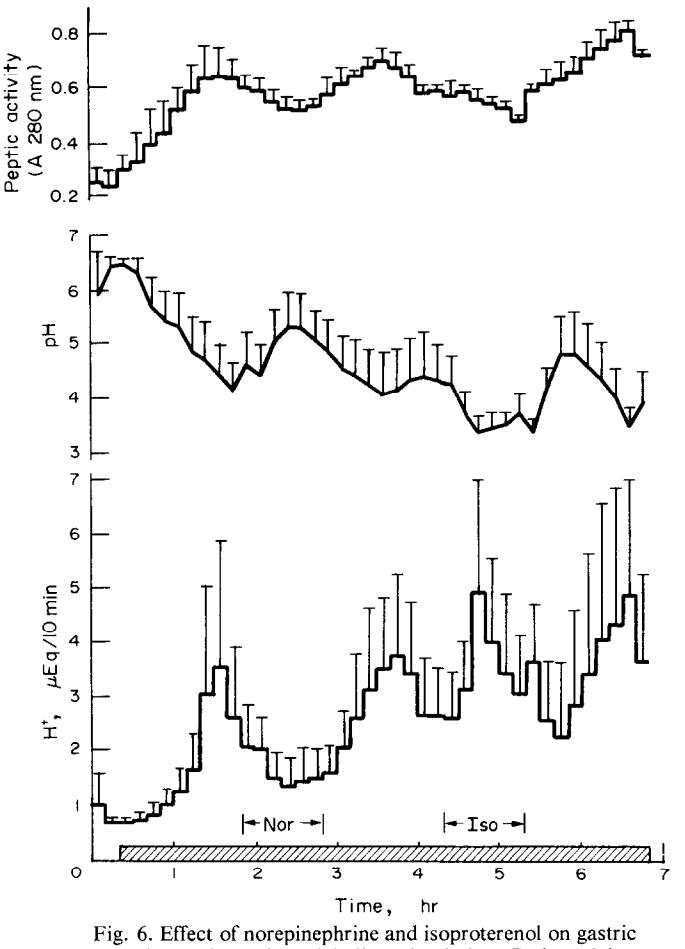


Fig. 6. Effect of norepinephrine and isoproterenol on gastric secretion under carbamylcholine stimulation. Carbamylcholine (2 μg/kg/hr) was infused during the period indicated by the hatched block. Norepinephrine (100 μg/kg/hr) and isoproterenol (100 μg/kg/hr) were infused during the time indicated by Nor and Iso respectively. The values represent the mean ± S.E. from four rats.

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## Lack of correlation between cortisol-induced precocious maturation of the fetal rabbit lung and drug metabolism

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Precocious lung maturation produced by prenatal administration of glucocorticoids has received considerable attention since it appears that these steroids may play an important role in the prevention of respiratory distress syndrome (hyaline membrane disease) in premature infants [1–3]. The syndrome is thought to be the result of a deficiency in pulmonary surfactant which is vital for stabilizing the lungs against collapse during expiration [3]. Morphological and biochemi-

cal studies in fetal rabbits have shown that glucocorticoids accelerate maturation of alveolar type II cells and induce enzymes involved in pulmonary phospholipid biosynthesis, thereby increasing pulmonary surfactant [2–10]. Glucocorticoid-mediated enzyme induction has also been demonstrated in fetal rat organs [2], and recently Wishart and Dutton [11, 12] have shown that hepatic UDP-glucuronyltransferase activity is increased by both cortisol and dexamethasone pretreatment. Developmental studies in the field of hepatic drug metabolism or mixed function oxidation (MFO) have demonstrated that MFO activities are very low in livers of fetal and newborn animals [13] and increase as hepatocytes mature morphologically [14, 15] to reach adult levels at some point near sexual maturation [16, 17]. Hence, the question we posed was: in view of the correlation between hepatocellular maturation and the magnitude of hepatic MFO activity, might the premature maturation of pulmonary type II cells produced by prenatal glucocorticoids be reflected by a precocious increase in pulmonary MFO activity?

Pregnant New Zealand white rabbits (3.4 to 4.5 kg; 20 days gestation ± 2 hr) were purchased from Dutchland Laboratory Animals, Inc., Denver, PA, and maintained on Purina laboratory chow and water *ad lib.* At 25-days gestation, the animals were anesthetized with ether and a midline laparotomy was performed. The fetuses in both uterine horns were injected intraperitoneally through the uterine wall with either 1 mg cortisol (Solu-Cortef, The Upjohn Co., Kalamazoo, MI) in 0.1 ml of 0.85% NaCl or an equal volume of NaCl solution. There was a mean of eight fetuses (range three to fifteen) per doe. The total duration of anesthesia was approximately 30 min.

