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FETAL LUNG DEVELOPMENT AND THE INFLUENCE OF GLUCOCORTICOIDS ON PULMONARY SURFACTANT

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SUMMARY

The fetal lung undergoes extensive anatomic and histologic differentiation during gestation in preparation for its role as an organ for gas exchange in the neonate. To successfully adapt to the air breathing state, it must also mature physiologically and biochemically and develop the capacity to produce essential pulmonary surfactants. The surfactants are composed primarily of phospholipids, especially lecithins, and serve the vital function of maintaining alveolar stability on expiration. This role is demonstrated experimentally with the classical pressure–volume apparatus. It has been observed that when a fetal lung is inflated with air, a threshold pressure of approximately 15 cm water is required to increase lung volume, and that maximum inflation is achieved between approximately 30 and 35 cm water. During the deflation phase, an immature lung will readily collapse, whereas a mature lung will show resistance to collapse when the pressure is lowered. With advancing gestation, progressively improved deflation stability is achieved, signalling the presence of adequate pulmonary surfactant in the fetal airway. Biochemical approaches to the study of lung development depend on assessment of phospholipid synthesis by determination of enzyme activities, pathway rates, and the concentration of phospholipid products. The attention of investigators has centered specifically on the biosynthesis of lung lecithin. There are two pathways for *de novo* formation of lecithin: the choline incorporation pathway (I) and phosphatidylethanolamine methylation (II). Recent studies with isotopic techniques have demonstrated that pathway I is the predominant mechanism. There are three enzymes in this pathway, each of which shows significantly increased activity during late gestation in fetal rats. Shortly after the rise in enzyme activity, an increase in overall choline pathway rate has been identified, and subsequently, the concentration of lung lecithin is likewise increased.

Hormonal regulation of fetal lung development has been studied with a number of techniques in several species of lower animals. Using the anatomic approach, investigators have demonstrated that 48–72 h following administration of glucocorticoids, increased potential air space and greater "alveolarization" is apparent. Physiologic evidence of enhanced maturation following corticosteroid administration to fetal animals includes greater distensibility, greater deflation stability, and an earlier appearance of surface active material in lung extracts. The biochemical effects of glucocorticoids with respect to lung development are an increased synthesis of lecithin, an enhanced rate of choline incorporation, and increased activities of various enzymes. The net biochemical effect is to enhance the capacity of the fetal lung to produce the surface active phospholipids of the alveolar lining layer. Because of exogenous corticosteroid, this capacity is developed at an earlier time in gestation than would normally be found. The hormone therefore acts as a stimulus capable of changing the *timing* of lung development such that the maturation process is accelerated.

INTRODUCTION

The study of fetal lung development is a rapidly emerging field. As recently as five years ago, it would have been impossible to present a comprehensive discussion of this topic. At the present time, in contrast, one cannot begin to describe all of the important experimental advances relating to lung maturation, nor cite all the work of the many excellent scientists who have generated key data. Nonetheless, it is appropriate to acknowledge the contributions of two investigators, Drs. G. C. Liggins and M. E. Avery, for their laboratories were instrumental in gathering the initial data which demonstrated that the fetal lung could be a target tissue for corticosteroids [1, 2].

The objectives of this article are two-fold: (1) to present an overview of the normal process of fetal lung maturation, as discussed from the anatomic, physiologic, and biochemical viewpoints; (2) to review the documented effects of glucocorticoids on lung development. The experimental observations to be described have come primarily from studies with lower animals such as rats, rabbits and sheep and also from evaluation of fetal Rhesus monkeys. Selected results from the study of all of these animals will be presented in an effort to provide as broad a picture as possible of the process of lung development as related to fetal endocrinology. One should not assume, however, that the events leading to lung maturation are identical in the various animal species studied to date. Rather, there is now evidence that species specificity may exist with regard to control mechanisms for surfactant production.

