1 Physiologic Principles of the Respiratory System

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A central issue in caring for ill or premature infants is the successful management of respiratory status. Management can be complicated by the relative lack of development of these infants' respiratory structures and the functional immaturity of their respiratory systems. This chapter therefore begins with a review of the developmental and functional anatomy of the lungs and associated structures. Subsequent sections address lung mechanics, the synthesis and roles of pulmonary surfactant, and the physiology of lung fluid and of fetal breathing. The events of transition and other elements of neonatal respiratory physiology are also discussed. Particular attention is given to those issues that are unique to the neonate, with the hope that this information will assist the clinician in understanding and optimally facilitating respiration in the newborn infant.

Respiratory System Development

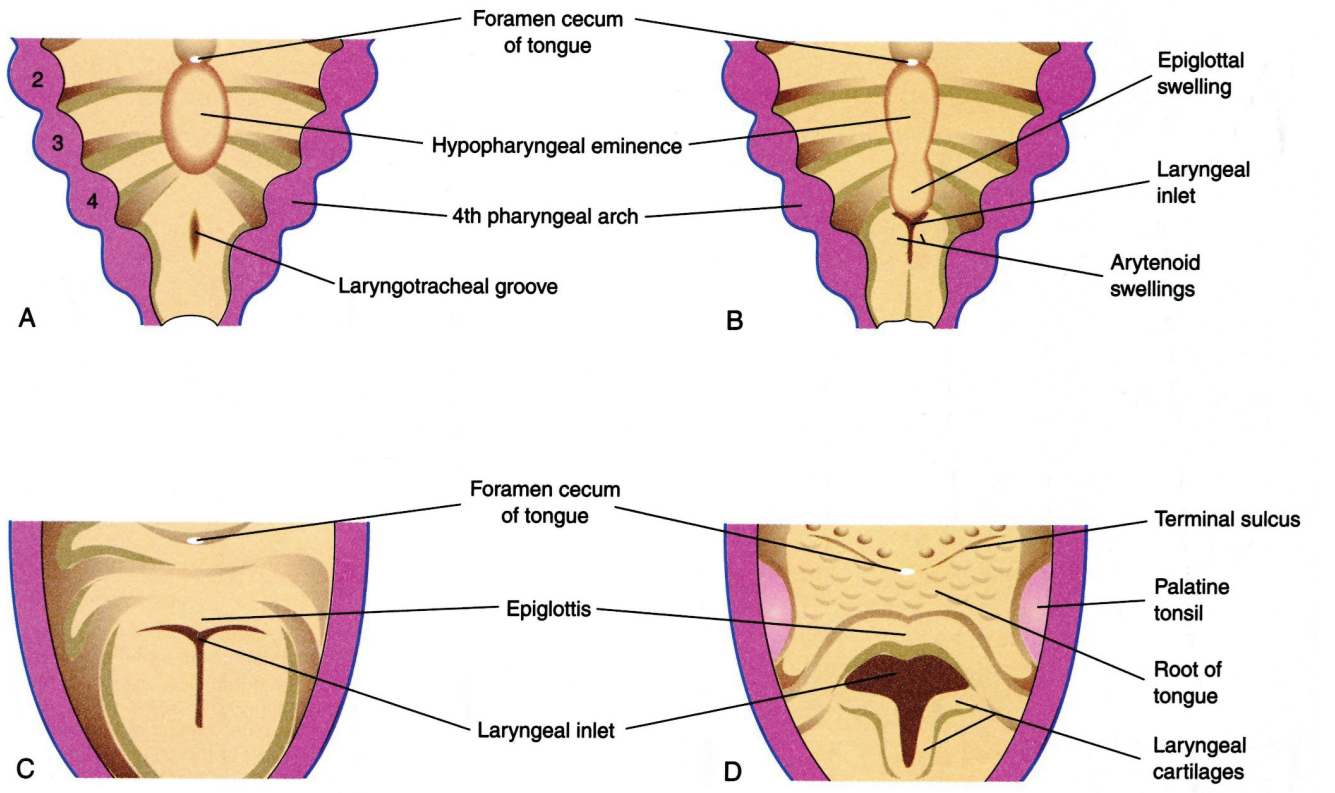
The organs of the lower respiratory system, which include the larynx, trachea, bronchi, and lungs, begin to develop during the embryonic period, in the fourth week following conception. Once the basic rudiments of the respiratory system have been established, they undergo considerable refinement during the subsequent fetal period of life. Even after parturition, respiratory structures, most notably the lungs, continue to mature and change both structurally and functionally. The focus of this section of the chapter is lung development during the embryonic, fetal, and postnatal periods. Various mechanical and biochemical signals control these developmental events.[1] These mediators are discussed later in the chapter.

Embryonic Lung Development

Late in the embryonic period (four weeks postconception), the rudiments of the respiratory system are established. The lower respiratory structures, which include the larynx, trachea, bronchi, and lungs, begin to form in this period.[2] The anatomic precursor of the future respiratory system is the laryngotracheal groove, an outgrowth of the primordial pharynx, which is visible by approximately day 24 of embryonic development (Figure 1-1). This groove extends downward and is gradually separated from the future esophagus by a septum. Failure of the septum to develop completely results in a tracheoesophageal fistula. Several other congenital anomalies of the respiratory system can develop during the embryonic and/or early fetal periods; they are summarized in Table 1-1.

Most of the anatomic rudiments of the respiratory system are laid down during the eight weeks comprising the embryonic period. Between days 26 and 28 postconception, the first dichotomous branches of the lung (bronchial) buds can be seen (Figure 1-2). By 35 days of embryonic development, secondary bronchi are evident; at 56 days postconception, three divisions are distinguishable on the right and two on the left (lobar and segmental bronchi). While these buds are dividing, the trachea is forming through the elongation of the upper portion of the lung bud. By the end of the embryonic period, the conductive lung pathways have been formed and need only to lengthen and increase in diameter.[3,4] The portions of the lungs related to gas exchange have not yet been elaborated, however, and must develop before the fetus can survive.

FIGURE 1-1
Development of the larynx at (A) 4 weeks, (B) 5 weeks, (C) 6 weeks, and (D) 10 weeks.



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Fetal Lung Development

There are four stages in lung maturation, which span the end of the embryonic and all of the fetal period of development (weeks 6–40). These include the pseudoglandular, canalicular, terminal saccular, and alveolar stages. Lung maturity, both anatomic and functional, is key to successful transition to the extrauterine environment, and these stages are discussed in that context.