On day 27 of gestation the doe was killed by a sharp blow to the neck. The fetuses were delivered by hysterotomy and killed immediately by cervical dislocation. Lungs and livers were quickly excised and rinsed in cold KCl–Tris buffer (150 mM KCl–50 mM Tris), pH 7.4. The lungs and livers of fetuses from two does were pooled and the fetal and maternal organs were blotted dry and weighed. No attempt was made to separate the fetuses by sex. Microsomes and cytosol (post-microsomal supernatant fraction) were then prepared essentially as described by Gram *et al.* [18] using an initial 5000 *g* (20 min) centrifugation of the homogenate.

All enzyme assays were conducted aerobically at 37° under zero-order kinetics regarding cofactor and substrate concentrations, and activities were linear with respect to enzyme concentrations and incubation time. Cytochrome P-450, protein and enzyme activities were determined as described previously [19, 20]. The lower left lobes of the lungs of 16 control and 19 cortisol-treated fetuses were fixed in Telly's solution (70% ethanol, 2% formaldehyde and 5% acetic acid), embedded in paraffin, and stained in 6-$\mu$ sections with hematoxylin and eosin for morphometric analysis. Each lung section was photographed at a magnification of 54 times, and a point grid was placed on at least four representative areas to quantitatively assess histologic maturation by determining the percentage of alveolar air space [21].

Administration of cortisol accelerated fetal lung maturation as indicated by an overall 16 per cent increase in alveolar air space (cortisol-treated: 32.9 ± 3.7 per cent vs control: 28.3 ± 4.2 per cent, $P < 0.002$). This confirms the morphologic effects of cortisol on prenatal lung previously reported by Kikkawa *et al.* [7]. However, no significant differences were found between cortisol and saline-treated fetal rabbits in pulmonary or hepatic drug-metabolizing enzymes (Table 1). It is noteworthy that, while this manuscript was in preparation, Possmayer *et al.* [22] also reported that cortisol was without effect on the NADPH-cytochrome *c* reductase activity in fetal rabbit lung.

In the present study employing fetal rabbits, the lack of induction of UDP-glucuronyltransferase either as "native" or "activated" enzyme activity contrasts with the reported induction of hepatic UDP glucuronyltransferase activity in fetal rats [11, 12]. In both adult and fetal rabbits, "native" and "activated" UDP-glucuronyltransferase activities in the lung were essentially undetectable as compared to the liver, where activation by UDP-*N*-acetylglucosamine increased activity approximately 2-fold (Table 1). Generally, enzyme activities were significantly greater in fetal livers than in fetal lungs with one exception; pulmonary *N*-acetyltransferase activity was 2- to 3-fold greater than in the liver. This is in contrast to adult male [23] and female (see Table 1) animals in which the *N*-acetyltransferase activities of the two organs are very similar. Cytochrome P-450 and aminopyrine *N*-demethylase and biphenyl 4-hydroxylase activities were undetectable in fetal rabbit lung and liver at 27-days gestation. There were no differences in the enzyme activities of maternal organs between the cortisol and saline-treated groups.

Chatterjee *et al.* [24] have reported that microsomal vesicles from fetal rat liver sediment between 200 and 8000 *g*, that is, at lower forces than are required to sediment vesicles from adult rat liver (9000–105,000 *g*). In order to rule out this possibility as a complication in the present investigation, an additional experiment was conducted. Aliquots of the fetal lung and liver homogenates were centrifuged initially at either 600, 5000 or 9000 *g* and the supernatant fractions were centrifuged at 105,000 *g* (60 min) to prepare microsomes and cytosol. Enzyme activities were determined as described above. There were no differences in the specific activities of NADPH-cytochrome *c* reductase, *N*-acetyltransferase and glutathione *S*-aryltransferase among the three methods of preparation. Cytochrome P-450, aminopyrine *N*-demethylase and biphenyl 4-hydroxylase remained undetectable in any of the preparations.

