PREFACE

The seminal observation by Liggins in 1969 that glucocorticoid treatment of fetal lambs resulted in enhanced lung maturation initiated the concept that the fetal lung is a hormonally responsive organ. During the past thirty years, great progress has been made in defining the roles of steroid, peptide, and polypeptide hormones in lung branching morphogenesis, differentiation of specialized cell types, and surfactant synthesis. In addition to glucocorticoids, it is apparent that the sex steroids, retinoids, catecholamines, prostaglandins, and peptide and polypeptide hormones, including a number of growth factors and cytokines, influence lung growth and differentiation as well as surfactant synthesis. Whereas the steroids and certain polypeptide hormones are delivered to lung through the systemic circulation, growth factors are produced locally by mesenchymal cells surrounding the developing lung buds, by type II epithelial cells, or by their precursors. Additionally, a variety of bioactive peptides are produced by innervated clusters of neuroendocrine cells that lie within the primitive airway epithelium.

Endocrinology of the Lung: Development and Surfactant Synthesis contains contributions from investigators studying the actions of the various classes of endocrine, paracrine, and neuroendocrine factors on lung development and surfactant synthesis. The model systems used in their studies range from whole animals to organ and cell culture and to transgenic, genetically altered, and gene-targeted mice. The first seven chapters are devoted to the actions of glucocorticoids on lung development and on the synthesis of surfactant glycerophospholipids and the surfactant proteins—SP-A, SP-B, and SP-C. Included in this group is a chapter on the role of the major histocompatibility complex (MHC) locus in glucocorticoid responsiveness, as well as one that addresses the role of corticotropin-releasing hormone (CRH) and glucocorticoids in lung development and surfactant synthesis using CRH gene-targeted mice. Two chapters address the actions of hormones that bind to other members of the nuclear receptor family; one is concerned with mechanisms that underlie the sexual dimorphism of fetal lung maturation and sex differences in responsiveness to perinatal glucocorticoid administration, while the other is concerned with the roles of retinoids and their receptors in lung development, surfactant synthesis, and the repair of lung injury in human premature newborns. Another chapter deals with fetal lung maturation and surfactant synthesis in the diabetic pregnancy and the effects of insulin on the synthesis of surfactant lipids and proteins. The remaining six chapters review the importance of cell–cell interactions and elaborate on various growth factors and bioactive peptides in lung branching morphogenesis, cell differentiation, gene expression, and pulmonary pathophysiology. The use of transgenic and gene-targeted mice to define the roles of members of a number of growth-factor families and their receptors in the regulation of lung morphogenesis and cellular differentiation also is addressed.

It is therefore apparent that lung growth, differentiation, and surfactant production are controlled by a variety of circulating and locally produced hormones and growth factors that exert their effects via endocrine, paracrine, autocrine, neuroendocrine, and possibly intracrine mechanisms. In light of the importance of circulating hormones and of growth

factor-mediated cellular interactions in lung growth, cell differentiation, function, and pathophysiology, it is my hope that *Endocrinology of the Lung: Development and Surfactant Synthesis* will have appeal, not only to pulmonary biologists, but also to those working in the areas of hormone action, and developmental and cell biology of other organ systems.

I would like to express my sincere appreciation to the contributors who have collaborated to make this a comprehensive review of the lung as an endocrine-responsive organ.

Carole R. Mendelson

2 Fetal Responses to Glucocorticoids

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Contents

ANTENATAL GLUCOCORTICOIDS FROM A CLINICAL PERSPECTIVE

This chapter focuses primarily on the 30 years of often conflicting responses of intact fetuses to glucocorticoid exposure following Liggins' observation in 1969 that antenatal glucocorticoids induced early lung maturation in preterm sheep *(1)*. Although the emphasis is on the surfactant system, other effects of glucocorticoid treatments on the fetal lung need to be discussed to dissociate them from effects on the surfactant system. The use of antenatal glucocorticoids is now routine for fetuses at risk of preterm delivery *(2)*. A clinical perspective on the efficacy of antenatal glucocorticoids in humans is useful to focus the interpretation of the experimental data on the important clinical questions.

Following the initial randomized controlled clinical trial of antenatal betamethasone to decrease the incidence of respiratory distress syndrome (RDS) published in 1972 *(3)*, 14 other randomized and controlled trials were included in a metaanalysis in 1995 *(2)*. Antenatal glucocorticoids decrease the incidence of RDS and death by about 50%. The benefits seem to occur at very early gestational ages, but there are limited clinical data on this point. There may be benefit for glucocorticoid treatment to delivery intervals of less than 24 h, although the 0-to 24-h interval has not been examined as to the minimal interval for effects on the incidence of RDS. There are minimal data suggesting that the beneficial effects of antenatal glucocorticoids may be lost if the treatment to delivery interval extends beyond 7 to 10 d *(4)* . Although repetitive courses of antenatal glucocorticoids are frequently used, there are no randomized and controlled trials evaluating the safety or efficacy of this approach.