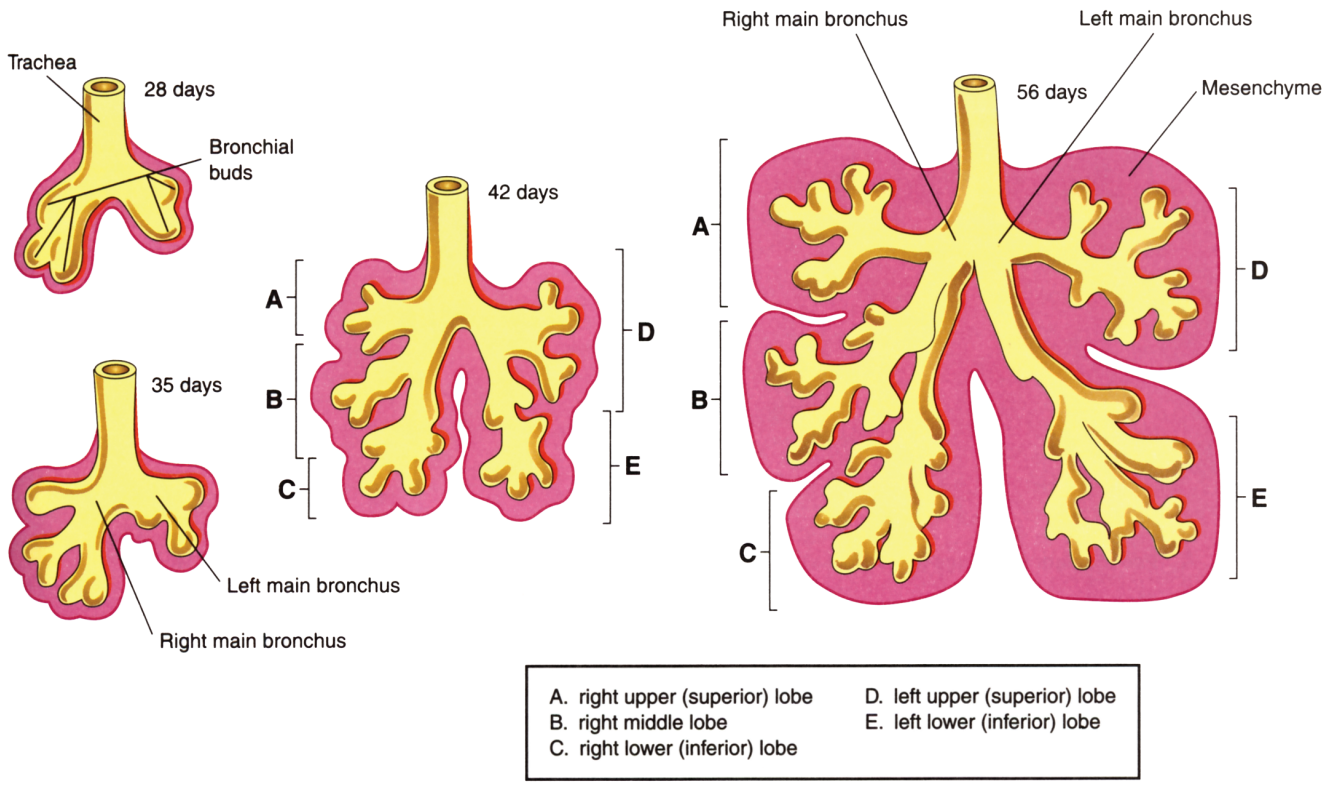
Pseudoglandular Stage (6–16 weeks)

From 5 to 17 weeks postconception, a tree of narrow tubules forms. New airway branches arise through a combination of cell multiplication and necrosis.[5] These tubules have thick epithelial walls composed of columnar or cuboidal cells. This morphology, along with the loose mesenchymal tissue surrounding the tree, gives the lungs the appearance of an exocrine gland. The ends

TABLE 1-1
Congenital Anomalies of the Respiratory System

Anomaly	Origin	Time Frame/Incidence
Pulmonary atresia (single lung or lobe)	Failure of primitive foregut branches to develop	Four to six weeks
Tracheoesophageal fistula	Failure of foregut (tracheoesophageal) septum to completely divide the esophagus and trachea	Four to five weeks Incidence: 1 in 2,500 births
Tracheal stenosis/atresia	Unequal division of foregut into trachea and esophagus	Four to five weeks Incidence: Rare
Diaphragmatic hernia	Failure of fusion of the septum transversum, pleuroperitoneal membranes, lateral body wall, and dorsal mesentery of esophagus	Six to ten weeks Incidence: 1 in 2,000 births

FIGURE 1-2
Development of the bronchial buds, bronchi, and lungs.



From: Moore KL, and Persaud TVN. 2008. *The Developing Human: Clinically Oriented Embryology,* 8th ed. Philadelphia: Saunders, 203. Reprinted by permission.

of the tubules are terminal bronchioles (Figure 1-3A), which are too thick to permit gas exchange. Fetuses born during this period are therefore unable to survive. The conductive portion of the tracheobronchial tree (trachea to terminal bronchioles) is now well established, and rudimentary forms of cartilage, connective tissue, muscle, blood vessels, and lymphatics can be identified.[6] One of the primary developmental anomalies during this period is diaphragmatic hernia (see Table 1-1).

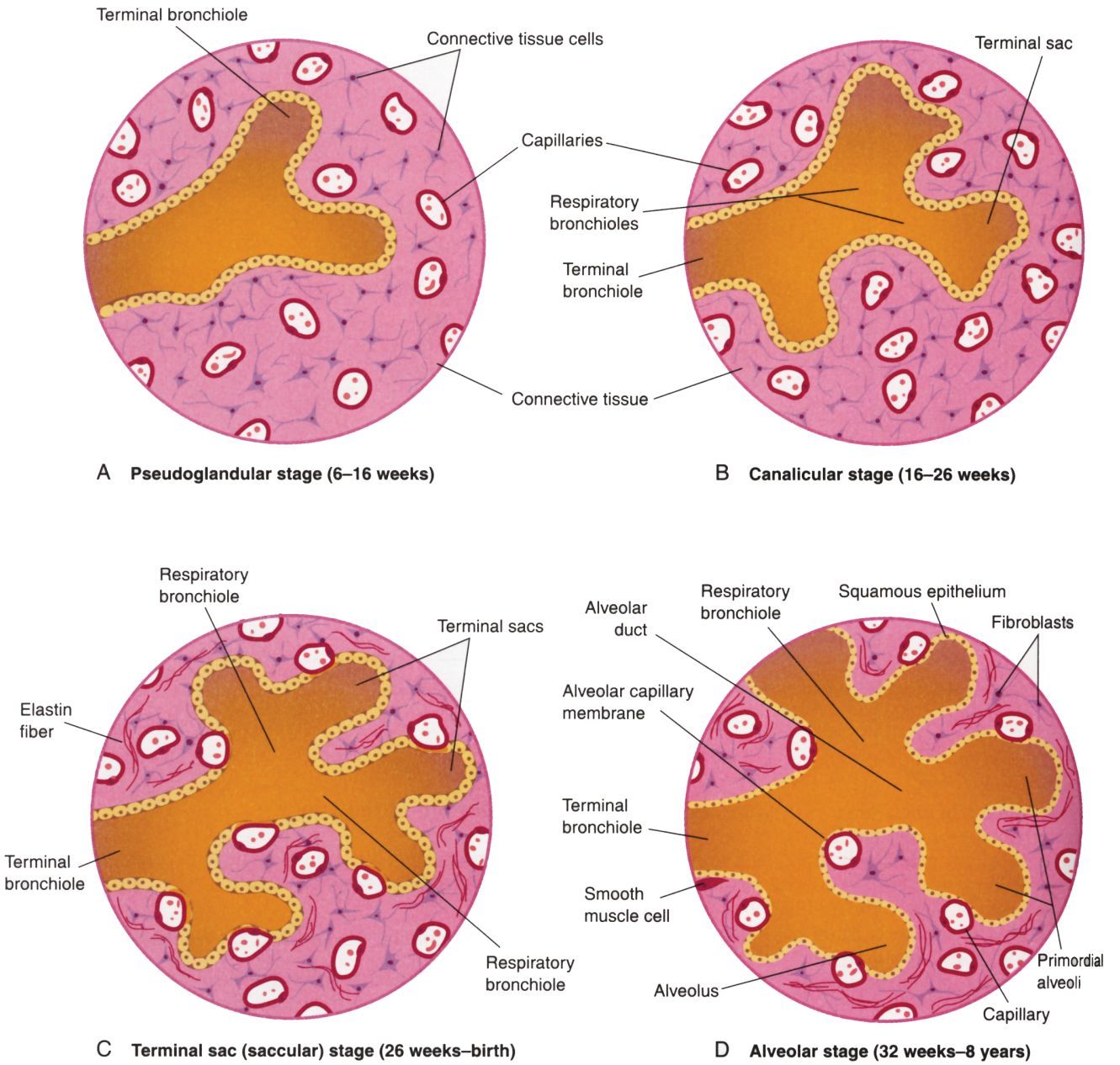
Canalicular Stage (16–26 weeks)

