Lung Development

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Human lung development is divided into five stages: embryonic, pseudoglandular, canalicular, saccular, and alveolar. The boundaries between these stages are not sharp; rather, overlap occurs between various gestational ages and individuals. The anatomic and morphological characteristics of each stage are described; general principles of lung development and cytodifferentiation of type I and type II pneumocytes are discussed. The complex phenomenon of lung development incorporates two processes—lung growth and lung maturation. Although these processes are developmentally related, they appear to be separately controlled. Lung growth seems to be influenced primarily by physical factors such as intrathoracic space, lung liquid volume and pressure, and amniotic fluid volume among others. Special attention is given to fetal lung liquid dynamics and the effects of its manipulation on lung growth, particularly by tracheal occlusion. Lung maturation has two components—structural and biochemical (ie, surfactant). Structural lung maturation appears to be regulated by physical factors. Physical factors that produce hypoplasia produce structurally immature lungs, whereas physical factors that produce hyperplasia produce structurally mature lungs. Biochemical maturation appears to be hormonally regulated by several endocrine organs (pituitary, adrenal, thyroid) and a host of endocrine factors including corticotropin, cortisol, thyroid hormones, and others.

LUNG DEVELOPMENT is a complex process with a host of regulatory factors. This article first details the five stages of lung development and then describes the various factors affecting the developmental process. Lung development will be considered as a combination of two processes—lung growth and lung maturation. Although these processes are related, they appear to be separately controlled—lung growth primarily by physical factors; lung maturation primarily by hormonal factors.

STAGES OF LUNG DEVELOPMENT

Lung development is divided into five stages: embryonic, pseudoglandular, canalicular, saccular, and alveolar. The boundaries between these stages are not sharp; rather, the different phases blend into one another with considerable overlap between various areas within the lung and also with variation from individual to individual.[1]

Embryonic Lung Development

The human fetal lung originates in the 3-week-old embryo as a ventral diverticulum that arises from the caudal end of the laryngotracheal groove of the foregut.[2] This diverticulum grows caudally to form the primitive trachea. By 4 weeks, the end of the diverticulum divides forming the two primary lung buds. These lung buds then develop lobar buds that correspond to the mature lung lobes, ie, three on the right and two on the left. By about 6 weeks of gestation, the lobar buds have further subdivided and formed the bronchopulmonary segments.

The primitive lung bud is lined by an endodermally derived epithelium that differentiates into both the respiratory epithelium, which lines the airways,[3] and the specialized epithelium, which lines the alveoli and permits gas exchange.[4] The lung bud grows into a rounded mass of mesodermal cells from which the blood vessels, smooth muscle, cartilage, and other connective tissues that form the framework of lung differentiate.[5] Ectoderm contributes to the innervation of the lung.[6]

The Pseudoglandular Phase of Lung Development

From the seventh to the 16th week of gestation, conducting airways are formed by repeated dichotomous branching, resulting in 16 to 25 generations of primitive airways.[2] During this phase, the lung has a distinctly glandular appearance created by small epithelial-lined tubules surrounded by abundant mesenchyme, hence the term pseudoglandular.[5] By the 16th week of gestation, all bronchial airways have been formed.[7-9] After this time, further growth occurs only by elongation and widening of existing airways and not by further branching. In this period, the respiratory epithelium begins to differentiate, cilia appear in proximal airways at 13 weeks, and cartilage begins to form.

During the pseudoglandular phase, mesenchymal-epithelial interactions are necessary for normal lung airway morphogenesis.[10] Spooner and Wessels[11] showed that endodermal lung buds will undergo normal bronchiolar branching in vitro only if exposed to bronchial mesoderm (no other mesoderm, including tracheal, would substitute). Moreover, the rate and extent of bronchial branching appear directly proportional to the amount of mesenchyme present.[10]

In addition to bronchial branching, it appears that

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mesenchyme is necessary for bronchial cytodifferentiation. In fact, with increasing amounts of mesenchyme, epithelial differentiation is shifted from bronchial (ciliated columnar cells and goblet cells) to alveolar (primarily type II pneumocytes).[10] Similarly, differentiation of lung mesenchyme into smooth muscle, blood vessels, and cartilage will occur only when lung epithelium and lung mesenchyme are cocultivated.[12]

The Canalicular Phase of Lung Development

The canalicular phase of lung development takes place from the 16th to the 24th week of gestation. During this time, the basic structure of the gas-exchanging portion of the lung is formed and vascularized.

Early in the canalicular period the lungs have a simple airspace configuration. Potential gas-exchanging structures are smooth-walled, blind-ending channels lined by cuboidal epithelium and supported by abundant loose interstitium and scattered small blood vessels. As the canalicular period progresses, interstitial tissue decreases, capillary growth increases, and these "channels" take on a more complex irregular pattern.