It is thought that, of more than 40 different cell types in the

Table 1. Pulmonary and hepatic drug-metabolizing enzyme activities following cortisol administration to fetal rabbits *in utero* *

| Parameter | Saline-treated fetuses | | Cortisol-treated fetuses | | Maternal organs | |
|---|---|---|---|---|---|---|
| | Lung+ | Liver | Lung+ | Liver | Lung | Liver |
| Protein | | | | | | |
| Microsomal | 5.5 ± 1.1 | 14.0 ± 3.0 | 5.9 ± 1.3 | 13.9 ± 2.8 | 8.7 ± 0.6 | 28.6 ± 7.6 |
| Supernatant | 32.9 ± 8.2 | 58.1 ± 1.6 | 38.6 ± 4.8 | 59.0 ± 9.0 | 58.3 ± 6.9 | 76.5 ± 5.6 |
| NADPH-cytochrome *c*-reductase | 14.1 ± 2.7 | 24.6 ± 1.0 | 17.1 ± 3.1 | 27.8 ± 4.4 | 98.1 ± 30.8 | 114.5 ± 46.9 |
| *N*-acetyltransferase | 0.63 ± 0.20 | 0.21 ± 0.14 | 0.47 ± 0.14 | 0.19 ± 0.02 | 0.97 ± 0.18 | 1.38 ± 0.19 |
| UDP-glucuronyltransferase | | | | | | |
| "Native" | 0.05 ± 0.03 | 0.59 ± 0.04 | 0.02 ± 0.02 | 0.51 ± 0.10 | 0.05 ± 0.05 | 1.78 ± 0.46 |
| "Activated" | 0.07 ± 0.04 | 1.07 ± 0.13 | 0.09 ± 0.08 | 0.96 ± 0.18 | 0.09 ± 0.04 | 4.1 ± 0.8 |
| Glutathione *S*-aryltransferase | 2.5 ± 0.6 | 3.4 ± 0.3 | 2.1 ± 0.03 | 3.5 ± 0.2 | 5.1 ± 1.0 | 12.6 ± 1.8 |

* Data are reported as the mean ± S.D. of four determinations. Values are expressed as mg protein/g of tissue for the protein concentration and as nmoles product formed/mg of protein/min at 37° for the enzyme activities.

+ All values are significantly different from corresponding values for liver ($P < 0.05$).

lung [25], the drug-metabolizing enzymes probably reside in either the type II alveolar cells or the Clara cells, since these have abundant endoplasmic reticulum [26]. Following cortisol administration, the type II cells mature earlier, and this correlates with increased lung phospholipid content [7]. In contrast, Wang *et al.* [6] report that at the same gestational age the Clara cells in fetal lungs are not well developed, do not respond to glucocorticoid treatment and are less likely to be involved in secreting surface-active compounds. It appears that, in the 27-day fetal rabbit, cortisol acts to induce enzymes involved in lung phospholipid synthesis specifically in type II cells without influencing the drug-metabolizing enzymes. Thus, the enzymes responsible for metabolizing drugs may not reside in the type II cells at all, or the appearance of these enzymes in fetal rabbits is under some other control mechanism. Localization of drug-metabolizing enzymes within Clara cells, however, cannot be ruled out on the evidence presently available. In this regard, it is interesting that Clara cells have been implicated recently as the site of oxidative metabolism in the lung of 4-ipomeanol [27]. Whether the pulmonary drug-metabolizing capacity is confined to the Clara cells and whether these cells are capable of metabolizing other xenobiotics have yet to be determined. Experiments in progress in our laboratory are aimed at determining the ability of isolated pulmonary type II cells to metabolize drugs.

The results presented in this communication indicate strongly that, although cortisol plays a role in accelerating lung maturation in the rabbit fetus, it does not induce fetal pulmonary or hepatic drug-metabolizing enzymes in this species.

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## Effects of prazosin on cyclic nucleotide content and blood pressure of the spontaneously hypertensive rat

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The involvement of abnormal levels of cyclic nucleotides in the pathophysiology of hypertension has been suggested by several workers [1–7]. This idea fits the concept that intracellular levels of cyclic nucleotides are one of the determinants of vascular smooth muscle tone [8]. Prazosin, a potent antihypertensive agent, was developed as an inhibitor *in vitro* of cyclic 3′,5′-nucleotide phosphodiesterase (EC 3.1.4.17) (PDE) [9]. It has been proposed that this drug leads to a reduction of blood pressure by raising vascular smooth muscle intracellular cyclic 3′,5′-adenosine monophosphate (cyclic AMP). The development of prazosin has recently been cited as an example of rational drug development [10]. However, the effects of this drug on cyclic nucleotide levels *in situ* have not been reported. We have studied the effects of prazosin on cyclic nucleotide levels in the aorta and aortic smooth cells in tissue culture from the