In addition to a review of animal studies, brief discussion will be presented of pertinent clinical investigations. These offer encouraging results which indicate that fetal lung development in humans can in

some instances be promoted by antenatal corticosteroid administration. Clinical trials with glucocorticoids have thus bridged the gap between the laboratory and the bedside and seemingly promise to improve the outcome of premature infants relative to respiratory function.

THE STUDY OF LUNG DEVELOPMENT: APPROACHES AND SIGNIFICANT OBSERVATIONS

It has long been appreciated that the fetal lung undergoes extensive anatomic and histologic differentiation during gestation in preparation for its role as an organ for gas exchange in the neonate. To successfully adapt to the air breathing state, it must also mature physiologically and biochemically and develop the capacity to produce the surface active material (SAM) of the alveolar lining layer. This material has been termed "pulmonary surfactant" and serves the critical function of lowering surface tension at the air-tissue interface. Such a phenomenon prevents collapse of the respiratory units or alveoli on expiration. The clinical consequence of diminished pulmonary surfactant in the neonate is respiratory distress syndrome or hyaline membrane disease. This is a disorder of premature infants characterized by general collapse of the air sacs, leading to ventilatory insufficiency.

Because of its cellular heterogeneity, connective tissue component, and unique functional role, studies on lung development are more complex than those conducted on other organs such as fetal liver. For this reason, a multidisciplinary approach has been utilized to gather fundamental information and correlates. Among the disciplines brought to bear on the problem are anatomy, physiology, and biochemistry. As listed in Table 1, the anatomic approach includes assessment of gross morphology and parenchymal architecture, examination of histology and cytology, and various cytochemical techniques.

The architecture and histology of the lung can be appreciated from Fig. 1 which shows structural changes in fetal rat lung from 16 days gestation to term (22 days) and at birth, as reported by Blackburn *et al.* [3]. A glandular phase is shown to be present at 16, 17 and 18 days gestation; canaliculi are evident at 20–21 days gestation and alveoli at 21–22 days. Electron microscopy demonstrates that during late gestation, cytologic changes occur with appearance of osmiophilic lamellar bodies in the cuboidal shaped type II alveolar pneumonocytes. These lamellar bodies presumably represent storage sites for surfactant, and make their appearance during the last 10–20% of gestation in rats, rabbits, and primates.

Table 1. Approaches to the study of lung development

I.	Anatomic:
	A. Gross morphology
	B. Parenchymal architecture
	C. Histology/cytology
	D. Cytochemistry
II.	Physiologic:
	A. P-V apparatus (alveolar surfactant)
	B. Surface balance (total SAM)
III.	Biochemical (assessment of phospholipid biosynthesis):
	A. Enzyme activities
	B. Pathway rates
	C. Product concentration in
	1. Lung parenchyma
	2. Lung lavage fluid
	3. Amniotic fluid

In addition to anatomy and histology, a further approach to the study of lung development involves physiologic techniques. As listed in Table 1, these utilize the pressure–volume apparatus for measurement of alveolar surfactant, and the Wilhelmy surface balance to detect the presence of surface active material (or surface tension lowering compounds) in lung extracts spread as a surface film. To describe the first of these methods in more detail, a diagramatic representation of a pressure–volume apparatus is shown in Fig. 2. The equipment employed for determining pressure–volume relationships and the amount of alveolar surfactant is as simple as a pressure gauge and a burette. The lung is attached to this closed system and filled with, or emptied of, gas in a stepwise fashion. By relating the change in volume to the change in pressure necessary to achieve a given change in volume (at points of no gas flow), static compliance can be calculated. It has been observed experimentally that when a fetal lung is inflated with air, a threshold pressure of approximately 15 cm water is required to increase lung volume, and that maximum inflation is achieved between approximately 30 and 35 cm water. During the deflation phase, a mature lung will show resistance to collapse when the pressure is lowered in increments of 5 cm water. Thus, when the pressure has been reduced to 0, a mature lung maintains a residual volume. The portion of the curve describing the response to emptying of gas is called the deflation limb; stability upon deflation reflects the presence of alveolar surfactant. When such an experiment is performed with saline rather than air, surface active material at the alveolar interface can not come into play, and consequently the inflation and deflation characteristics are entirely different. The lung readily collapses, as the pressure generated by saline is lowered. The marked separation of inflation and deflation segments during experiments with air, but not saline, is termed pressure–volume hysteresis or simply hysteresis.