At about 20 weeks of gestation, differentiation of the primitive epithelial cells lining the potential gas-exchanging spaces begins. The first morphological evidence of this phase of differentiation is the growth of capillaries beneath the epithelial cells lining the primitive gas-exchanging channels. In one population of overlying epithelial cells, capillary ingrowth results in thinning of the cytoplasm, narrowing of the air-blood interface, and differentiation into type I pneumocytes—the cells ultimately responsible for gas exchange. In other overlying epithelial cells, the lamellar bodies associated with surfactant synthesis begin to appear, identifying the type II cells that will ultimately be responsible for surfactant production. Although some investigators have concluded that the progenitor of type I cells is an undifferentiated epithelial cell, there is a more convincing body of evidence suggesting that type I cells develop from differentiated type II cells.[13-17] In either case, the growth of capillaries beneath the epithelial cell (either undifferentiated or type II) seems to be the stimulus for type I cell differentiation. By the end of the canalicular period, structural development of the lung has progressed to the point where gas exchange is possible.

The Terminal Saccular Phase of Lung Development

The terminal saccular phase of lung development takes place from 24 weeks of gestation until term and is associated with striking changes in the appearance of the lung. There is a marked decrease in the prominence of the interstitial tissue and a marked thinning of the airspace walls. Tissue projections into the distal airspace regions divide the distal airspaces into saccules. In these saccules, capillaries generally are exposed to only one respiratory surface. Later, in the mature alveolus, each capillary is exposed to at least two alveoli simultaneously.[18]

The cells lining the saccules of human fetal lung at this stage of development are recognizable type I and type II pneumocytes. Morphologically, they are indistinguishable from the corresponding cells described in neonatal or adult human lung tissue. However, biochemically the surfactant produced by the early fetal lung differs from that produced later in gestation. Despite no apparent morphological differences in their lamellar bodies, immature lungs produce surfactant rich in phosphatidylinositol, whereas lungs late in gestation produce surfactant rich in phosphatidylglycerol.[19]

Postnatal or Alveolar Lung Development

The three distinguishing features of an alveolus are (1) it is an open outpouching of an alveolar duct; (2) it is lined almost exclusively by the thin processes of type I pneumocytes; and (3) its interstitial capillaries are exposed to at least two alveoli simultaneously. The barrier between the gas in the alveoli and the blood in the capillaries is normally composed of three layers—the thin processes of the type I cells; a basement membrane that appears to be common to both the endothelial and alveolar cells; and the thin extensions of the endothelial cells. Because the nuclei of all cells are located away from the gas-exchange surface, the barrier to gas exchange usually is only a few nanometers thick.[1] The type I cell is responsible for gas exchange; the type II cell is responsible for surfactant synthesis and secretion. Pringle[1] observed in the lamb lung that there is generally one type II cell per alveolus.

Mature alveoli are not present at birth but begin to appear approximately 5 weeks after birth.[20,21] Boyden[2] used the term "primitive saccule" to describe the simple airspaces found at the lung periphery at birth. The growth and remodeling of the lung periphery in the last third or so of intrauterine growth (terminal saccular phase) result in approximately 20 million of these primitive terminal sacs present in the lung at birth.[22,23] They are lined by mature alveolar epithelium, but in shape they are large shallow cups.[8] These 20 million primitive terminal sacs eventually will develop into 300 million alveoli by age 8 years, with the fastest spurt of multiplication before the age

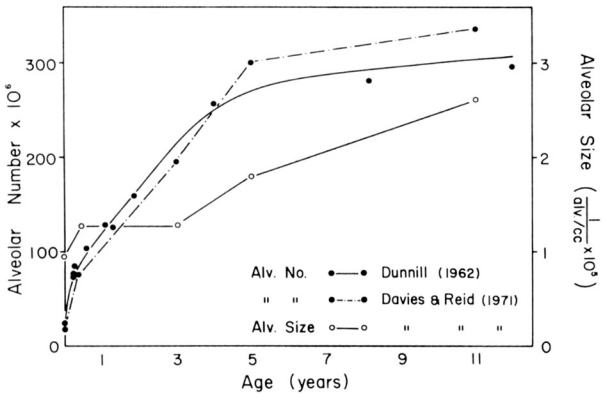

Fig 1. The increase in number and size of alveoli with age using quantitative analysis. Curve of Dunnill's study[22] is fitted from his figures. Figures for number and size from Davies and Reid[23] are shown; size is shown as a reciprocal. (Reprinted with permission.[8])

of 4 (Fig 1).[22-24] Although the "8-years" rule is most widely held, other investigators concluded that alveolar formation is complete by 2 years of age.[25]

In the early years when alveolar multiplication is fastest, there is little change in alveolar size. Then, as the thoracic cage increases relatively faster than multiplication, the alveolar size increases (Fig 1).[23]

Pulmonary Arterial Growth

The pattern of pulmonary artery growth differs depending on the location of the artery relative to the acinus. The preacinar region refers to the conducting airways and includes the trachea, major bronchi, and bronchial branches to the level of the terminal brochiolus. The acinus refers to the functional respiratory unit of the lung and includes structures distal to the terminal bronchiolus (specifically, the respiratory bronchioli, alveolar ducts, and alveoli). In the preacinar region, the pulmonary artery gives off a branch to accompany each airway branch—a "conventional" artery. These conventional arteries ultimately provide terminal branches to supply the acini. In addition, numerous branches arise from the conventional arteries and pass directly into adjacent respiratory tissue to supply the peribronchial parenchyma; these are called "supernumerary" arteries.[26,27]

Just as the branching of bronchial airways is complete by 16 weeks of gestation, all preacinar arteries, conventional and supernumerary, are present by this time as well.[27,28] Subsequent changes in the preacinar arteries are in size only, not in number (again similar to airway growth). In the intraacinar region, terminal branches of the conventional pulmonary arterioles supply the capillary bed. Concurrent with alveolar development, these small vessels of the lung multiply rapidly only after birth to keep pace with alveolar multiplication.