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Liggins *(1)* proposed that the beneficial effect of antenatal glucocorticoids to decrease RDS resulted from early induction of surfactant synthesis, because the contemporary research at the time showed a close link between RDS and surfactant deficiency *(5)*. Although not supported by a number of reports *(6)*, the concepts that RDS is the result primarily of surfactant deficiency and that the primary effect of antenatal glucocorticoids is induction of surfactant synthesis continue to be generally accepted. A goal of this chapter is to develop the thesis that glucocorticoid effects on the fetus and the fetal lung are much more complex.

Although antenatal glucocorticoid treatments are a major therapeutic benefit for the fetus at risk of preterm delivery, the clinical data also provide indications of the complexities of fetal responses *(2)*. From the evolutionary perspective, the responses of the fetal lung to elevated glucocorticoids are a survival strategy to a stress signal that may protect the fetus if preterm delivery occurs. However, fetal growth restriction, premature rupture of membranes and preeclampsia can severely stress the fetus without causing early lung maturation *(7–9)*. Antenatal glucocorticoids also are not uniformly effective because optimal treatment will decrease the incidence of RDS by about 50% *(2)*. Repetitive courses of antenatal glucocorticoids may not further decrease the incidence of RDS *(10)*. Why are some fetuses apparently resistant to glucocorticoid stimulation of lung maturation? The fetal lung at 12–20 wk gestation is in glandular and then in canalicular developmental stages without terminal air spaces or a mature airway epithelium. Following explant into organ culture, the human fetal lung will develop a mature epithelium with typical type II cells, intracellular lamellar bodies, and surfactant proteins within a few days *(11,12)*. Although this process can be hastened with glucocorticoids, the rapid terminal differentiation of the lung occurs without glucocorticoids at an early point in gestation when maturation of the surfactant system does not normally occur. Is something promoting fetal lung growth and interfering with glucocorticoid responsiveness in vivo? These are clinically derived questions for which there are no answers.

GLUCOCORTICOID EFFECTS ON THE FETAL LUNG

Rodents and Rabbits

Following Liggins' observations of better aeration of the lungs of lambs that delivered prematurely after 2 to 4 d of fetal abdominal infusions of cortisol *(1)*, Kotas and Avery *(13)* demonstrated in 1971 that a combined fetal intramuscular (IM) and intraperitoneal injection of 9-fluoroprednisolone (equivalent to 75–100 mg/kg cortisol) increased lung gas volumes and deflation stability of preterm rabbit lungs. Kikkawa et al. *(14)* gave intraperitoneal and amniotic cortisol (dose estimate of about 60 mg/kg) to fetal rabbits and described early maturation of the pulmonary epithelium with anatomic evidence of surfactant production. Neither study reported fetal growth restriction after fetal glucocorticoid treatments. Kotas and colleagues *(15)* found that about 120 mg/kg cortisol given by fetal injection decreased lung cell number but did not decrease birth weight in rabbits. These early studies of direct fetal therapy are of interest because fetal growth effects of glucocorticoids were not found, despite very high doses of glucocorticoids.

Multiple investigators have consistently noted fetal growth restriction and lung maturation after maternal glucocorticoids in rodents and rabbits *(16)*. In an early and thorough study, Kauffman *(17)* reported in 1977 that low-dose maternal dexamethasone caused increased airspace volume density and no growth restriction in fetal mice. At

higher doses, dexamethasone caused growth restriction and increases in lamellar bodies in type II cells. The increased airspace volume density was detected within 14 h of fetal dexamethasone exposure, while the effects on type II cells required 24 h. This anatomic study was important because it separated the more rapid changes in airspace volume from the delayed appearance of indicators of surfactant induction. The airspace components of maturation also were separated from glucocorticoid induced fetal growth restriction in mice at low doses. We and others subsequently found that in fetal rabbits the major effects of maternal glucocorticoids were increased lung gas volumes that correlated with improved compliances and decreased plasma to alveolar albumin leaks after preterm delivery and ventilation *(18,19)*. However, the fetal rabbits had no increase in alveolar or lung surfactant phospholipid pools at gestational ages from 27 d to term at 31 d *(20)*. Low-dose maternal glucocorticoids caused fetal growth restriction without improved postnatal lung function *(21)*. Enhanced lung maturation could not be demonstrated in preterm rabbits without concomitant growth restriction *(22)*. By contrast, Snyder et al. *(23)* found that maternal betamethasone caused not only an increase in airspace volume but an increase in type II cell number, phospholipid synthesis, and SP-A but with no effects on lamellar body volume density. Interpretation of these and other studies of antenatal glucocorticoid effects on the preterm fetal lung in rodents and rabbits are complicated by the fetal growth effects of maternal glucocorticoids (perhaps not with fetal treatments), the short gestation and rapid fetal growth in these species.

