Development and Growth of the Lung

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I. OVERVIEW
The lung is designed to provide a large internal surface area in which the inspired air and capillary blood get in intimate contact to each other and thus allow for an efficient exchange of gases. This goal is achieved by a sequence of distinct but overlapping developmental processes (Fig. 5-1; Table 5-1). Organogenesis (embryonic stage) starts with a ventral outpouching of the foregut giving rise to the left and right lung buds. The following development of the airways and gas exchange structures is based on two different mechanisms. Starting from the lung buds, a continuous process of branching and growth into the surrounding mesenchyme preforms the whole conducting airway tree and probably also parts of the respiratory airways (branching morphogenesis). Most of this process takes place during the pseudoglandular stage. The tremendous increase of the gas exchange surface area in late fetal and postnatal lung development is brought about by a process of repetitive airspace septation leading to the formation of alveoli (stage of alveolarization). The canalicular and saccular stages may be considered as intermediate stages between both developmental principles. In the canalicular stage the first air-blood barriers are formed and surfactant production starts. During the saccular stage the switch from branching to septation occurs.

IV. POSTNATAL LUNG DEVELOPMENT
Alveolar Stage (Week 36 to 1–2 Years)
Microvascular Maturation Stage (Birth to 2–3 Years)

V. GROWTH OF THE LUNG
Transition from Development to Growth
Growth of the Lung Microvasculature
Dimensions of the Adult Lung

Following bulk alveolarization, which in humans occurs mainly after birth, the interalveolar septa and their capillary networks are remodeled to optimize gas exchange during the stage of microvascular maturation. At this point lung development is viewed as finished; it is followed by normal growth of the organ. However, we assume today that some form of late alveolarization at a slow rate may exist. Relative to lung development, the time point of birth varies greatly among mammalian species. In humans, birth occurs early in the alveolar stage.

The staging of lung development as described traditionally is highly descriptive. It is based on light microscopic observations of morphologic changes in the developing lung (Figs. 5-1 and 5-2). Large overlaps exist between subsequent stages for two reasons. First, lung development proceeds metachrotically from the proximal to the peripheral portions of the airway tree. Second, some developmental steps and maturation processes defining the stages may run in parallel in neighboring areas. The beginning of pulmonary development is relatively well defined by the first appearance of the future trachea and the lung buds in the region of the foregut. There is no such clear limit, however, as to when it ends during childhood. Thus, lung development slowly blends into lung growth and it boils down to a question of sophisticated definitions when development ends and growth starts.

OVERVIEW

The lung is designed to provide a large internal surface area in which the inspired air and capillary blood get in intimate contact to each other and thus allow for an efficient exchange of gases. This goal is achieved by a sequence of distinct but overlapping developmental processes (Fig. 5-1; Table 5-1). Organogenesis (embryonic stage) starts with a ventral outpouching of the foregut giving rise to the left and right lung buds. The following development of the airways and gas exchange structures is based on two different mechanisms. Starting from the lung buds, a continuous process of branching and growth into the surrounding mesenchyme preforms the whole conducting airway tree and probably also parts of the respiratory airways (branching morphogenesis). Most of this process takes place during the pseudoglandular stage. The tremendous increase of the gas exchange surface area in late fetal and postnatal lung development is brought about by a process of repetitive airspace septation leading to the formation of alveoli (stage of alveolarization). The canalicular and saccular stages may be considered as intermediate stages between both developmental principles. In the canalicular stage the first air-blood barriers are formed and surfactant production starts. During the saccular stage the switch from branching to septation occurs.
**Prenatal Lung Development**

**Embryonic Period (Weeks 4–7)**

**Organogenesis**
Following fertilization, the germ cells soon segregate into a cluster of trophoblastic cells to which a few embryoblastic cells adhere. The trophoblastic cells may be viewed as the future placenta, whereas the embryoblastic cells, after differentiation into the three germ layers, will form the human embryo.

**Lung Anlage**
The organs of the body are laid down by differentiation from the germ layers during the so-called embryonic period, which encompasses the first 7 weeks after fertilization. The lung anlage appears at day 26 as two ventral buds of the foregut at the caudal end of the laryngotracheal sulci (Figs. 5-3 and 5-4; Table 5-1). It will give rise to the left and right lung. Both buds elongate, grow into the surrounding mesenchyme, and form the left and right main bronchi (day 32) (Figs. 5-3 and 5-4). The terminal ends of the growing bronchial tree start a repetitive process of growth and mainly dichotomous branching. By day E37 the future conducting airways are preformed to the lobar, by day E41 to the segmental, and by day E48 to the subsegmental bronchi (Figs. 5-3 and 5-4). An early stage of a budding mouse lung is illustrated in Fig. 5-4.