In the adult, complete muscularization of pulmonary arteries is found throughout the acinus, even in the walls of alveoli just under the pleura. However, in the fetus, completely muscularized arteries are proximal to or with terminal bronchioli. Consequently, only partially muscular or nonmuscular arteries are found within the acinus itself. As new alveoli appear during early childhood, so do their accompanying intraacinar arteries. However, muscularization of these arteries is slow. The level within the acinus to which muscular arteries penetrate at various ages is shown in Fig 2.[8]

In summary, although the structural development of the lung is complex, the above information illustrates three "Laws of Lung Development" put forth by Reid. Although these are generalizations, they offer a useful framework in the understanding of lung growth and development.[8,9]

Airway. The bronchial tree is fully developed by week 16 of intrauterine life.

Alveoli. Alveoli develop after birth, increasing in number until the age of 8 years. Size increases until the growth of the chest wall finishes with adulthood.

Blood vessels. The preacinar vessels (arteries and veins) follow the development of the airways; the intraacinar vessels follow that of the alveoli. Muscularization of the intraacinar arteries does not keep pace with the appearance of new arteries.

EVALUATION OF LUNG DEVELOPMENT

Lung development incorporates two processes—growth and maturation. Normal lung growth refers to

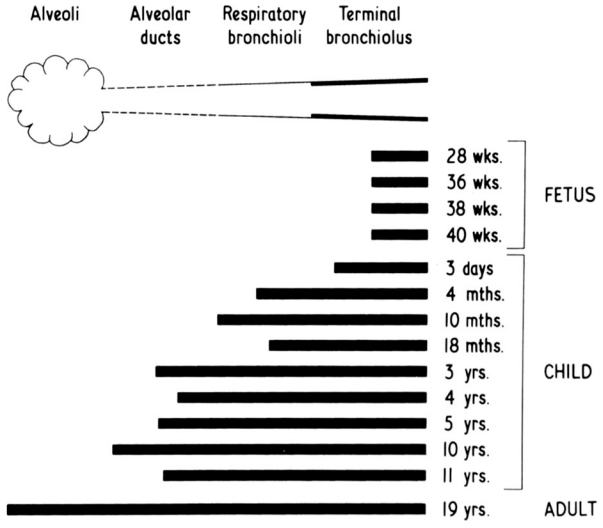

Fig 2. Bar graph showing progression of muscle in the walls of arteries within the acinus. In fetuses there is no muscle within the acinus. With age, there is a gradual extension into the acinar region but, even at 11 years, muscular arteries have not reached the alveolar wall, where they are found in adults. (Reprinted with permission.[27])

cell multiplication. Lung maturation refers to the distensibility or compliance of the lung and is divided into two components—structural and biochemical. Because it appears that lung growth and lung maturation are separately controlled, the terms hypoplasia and hyperplasia will refer only to decreased or increased tissue growth with no implications toward maturation.

LUNG GROWTH

Lung growth is evaluated by the following parameters: lung weight, lung volume, and their ratios to body weight; amount and/or concentration of DNA in the lung; total alveolar surface area and total alveolar number.

Physical Factors Affecting Lung Growth

Intrathoracic Space

Evidence that adequate intrathoracic space is necessary for normal lung development comes primarily from studies that created or simulated fetal diaphragmatic hernia. The first animal model for fetal diaphragmatic hernia (DH) was constructed in 1967 by de Lorimier et al[29] who surgically created DH in fetal lambs. The resulting lungs were hypoplastic by histological appearance and lung weights. Confirmatory studies by Burrington and Olley in 1970[30] and Kent et al in 1972[31] showed similar changes; the latter also demonstrated alterations in hemodynamics, ventilation, and compliance. A model of congenital DH (CDH) in fetal lambs using intrathoracic balloon inflation to simulate herniated viscera first described by Haller et al in 1976[32] was later shown by Harrison et al to produce fatal pulmonary hypoplasia.[33]

Lung Liquid

Experiments of nature suggest that maintenance of lung liquid volume is important to lung growth and maturation and that disruption might be the cause of pathology. Information from laboratory experiments involving fetal tracheal ligation and tracheostomy combined with physiological studies of fetal lung liquid secretion rate, intrapulmonary pressure, and rates of fetal lung growth supports this hypothesis. Unless otherwise stated, all studies discussed below involve fetal sheep.