Monkeys

Because of presumed comparability of lung maturation in baboons, monkeys, and humans, the effects of maternal glucocorticoids on fetal lung development were explored. Johnson and his colleagues *(6,24,25)* reported the effects of timing of maternal glucocorticoids on the fetal monkey lung. Daily antenatal maternal treatments with about 0.3 mg/kg betamethasone for 3 d before preterm delivery increased maximal lung gas volumes almost twofold *(6,25)*. Lung phosphatidylcholine expressed per lung weight increased significantly by about 20%, but surface tensions of lung extracts or deflation stability on pressure–volume curves did not demonstrate enhanced surfactant function. The same dose of betamethasone given for 13 d caused fetal growth restriction and increased lung volumes measured with gas or saline but not physiologic indications of increases in surfactant *(6)*. Subsequent measurements using the 13-d treatment course begun at 120 d gestation again demonstrated fetal growth restriction at preterm delivery at 133 d and at term delivery at 160 d gestation. The lung gas volumes of the glucocorticoid exposed fetuses were higher than controls at 133 d gestation but lower than controls at term *(6)*. The explanation for this result was that the glucocorticoids had an acute effect on the lung to decrease the lung interstitium and to increase airspace but subsequently alveolar number and lung surface area were decreased. The physiologic changes that resulted in induced maturation were interpreted to be primarily changes in lung connective tissue and not the surfactant system *(24)*. Bunton and Plopper *(26)* extended these initial observations on the effects of glucocorticoids on alveolarization in monkeys. They demonstrated that a high dose of triamcinolone acetonide given for 3 consecutive days in the midpseudoglandular (63–65 d gestation) or midcanalicular (110–112 d gestation) phases of lung development resulted in lungs that appeared more mature because less interstitial tissue was present. However, the alveoli were less numerous and larger, resulting in an "emphysematous" lung. These experiments with monkeys demonstrated

large and persistent effects on lung structure, resulting from high-dose and/or prolonged fetal exposures to maternal betamethasone, even at early gestational ages. By contrast in a study focused on the surfactant system, Kessler et al. *(27)* treated pregnant monkeys with 0.2 mg/kg daily dexamethasone for 3 d before delivery and ventilation of the preterm monkeys. The antenatal glucocorticoid exposed animals had less severe respiratory distress syndrome and multiple indications of increased surfactant in the lung tissue and alveolar lavages. A recent report from Edwards et al. *(28)* did not demonstrate improved postnatal lung function of preterm monkeys after antenatal glucocorticoid treatments or increased surfactant. We found no effects of fetal or maternal treatments on postnatal lung function or on postnatal surfactant metabolism of premature baboons *(29,30)*. However, the high levels of maternal cortisol that resulted from handling the animals crossed into the fetal circulation, which may have masked any effects of the antenatal treatments. These experiments in primates demonstrated that antenatal glucocorticoids even at early gestational ages had profound effects on lung anatomy. However, consistent maturational effects of antenatal glucocorticoids on the surfactant system have not been reported in primates.

Sheep

Following Liggins' initial report of improved lung aeration in lambs that delivered prematurely after an intraabdominal infusion of cortisol *(1)*, Taeusch and coworkers *(31)* used a long acting preparation of methylprednisolone (about 20 mg/kg) given intramuscularly to the surgically exposed fetus and reported preterm delivery within 85 h but no increase in fetal lung volumes or surface tensions. The explanation for no fetal lung response was not evident. Subsequently Mescher et al. *(32)* measured the amount of surfactant in tracheal fluid from 120 d gestation to term (term is 150 d) and found that surfactant increased before the normal large increase in endogenous cortisol before term. This result suggested that endogenous glucocorticoids were not responsible for the normal increases in surfactant before delivery. Hypophysectomy in sheep did delay lung maturation, an effect that was reversed by ACTH or cortisol *(33)*, demonstrating that some cortisol was required for lung maturation. Platzker et al. *(34)* found that intraperitoneal dexamethasone (about 0.2 mg/kg fetal weight) increased the amount of surfactant in tracheal fluid about 13-fold from before 120 d gestation to 134 d gestation, demonstrating that glucocorticoids could increase surfactant at quite early gestational ages.