Deflation segments of pressure–volume curves reported by Kotas and Avery [4] for developing fetal rabbits are shown in Fig. 3. The lines shown are from 25, 27, and 31 day control fetuses (term gestation = 31 days). With increasing gestation, it is evident that stability upon deflation is achieved and an increasing residual volume maintained by the fetal lung. A helpful measure of deflation stability or alveo-

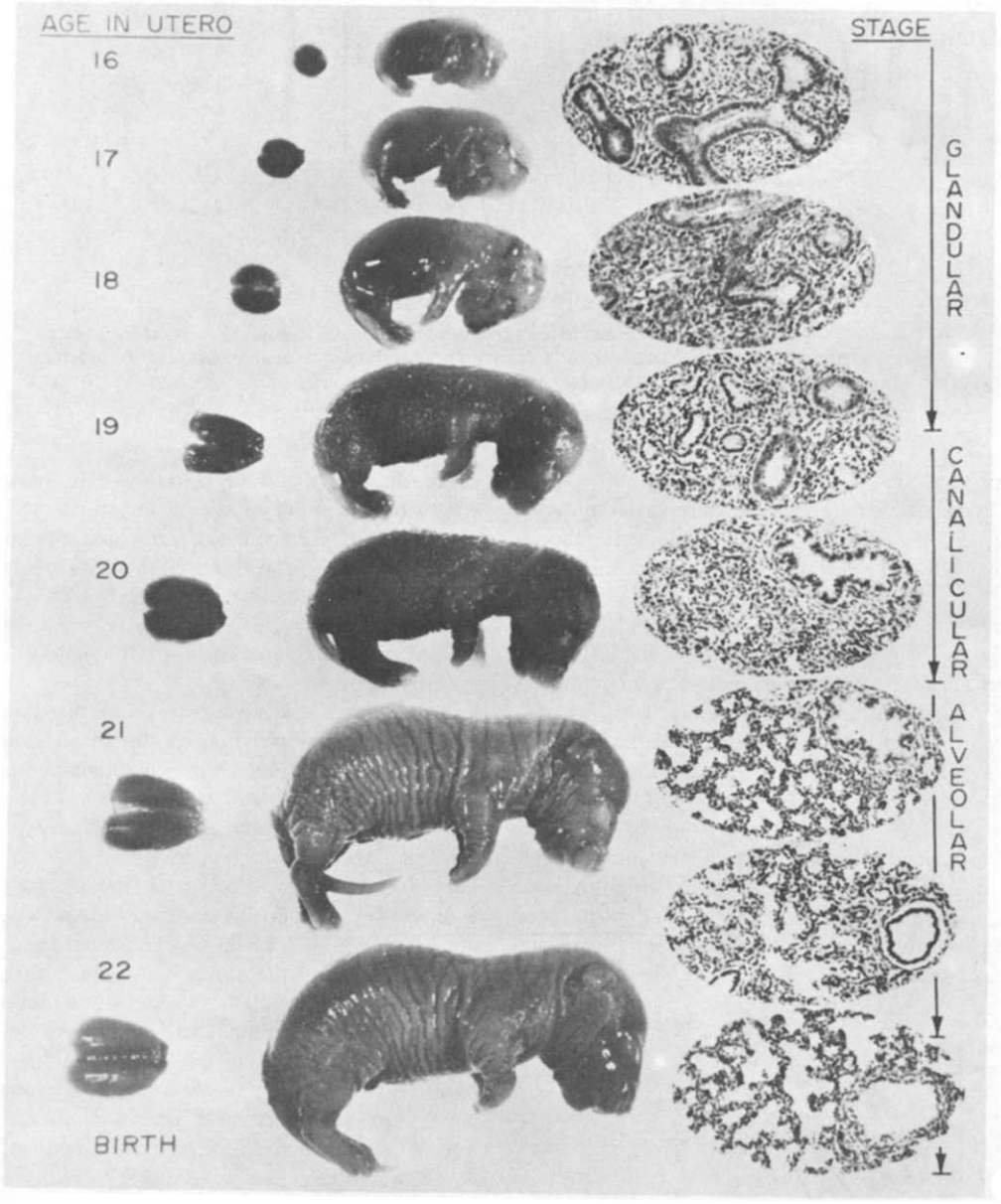

Fig. 1. The growth and morphologic development of the fetal rat lung. (Illustration kindly provided by Dr. Will Blackburn, Departments of Pediatrics and Pathology, University of South Alabama.)

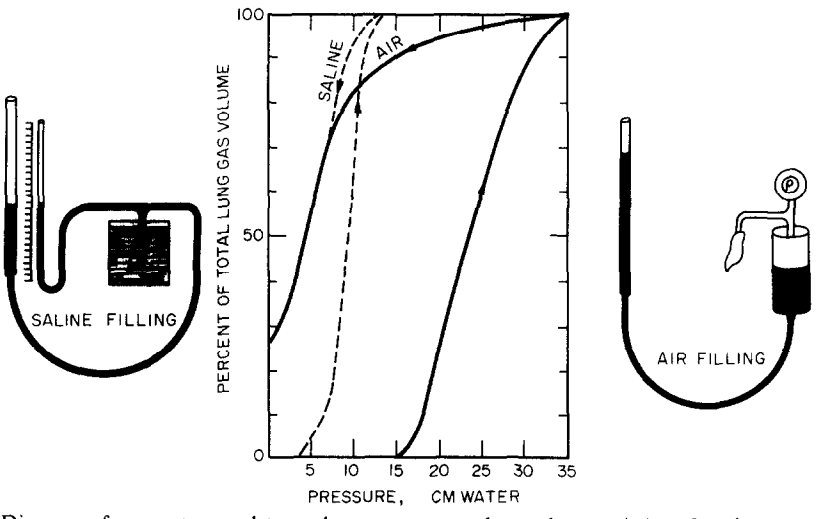

Fig. 2. Diagram of apparatus used to evaluate pressure–volume characteristics of excised lungs. Lung volume is expressed as percent of total lung capacity and inflation measurements are to the right of deflation measurements. Note the stability upon deflation with air (solid line) exhibited by this mature fetal lung. Taken from Farrell and Kotas [21].

lar surfactant is the percentage of maximum volume remaining in the lung at a pressure of 10 cm water (% V_{10}). As shown, % V_{10} approximately doubles between 25 and 27 days gestation, and then more than doubles between 27 days and term.

In addition to an increased amount of alveolar surfactant producing improved deflation stability, other readily detectable physiologic indices of lung development include the appearance of surface tension lowering material (producing minimum surface tension values of less than 10 dynes/cm), and increased lung distensibility.

Biochemical approaches to the study of lung development depend on assessment of phospholipid synthesis by determination of enzyme activities, pathway rates, and the concentration of phospholipid products (Table 1). Prior to discussing the biosynthesis of pulmonary surfactant, it is useful to review briefly the