The canalicular stage overlaps the pseudoglandular stage because the superior lung segments develop before the inferior segments. The epithelial cells of the distal air spaces (future alveolar lining) flatten sometime between weeks 13 and 25, signaling the beginning of the canalicular stage (Figure 1-3B). A rich vascular supply begins to proliferate, and with the changes in mesenchymal tissue, the capillaries are brought closer to the airway epithelium. By week 24 of gestation, terminal bronchioles give rise to two or more respiratory bronchioles, which in turn develop into alveolar ducts. Toward the end of this crucial developmental period, primitive alveoli called terminal saccules develop at the tips of the respiratory bronchioles. These structures are thin walled, thereby permitting gas exchange. The increased vascularity evident by this time, along with the development of the terminal saccules, makes it possible for infants born at the end of this stage to survive with intensive care, depending on the degree of saccular-capillary coupling.[7]

Terminal Saccular Stage (26 weeks–birth)

Two critical changes occur during the terminal saccular stage: Many more terminal saccules develop, and their epithelium becomes increasingly thin. Capillaries invade what are now developing alveoli, and the blood-air interface becomes more elaborate (Figure 1-3C). This involves a close physical association between thin alveolar epithelial cells and capillary endothelial cells, which in many regions share a fused basement membrane, greatly facilitating gas exchange. Early in this period, most cells lining the alveoli are squamous cells, called

FIGURE 1-3
Progressive stages of lung development (illustrated using histologic sections).



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type I alveolar cells, or pneumocytes. Increasingly, rounded pneumocytes, or type II cells, also populate the primitive alveoli. These cells secrete pulmonary surfactant, a complex mixture of phospholipids, which forms a monomolecular layer over the internal surface of the terminal saccules. The biochemistry of surfactant and its role in lung compliance are discussed later in this chapter. Birth during this phase may result in several conditions, including respiratory distress syndrome (RDS).

Alveolar Stage (32 weeks–8 years)

Structures resembling alveoli are usually present on the terminal saccules by 32 weeks gestation. At the beginning of this period, respiratory bronchioles end

in several thin-walled saccules that are surrounded by connective tissue. The squamous epithelial cells lining the terminal sacs acquire a thin, stretched appearance, and adjacent capillaries encroach into the terminal saccules. These primitive alveoli are recognizable as small bulges on the walls of terminal saccules and respiratory bronchioles (Figure 1-3D). Mature alveoli typically do not form until after birth. At birth, primordial alveoli enlarge as the lungs expand. The main contributor to the increase in lung volume that is seen after birth, however, is a growth in the number of respiratory bronchioles and alveoli; approximately 95 percent of all alveoli develop after birth.

Genetic and Environmental Influences on Prenatal Lung Development

The four fetal development stages and their associated morphologic changes are tightly regulated. The developmental program that guides these events is complex, but we are now beginning to understand the molecular genetic control mechanisms, which include multiple tissue interactions, transcription factors that decode specific genes, and growth and signaling factors that are expressed or become active at specific time points. A detailed review of these genes and molecules is beyond the scope of this chapter. An overview of the subject is useful, however, in that knowledge of the molecular controls of lung development will likely yield treatment strategies for respiratory problems associated with premature birth or abnormal lung development.[8]

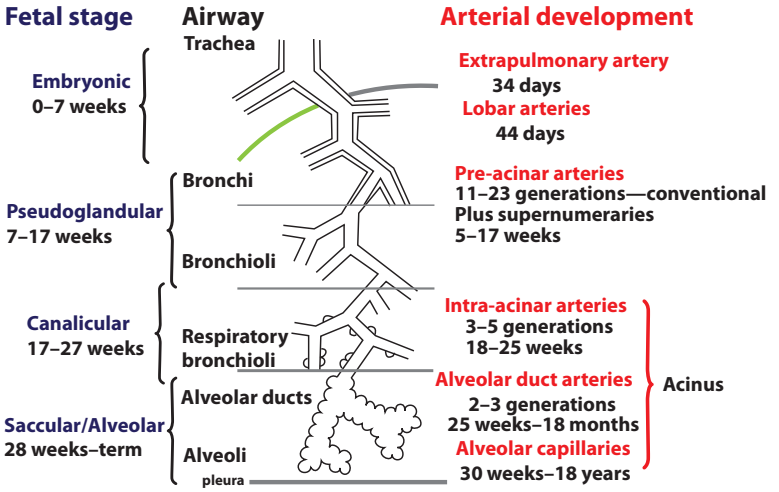
During the embryonic stage, when the trachea and lungs originate from endoderm, two key transcription factors are implicated: hepatocyte nuclear transcription factor (HNF-3β) and thyroid transcription factor (TTF-1). If the gene coding for HNF-3β is experimentally ablated in mice, the foregut does not form, which leads to the absence of lungs.[9] Furthermore, HNF-3β controls the expression of surfactant protein B and Clara-cell–specific protein.[10] As for TTF-1, mice lacking this gene exhibit tracheoesophageal fistulae and hypoplastic lungs.[11] Still other genes, such as sonic hedgehog (Shh), fibroblast growth factor-8, and N-cadherin, modulate the expression of the transforming growth factor-8 family of genes, resulting in left-right asymmetry of the lungs.[8] Many of these genes have multiple functions and are expressed at specific locations. For example, Shh is expressed at the tips of developing lung branches and appears to control pulmonary cell proliferation.[12]

During the pseudoglandular stage, when preacinar airways and blood vessels develop and the branching pattern of the bronchial tree is formed, two genes appear to be especially important: Gata-6 and N-myc.[13,14] Mutations in and/or knockouts of these genes result in reduced branching and lung hypoplasia. Many other gene products and receptors are implicated in this process, yet the essential question that underpins lung development in the fetus is "What specifies when and where these genes get 'turned on'?" The answer must, in part, lie in the physical environment of the lung.