More recent reports make a consistent interpretation of the lung maturational effects of glucocorticoids difficult. Schellenberg et al. *(35)* evaluated lung maturation after fetal cortisol, epinephrine, T_3, and epidermal growth factor infusions separately and in combination for 84 h. The only single agent to increase lung gas volume was cortisol (1 mg/kg · h infusion), although there were no increases in lung tissue or alveolar phospholipids after delivery at 127 d gestation. A subsequent report from the same group found no effect of fetal cortisol infusions on lung gas volumes or surfactant phospholipids *(36)*. By contrast, Warbarton et al. *(37)* found that 48 h cortisol infusions (0.45 mg/kg · h) followed by delivery at about 132 d gestation resulted in large effects — lung gas volume and lung tissue saturated phosphatidylcholine almost doubled, and alveolar saturated phosphatidylcholine increased threefold. Ikegami et al. *(38)* reported yet another pattern of lung responses in catheterized fetal sheep infused with 0.75 mg/kg · h cortisol for 60 h before preterm delivery at 128 d gestation. Lung and alveolar-saturated phosphatidylcholine and alveolar SP-A did not increase, but lung compliance increased almost

twofold, lung gas volumes measured by static pressure–volume curves doubled, and the vascular to alveolar leak of albumin decreased. This series of reports demonstrated that both lung volume increases and surfactant effects could be found after cortisol infusions, but the responses were not consistent across reports.

This lack of consistency of reponses to glucocorticoids has been explained by type of glucocorticoid, duration, and dose of treatment, and gestational age at treatment. While these and other factors may contribute to the varied responses, the responsiveness of the fetus as a result of its prior history may be the major variable. Most of the research in fetal sheep has utilized surgically placed catheters, and the maternal anesthesia and surgery may alter fetal responsiveness to either endogenous or exogenous hormones. Tabor et al. *(39)* found that an interval from catheterization to cortisol infusion of 4 d before preterm delivery 60 h later at 128 d resulted in improved compliance, but no significant increase in lung tissue or alveolar saturated phosphatidylcholine or alveolar SP-A (Fig. 1). By contrast, an interval of 11 d from catheterization to the initiation of the cortisol infusion resulted in control animals that had compliance values equivalent to the cortisol-treated animals after the 4-d catheterization to treatment interval. The cortisol further augmented compliance, almost doubled alveolar and total lung-saturated phosphatidylcholine, and increased SP-A levels in alveolar wash. Although the mechanisms responsible for altered fetal responses are not known, this study demonstrated that fetal lung responses to the same glucocorticoid treatment varied strikingly.

Unstressed Fetal Sheep

Interpretation of glucocorticoid effects on the fetal lungs is complicated by global fetal growth restriction in rodents and rabbits, by stress of handling and maternal to fetal cortisol transfer in primates, and by the stress of catheter placement in sheep to variable degrees in the different experiments. The responses may represent glucocorticoid effects that are augmented variably by the response state of the fetus. To avoid these problems and to evaluate the pure response of the unstressed fetus to glucocorticoids, Jobe et al. *(40)* gave fetal sheep glucocorticoid injections using an ultrasound-guided intramuscular injection technique that did not alter fetal catecholamine or cortisol levels *(41)*. Sheep were used because the placenta is impermeable to the cortisol, and as farm animals they tolerate handling without undue stress. Betamethasone (0.5 mg/kg) given by intramuscular injection to fetuses as a mixture of the acetate and sodium-phosphate salts caused consistent improvements in postnatal lung function after preterm delivery. The preterm lambs had improved lung compliances, improved gas exchange, a doubling of lung gas volume measured using static pressure–volume curves, and decreased protein losses from the vascular space into the lungs *(40,42)*. These effects on lung physiology after preterm delivery occurred after a 15-h fetal treatment to delivery interval but not within 8 h of fetal treatment *(43)*. The alveolar-saturated phosphatidylcholine pool size was very low at 121–128 d gestation (<1 μmol/kg vs about 100 μmol/kg at term), and no increases were detected for glucocorticoid treatment to delivery intervals less than 7 d *(44)* . By combining measurements from several protocols to permit comparisons of more than 20 glucocorticoid treated and 20 control fetuses for a betamethasone treatment to delivery interval of 48 h, alveolar-saturated phosphatidylcholine increased significantly from 0.9 μmol/kg to 1.8 μmol/kg without a change in saturated phosphatidylcholine in lung tissue *(45)*. This small increase may result from the more efficient lung lavage that was possible because the glucocorticoid exposed lungs were more compliant and had larger gas volumes. Saturated

