REFERENCES

1. A. Baisset, P. Foulhoux and P. Montastruc, *Therapie* **31**, 659 (1977).
2. M. C. Auclair, S. Dechezlepretre, H. Lauressergues, M. Lemeignan and P. Lechat, *Therapie* **31**, 647 (1976).
3. A. Di Pietro, C. Godinot, C. Vial, D. Gautheron and P. Foulhoux, *Biochem. Pharmac.* **26**, 1775 (1977).
4. A. Di Pietro, C. Godinot, D. Gautheron and P. Foulhoux, *Biochem. Pharmac.* **26**, 1021 (1977).
5. J. B. Chappell, *Biochem. J.* **90**, 225 (1964).
6. J. Lenard, C. Wong and R. Compans, *Biochem. biophys. Acta* **332**, 341 (1974).
7. J. Raison, *Bioenergetics* **4**, 285 (1973).
8. J. Raison and E. McMurchie, *Biochem. biophys. Acta* **363**, 135 (1974).
9. P. Overath, L. Thilo and H. Träuble, *Trends Biochem. Sci.* **1**, 186 (1976).
10. C. Hockenbrock, M. Höchli and R. Chau, *Biochem. biophys. Acta* **455**, 466 (1976).

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Effect of dexamethasone treatment on *N*,*N*-dimethylaniline demethylation and *N*-oxidation in pulmonary microsomes from pregnant and fetal rabbits

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In a previous report [1] we looked at the effects of pregnancy and the administration of certain steroids to nonpregnant adult rabbits on *N*,*N*-dimethylaniline (DMA) metabolism by rabbit liver and lung microsomes. In that study, we showed that microsomal DMA *N*-oxidase and demethylase activities in lung were higher in pregnant rabbits than in adult nonpregnant controls. We are reporting here the effects of animal pretreatment with dexamethasone on pulmonary microsomal DMA demethylase and *N*-oxidase activity in pregnant and fetal rabbits. In this study, we wanted to determine if the elevated levels of drug metabolism that we saw in pulmonary microsomes from pregnant rabbits would be affected by steroid treatment. Our effort was also directed toward determining if fetal lung drug metabolism would be affected by steroid treatment of the pregnant doe, and, if so, at what stages of gestation. We used the steroid dexamethasone, which is known to affect lung maturation [2, 3], and which we had shown to have a stimulatory effect on DMA metabolism in pulmonary microsomes from adult nonpregnant rabbits [1].

Dutch Belt rabbits (Arrow Farms, Statesville, NC) were used in this study. The rabbits, housing conditions, and microsomal preparations were the same as reported previously [4].

N,*N*-dimethylaniline was obtained from Fisher Scientific Co. (Pittsburgh, PA) *N*,*N*-dimethylaniline *N*-oxide (DMAO) was prepared by the method of Craig and Purushothaman [5]. Solutions of DMA and DMAO were made up as described previously [1]. Dexamethasone acetate was obtained from Sigma Chemical Co. (St. Louis, MO). Emulphor EL 620 (polyethoxylated vegetable oil) was supplied by GAF Corp. (New York, NY). All other chemicals were reagent grade and were obtained from commercial sources.

Dexamethasone acetate was administered to pregnant rabbits in three daily s.c. doses or a single s.c. dose of 2 mg/kg in Emulphor–ethanol–water (3:3:4). Control rabbits received Emulphor–ethanol–water (3:3:4) in a dose of 0.5 ml/kg (the same as treated animals). The animals were killed on day 4. In one set of experiments, pregnant rabbits were treated with dexamethasone on day 21 or days 21, 22 and 23 of pregnancy and then killed on day 24 of pregnancy. In the other set of experiments, the steroid was administered to the rabbits on days 24, 25 and 26 of pregnancy and the rabbits were killed on day 27.

The amount of protein in the microsomal fractions was measured by the method of Lowry *et al.* [6]. The methods for assaying DMA demethylase and *N*-oxidase activities have been reported previously [4].

The experimental data were analyzed statistically utilizing two-sided Student's *t*-tests.

The effect of dexamethasone acetate pretreatment of animals on DMA *N*-oxidation and demethylation in pulmonary microsomes from 24-day pregnant rabbits and 24-day fetal rabbits is shown in Table 1. Dexamethasone treatment had no effect on DMA demethylase or *N*-oxidase activities in pulmonary microsomes of the pregnant rabbits. Treatment of pregnant does with dexamethasone had little or no effect on the low microsomal DMA metabolism seen in the 24-day fetal rabbit lung. In an earlier report [7], we showed the first appearance of measurable DMA demethylase and *N*-oxidase activities in fetal pulmonary microsomes at about 24 days of gestation.

We have shown previously that maternal pulmonary microsomes from 27- to 28-day pregnant rabbits have DMA *N*-oxidase and demethylase activities about 50 per cent above control levels [1]. Dexamethasone pretreatment of pregnant does did not alter these elevated enzyme activities in the maternal lung microsomes of the 27-day pregnant rabbits (Table 1). However, these data show that dexamethasone treatment caused significant increases in DMA *N*-oxidase and demethylase activities in pulmonary microsomes of 27-day fetal rabbits; pulmonary demethylase activity was increased 2.8 times and *N*-oxidase was increased 1.5 times the control activity (fetal rabbits from untreated pregnant rabbits).