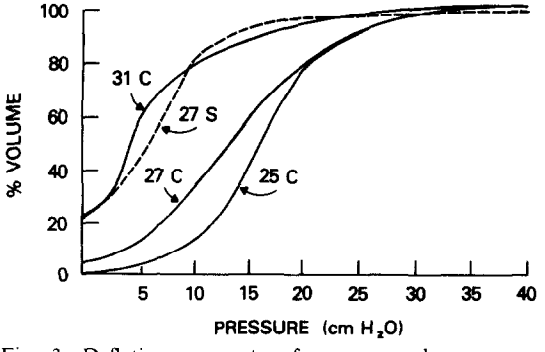

Fig. 3. Deflation segments of pressure–volume curves obtained in studies of fetal rabbit lungs. Results are shown for control fetuses of 25, 27, and 31 days gestation (25C, 27C, 31C) and for a corticosteroid treated fetus of 27 days gestation (27S-broken line). Note the greater retention of air on deflation with advancing gestation in controls. Also evident is the increased deflation stability of lungs 48 h after *in utero* administration of glucocorticoid (27S). Adapted from the data of Kotas and Avery [4].

composition of this material. On a mass basis, it is clear from the work of several investigators [5] that phospholipids are the major surfactant components. More specifically, however, lecithins with highly saturated fatty acid esters, account for two-thirds of the compounds present. These highly saturated species are not only abundant, but also are essential for the lowering of surface tension.

The attention of investigators has therefore centered on biosynthesis of lung lecithin or phosphatidylcholine (PC). Structurally, this compound is comprised of a glycerol backbone with the first two carbon atoms esterified to fatty acids (particularly palmitic acid) and the third carbon to phosphoric acid; the latter is in turn esterified to the quaternary amine, choline. Key components in the synthesis of lecithin are 1,2-diacylglycerol, which is generated from phosphatidic acid, and the nitrogenous base, which is introduced either as choline *per se* or as the non-methylated base, ethanolamine. There are two pathways for *de novo* synthesis of lung lecithin: the choline incorporation pathway (I) and phosphatidyl ethanolamine methylation (II). Mechanism I involves phosphorylation of choline, "activation" to the CDP-choline derivative, and final transfer of the phosphorylcholine portion of the activated derivative to diacylglycerol. In the second pathway, ethanolamine is converted to phosphatidylethanolamine by an analogous series of reactions; PE is then trimethylated in three steps to form the final choline phosphoglyceride product. Recent studies with isotopic techniques [6, 7] have demonstrated that pathway I is the primary *de novo* mechanism of lung PC biosynthesis [5, 8].

Recent studies have therefore focused on the choline incorporation pathway. There are three enzymes in this pathway: choline kinase, cytidyl transferase, and choline phosphotransferase. Each of these shares features in common with well studied regulatory