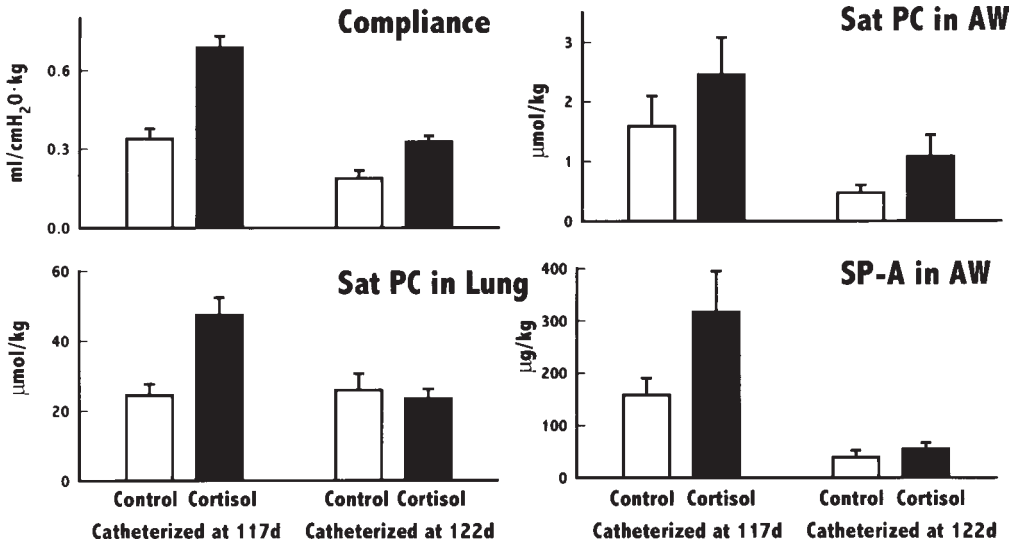

Fig. 1. Effect of time of catheterization on subsequent responses of the fetal lung to 60 h cortisol infusion (0.75 mg/kg · h). Preterm lambs were catheterized at 117 d or 122 d gestation and subsequently randomized to cortisol (0.75 mg/kg · h) or saline infusions for 60 h before preterm delivery at 128 d gestation. The measurements of lung compliance, alveolar saturated phosphatidylcholine (Sat PC in AW) and SP-A (SP-A in AW) were higher for the controls catheterized at 117 d gestation than for the controls catheterized at 122 d gestational age. The increases in compliance, alveolar and tissue Sat PC, and SP-A also were higher in the cortisol-infused fetal sheep catheterized at 117 d gestation. The responses of the fetal lung differed based on interval from catheterization to initiation of the cortisol infusion. (Data from ref. *22*.)

phosphatidylcholine in lung tissue was not increased up to 4 d after fetal betamethasone treatment *(44)*. The surfactant proteins SP-A and SP-B do not increase in lung tissue or alveolar washes for treatment to delivery intervals of 48 h or less *(46)*.

Antenatal glucocorticoids given directly to the fetus caused a decrease in alveolar thickness and an increase in aerated parenchyma without a change in alveolar size within 48 h of treatment *(47)*. Type II cells sampled from lungs of lambs that had physiologic responses to betamethasone demonstrated no changes in subcellular organelle volume densities. Low values for lamellar bodies (10% for controls and 12.5% for glucocorticoid treated) and high values for glycogen (28% in controls and 25% in glucocorticoid treated) indicated immaturity of the type II cells (K. Pinkerton, A. H. Jobe, and M. Ikegami, *unpublished observations)*. These anatomic results and the subtle or lack of effect of antenatal betamethasone on saturated phosphatidylcholine, SP-A and SP-B pool sizes in alveolar washes and lung tissue demonstrated no physiologically important effects of glucocorticoids on surfactant pools in this animal model.

However, fetal treatments of sheep with glucocorticoids do result in more delayed effects on the surfactant system. When the fetal glucocorticoid treatment to delivery interval was extended to 7 d, the alveolar saturated phosphatidylcholine pool increased about sixfold in one experiment and doubled in another *(44,48)*. The total lung-saturated phosphatidylcholine pools increased by 42%, demonstrating a potent but delayed effect on surfactant phospholipids in lung tissue *(49)*. In other experiments, repetitive maternal glucocorticoid treatments given at 7-d intervals beginning at 104 d gestation resulted in

large increases in alveolar and lung tissue saturated phosphatidylcholine as well as increases in alveolar and lung tissue pools of SP-A and SP-B *(46,48)* (Fig. 2). These increases in surfactant components correlated with improved postnatal lung function after preterm delivery. The effects of maternal glucocorticoid treatments on SP-A, SP-B, and SP-C mRNA levels in this in vivo model did not parallel the changes in protein levels *(50)*. The three mRNA species for the surfactant proteins were increased 24 and 48 h after fetal glucocorticoid exposure (when protein levels were unchanged) but had decreased to control levels for a treatment to delivery interval of 7 d.