During the pseudoglandular stage of development, fetal breathing, peristaltic airway contraction, and the beginning secretion of lung fluid exert physical forces on the lungs.[8] If these forces are disrupted through surgical or mechanical means, lung hypoplasia results. For example, chronic deflation of a lung results in hypoplasia, whereas chronic overexpansion produces hyperplasia.[15] Although the notion that mechanical forces playing a role in lung development may seem intuitive, it is important to remember that this process is regulated at the molecular level. The question then becomes "How do these mechanical forces generate molecular signals?" An emerging body of evidence suggests that physical stress can stimulate growth factor and growth factor receptor expression. The clinical relevance is that, as we understand more about these signals and forces, it may be possible to therapeutically enhance them through targeted gene expression.

In addition to the genetically programmed events of lung development discussed in the following sections, the environment of the uterus itself likely influences growth. Fetal hypoxemia, which has been studied extensively in sheep, is known to affect a variety of physiologic parameters, lung growth and development included. For example, moderate degrees of hypoxemia affect the hypothalamic-pituitary-adrenal axis in sheep, even when pH and arterial carbon dioxide tension ($PaCO_2$) level are controlled.[16] These effects of hypoxemia include increased plasma adrenocorticotrophic hormone (ACTH) and cortisol, the latter being critical for the structural and functional maturation of surfactant-secreting type II alveolar cells. Furthermore, surfactant protein A messenger RNA (mRNA) is increased in hypoxemic fetuses, whereas insulin-like growth factor-I and its binding protein-5 mRNA are decreased. Other work with fetal lung explants subjected to hypoxia has shown the suppression of several biochemical and morphologic markers of lung cell differentiation.[17] Other uterine environment (maternal) factors are also likely to elicit changes in prenatal lung development and may present opportunities for therapeutically modulating fetal lung maturation.

FIGURE 1-4
Development of the intra-acinar arteries.



From: Hislop A. 2005. Developmental biology of the pulmonary circulation. *Paediatric Respiratory Reviews* 6(1): 35. Reprinted by permission.

Postnatal Lung Development: Genetic and Environmental Influences

Lung development continues for a considerable time after birth; the developmental program spans not only embryonic and fetal life, but also infancy and early childhood. In addition to genetic factors, the extrauterine environment also comes into play, and exogenous factors help to shape lung growth and development. These forces may be classified as either mechanical or nonmechanical. With respect to the former, septal wall strain, which can be induced by growth of the thorax and/or hyperinflation, is known to influence lung growth and maturation.[18] Capillary distention and shear forces can also affect lung growth. Ligation of one pulmonary artery increases alveolar growth of the contralateral lung in newborn piglets.[19] We also know that pulmonary venous hypertension in humans can cause thickening of the endothelium and epithelium of the alveolar-capillary barrier.[20] Acute interstitial edema in the lungs increases the synthesis and deposition of collagen and glycosaminoglycans.[21]

How these mechanical signals are transduced into structural/biochemical alterations in lung tissue is a broader question. Without doubt, biochemical mediators are involved. Many nonmechanical mediators of lung growth have been identified. They include hormones, growth factors, and cytokines.

Pulmonary Vasculature

Pulmonary vasculature develops in conjunction with the branching of the bronchial tree.[22] This process involves both vasculogenesis, or new growth of blood vessels from embryonic angioblasts, and angiogenesis, or the "sprouting" of blood vessels from existing vessels.[1] As the preacinar airways develop, bronchial or systemic vessels also differentiate, dividing into the conventional and supernumerary tributaries that supply the peripheral acini. This development is complete by approximately 16 weeks of gestation, but the vasculature continues to grow in length and diameter to accommodate lung growth. Generally, if there is a decrease in the number of preacinar airways, there is a concomitant decrease in conventional and supernumerary arteries.[23]

As development progresses into the canalicular and terminal saccular stages, intra-acinar arteries appear; they will continue their development during the postnatal period (Figure 1-4). The conventional arteries continue their development for the first 18 months of life, and supernumerary arteries continue to be laid down for the first eight years.[24] These late-developing supernumerary vessels are smaller and more numerous than their precursors, servicing the alveoli directly.[25] If blood flow is reduced or blocked through the conventional arteries, the supernumerary arteries may serve as collateral circulation, maintaining lung function during periods of ischemia or increased pulmonary vascular resistance.[26] Postnatally, the intra-acinar vessels multiply rapidly as alveoli appear.[22]

The pulmonary veins develop more slowly. By 20 weeks gestation, however, preacinar veins are present.[24] The structural development of veins parallels that of the arteries and conducting airways, although supernumerary veins outnumber supernumerary arteries. Interestingly, both acinar and supernumerary veins appear simultaneously.[6] The development of additional veins, as well as the lengthening of existing veins, continues postnatally.

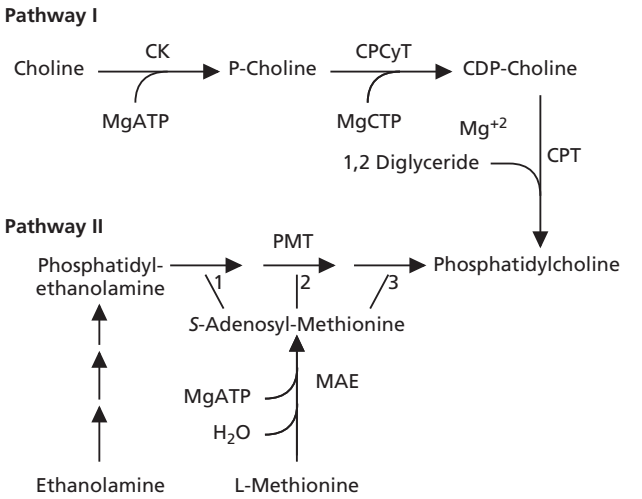
Further development of the pulmonary circulation is related to changes in muscle wall thickness and muscle extension into arterial walls. The pulmonary artery wall is relatively thick at birth, as a result of the low oxygen tension in the intrauterine environment. The wall thins as oxygen tension rises at birth, and the medial-layer

TABLE 1-2
Surfactant Composition

Composition by Weight (Percent)		
Phospholipids		85
Saturated phosphatidylcholine	60	
Unsaturated phosphatidylcholine	20	
Phosphatidylglycerol	8	
Phosphatidylinositol	2	
Phosphatidylethanolamine	5	
Sphingomyelin	2	
Others	3	
Neutral lipids and cholesterol		5
Proteins		10
Contaminating serum proteins	8	
Surfactant protein 35 (32–36,000 daltons)	~1	
Lipophilic proteins (6–12,000 daltons)	~1	

From: Jobe A. 1987. Questions about surfactant for respiratory distress syndrome (RDS). In *Mead Johnson Symposium on Perinatal and Developmental Medicine.* Evansville, Indiana: Mead Johnson, 43. Reprinted by permission.