**Epithelial-Mesenchymal Interactions**
As is evident from the preceding description, the high columnar epithelium of the tubular sprouts is of endodermal origin, whereas the mesenchyme is derived from the third germ layer, the mesoderm. This double origin of the lung tissues is important: many processes of lung development are dependent on the interaction between epithelium and mesenchyme. Classical transplantation experiments have shown that a cross-talk between the endodermal epithelium and the mesodermal mesenchyme is needed for the control of branching morphogenesis and cytodifferentiation, e.g., after removal of the mesenchyme at the growing tip further branching of the epithelial tubules is prevented. But when the mesenchyme of the growing tip is transplanted next to the prospective trachea, an abnormal outgrowth of bronchial branches was observed in this region.

During the last decade some of the mechanisms involved have been deciphered. Briefly, the epithelium of the growing terminal bud differs from the more proximal epithelium and the epithelium of the forming cleft, which represents the depression between the two buds. The proliferation rate is significantly elevated at the growing end buds as compared to the epithelium directly proximal to it and to the one of the cleft. The basement membranes are also different. Although nidogen-1 (also known as entactin-1), collagen IV, and fibronectin are absent in the basement membrane at the growing end buds, tenasin-C is present and the latter takes part in the control of the number of branches.

In addition, a large set of factors which are secreted into the mesenchyme contribute significantly to the epithelial-mesenchymal cross-talk. These factors are produced in both the epithelium and mesenchyme and have a strong influence on the behavior of the epithelium. They include transcription factors like TTF-1, Gli2, and Gli3; as well as growth factors like FGF-10, TGF-β, BMP-4, SHH, EGF, and VEGF. No growth
### Table 5-1

<table>
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<tr>
<th>Period</th>
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| Embryonic     | Embryonic                 | Rabbit: n.d.–E18
Sheep: E17–E30
Human: E26–E49 (4–7 Weeks)
Mouse: E9.5–E12
Rat: E11–E13 | Start of organogenesis; formation of major airways |
| Fetal         | Pseudoglandular           | Rabbit: E18–E24
Sheep: E30–E85
Human: E35–E119 (5–17 weeks)
Mouse: E12–E16.5
Rat: E13–E18.5 | Formation of bronchial tree and large parts of prospective respiratory parenchyma; birth of the acinus |
|               |                           | Canicular Rabbit: E21–E27
Sheep: E80–E120
Human: E112–E182 (16–26 Weeks)
Mouse: E16.5–E17.5
Rat: E18.5–E20 | Completion of conducting airways; epithelial differentiation; first airblood barrier; appearance of surfactant |
|               |                           | Saccular or terminal sac Rabbit: E27–E30
Sheep: E110–E140
Human: E168–E266 (24 weeks-term)
Mouse: E17.5–P4
Rat: E21–P4 | Expansion of airspaces |
| Postnatal     | Alveolar                  | Rabbit: E30–term (E31)
Sheep: E120–term (E145)
Human: E252 (36 weeks preterm): 1–2 years
Mouse: P4–P14
Rat: P4–P14 | Alvolarization by formation of secondary septa (septation) |
|               |                           | Microvascular maturation Rabbit: unknown
Sheep: unknown
Human: 0–3 years
Mouse: P14–P21
Rat: P14–P21 | Remodeling and maturation of interalveolar septa and of the capillary bed |
|               | Normal growth             | Rabbit: Birth–adulthood
Sheep: Birth–adulthood
Human: 2nd year–adulthood
Mouse: 4 weeks–adulthood
Rat: 4 weeks–adulthood | Normal growth of the lungs |

The described duration of the stages represents the time where the bulk of a particular developmental alteration takes place. Stages are overlapping, in particular the alveolar stage and the stage of microvascular maturation. In addition, regional differences are common, especially between central and peripheral regions. Litter size and nutrition also have an influence on the exact timing of development. E = embryonic day (days post-coitum); n.d. = not determined; P = postnatal day.
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Figure 5-2  Development of the airways and arteries. The stages of lung development (blue) are correlated to the development of the bronchial tree (black) and the arteries (red). An average value of the generations formed by each category of airways is given in parentheses. On average an airway of the human lung ends after 23 generations in an alveolar sacule. In reality this number ranges from about 18 to 30. In the lung pathways are of varying lengths, partly due to space constraints in the thoracic cage. Pre-acinar arteries start as a capillary plexus surrounding the growing lung buds (vasculogenesis). Intra-acinar arteries grow by angiogenesis. (Based on Hislop A: Developmental biology of the pulmonary circulation. Paediatr Respir Rev 6:35–43, 2005.)