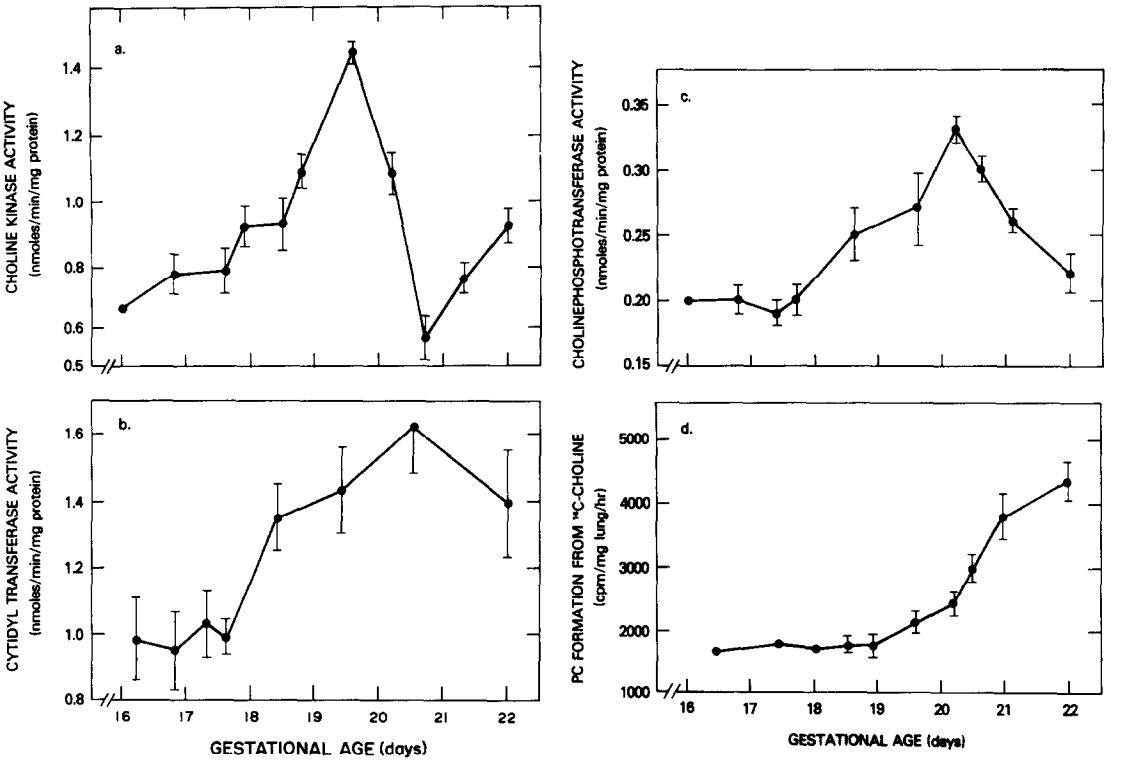

Fig. 4. Specific activities of choline pathway enzymes in developing fetal rat lung (a, b, c), as compared to the apparent rate of the overall pathway (d). Phosphatidylcholine (PC) formation was measured in lung slices incubated at pH 7.4 in the presence of trace [^{14}C]-choline. Part of these data have been reported by Farrell *et al.* [8].

enzymes of other pathways. It is conceivable, in fact, that each could be a key catalyst of potential significance in controlling the rate of the overall sequence of reactions. These three catalysts have been measured by Farrell *et al.* [8] in lung samples from developing fetal rats as shown in Fig. 4. All three enzymes have been found to show a significant rise in activity at approximately 18.5–19 days of gestation. In each case, a peak of activity is reached and this is followed by a decline. Shortly after the rise of enzyme activity, an increase in pathway rate has been identified. Figure 4 also demonstrates that the overall conversion of choline to phosphatidylcholine is augmented beyond 20 days gestation; subsequently, the concentration of lecithin in lung parenchyma is likewise increased in fetal rat lungs. A similar increase in the conversion of choline to PC has been detected in lung samples from fetal Rhesus monkeys [6]. This is followed by an increase in the concentration of lung lecithin and a surge in the lecithin/sphingomyelin ratio of amniotic fluid. Biochemical features of lung development therefore include changes in enzyme activities, increases in pathway rates, and an increase in the concentration of lecithin in lung tissue and fluids derived in part from the respiratory epithelium.

EFFECTS OF GLUCOCORTICOIDS ON LUNG DEVELOPMENT

With the foregoing as background information, consideration can be given to the effects of glucocorticoid on lung development. From the list presented in Table 1, it is apparent that agents which influence the fetal lung can be studied with a number of experimental techniques. All these have been utilized in recent investigations where glucocorticoids, generally potent analogs of cortisol such as dexamethasone, have been administered to the immature fetus *in utero* and changes in the rate of lung development assessed.

Using the anatomic approach, several investigators [5] have demonstrated that 48–72 h following administration of glucocorticoids, increased potential air space and greater "alveolarization" is apparent. As listed in Table 2, this is manifested primarily by attenuation of alveolar cells and narrowing of septae. In the rabbit, for instance, instead of the usual phenomenon of complete "alveolarization" at day 28, these maturational changes can be demonstrated at day 26 if steroids are injected at 23–24 days of gestation [9]. In addition, prominent type II pneumonocytes are also evident within 2 days of corticosteroid injection to immature fetuses.

