has been shown to cause methemoglobinemia in mammals (18). Placental formation of DMA-N-oxide may have toxicologic implications to the fetus since fetal hemoglobin has been shown to be more readily oxidized to methemoglobin than is adult and the fetus is recognized to be deficient in NADH-methemoglobin reductase (19).

The data on the changes in the levels of hepatic microsomal MFMO, DMA N-demethylase, and AP N-demethylase activities are given in Table 2. Although as compared to nonpregnant mice the mean MFMO activity appears to increase at day 12 of gestation, the difference is not statistically significant. Likewise, there was no change in DMA N-demethylase activity during pregnancy. This is in contrast to AP N-demethylase activity which was decreased significantly (20 per cent) in pregnant mice on day 12 and 18 of gestation as compared to nonpregnant mice. Earlier, Feuer and Kardish (9) as well as Dean and Stock (6) also observed a decrease in AP N-demethylation in pregnant rats. Although purportedly a cytochrome P-450-dependent reaction, it appears that the particular species of cytochrome P-450 responsible for the demethylation of DMA may not be affected by pregnancy. Alternatively, in mouse liver microsomes, formation of the N-oxide by MFMO prior to demethylation (12) may be the rate-limiting step for the release of formaldehyde to the extent that changes in the cytochrome P-450-dependent demethylase may not affect the overall demethylation of DMA.

Turcan *et al.* (20) have shown a significant decrease in the proportion of cytochrome P-450 in the high spin state in microsomes from pregnant rats. The spin state of the cytochrome P-450 is important in overall metabolism since a shift to a lower spin state may alter the redox potential of the NADPH cytochrome P-450 reductase-hemoprotein coupling causing a reduction in electron flow to the hemoprotein and decreased metabolism of substrates (21). In contrast to the cytochrome P-450, the MFMO may not be sensitive to those modulators that changes the spin state of the cytochrome P-450 hemoprotein.

There are several reports on the effect of pregnancy-induced changes in the hepatic microsomal phospholipid composition that leads to altered rates of cytochrome P-450-mediated xenobiotic metabolism (9, 22). The importance of phospholipids in cytochrome P-450-dependent oxidative reactions is well established (23, 24). In contrast, phospholipid is not an absolute requirement for activity of MFMO (25). Hence pregnancy-related changes in phospholi-

pids could conceivably affect cytochrome P-450-related metabolism but not that mediated by MFMO.

An elevation of serum levels of progestational hormones has been noted during pregnancy (22). Evidence that steroid hormones play an important role in regulating hepatic microsomal cytochrome P-450 dependent xenobiotic metabolism is also available. Inhibition of oxidative metabolism of AP, aniline, and hexobarbital (26, 27) as well as stimulation of biphenyl 2-hydroxylation (28) is known.

Our work suggests that, unlike the cytochrome P-450 system, MFMO may not be inhibited by high in vivo concentrations of progestational steroids. This is consistent with the report of Duffel et. al. (29) that progesterone treatment of gonadectomized female mice did not elevate microsomal DMA N-oxidase activity in either kidney, liver, or lung above that of gonadectomized controls. Exactly how high serum levels of the progestational hormones present during pregnancy relate to the effects we see in vitro is uncertain. Devereaux and Fouts (10) noted that microsomal progesterone levels did not differ between pregnant and non pregnant rabbits, however they did not measure levels of progesterone precursors or its metabolites. Indeed some of the progesterone metabolites have been shown to be more effective inhibitors of cytochrome P-450 catalyzed reactions than progesterone itself (27). Whether the same is true in the mouse is presently unknown and further studies are necessary to clarify this point.

Hepatic microsomes possess high affinity binding sites for steroid hormones (30, 31) and it is suggested that the observed binding does not reflect binding to steroid metabolizing enzymes; rather it represents an interaction with effector sites which may modulate microsomal metabolism (3). Our preliminary observations that in vitro addition of either pregnenolone or progesterone results in stimulation rather than inhibition of hepatic microsomal MFMO activity (unpublished results) are consistent with this view.

In conclusion, our data suggest that:

- a. The depression in cytochrome P-450-mediated metabolism of xenobiotics reported previously (5-9) is not a general phenomenon but is specific for certain substrates.
- b. MFMO activity of different tissues does not decline during pregnancy, hence overall in vivo oxidation of the compounds metabolized by both MFMO and cytochrome P-450 may not alter in pregnant animals.
- c. MFMO is present in the mouse placental microsomes and its titer increases toward term. Therefore, metabolism by MFMO may represent one of the important determinants of fetal exposure to environmental chemicals.

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