During later stages of prenatal and postnatal lung development, and even in the adult lung, the interplay between differentiating pneumocytes, mesodermally derived interstitial cells, and extracellular matrix continues to be decisive for cell maturation and cell function regulation.

Development of Esophagus and Trachea

The laryngotracheal sulci of the lateral wall of the foregut are deepening and joining in parallel to the appearance of the first airway divisions. As a result the foregut is divided into the prospective trachea and the esophagus by the esophageal-tracheal septum (Fig. 5-3). The formation of the trachea appears to be independent of the formation of the lung buds. Because FGF10 null mice develop a trachea, but no lung buds, we must assume that trachea formation is independent of the formation of the lung buds. Toward the end of the embryonic period mesenchymal cells surrounding the prospective trachea condense focally and differentiate into cartilage precursors. Proximally, embryonic cartilage is found at the end of week 7. Cartilage formation moves distally along the future airways. Cartilage is commonly found in main bronchi around week 10 and in segmental bronchi in week 12 of gestation. However, cartilage formation continues almost until the end of the canalicular stage until it reaches its completion around the smallest bronchi (week 25). In animal studies and in the human lung, Sparrow and co-workers have demonstrated in beautiful confocal laser scanning microscopic studies that, as the primitive airways and vessels grow and divide,
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Figure 5-4  Lung organogenesis. Freshly explanted mouse lung is shown at days E11.5 (A) and E12.5 (B) toward the end of the embryonic period (E11.5/A) the visceral pleura and main bronchi of the lung lobes are formed. In mice the right lung consists of four lobes (cranial, middle, caudal, and accessory) and the left lung of only one. At the beginning of the pseudoglandular stage (E12.5/B) the lungs are already subdivided into definitive lobes. As seen in both lungs, the branching pattern of the bronchial tree is not dichotomous, but monopodial in mice. LL, left lung; LR, right lung. Bar = 0.5 mm.

As early as the pleural mesenchyme has formed invaginations of the pleura start to separate the lobar bronchi and give rise to the lobar fissure and the lung lobes (Fig. 5-4). Little is known about the mechanisms involved. It appears, however, that the basement membrane of the visceral pleura plays a role, because mice lacking the nidogen-binding domain of the laminin-γ1-chain or the laminin-α5-chain show defective lobar septation and visceral pleura basement membrane formation.

At around 7 weeks, the period of organogenesis can be considered to merge imperceptibly with the period proper of lung development.

Vasculogenesis of the Pulmonary Circulation

In the adult lung, the pulmonary arteries are found in close proximity to the branches of the airways and they distribute the blood to the alveolar capillary bed. The pulmonary veins are independent of the bronchial tree. They are found roughly in the middle between the pairs of bronchi and arteries. They maintain a similar number of branches and return the blood from the capillary bed to the heart. The close proximity of the circulation and the airways suggests that their development is linked to each other.

The mesenchyme surrounding the lung buds contains a number of cells staining positively for markers of endothelial cells. By day 34 of gestation each prospective main bronchus possesses a capillary network that connects cranially to the aortic sac and caudally to the left atrium (Fig. 5-5). After establishment of these connections there is evidence of circulating blood cells. Apparently, the earliest pulmonary vessels form by vasculogenesis—a process in which vessels form de novo due to a differentiation of mesenchymal cells. The newly formed endothelial cells are connecting to each other to form first capillary tubes. These capillaries coalesce to form small blood vessels alongside the airways. During branching of the future airways a new plexus forms as a halo around each newly formed bud. Each plexus adds to the peripheral circulation and extends the arteries and veins. Thus there is sustained addition of the newly formed tubules to the existing vessels and the airways act as a template for the development of blood vessels.
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Figure 5-6  Morphology of pseudoglandular stage of lung development in human and rat. A. Human fetal lung, gestational age about 15 weeks. Bright bands of loose mesenchyme containing veins (v) indicate subdivision of lung into segments and lobules. Denser mesenchyme surrounds the tubular sprouts. B. Subpleural region of rat lung, gestational day 18.5. Zone I (I) is characterized by a loose arrangement of mesenchymal cells immediately below the pleura. In zone II (II) epithelial tubes are enwrapped by a more densely packed network of interstitial cells. The zone I to II boundary is the site of formation, growth, and differentiation of the gas exchange region. The future conducting airways are located in zone III (III), which is characterized by epithelial tubes with an outer layer of smooth muscle cell precursors (smc). Pa, pulmonary artery; br, bronchus; light microscopical image, 65 x (A), 150 x (B).