Physiologic evidence of maturation following corticosteroid administration to fetal animals includes greater distensibility, greater deflation stability, and an earlier appearance of surface active material (Table 2). In other words, there is an acceleration in the appearance of SAM and a shifting "backward" of the time when deflation stability is evident. Figure 3 illustrates the influence of glucocorticoids on deflation stability as studied in fetal rabbits by Kotas *et*

Table 2. Effects of glucocorticoid on lung development

I.	Anatomic:
	A. Increased potential airspace
	1. Attenuation of alveolar cells
	2. Narrowing of septae
	3. Greater 'alveolarization'
	B. Increased prominence or numbers of type II pneumonocytes
II.	Physiologic:
	A. Greater distensibility
	B. Greater deflation stability
	C. Earlier appearance of SAM
III.	Biochemical:
	A. Increased concentration and/or degree of saturation of phosphatidylcholine in
	1. Lung parenchyma
	2. Lung lavage fluid
	B. Increased conversion of choline to PC, i.e. the apparent rate of the choline pathway is enhanced
	C. Increased activities of cholinephosphotransferase, glycerolphosphate phosphatidyltransferase, and lipoprotein lipase

al. [2, 4]. The volume of gas remaining in the lungs of a 27-day control fetus (27C) is seen to decrease somewhat precipitously upon lowering of the pressure. In contrast, improved deflation stability is noted with lungs of a 27-day fetus administered glucocorticoid three days earlier. Thus, the percent volume remaining at 10 cm pressure (% V_{10}) is more than two-fold greater in the lungs of the 27-day steroid treated animal, as compared to the corresponding control.

The biochemical effects of glucocorticoids with respect to lung development are also listed in Table 2. Following the demonstration in 1971 that alveolar surfactant is augmented in fetal rabbits administered glucocorticoid *in utero* [2, 4], a number of investigators have measured the concentration and/or fatty acid composition of lung phosphatidylcholine. Farrell and Zachman [10], for instance, reported that the PC concentration in rabbit lung was increased 72 h after administration of 9-α-fluoroprednisolone acetate. More recently, Ekelund *et al.* [11] reported that cortisol promotes the accumulation of phospholipids in cultured human fetal lung. In addition, Rooney *et al.* [12] have reported that pulmonary fluid obtained by saline lavage contains increased amounts of phosphatidylcholine when fetal rabbits are given cortisol *in utero*. Measurements of lecithin concentration in amniotic fluid also suggest, but have not conclusively demonstrated, that lung-derived phospholipids in the amniotic space are increased following glucocorticoid treatment [5].

To illustrate the influence of corticosteroids on the concentration of lecithin in lung parenchyma, data from studies [10] with fetal rabbits are presented in Fig. 5. From this graph, it is evident that the concentration of lecithin in lungs from control fetuses begin to increase after 24–25 days gestation. The mean concentration approximately doubles, from 40–80 mg/g dry lung, during the interval of 24–28 days gestation. Fetal rabbits given an injection of glucocorticoid at 24 days gestation showed significantly greater lung lecithin at 27 days in comparison to saline injected controls. In fact, the mean value of 96 mg/g in the 27-day steroid treatment group is even slightly higher than the value found in control fetuses at term. Thus, glucocorticoid has apparently promoted the accumulation of lecithin in fetal lung and has shortened the time required for development of a phospholipid reservoir in lung parenchyma.

A further, and perhaps more primary, biochemical effect of glucocorticoid on lung development is an increased conversion of choline to phosphatidylcholine, that is an enhanced rate of choline pathway activity. Such an effect was first demonstrated by Farrell and Zachman [10] when lung slices were utilized to measure the incorporation of [^{14}C]-choline into lecithin, as well as the conversion of [^{14}C]-methionine to the final product. With this approach, it was found that lungs of corticosteroid treated rabbit fetuses are approximately 50% more active in terms of choline pathway rates than those of controls. In contrast, phosphatidylethanolamine methylation rates were not influenced significantly by injection of glucocorticoid. Subsequently, similar changes in choline pathway activity after steroid treatment have also been demonstrated by Barrett and associates [13] in rabbits by Russell *et al.* [14] in fetal rats, by Ekelund *et al.* [11] with lung explants from human fetuses, and by Smith and Torday [15] using lung cells in tissue culture.