Normal Fetal Lung Liquid Dynamics

Regulation of lung liquid volume and pressure. In the normal sheep fetus, lung liquid is actively secreted by the pulmonary epithelium.[34-38] This liquid is retained in the intrapulmonary space and maintained at a specific volume and pressure by the regulatory activity of the upper airway, specifically by changes in the efflux of lung liquid from the intrapulmonary space to the amniotic space.[39] When lung liquid volume is experimentally decreased, efflux of lung liquid from the trachea will spontaneously decrease until lung liquid volume and intratracheal pressure are restored to normal. Likewise, an increase in lung liquid volume increases the efflux of liquid until lung liquid volume and intratracheal pressure are restored to normal.[39] The larynx itself seems to be the major sight of this regulation, because decreases in laryngeal resistance by initiation of fetal breathing movements[40-42] and by sectioning of the recurrent laryngeal nerve[43] both lead to an increase in the rate of efflux of lung liquid in fetal lambs.

Lung liquid secretion rate. In the sheep fetus, the earliest gestational measurements of lung liquid secretion rate are 1.6 mL/kg/h at 74 days, increasing to 2.0 mL/kg/h at 84 days.[44] From 85 to 115 days, this rate of 2 to 3 mL/kg/h is maintained.[45] At 115 days, there is a significant increase in the rate of fluid production with reported rates of 4.3 to 5.5 mL/kg/h from 115 to 142 days.[46-48] Several days before delivery, lung liquid secretion rates return to their pre-115-day values,[46,47,49] and during spontaneous labor, the fetal sheep lung actually changes from a secretory to an absorptive state.[46] This slowing of secretion and ultimate reabsorption of lung liquid appears to be due to epinephrine-induced active transport of Na^+ from lung lumen to plasma.[50,51]

Intratracheal pressure. The earliest measurements of in utero intratracheal pressure (ITP) were made by Dickson and Harding[52] who reported values of 0.75 to 1.0 mm Hg from 105 to 115 days of gestation. At 115 days, ITP doubled to 2.0 to 2.5 mm Hg and was maintained at that level until 130 days when measurements were discontinued. Vilos and Liggins[38] documented that ITP remained at approximately 2.0 mm Hg from 120 to 145 days (full term).

Rate of lung growth. In 1981, Alcorn et al[53] outlined fetal lamb lung development from 60 days of gestation to term and noted a doubling in dry lung weight from 108 to 133 days. In 1991, Docimo et al[54] determined that the greatest increase in lung volume and total alveolar number occurred between 112 and 124 days of gestation, with each of these parameters growing exponentially with respect to body weight.

Summary. At approximately 112 to 115 days of gestation, there is a significant change in fetal lung liquid dynamics that coincides with significant increases in lung growth. During this time, the rate of lung liquid production increases substantially as does the intratracheal/intrapulmonary pressure. More significantly, these changes coincide with the most rapid period of lung growth; dramatic increases in lung

volume and total alveolar number occur in the 12 to 14 days following these changes in lung liquid dynamics.

Disruption of Normal Fetal Lung Liquid Dynamics

Experiments of nature. Naturally occurring airway occlusions in humans resulted in large, fluid-filled lungs that histologically appeared to have either normal or slightly distended alveoli.[35,55-58] In other instances, intrauterine airway occlusion resulted in large lungs despite the presence of other anatomic abnormalities that normally would have led to pulmonary hypoplasia. In one instance of Fraser syndrome, an infant with bilateral renal agenesis (associated with pulmonary hypoplasia in Potter's syndrome[59]) and concurrent laryngeal atresia had enlarged lungs.[60] In 1941, Potter[61] reported an infant with left-sided DH and a left lower accessory lobe with no connection to the airway. The normally connected left lung was hypoplastic as would be expected in CDH. However, the accessory lobe, which had no bronchial exit for lung liquid, was expanded, fluid-filled, and its architecture was normal. These findings suggested that maintenance of lung liquid volume was important to lung development and that disruption might be the cause of pathology.

Laboratory experiments. The first experimental tracheal occlusion was reported in 1948 by Jost and Policard[62] who decapitated fetal rabbits and occluded the trachea as part of the operative procedure. On delivery, histological examination of the fetal lungs showed alveoli that were "larger and more dilated than controls." In several animals, the trachea was left in open communication with the amniotic cavity resulting in alveoli that were "small and collapsed." These findings were later confirmed in fetal rabbits[63] and fetal sheep.[64,65]

In 1977, a landmark study by Alcorn et al[66] with fetal sheep showed that tracheal ligation led to increased lung growth, whereas chronic drainage of lung liquid led to pulmonary hypoplasia. Histologically, Alcorn interpreted the "thinning of the future intraalveolar walls and better defined alveolar formation" as being "generally postnatal events" implying precocious maturation in the ligated lungs. She also noted significant differences in ITP with a positive ITP of 6.4 mm Hg in the large ligated lungs and a negative or undetectable ITP in the drained hypoplastic lungs. These effects were later confirmed by other investigators.[67,68]

In 1990, Moessinger et al,[69] using a novel model, continually drained fetal lung liquid of the right lung while simultaneously increasing lung liquid volume of the left by mainstem bronchus ligation. Lung growth evaluated by dry and wet lung weights and DNA content showed hypoplasia of the drained right side and hyperplasia of the ligated left side. However, there was no difference in biochemical indices of lung maturation (total phospholipids, phosphatidylcholine, and disaturated phosphatidylcholine) among tissue from hypoplastic, hyperplastic, and control lungs. This led to the conclusion that although fetal lung cell multiplication was influenced by local distension with lung liquid, the biochemical maturation of fetal lung surfactant was under systemic control.