Pseudoglandular Stage (Weeks 5–17)

From the fifth week to the 17th week, the developing lung shows the characteristics of a tubular gland, giving rise to the name for this stage (Fig. 5-6). Until the end of this stage, the tubular tree preforms, through growth and branching, all the conductive airways down to their last generations—i.e., the future terminal bronchioles. Therefore, this stage has often been referred to as the stage of conductive airway formation. Although Boydelen claimed that the transition to the next stage was marked by the first appearance of the pulmonary acinus (which means that the prospective lung parenchyma appears at the periphery of the developing lung), the amount of future gas exchange tissue present during and at the end of the pseudoglandular stage has been severely underestimated. Already in the early 1980s, Ten Have–Opbroek had demonstrated by immunohistochemical techniques that epithelial cells at the periphery of the airway tree in the pseudoglandular stage had to be considered precursors of the later alveolar epithelium. In morphologic and morphometric studies of fetal rat lungs, we found that half of the parenchymal epithelial cell mass in the saccular stage before birth was already present in the late pseudoglandular stage. Furthermore, Kitaoka and co-workers concluded from counts of the number of end segments in human lungs in the pseudoglandular and canalicular stages that all the airway divisions down to the level of alveolar ducts were present toward the end of the pseudoglandular stage. For these reasons, the view that the pseudoglandular stage is almost exclusively the stage of conducting airway development can be maintained no longer.

Although the appearance of the acinar structures apparently provides a well-defined morphologic characteristic for the transition to the next stage of development, a precise estimate of gestational age cannot be made from it. Differentiation usually proceeds centrifugally, and the speed of growth varies during a developmental period, with acceleration toward the end of a stage. Furthermore, animal studies have demonstrated that upper lobes develop faster than lower lobes. If the time differences in development observed between lobes in rabbits are transposed to the human lung, the differences in lobar development could amount up to 2 weeks in view of the much longer gestation period. Such differences, however, have never been assessed. Recently it was postulated that human lung development proceeded in a relatively homogeneous manner.

Epithelial and Smooth Muscle Cell Differentiation

During the pseudoglandular stage, the airway tubes are proximally lined by a very high columnar epithelium (Figs. 5-6 and 5-7D). The height of the cells decreases continuously toward the periphery, to reach a cuboidal shape in the terminal branches. The epithelium of the terminal buds maintain their cuboidal undifferentiated state until branching is completed. Mitotic figures are frequent. The cytoplasmic organelar machinery looks relatively simple: mitochondria, many free ribosomes and a little rough endoplasmic reticulum, some lipid droplets, and large patches of glycogen. Remarkably, the epithelial barrier appears to be tight from the early stages of development. In freeze-fracture preparations, the morphology of the junctional complexes does not differ during development to full term; conversely, gap junctions are present early in gestation and disappear during the canalicular stage as the...
Figure 5-7 Changes in lung parenchymal morphology in the pseudoglandular, canalicular, and saccular stages. A. During the pseudoglandular stage, the epithelial tubules branch constantly and penetrate into the surrounding mesenchyme (open arrow, branching point). A loose three-dimensional capillary network is located in the mesenchyme. B. The canalicular stage is characterized by: (1) a widening of the future airways; (2) a differentiation of the tall columnar epithelial cells of the pseudoglandular stage (D) into prospective lining and secretory cells (type I and II epithelial cells, e→f); (3) a multiplication of the capillaries and their first close contacts to the epithelium; and (4) the formation of first air-blood barriers (e→f). C. Throughout the saccular stage the mesenchyme condenses to form thick inter-airway septa that contain a capillary layer on either side of the septum. The widened terminal ends of the bronchial tree are recognized as sacculae (asterisks) (At modified from Caduff HH, Fischer LC, Burri PH: Scanning electron microscopic study of the developing microvasculature in the postnatal rat lung. Anat Rec 216:154–164, 1986; D and E from Burri PH, Weibel ER: Ultrastructure and morphometry of the developing lung, in Hodson WA (ed). Development of the Lung. New York, Dekker, 215–268, 1977.)

epithelial cells differentiate. Therefore, it may be that electrical coupling between cells plays a role in cellular differentiation.