FIGURE 1-5
Pathways for phosphatidylcholine synthesis.



From: Farrell PM, and Ulane RE. 1981. The regulation of lung phospholipid metabolism. In *Physiological and Biochemical Basis for Perinatal Medicine,* Minkowski A, and Monset-Couchard M, eds. Basel, Switzerland: S. Karger AG, 31. Reprinted by permission.

elastic fibrils become less organized. The pulmonary vein is deficient in elastic fibers at parturition and progressively incorporates muscle and elastic tissue during the first two years of life.[24]

The intrapulmonary arteries also have relatively thick walls. The smaller arteries have increased muscularity and dilate actively as oxygen tension increases postnatally.[23] There is a concomitant fall in pulmonary vascular resistance.[6] Between 3 and 28 days postnatally, these vessels achieve their adult ratio of wall thickness to external diameter. The larger arteries take longer, increasing to adult thickness between 4 and 18 months postparturition.[24]

The systemic arteries of the fetus are also more muscular than those of the adult or child. The ratio between muscle thickness and external diameter of the systemic arteries decreases postnatally.[24] Muscle distribution changes following birth (with the muscle extending peripherally) and continues to change during the first 19 years of life.

Alveolar Epithelium

The respiratory portion of the lung has a continuous epithelial lining composed mainly of two cell types: type I and type II pneumocytes. The type I cells are squamous, or flattened, and cover approximately 95 percent of the alveolar surface via numerous interdigitating cytoplasmic extensions.[27,28] The thinnest area of the alveolus is composed of these extensions, and gas exchange occurs here most rapidly. The type II pneumocyte, although more numerous than the type I pneumocyte, occupies less than 5 percent of the alveolar surface.[27] Osmiophilic, lamellated bodies are characteristic of the type II cells, which are under the control of numerous hormonal axes and which will ultimately produce pulmonary surfactant. The first type II cells are seen in the fetus between 20 and 24 weeks gestation. Surfactant secretion is detectable between 25 and 30 weeks gestation, although the potential for alveolar stability is low.

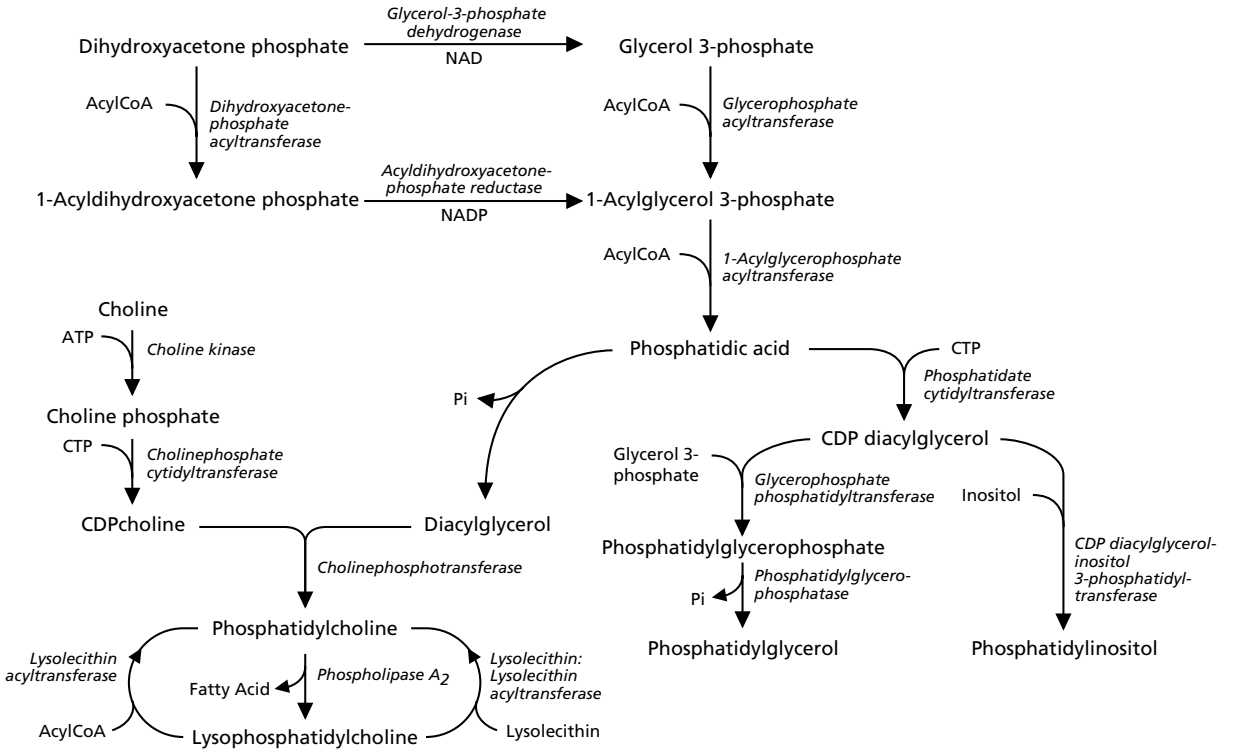
Pulmonary Surfactant

As indicated previously, surfactant coats the inner surface of the alveoli and is a key determinant of inward-directed collapsing pressure in the lungs. It is therefore also important with respect to compliance. This section discusses both the composition and synthesis of surfactant, as well as the influence of hormones on surfactant biosynthesis and secretion.

Composition and Synthesis

The composition of pulmonary surfactant is shown in Table 1-2. Surfactant is a lipoprotein, with 90 percent of its dry weight composed of lipid.[29] The majority of this lipid (60 percent) is saturated phosphatidylcholine (PC), of which dipalmitoylphosphatidylcholine (DPPC) is the most abundant. DPPC is the component responsible for

FIGURE 1-6
Biosynthesis of phospholipids.



From: Jobe A. 1987. Questions about surfactant for respiratory distress syndrome (RDS). In *Mead Johnson Symposium on Perinatal and Developmental Medicine.* Evansville, Indiana: Mead Johnson, 13. Reprinted by permission.

decreasing the surface tension to almost zero when compressed at the surface during inspiration.