An unanticipated aspect of the fetal response to antenatal glucocorticoids in sheep was the effect of the route of fetal exposure. Moraga et al. *(51)* found that maternal betamethasone (12 mg IM) given 48 and 24 h before preterm delivery at 125 d gestation doubled lung gas volume but did not significantly increase surfactant phospholipids. This result was similar qualitatively to the effects of fetal glucocorticoid treatment *(40)*. However, when directly compared, using the same dose of 0.5 mg/kg betamethasone based on maternal weight or fetal weight, maternal betamethasone resulted in a larger maturational response of the fetal lungs characterized by better compliances, improved gas exchange, larger lung volumes, and larger increases in saturated phosphatidylcholine after multiple doses given at 7-d intervals *(52)* (Fig. 3). Single or repetitive maternal betamethasone treatments resulted in proportionate fetal growth restriction following preterm delivery at 125 days gestation and at term *(48, 53)*. By contrast, single or repetitive fetal treatments did not cause growth restriction *(52)*,even though fetal betamethasone treatments result in fetal plasma levels about threefold higher than fetal plasma betamethasone levels after maternal treatments *(54)*. In this model the acute physiological lung maturational responses and the delayed increases in surfactant occurred without fetal growth restriction, disassociating these two glucocorticoid effects on the fetal sheep.

ALTERATIONS OF GLUCOCORTICOID FUNCTION IN TRANSGENIC MICE

Recent experiments designed to alter endogenous glucocorticoid responsiveness in mice provide important insights into lung maturation in vivo. Disruption of the corticosteroid-releasing hormone (CRH) gene resulted in very low plasma corticosterone levels in mice *(55)* (*see* Chapter 7). The mice survived normally but required corticosterone supplementation to reproduce. Fetuses of the mating of CRH–/– mice died after birth of respiratory failure unless supplemental corticosterone was provided to the dam. The lungs were cellular and appeared to have an arrest in thinning of the saccules. The mRNAs for SP-A and SP-B were decreased on 17.5 d, but were similar to wild-type by 18.5 d; other components of the surfactant system that were evaluated appeared to be normal (*see* Chapter 7). Corticosterone supplementation in the water of the dam prevented the delayed lung development, presumably because small amounts of glucocorticoids leaked from dam to fetus**.** Very low levels of fetal glucocorticoid exposure were sufficient to support normal lung maturation, based on the stressed CRH–/– mice. Therefore, glucocorticoids probably are "permissive" for normal lung maturation, but large increases in fetal glucocorticoid levels are not required. In another model, targeted disruption of the glucocorticoid receptor in mice resulted in delayed anatomic maturation of the lung after about 15.5 d gestation and death after delivery *(56)*. The expression of SP-A, SP-B, and

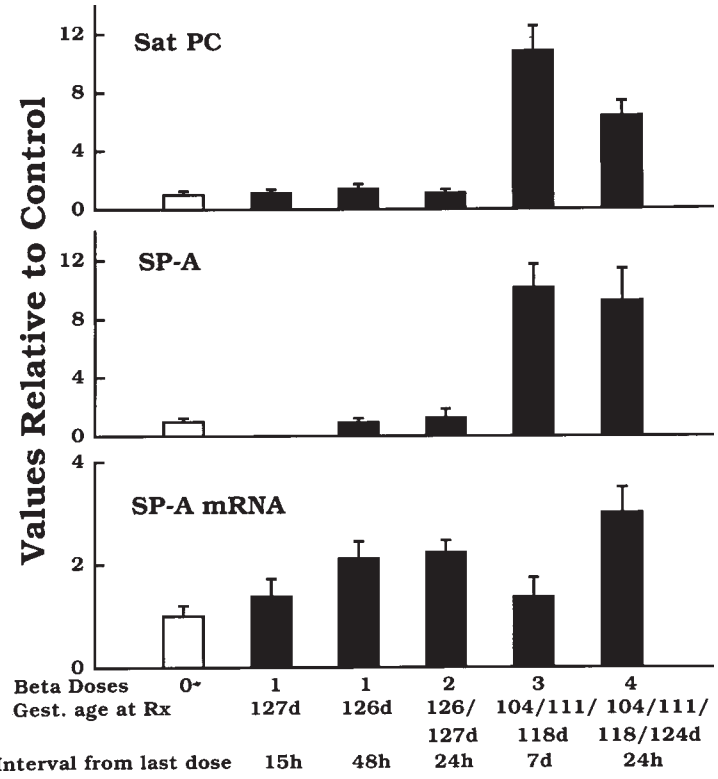

Fig. 2. Changes in saturated phosphatidylcholine (Sat PC), SP-A, and SP-A mRNA following betamethasone treatments. All values are expressed relative to values for saline injected controls. Alveolar Sat PC and SP-A did not increase for the short-term treatment to delivery intervals. Large increases in alveolar Sat PC and SP-A occurred after repetitive treatments. The mRNA for SP-A increased within 48 h of treatment but decreased to control levels even after multiple retreatments unless the last treatment was close to the time of delivery (the 4-dose beta group). (Data from refs. *46* and *51*.)