In 1993, we recreated the well-documented natural experiment of bilateral renal agenesis (Potter's syndrome) using bilateral fetal nephrectomy as the stimulus for pulmonary hypoplasia.[70] This model eliminated fetal urine contribution to amniotic fluid volume and any renal modulation of pulmonary growth. Histologically, lungs with neprectomy (Nx) were hypoplastic; those with nephrectomy and tracheal ligation (Nx/TL) were similar to controls. Total lung volume (normalized to body weight), total alveolar surface area, and total alveolar number were elevated in Nx/TL animals over both Nx alone and controls. Total DNA, total protein, and DNA/protein ratios confirmed lung growth by hyperplasia. These data indicated that fetal TL could not only prevent the pulmonary hypoplasia usually seen in anephric sheep, but could actually accelerate lung growth even beyond normal limits.

Next we determined the effects of tracheal ligation in fetal lambs with surgically created DH.[71] In all DH animals, the left side of the chest was occupied by the stomach, spleen, and small and large bowel, and the lungs were markedly reduced in size (Fig 3A). In contrast, in all animals with DH and tracheal ligation (DH/TL), herniated viscera were completely reduced from the chest by enlarged lungs that had grown through the diaphragmatic defect and into the abdominal cavity (Fig 3B).

Total lung volume (normalized to body weight), total alveolar surface area, and total alveolar number were elevated in the DH/TL animals indicating dramatically increased lung growth over both DH animals and controls. Total lung DNA, total lung protein, and DNA/protein ratios confirmed growth by hyperplasia rather than hypertrophy. Histologically, compared with controls, DH lungs appeared structurally immature with marked thickening of alveolar walls and decreased airspace. In contrast, DH/TL lungs were similar to controls with normal appearing alveoli and thin alveolar septae. Airspace fraction and alveolar numerical density determinations confirmed normal structural maturation in the DH/TL lungs.