In a previous study [1] we showed that treatment of adult nonpregnant rabbits with dexamethasone caused significant increases in DMA demethylase and *N*-oxidase activity in pulmonary microsomes. However, the treatment of pregnant rabbits with dexamethasone, as seen in these experiments, had no further effect on the already elevated levels of these enzymes.

In the experiments with fetal rabbits, it was shown that treatment of the pregnant rabbit by dexamethasone caused

Table 1. Effect of dexamethasone acetate pretreatment of animals on DMA *N*-oxidation and demethylation in lung microsomes from 24-day and 27-day pregnant rabbits and 24-day and 27-day fetal rabbits*

Expt.	Origin of lung microsomes	Demethylase (nmoles HCHO/mg protein/min)	*N*-oxidase (nmoles DMAO/mg protein/min)
I	24-day pregnant rabbits, untreated	9.5 ± 1.8 (4)	11.3 ± 1.2 (4)
	24-day pregnant rabbits, treated	9.5 ± 1.5 (4)	12.6 ± 0.9 (4)
II	24-day fetuses, mother untreated	0.12 ± 0.02 (0.13, 0.10)	0.40 ± 0.28 (0.60, 0.21)
	24-day fetuses, mother treated	0.15 ± 0.03 (0.17, 0.13)	0.47 ± 0.10 (0.40, 0.54
III	24-day fetuses, mother untreated	0.16 ± 0.02 (3)	0.31 ± 0.04 (3)
	24-day fetuses, mother treated	0.16 ± 0.05 (3)	0.36 ± 0.04 (3)
IV	27-day pregnant rabbits, untreated	13.7 ± 1.1 (4)	14.8 ± 2.2 (4)
	27-day pregnant rabbits, treated	15.3 ± 2.6 (4)	15.1 ± 1.3 (4)
V	27-day fetuses, mother untreated	0.29 ± 0.06 (4)	0.80 ± 0.17 (4)
	27-day fetuses, mother treated	0.81 ± 0.05† (4)	1.22 ± 0.27‡ (4)

* Dexamethasone acetate treatment—Exp. I and II: 2 mg/kg (4 mg/ml) s.c. in Emulphor-alcohol-water (3:3:4), 0.5 ml/kg on days 21, 22 and 23 of pregnancy; rabbits were killed on day 24. Exp. III: same dose but administered on day 21 of pregnancy only; rabbits were killed on day 24. Exp. IV and V: 2 mg/kg (4 mg/ml) s.c. in Emulphor-alcohol-water (3:3:4), 0.5 ml/kg on days 24, 25 and 26 of pregnancy; rabbits were killed on day 27. Values are mean ± S.D. Numbers in parentheses are numbers of samples with actual values given for Exp. II. Individual adult lungs or pools of 6–11 fetal lungs were used.

† Treated is significantly different from control ($P < 0.01$).

‡ Treated is significantly different from control ($P < 0.05$).

induction of drug metabolism in the 27-day fetus, but not in the 24-day fetus. As binding receptors for dexamethasone are known to be present in rabbit fetal lungs as early as 21 days of gestation [3], the binding of dexamethasone does not seem to be the only factor necessary for its effect on drug-metabolizing activity. Since DMA metabolism is not measurable in fetal pulmonary microsomes by these methods until day 24 of gestation, the enzyme systems involved may not be adequately developed to respond to the inductive effect of dexamethasone. It is possible that the receptors responsible for the inductive effect of dexamethasone may be different from the dexamethasone receptors that result in lung maturation; prior to day 24 of gestation, the receptors for enzyme induction may not be responsive to dexamethasone.

In summary, we have shown that treatment of pregnant rabbits with dexamethasone can increase the microsomal drug-metabolizing enzyme activities of fetal lung late in its development. However, the already elevated levels of drug-metabolizing enzymes from maternal pulmonary microsomes seen during late pregnancy are unchanged by this treatment. This study suggests that hormone balance in the pregnant animal can be important to the chemical metabolism of the lungs of the developing fetus. Disturbances in this hormone balance, while possibly not affecting the mother, could seriously affect the fetus during certain parts of its development.

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REFERENCES

1. T. R. Devereux and J. R. Fouts, *Drug Metab. Dispos.* **3**, 254 (1975).
2. J. Giannopoulos, *J. biol. Chem.* **248**, 3876 (1973).
3. J. Giannopoulos, *Endocrinology* **94**, 450 (1974).
4. T. R. Devereux and J. R. Fouts, *Chem. Biol. Interact.* **8**, 91 (1974).
5. J. C. Craig and K. K. Purushothaman, *J. org. Chem.* **35**, 1721 (1970).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
7. J. R. Bend, M. O. Janes, T. R. Devereux and J. R. Fouts, in *Basic and Therapeutic Aspects of Perinatal Pharmacology* (Eds P. L. Morselli, S. Garattini and F. Serini), p. 229. Raven Press, NY (1975).