SP-C genes at birth were normal. These experiments demonstrated that the fetal lung required glucocorticoids to achieve the anatomic maturation characterized by late gestation loss of cellularity and thinning of saccules. There may be no requirement for glucocorticoid for development of the surfactant system, although this point is not clear. Glucocorticoid receptor binding to DNA was not required for normal lung maturation because disruption of the dimerization required for receptor binding to glucocorticoid response elements did not interfere with normal lung development *(57)*.

A UNIFIED VIEW OF GLUCOCORTICOID EFFECTS ON LUNG MATURATION

In trying to integrate the multiple and often inconsistent observations about fetal lung responses in animals and in clinical practice, we will utilize a Venn diagram that separates glucocorticoid responses into three components — normal lung development, stresses on the pregnancy and the pharmacologic effects of antenatal glucocorticoids (Fig. 4). Normal lung development requires endogenous glucocorticoids at low levels to achieve anatomic maturation *(33,55)*, but the normal increase in endogenous glucocorticoids just before

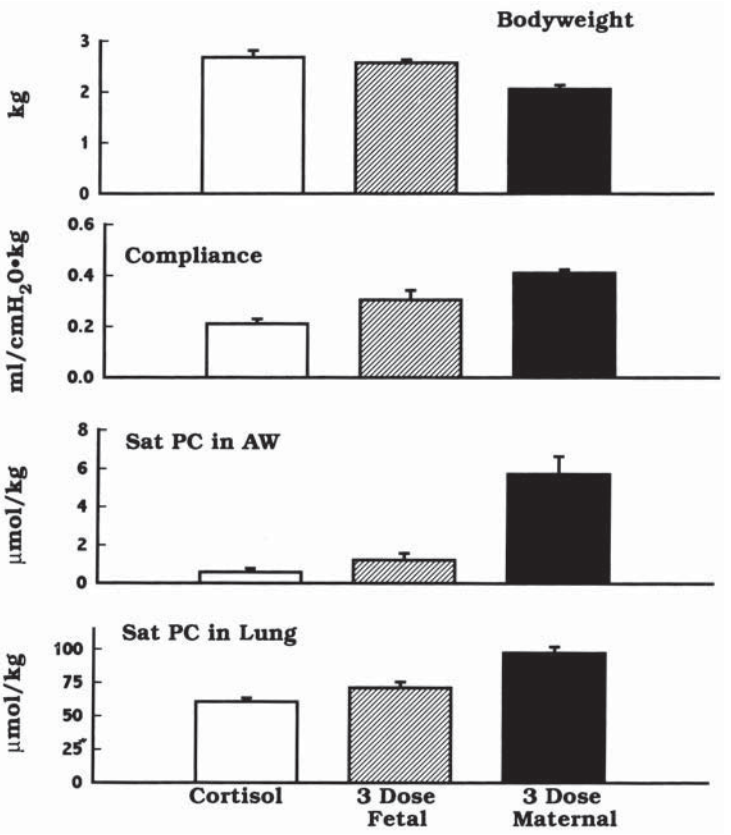

Fig. 3. Effect of fetal or maternal repetitive glucocorticoid treatments on fetal weight, compliance, and saturated phosphatidylcholine (Sat PC) in alveolar washes (AW) and the total lungs of lambs delivered prematurely at 125 d gestation. The dosing schedule was 0.5 mg betamethasone/kg maternal or fetal weight at 104, 111, and 118 d gestation. The fetal doses had less effect on compliance and Sat PC than did the maternal doses. The maternal doses decreased fetal weight. (Data from ref. *53*.)

birth are not required for either anatomic maturation or maturation of the surfactant system *(32)*. Although the information is incomplete, glucocorticoids may not be required for normal maturation of the surfactant system. Infants without adrenal function can have normal lung maturation. Transgenic mice with abnormalities in glucocorticoid function demonstrate that the major effects of endogenous fetal glucocorticoids is to permit structural maturation of the lung.

We think the fetal sheep model is the best model to date in which to evaluate glucocorticoid responses on unstressed fetuses. Maternal or fetal routes of exposure of the fetus to betamethasone cause large improvements in postnatal lung function that result from the rapid maturation of alveolar architecture *(47,52)*. High-dose or more prolonged treatments in monkeys and rats can result in permanent alterations in alveolar development that can result in decreased alveolar numbers *(6,26,58)*. In mice, rabbits, sheep, and monkeys the initial effects of antenatal glucocorticoid treatments are on lung anatomy and those effects can occur early in gestation and persist to term.