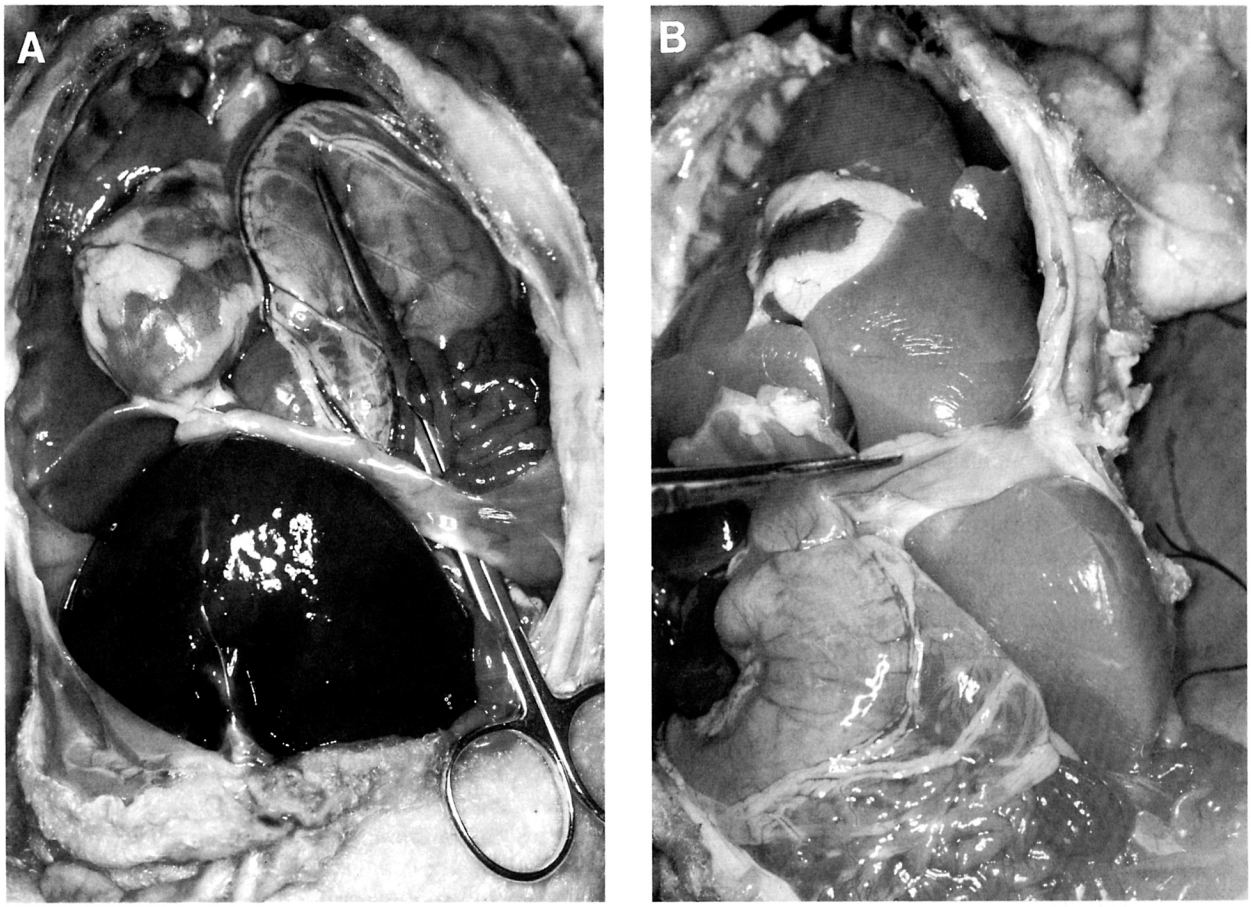

Fig 3. (A) DH animal, chest open via median sternotomy. Scissor passes through left diaphragmatic defect. Abdominal contents are present in left side of chest; lungs are small and not visible. (B) DH/TL animal, chest open via median sternotomy. Forceps are grasping the diaphragm. The enlarged lung has completely reduced the herniated viscera, and has grown into the abdominal cavity. (Reprinted with permission.[71])

On delivery, the DH/TL group showed a positive ITP of 6.14 mm Hg and a dramatic increase in lung liquid volume. Conversely, there was no detectable ITP in the DH or control groups, and lung liquid volume in the DH animals was significantly reduced.

When ventilated, the DH animals exhibited poor gas exchange with a mean highest Pa_{O_2} of 51 mm Hg and a mean lowest Pa_{CO_2} of 143 mm Hg. DH/TL animals had improved function achieving a mean highest Pa_{O_2} of 360 mm Hg and a mean lowest Pa_{CO_2} of 41 mm Hg. Air pressure-volume measurements performed during ventilation showed that lungs in the DH/TL group were 3.5 times more compliant than those in the DH group.

Although the specific mechanisms responsible for lung growth are unknown, we believe increased intratracheal/pulmonary pressure may be involved. The ITP values of 6 to 7 mm Hg consistently found in the DH/TL group are well above the 1.8 to 2.0 mm Hg values seen in normal fetal lambs of similar gestational age in utero[38] and concur with the 6.4 mm Hg ITP found by Alcorn et al in ligated animals.[66] Furthermore, as previously discussed, there are normally significant increases in lung liquid secretion rate and ITP that coincide with the period of maximal lung growth. These data all support our hypothesis that tracheal ligation corrects pulmonary hypoplasia by enhancing normal mechanisms of fetal lung growth.

We further hypothesize that mechanical alveolar distension may promote pulmonary growth through elaboration of local growth factor(s). The study by Moessinger et al[69] of right lung liquid drainage and left lung bronchial occlusion refutes the concept of a systemic growth factor. If this were the case, hypoplasia of the drained right lung in that study should have been prevented by concurrent left mainstem bronchus ligation, and it was not.

Oligohydramnios/Amniotic Fluid Volume

Although the association between oligohydramnios and pulmonary hypoplasia is well known, the mechanisms of this phenomenon remain controversial. Sev-

eral theories including fetal compression by the uterine wall and inhibition of fetal breathing movements have been entertained. However, the bulk of evidence suggests that the pulmonary hypoplasia seen in oligohydramnios is caused by increased efflux of lung liquid from the intrapulmonary space to the amniotic space. The resulting net loss of intrapulmonary pressure, which may be essential to normal lung growth, leads to hypoplasia.

In 1989, Dickson and Harding[72] showed that prolonged oligohydramnios causes decreases in lung liquid volume, secretory rate of lung liquid, and flow rates of tracheal fluid and concluded that fetal lung hypoplasia associated with oligohydramnios may be the result of a prolonged reduction in lung liquid volume. Moreover, fetal breathing movements, previously shown to be associated with increased loss of lung liquid,[73] were also increased.

Summary

There is now a large body of evidence supporting the role of lung liquid in normal and experimental fetal lung growth. It is clear that tracheal ligation causes retention of lung liquid, increased ITP, and lung hyperplasia, and that this hyperplastic effect can reverse the experimental pulmonary hypoplasia of clinically relevant entities such as oligohydramnios and CDH.

Hormonal Factors Affecting Lung Growth

Studies of hormonal influences on lung growth are relatively few. We know that during the last month of gestation in the fetal sheep, lung growth is proportional to the growth of the fetal body. During the last month of gestation, when marked endocrine changes are occurring in the fetus, the constant relationship of lung weight to body weight[74] and the constant concentration of DNA in the lung[75] suggest that hormonal factors play no major role in lung growth. This concept is supported by ablation studies of fetal endocrine organs. Although fetal thyroidectomy[76] and hypophysectomy[77] retard lung growth, they also retard growth of the fetus in general; thus, lung weight is normal when corrected to fetal body weight. Moreover, human infants with pituitary or thyroid aplasia typically are not born with hypoplastic lungs. Although growth factors such as epidermal growth factor (EGF) stimulate growth and budding of the embryonic lung,[78] they have not been shown to affect fetal lung growth after this early stage of lung development.

LUNG MATURATION

Whereas lung growth refers to the "size" of the lung (volume, weight, cell number), maturation generally refers to the distensibility of the lung. The development of a stable distensible lung depends in part on changes in structure of the walls of the airspaces, which become increasingly numerous as septa form and increasingly thin-walled as the epithelium flattens and the mesenchyme thins. This structural aspect of maturation is evaluated by histological appearance and the morphometric parameters of airspace fraction (the percentage of lung occupied by air as opposed to tissue) and alveolar numerical density (the number of alveoli per cubic centimeter of lung tissue), which allow determination of alveolar size.