Phosphatidylglycerol (PG) accounts for another 8 percent of the phospholipid in surfactant, a ratio that is unique to lung cells. PG is the last phospholipid to develop in surfactant. Because fetal lung fluid flows into the amniotic cavity, the presence of PG in the amniotic fluid is a good marker for the presence of surfactant and, hence, lung maturity. The rest of the compound is involved in intracellular transport, storage, exocytosis, adsorption, and clearance at the alveolar lining.[27,29]

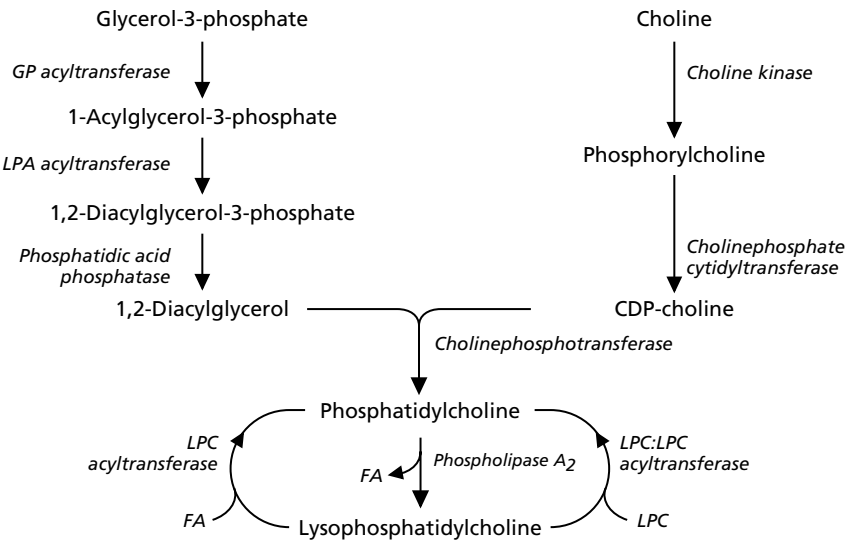
Surfactant synthesis involves a series of biochemical events that includes synthesis and integration of surfactant components in the membranes of the smooth and rough endoplasmic reticulum and multivesicular bodies of the type II pneumocyte.[27] Once assembled, surfactant is transported intracellularly to the Golgi apparatus and then on to the lamellar bodies.[30] The biosynthesis of surfactant is discussed in detail in several publications.[27,29] Chapter 11 also contains more information on surfactant.

Before it is biochemically functional, surfactant is transformed into a lattice-shaped structure known as tubular myelin. This transformation is influenced to a large extent by surfactant protein D.[31] This substance enhances surfactant spreadability and adsorption. Surfactant is then stored in the lamellar bodies of type II pneumocytes. Secretion occurs by exocytosis; however, surfactant must diffuse to the surface of the liquid layer to be physiologically functional.[27]

There are two major pathways for PC synthesis (Figure 1-5). Key precursors for PC synthesis include glycerol, fatty acids, choline, glucose, and ethanolamine.[32] The primary synthetic pathway for PC is the cytidine diphosphate choline system. The other biosynthetic pathway—the methyltransferase system—leads to phosphatidylethanolamine formation. The latter has minor significance in the adult lung and seems to play a relatively insignificant role in fetal lung development.[32]

The biosynthesis of PC, phosphatidylinositol, and PG is depicted in Figure 1-6. The schematic illustrates the importance of phosphatidic acid biosynthesis. The increased production of phospholipids seen in late gestation depends on the increased synthesis of this acid.

FIGURE 1-7
Biosyntheses and remodeling of phosphatidylcholine.



From: Farrell PM, and Ulane RE. 1981. The regulation of lung phospholipid metabolism. In *Physiological and Biochemical Basis for Perinatal Medicine,* Minkowski A, and Monset-Couchard M, eds. Basel, Switzerland: S. Karger AG, 33. Reprinted by permission.

The majority of phospholipid produced is PC; Figure 1-7 represents the biosynthesis and remodeling of this critical phospholipid.[29]

Figure 1-7 also demonstrates the interaction of the choline pathway and the diglyceride synthesis mechanisms that yield increased PC synthesis during late gestation. Although this interaction produces increased quantities of PC, the PC is not the highly saturated version identified in the final surfactant compound. The remodeling of PC that occurs in the phosphatidylcholine-lysophosphatidylcholine cycle provides the dipalmitoyl-PC required for surfactant.[32]

As gestation advances, alveolar phospholipid content and saturation increase. This is accompanied by a growth in the number of osmiophilic inclusion bodies within type II pneumocytes. Choline incorporation, which is low in early gestation, has been reported to rise abruptly in Rhesus monkeys when gestation is 90 percent complete.[33] This suggests that this particular pathway is upregulated in advance to meet postnatal needs.

Enzymatic changes in the phospholipid synthesis pathway are discussed in several review articles.[29,32,34] The correlation of these changes with the surge in saturated PC and increase in PG and the concomitant decrease in phosphatidylinositol is not yet understood. Whether it is a change in concentration of enzyme or substrate, adjustment in catalytic efficiency, change in substrate affinity, or activation of latent enzymes is not known.

Hormonal Influences on Surfactant Secretion and Production

In addition to enzymes, hormones also regulate surfactant biosynthesis and secretion. Those hormones that have been implicated include glucocorticoids, ACTH, thyroid hormones, estrogens, prolactin, thyrotropin-releasing hormone, catecholamines, insulin, fibroblast pneumocyte factor, prostaglandins, and epidermal growth factor. Only glucocorticoids, thyroid hormones, catecholamines, and insulin are reviewed here because these endocrine factors are the primary axes affecting lung development.

Glucocorticoids

Glucocorticoids are probably the best known of the hormones affecting surfactant. Liggins' observations in 1969 set off a flurry of research in the area of hormonal control of fetal lung development that has continued to the present.[35] Glucocorticoids accelerate the normal pattern of fetal lung development by increasing the rate of glycogen depletion and phospholipid biosynthesis.

Depletion of glycogen leads to direct anatomic changes in alveolar structures, thinning the interalveolar septa while increasing the size of the alveoli. Other morphologic changes include increases in the numbers of type II pneumocytes and lamellar bodies within those cells. This occurs in conjunction with a functional maturation of these cells, leading to an accelerated synthesis of surfactant phospholipids.[29,36]

Glucocorticoids, like most steroid ligands, bind to intracellular receptors, forming a receptor-ligand complex that acts as a nuclear transcription factor. This ultimately results in the generation of a repertoire of new proteins, the identities of which are currently unknown. Dexamethasone and betamethasone have a higher affinity for the glucocorticoid receptor than do the natural corticoids (cortisol and cortisone), which favors the use of betamethasone to enhance lung maturation during preterm labor.[37]

The question of whether the triggering of protein synthesis accounts for

the increase in fatty acid synthetase, phosphatidic acid phosphatase, and choline-phosphate cytidyltransferase activity is yet to be answered. Conflicting evidence suggests that glucocorticoids could promote the synthesis of an enzyme activator that may influence the production of the heavier of the surfactant apoproteins.[38]

Several other questions have yet to be answered as well. It is evident that glucocorticoid action is centered on surfactant synthesis, not secretion. It is also clear that it affects more than surfactant synthesis. Acting directly on lung tissue, glucocorticoids increase the number of β-adrenergic receptors and enhance elastin and collagen production, which improves lung compliance.[39]