The list of biochemical effects of glucocorticoid on lung development presented in Table 2 gives an indication of enzymes showing increased activities in lung tissue upon corticosteroid administration. These include: (1) *choline phosphotransferase* [10], the terminal

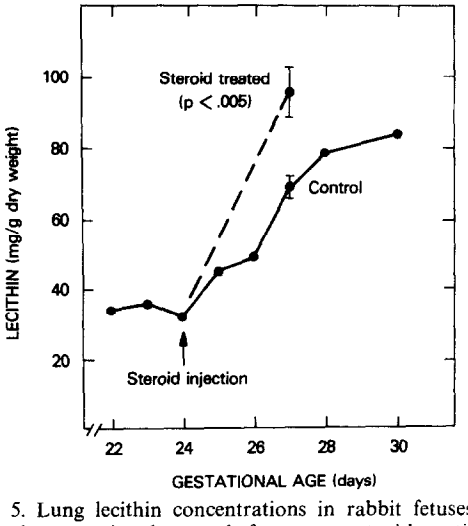

Fig. 5. Lung lecithin concentrations in rabbit fetuses as related to gestational age and after treatment with corticosteroid. Steroid treated fetuses received one injection of 9-α-fluoroprednisolone acetate at 24 days gestation. Adapted from Farrell [22].

enzyme of the choline pathway and a possible rate limiting catalyst; (2) *glycerolphosphate phosphatidyltransferase* [16], a key enzyme in phosphatidylglycerol production—another surface active phospholipid of the alveolar lining layer; and (3) *lipoprotein lipase* [17], which may play an important role in supplying fatty acid for phospholipid synthesis through hydrolysis of circulating triglyceride.

The net biochemical effect of glucocorticoid, through apparent induction of these enzymes, is to enhance the capacity of the fetal lung to produce the surface active phospholipids of the alveolar lining layer. Because of exogenous corticosteroid, this capacity is developed at an earlier time in gestation than would normally be found. The hormone therefore acts as a stimulus capable of changing the *timing* of lung development such that the maturation process is accelerated.

It should be mentioned that the effect of glucocorticoid on accelerating fetal lung development has been demonstrated in a number of species with varying lengths of gestation; these include the rat, rabbit, sheep, and monkey. Data obtained thus far suggest that the effects on lung lecithin concentration and alveolar surfactant are evident within 48 h.

Corticosteroid-mediated acceleration of lung development has been assessed indirectly in humans, where recent clinical trials suggest a potentially important medical use for glucocorticoids in the prevention of the respiratory distress syndrome. It has been shown in the studies of Liggins and Howie [18, 19] that antenatal administration of glucocorticoid leads to a significant decrease in the incidence of this disease. In their trial, betamethasone was administered intramuscularly to pregnant women in premature labor approximately 24 and 48 h before delivery. This potent steroid, crosses the placenta as demonstrated by Ballard and associates [20], and raises circulating glucocorticoid in fetal plasma to approximately three times that found prior to injection (expressed on the basis of cortisol equivalents). The preventative effect relative to respiratory distress syndrome—or as inferred, the ability to accelerate lung development—was particularly impressive in the most immature group of infants, i.e. those of 26–32 weeks of gestation. A number of smaller studies [21], albeit poorly controlled in some instances, have also supported the proposal that glucocorticoids lower the incidence of neonatal RDS. Currently, a multi-center trial of these agents is being organized in the United States by the National Heart, Lung, and Blood Institute.

CONCLUSION

Glucocorticoids influence the development of the fetal lung by: (1) changing the *timing* of pulmonary maturation, that is accelerating the process; (2) by increasing the synthesis of surface active phospholipids, which can then stabilize the respiratory units; and (3) by increasing the capacity of the lung for postnatal gas exchange, thereby facilitating extrauterine adaptation of the delivered newborn infant.

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