The fetal lung seems to respond to glucocorticoids primarily with anatomic maturation characterized by a thinning of the interstitium. Collagen may decrease, although

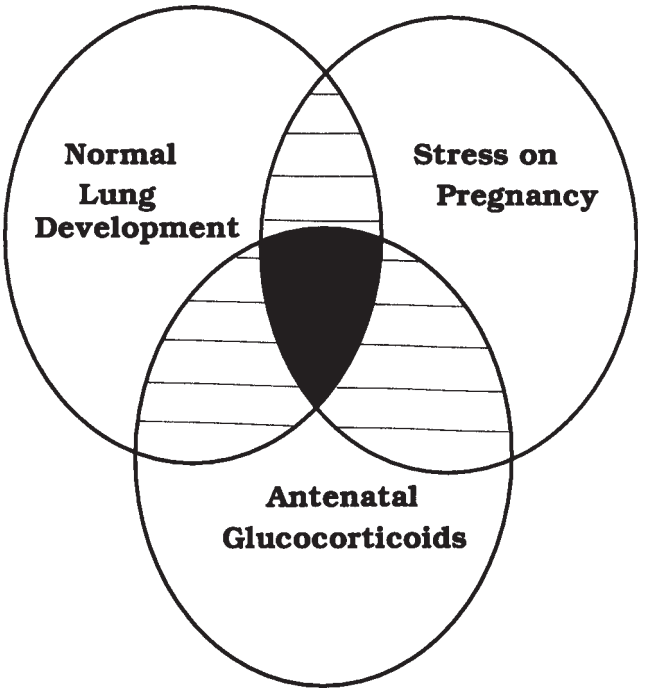

Fig. 4. Venn diagram of factors that can influence fetal lung maturation.

hyaluronan content does not change *(24,59)*. The mechanisms by which glucocorticoids can "accelerate" anatomic maturation are not known but they probably result from glucocorticoid regulation of transcription factors, because disruption of binding of the glucocorticoid receptor to glucocorticoid response elements does not alter anatomic maturation *(57)*. The characteristics of glucocorticoid induced early lung maturation are similar to the normal process of lung maturation in that the primary effect is on anatomic maturation. Increases in surfactant phospholipid pools and the amounts of the surfactant proteins are detectable after about 7 d in fetal sheep and may or may not occur in primates, depending on the experiment *(6,27,46)*. Surfactant proteins and their mRNAs have not been evaluated in primates, but the rapid induction of mRNA levels in sheep followed by a decrease to baseline parallels the reversible increases in SP-A mRNA induced by glucocorticoids in human fetal lung explants *(11,50)*. The more potent maturational responses after maternal than after fetal glucocorticoid treatments in sheep are consistent with the lung maturational response resulting from signals from the mother (placenta). The extreme interpretation of these results is consistent with glucocorticoids not being directly responsible for inducing early maturation of the preterm lung.

Stresses on a pregnancy can modulate both normal lung maturation and glucocorticoid induced early maturation. Large increases in surfactant phospholipids and proteins can occur quickly after glucocorticoid exposure of a stressed fetus *(39)*. In clinical practice, the lecithin : sphingomyelin ratio in amniotic fluid can increase rapidly with fetal stress, perhaps because of elevated glucocorticoid levels *(60)*. Infants with respiratory distress syndrome no doubt often have a combination of lung structural immaturity and surfactant deficiency, and the relative importance of each is not easily evaluated clinically. However,

very preterm infants can have quite mature lungs, demonstrating that anatomic maturation and induction of surfactant can occur by 24–25 wk gestation in the human fetus with sufficient fetal stress and/or glucocorticoid exposure. Fetal stress sufficient to cause growth restriction may occur without consistent effects on lung maturation *(9)*. Fetal stress is a nonspecific designation that probably includes multiple mechanisms that contribute to fetal compromise. In both humans and sheep multiple courses of antenatal glucocorticoids (even when associated with growth restriction) can result in a preterm newborn with lung immaturity *(10)*. This result suggests that some fetuses are unresponsive to antenatal glucocorticoids. Explants of human fetal lung at midgestation will "mature" anatomically and mature type II cells will appear within a few days *(11)*. Perhaps maturation is suppressed by a factor(s) that make that lung unresponsive to exogenous glucocorticoids. Many of the inconsistencies in the effects of glucocorticoids on fetal lungs in the literature probably are explained by modulating effects of maternal and/or fetal stress on the glucocorticoid responses of the fetal lung.

Evaluations of fetal lung responses in vivo inevitably provide few answers and raise many questions. However, these integrated responses are important because of their clinical relevance and because they direct investigators toward exploring the mechanisms that are central to the regulation of maturation. A major effort needs to be directed toward understanding how glucocorticoids (or secondary signals perhaps from the placenta) alter lung anatomic development.

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