What has until recently not been evident is whether glucocorticoids directly affect type II pneumocytes or whether they mediate their actions through other lung cells, such as fibroblasts. Smith suggested that, rather than having a direct impact on type II cells, glucocorticoids may act on fibroblasts (increasing the production of fibroblast pneumocyte factor), which then affects surfactant production.[40] More recently, however, experiments with cultured human type II pneumocytes have revealed that these cells produce at least two surfactant components in response to synthetic glucocorticoids. In experiments by Ramin and coworkers, type II cells were exposed to physiologic concentrations of either betamethasone or dexamethasone. Surfactant protein B mRNA production increased after 48 hours' exposure to steroids, as determined by quantitative reverse transcription-polymerase chain reaction.[41] This would imply that type II cells do not require the cooperation of other lung cells to respond to steroids. As determined by other researchers using cultured pneumocytes, this effect is likely limited to seven to eight days after exposure to dexamethasone.[42] Glucocorticoids also regulate the expression of at least one other surfactant gene in type II cells. Studies with a fetal rat type II cell line have established that glucocorticoids regulate the expression of the fatty acid synthase (FAS) gene.[43] The protein product of this gene is a key enzyme that is involved in *de novo* synthesis of fatty acids, which are integral components of pulmonary surfactant. Interestingly, glucocorticoids increased FAS activity, protein content, mRNA content, and rate of transcription in type II cells, but not in lung fibroblasts. Although these findings do not rule out positive cooperation between type II pneumocytes and other cells such as fibroblasts, they do suggest that type II cells can respond autonomously to glucocorticoids. Yet another gene, ABCA3, which is involved in membrane transport in type II cells, is also up-regulated by glucocorticoids.[44] Defects in this gene have been found in newborns with fatal surfactant deficiency.

Not only are glucocorticoids involved in the regulation of surfactant production; they also appear to regulate architectural changes in the newborn lung. During the canalicular stage of development (see **Canalicular Stage [16–26 weeks],** earlier in this chapter), septal wall thickness decreases, in part as a result of a lessening in the amount of fibronectin, an extracellular matrix protein. In rats treated with dexamethasone, fibronectin expression falls, a finding congruent with the notion that dexamethasone promotes alveolar-wall thinning.[45] The cellular target in this case is unknown, although it seems likely that alveolar fibroblasts are involved.

In all research involving cell cultures, an important caveat is that cultured cells are genetically different from their cells of origin, and they are not in the same cellular milieu as cells *in vivo* or in intact lungs. Despite these limitations, cell culture studies have provided important information that may be generalized to the neonate. For example, prenatal (maternal) betamethasone administration changes the phospholipid profile in tracheal aspirates of very preterm infants.[46] It is also generally accepted that glucocorticoids enhance both biochemical and structural maturation of the neonatal lungs.[47] Cell culture work has enabled us to more specifically identify the likely effectors of glucocorticoids. There are, however, controversies related to the choice of glucocorticoid, number and timing of doses, as well as side effects.[48] These topics are addressed in Chapter 11.

Thyroid Hormones

The idea that hormones may work in conjunction with other compounds or hormones is reinforced by observations of the actions of thyroid hormones. Thyroxine (T_4) and triiodothyronine (T_3) have been shown to increase the rate of phospholipid synthesis.[29,49–51] As do glucocorticoids, thyroid hormones enhance production of PC through choline incorporation. They do not, however, increase PG synthesis or stimulate the production of surfactant-specific proteins.[52,53] Although glucocorticoids increase fatty acid synthetase activity, thyroid hormones seem to decrease it.[54] These disparities suggest different sites of action for these hormones, as well as their need to act in conjunction with other hormones.[52]

Low maternal T_3 and T_4 levels have been associated with RDS in the neonate, although exact mechanisms are unclear.[32,36,51,55] It has also been shown that the effects of thyroid hormones are mediated by a specific thyroid receptor that is a potent phospholipid-synthesis

stimulator and to which T_3 has a higher affinity than T_4.[56]

Clinical application of this information is aimed at maximizing beneficial effects through the delivery of hormones or hormone-activating substances that cross the placenta. Naturally occurring thyroid hormones do not readily cross the placenta unless concentrations far exceeding normal levels are achieved. However, thyrotropin-releasing hormone does cross the placenta and stimulates the fetal pituitary gland to produce thyroid-stimulating hormone, resulting in increased production of PC.[36,57]

Continued investigation into the precise mechanisms of thyroid hormone action is essential. There seems to be little doubt that synergistic interaction between glucocorticoids and thyroid hormones occurs, apparently at the level of mRNA.[52] A significant increase in PC production occurs in a shortened period of time when these two hormones are used together prior to delivery.[50,51,56] These findings may have significant clinical implications, which may alter future therapeutic interventions. A review of the Cochrane database has shown, however, that antenatal coadministration of thyroid-releasing hormone with glucocorticoids does not improve any fetal, neonatal, or childhood outcomes.[58] Prenatal administration of TSH appears to have a number of adverse effects for both women and their infants, including increased likelihood of ventilation and lower five-minute Apgar scores for the neonate, and poorer outcomes when children are seen at follow-up.[58]

Catecholamines

Glucocorticoids and thyroid hormones play a role in enhancing the synthesis of phospholipids, whereas catecholamines stimulate the secretion of surfactant into the alveolar space. This appears to be a direct action of adrenergic compounds on type II cells.[59] The response is prompt, occurring in less than an hour.

Research has shown that catecholamines increase surfactant and saturated PC in the lung fluid and improve lung stability. This is demonstrated in an increased ratio of lecithin to sphingomyelin. An added benefit is a decrease in fetal lung fluid within the alveoli at the time of delivery. These two effects (increase in surfactant and decrease in lung fluid) work together to prepare the fetus for respiratory conversion.[36,60]

Insulin

Surfactant development appears to be inhibited in neonates born to diabetic mothers whose blood sugar levels are not well controlled. Whether this is caused by hyperglycemia, hyperinsulinemia, or both is unclear, and research continues to provide conflicting answers. Maturation of surfactant synthesis occurs at the same time glycogen is depleted from the lungs. Insulin inhibits glycogen breakdown, thereby decreasing the substrate available for PC synthesis as well as altering the natural anatomic changes that occur with glycogen depletion.[29,36] These alterations affect the ability of the lungs to perform respiratory functions.

Insulin also reduces the effect of cortisol on choline incorporation, even though it does not reduce cortisol effects on cell growth.[61] The biochemical interactions are complex; some researchers were unable to document evidence of an insulin influence or of any insulin antagonism of the usual dexamethasone response.[62] Others have reported a synergistic effect when cortisol and insulin were combined.[25]

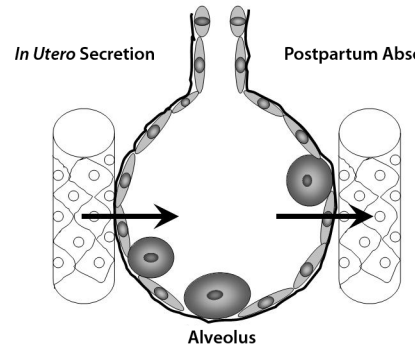
Insulin may antagonize glucocorticoids at the fibroblast level, affecting the production of the fibroblast pneumocyte factor.[63] Miakotina and colleagues have shown that insulin directly inhibits surfactant-associated peptides A and B expression in lung epithelial cells.[64] Clinically, this likely predisposes infants of diabetic mothers to RDS, which argues for stricter monitoring and control of maternal glucose levels.