Maturation also depends on the synthesis, storage, and secretion into the air spaces of surfactant, which increases distensibility by reducing alveolar surface tension. This biochemical aspect of maturation is evaluated by (1) morphological maturation of type II alveolar epithelial cells that produce surfactant and store it in the intracellular organelles known as lamellar bodies; (2) assay of saturated phosphatidylcholine (SPC)—the major surface active component of pulmonary surfactant—in lung tissue or lung liquid; (3) biochemical maturation of enzymes involved in the biosynthesis of SPC; namely, phosphatidate phosphohydrolase (PAPase); and (4) measurement of lung tissue glycogen—an important substrate in surfactant synthesis.

Because the ultimate question of whether a lung is mature depends on distensibility, the overall functional determination of lung maturity is made by pressure-volume or compliance curves that incorporate both structural and biochemical factors.

Physical factors affect the structural aspects of maturation, whereas hormonal factors have a more profound effect on biochemical maturation (ie, the surfactant system).

Physical Factors Affecting Lung Maturation

Physical Factors and Structural Lung Maturation

The effect of physical factors on structural maturation depends on whether the physical factor being examined produces hypoplasia or hyperplasia.

When pulmonary hypoplasia is induced by physical factors such as decreased intrathoracic space,[29,33,71,79] decreased lung liquid volume and pressure,[66,67,69] oligohydramnios,[68,70] and others,[80,81] the resulting lungs are structurally immature by histology[29,33,66-71,79-81] and by airspace fraction and alveolar numerical density.[66,67,70,71] Presently the only physical factor known

to produce lung hyperplasia is retention of lung liquid by tracheal occlusion. In these instances, structural maturation is normal by histological appearance,[66,68-71] airspace fraction,[66,70,71] and alveolar numerical density.[70,71]

Thus, physical factors that produce hypoplasia produce structurally immature lungs; physical factors that produce hyperplasia produce structurally mature lungs.

Physical Factors and Biochemical Maturation

The biochemical maturation of lung, (ie, the surfactant system), seems to be independent of physical factors regardless of whether these physical factors produce pulmonary hypoplasia or hyperplasia.

When pulmonary hypoplasia is induced by physical factors such as decreased intrathoracic space,[79] decreased lung liquid volume,[66,67,69] or alterations in chest wall or diaphragmatic muscle activity,[80-82] the biochemical maturation of the lung is completely normal by both saturated phosphatidylcholine in lung tissue and lavage fluid[67,69,80-82] and by evaluation of type II cell populations.[66,79] Lungs made hyperplastic by tracheal ligation are likewise biochemically normal.[66,69]

The normal biochemical maturation of experimentally hypoplastic lungs reinforces the concept that the decreased distensibility of hypoplastic lungs is due to altered structure rather than to a deficiency in biochemical surfactant system. The apparent contradiction of hypoplastic lungs that are structurally immature but biochemically mature fits nicely with the view by many investigators that the lung initially is a mass of type II cells from which the type I cells develop.[13-17] This explains the increased number of type II cells in the DH model by Pringle et al[79] and the normal surfactant production of other hypoplastic models.[67,69,80-82]

The presumed stimulus for type I cell differentiation is capillary growth beneath the epithelial cell (either undifferentiated or type II); also observed with this capillary ingrowth is a thinning of the overlying cytoplasm. If the thinning of cytoplasm itself was a stimulus for cytodifferentiation, the increased ITP and increased lung liquid volume of tracheal ligation[66,71] could enhance the differentiation of type I cells from type II cells by mechanically thinning cytoplasm. This would explain Alcorn et al's higher ratio of type II to type I cells in drained hypoplastic lungs and lower ratio of type II to type I cells in tracheally ligated hyperplastic lungs.[66] Indeed, changes in cell shape are already known to directly modulate cell growth and differentiation.[83-86]

Hormonal Factors Affecting Lung Maturation

Hormones and Structural Maturation

Hormonal influence on structural maturation has been given little emphasis compared to its effect on surfactant synthesis (biochemical maturation). Nonetheless, structural changes in the lung have been demonstrated by various hormonal manipulations. In the rhesus monkey, Beck et al[87] and Burton and Plopper[88] showed that maternal corticosteroid treatment caused pronounced structural changes, which were independent of surfactant secretion, in the fetal lung. Airspace fraction was elevated, septa were longer, thinner, and less cellular, interstitial tissue was reduced, and alveoli were more developed than controls.

Kauffman[89] found an increase in airspace fraction of dexamethasone-treated mice similar to that in rhesus monkeys. Maximal development of the changes was achieved with doses of corticosteroid that were too low to change lung weight or fetal weight or to induce the formation of lamellar bodies.

Suen et al[90] showed that in the lungs of rats with large CDH (induced by prenatal treatment with nitrofen), antenatal glucocorticoid treatment reduced the saccular septal wall thickness and increased the mean saccular size and volume fraction of saccules when compared with similar CDH rats not so treated.

Hormones and Biochemical Maturation (Surfactant)

In 1969, Liggins[91] reported that infusion of dexamethasone into fetal sheep accelerated the appearance of pulmonary surfactant. His theory was subsequently confirmed in 1970 by DeLemos et al in sheep[92] and in other species by several investigators. Since then a large body of experimental work has been directed toward elucidating the role of hormones in the control of surfactant metabolism.

Adrenal cortex/cortisol. During the last few days of fetal life in the sheep, there is a progressive and marked rise in fetal plasma cortisol concentration.[93] During the same period, there is a marked increase in the concentration of saturated phosphatidylcholine (SPC) in lung tissue and in lung lavage fluid. These increases in SPC concentration correlate closely with fetal plasma cortisol concentration—more closely, in fact, than with gestational age. Quantified distensibility of the lung with air and morphological maturation also correlate closely with the rise in prepartum fetal plasma cortisol suggesting that endoginous cortisol is an important physiological stimulus to fetal lung maturation.[74] Torday found that fetal rabbit lung cells

were incapable of maturation in vitro in the absence of cortisol.[94] Subsequently, Suen et al[90] were able to increase the SPC content of lungs in rats with nitrofen induced CDH by antenatal treatment with glucocorticoids. Cortisol exerts its effect by inducing fetal lung fibroblasts to produce a small protein-fibroblast pneumocyte factor (FPF) that in turn stimulates surfactant production by the fetal alveolar type II cell.[95]

Although the administration of glucocorticoids can increase surfactant production in fetal lung tissue, it is not clear that glucocorticoids are the stimulus for surfactant production in fetal lungs. The concentration of fetal plasma cortisol in several species does not increase until surfactant biosynthesis already has been initiated.[96]

Pituitary/corticotropin. In 1969, Liggins observed that infusion of corticotropin (ACTH) accelerated lung maturation in lambs.[91] The effect was later shown to be mediated through cortisol because corticotropin infusions into premature fetal lambs advanced lung maturity (by distensibility, SPC, and histology) were associated with a rise in fetal plasma cortisol, and because metopirone, which blocks the production of cortisol in the fetal adrenal gland, inhibited the maturational effect of corticotropin.[97] Furthermore, it was shown that fetal hypophysectomy lowered plasma cortisol and markedly retarded lung maturation. Conversely, hypophysectomy and infusion of corticotropin elevated plasma cortisol concentrations, increased lung distensibility, and increased SPC in lavage fluid. In a third group, hypophysectomy and infusion of hydrocortisone elevated plasma cortisol but did not increase lung distensibility or SPC concentration in lung tissue or lavage fluid.[77] These studies indicated that although cortisol is necessary for normal fetal lung maturation, it is not the only means by which corticotropin affects maturation.

Thyroid. Thyroid hormones also are necessary for normal lung maturation. Thyroidectomy in fetal sheep is associated with delayed lung maturation.[76,98] Conversely, administration of thyroxine to the fetal rat accelerates morphological development and increases surfactant synthesis.[99] The fact that thyroidectomy delays maturation but has no effect on plasma cortisol[76] suggests that both cortisol and triiodithyronine (T_3) are necessary for lung maturation. In fact, in cultures of fetal rat lung, dexamethasone and T_3 are additive in their stimulation of SPC.[100] Thyroidectomy also prevents the improvement in distensibility seen when corticotropin is infused into hypophysectomized fetuses,[77] suggesting that the effect of hypophysectomy is due in part to loss of thyroid-stimulating hormone (TSH) (and therefore of T_3), and that at the pituitary level, both TSH and corticotropin may be involved in lung maturation.

Insulin. Fetal insulin may inhibit formation and release of pulmonary surfactant in the fetal lung. In cultures of lung cells from fetal rabbits, insulin abolishes the stimulatory effect of cortisol on lecithin synthesis.[101] In fetal sheep, increased circulating concentrations of insulin produced either by insulin infusion or glucose infusion decreases the flux of surfactant in tracheal fluid.[102,103]

Epinephrine. Several studies in fetal rabbits show evidence of increased surfactant release after administration of β-adrenergic agents. Infusion of epinephrine into fetal sheep stimulates increased flux of surfactant in tracheal fluid, the effect being most pronounced near term.[104] Cortisol stimulates the enzyme that converts norepinephrine to epinephrine[105] and also stimulates formation of β-adrenergic receptors in the lung of fetal rabbits.[106] Thus, the rise in fetal plasma cortisol during the last several days of gestation may affect surfactant metabolism by direct effect on the lung as well as by stimulating production of epinephrine and formation of β-adrenergic receptors.

Epidermal growth factor. EGF has been found to stimulate the proliferation of alveolar type II pneumocytes[107]; when injected into rabbit fetuses, it accelerates lung maturation by increased production of surfactant.[108] Injection of EGF into fetal lambs causes epithelial hyperplasia of the conducting airways and also prevents the development of the idiopathic respiratory distress syndrome if the lambs are delivered prematurely by cesarean section.[109]

Other factors. Other factors proposed to influence surfactant production include prolactin, testosterone, estrogen, and prostaglandins. In summary, there is no single hormone or factor but rather a total hormonal milieu necessary for the normal processes of lung maturation.

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