At present, surfactant synthesis appears to be controlled by a complex interaction of several hormones and factors. Normal lung function clearly depends on the presence of surfactant, which permits a decrease in surface tension at end-expiration and an increase in surface tension during lung expansion. This prevents atelectasis at end-expiration and facilitates elastic recoil on inspiration. Surfactant provides the lung with the stability required to maintain homeostatic blood gas pressures while decreasing the work of breathing. For the preterm infant, endotracheal administration of exogenous surfactant has been unequivically established as an efficacious treatment to minimize respiratory morbidity and mortality. Currently, there are studies that suggest that intrapartum administration of surfactant (before the first breath is drawn) may be safe and effective, although more studies are required.[65]

Lung Fluid

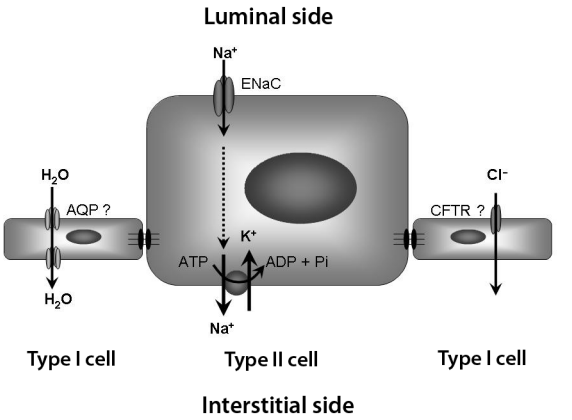
In utero, the lung epithelium secretes fluid into the air spaces, a process necessary for development of the alveoli.[66] A delicate balance of secretion and reabsorption of fluids occurs during lung development. The relative

FIGURE 1-8
Switch from net fluid secretion (fetus) to fluid absorption (neonate) as part of transition from placental to atmospheric oxygen delivery.



Courtesy of William Diehl-Jones.

FIGURE 1-9
Ion and water transport in production of alveolar fluid.



Key: ATP = adenosine triphosphate; ADP = adenosine diphosphate; AQP = aquaporin; CFTR = cystic fibrosis transmembrane conductance regulator; ENaC = epithelial sodium channel; Pi = inorganic phosphate.

Courtesy of William Diehl-Jones.

rates of these two processes shift between the prenatal and the postnatal periods. Alveolar fluid is secreted from the beginning of the canalicular stage until birth. In the newborn lamb at term, this fluid is continually secreted and reabsorbed, with complete turnover every ten hours. It is quite likely that lung fluid plays an important part in cell maturation and development, as well as in determining the formation, size, and shape of the developing air spaces. For example, alterations in fluid dynamics affect pulmonary cell proliferation and differentiation.[67]

At birth, however, the infant must make the transition from placental to atmospheric oxygen delivery. This necessitates a switch from net fluid secretion to absorption (Figure 1-8). As with secretion of many types of fluid, alveolar fluid production depends on sodium transport, which in turn drives osmotic water movement. To facilitate these fluxes, the appropriate ion and water transporters must be present. Lung fluid is believed to be derived from alveolar epithelial cells, and experimental evidence suggests that type II alveolar cells are the primary site of fluid secretion: They possess both amiloride-sensitive epithelial sodium channels (ENaCs) and water channels called aquaporins (AQPs).[67] The ENaC proteins allow vectorial transport of sodium ions, whereas AQPs facilitate water movement along the osmotic gradient established by sodium (Figure 1-9). A host of other ion channels, including potassium, chloride, and proton channels, are also found in types I and II alveolar cells; these permit the transport of ions that contribute to alveolar and tracheal fluid. Osmolarity, sodium, and chloride levels are lower in amniotic fluid than in tracheal fluid; pH, glucose, and protein are higher.[68] Some lung fluid is swallowed, and some moves into the amniotic fluid, although lung fluid's contribution to the amniotic fluid is not significant when compared with the volume secreted by the kidneys. The lung fluid volume is approximately equal to the functional residual capacity (FRC) and must be either expelled or absorbed at birth.[69,70]

Animal fetuses whose tracheas have been ligated develop relatively large but immature lungs. Fetuses whose lungs have been drained have thick alveolar walls, smaller lungs, and more abundant type II cells.[71] This is consistent with the finding that human infants who have experienced amniotic fluid leakage have reduced numbers of alveoli.[72] These data together suggest that reduced lung fluid production or leakage of amniotic fluid places the fetus at risk for lung hypoplasia.[73] Chronic tracheal obstruction leads to hyperplasia, with an increase in the number of alveoli, although they are functionally immature.[26]

As indicated earlier, concomitant with the increase in alveolar absorption as birth approaches, there is a lessening in the rate of secretion. The administration of epinephrine to lambs leads to a decrease in fluid secretion. This effect is mediated by β-adrenergic receptors in the alveolar epithelium and may either suppress chloride transport or activate a second transport process (such as amiloride-sensitive sodium channels) that enhances the absorption process.[74] The absorption rate is known to grow as gestation progresses; this increase in absorption can be correlated with a gain in catecholamine levels.[75] During gestation, the fetal adrenal glands are

probably not stimulated to produce sufficient amounts of catecholamines to trigger the absorption process; labor, however, provides sufficient stimulus to the glands to release enough epinephrine to prompt the switch from secretion to absorption.[76] The catecholamine surge that occurs at delivery is probably the final mechanism to assure that the change from secretion to absorption is completed.[68]

The drop in pulmonary vascular resistance with aeration and the rise in oxygen tension increase the number of alveolar capillaries perfused, enhancing blood removal capacity. Between the enlarged lymphatic flow and the dramatic change in pulmonary blood flow, lung fluid is dispersed within the first few hours following delivery.

with gestational age and becoming more organized and vigorous.[68] Even with these gestational changes, tracheal fluid shifts are negligible, the pressure generated being no more than 25 mmHg.[80–82] Fetal maturation leads to the appearance of cycles, with a growth in the number of fetal breathing movements during daytime hours.[77–83] Fetal breathing movements peak in late evening and reach their nadir in the early morning hours.[84]

Abnormal breathing patterns can be seen during periods of hypoxia. Mild hypoxemia decreases the incidence of fetal breathing movements; severe hypoxemia may cause them to cease for several hours. The onset of asphyxia leads to gasping that persists until death.[85] Interestingly, the onset of mild hypoxemia (as with umbilical artery occlusion of short duration) may lead to quiet sleep, which for the fetus decreases activity, energy expenditure, and oxygen consumption.[77] Although paradoxical in nature, this conservation mechanism may save the fetus until cardiac output is redistributed toward the placenta.

A reduction in fetal breathing movements before delivery coincides with the increase in prostaglandin E concentrations seen during the final days of gestation. These factors play a role in respiratory conversion at birth.[86] Furthermore, the placenta may be a key source of inhibitory prostaglandins. Infusion of the prostaglandin biosynthesis inhibitors aspirin and indomethacin with placental extracts significantly inhibits fetal breathing in sheep.[87] That finding further implicates prostaglandins as one of the factors regulating fetal breathing. Why irregular fetal breathing movements lead to the sustained respirations of postnatal life remains unknown.