The first ciliated, goblet, and basal cells appear in the central airways. Mucous glands and goblet cells appear almost simultaneously in the airway epithelium. They develop from solid epithelial sprouts that invade the mesenchyme underneath the epithelium. At around weeks 12 to 13, mucous glands are found in bronchi; at week 14, mucus formation can be detected in the trachea.

A continuous layer of contractile \( \alpha \)-smooth muscle actin positive cells surrounds the larger future airways in the proximal part of the bronchial tree. These cells are defined as smooth muscle cell precursors, because morphologically they are not yet fully differentiated. The layer of smooth muscle cell precursors becomes step by step discontinuous in the more distal parts and ends in front of the terminal buds. Until birth these contractile cells perform spontaneous contractions, which start centrally and travel like a peristaltic wave into the periphery. The resulting wave of intrabronchial liquid pushes liquid into the terminal buds and extends them rhythmically. It was postulated that branching is stimulated by this mechanical signal via mechanical transduction.

Arteries and Veins

During the pseudoglandular stage, the vascular system develops along with the bronchial tree, so that by the end of this stage, all the preacinar vessels, arteries, and veins, are laid down in the characteristic pattern of the adult lung. However, the number of generations in the arterial tree is greater than in the airway system: On average, there are more than 28 generations in the arterial tree versus 23 in the airways. In addition to the conventional arteries that follow the bronchi and bronchioles, there are other branches (i.e., “supernumerary” arteries), which often split off at right angles. Usually these branches are smaller vessels that irrigate the “recurrent” gas exchange tissue adjacent to the conducting airways.

The veins follow different pathways. They run mainly interaxially in mesenchymal septa of the lung segments and subsegments. Verbeken et al. showed that as a general rule venous branches systematically follow the connective tissue septa that extend into the plane between each generation of airway branching. In the central areas the large venous branches join the arteries and airways to reach the hilum.

The generations of the three pulmonary trees—future airways, arteries and veins—develop in parallel. According to current estimations approximately five-sixths of the number of generations is laid down at the end of the pseudoglandular stage. However, due to the exponential growth by mostly dichotomous branching \( 2^n \) the total numbers behave quite differently. In a first approximation the total number of branches, the total length, and the total volume of the airways, arteries, and veins doubles with every additional generation.

Zones of Lung Development

Based on morphologic observation during rat lung development Burri and Moschopulos established a concept of zonal
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Figure 5-8  Schematic illustration of Zone concept. A. Early pseudoglandular stage: Zone I is made from a loose network of mesenchymal cells and capillaries into which the airway tubules of zone II penetrate. Zone III contains the prospective conducting airways and vessels. The conducting structures have no adventitial layer yet. No Zone IV is present anymore. B. Late pseudoglandular stage. All four zones have now developed. The peripheral tubules reach into zone I, which represents a layer of almost constant thickness despite lung volume increase. This means that it represents a kind of cambial or germinal layer for airway growth. Due to the recurrent growth of zone II (asterisk and arrow in A), zone I areas have reached the cuffs of airways and vessels. Part of airways and vessels of earlier zone III are now enwrapped by an adventitial layer and have therefore mutated to zone IV. C. Canalicular stage: Airspaces of zones II and III have widened, while the airway buds in zone I retain a cuboidal epithelium. D. Saccular stage. Zone I has now disappeared; there is no more budding. The lumina of airspaces and airways are wide. (From Burri PH, Moschopulos M: Structural analysis of fetal rat lung development. Anat Rec 1992; 234:399–418.)

development. Zone I is defined as a superficial mantle around the lobes and the future acini. It consists of primitive mesenchymal cells and represents a zone of growth and branching of the epithelial tubules. Zone I disappears at the beginning of the saccular stage when branching is completed. Zone II is mainly a zone of differentiation. In electron micrographs its interstitium stains intensely due to a dense population of dark cells. Zones III and IV contain the elements of the airway tree and vascular system, zone IV corresponds to the most proximal generations with an adventitial layer. For all differentiation processes a centrifugal directionality is manifested (Figs. 5-6 and 5-8), the most differentiated cells being in the first branching generations, whereas cellular multiplication is at work in the periphery.

Besides helping to understand developmental processes the zone concept is also interesting in terms of molecular aspects, e.g., many factors that are expressed in zone I contribute to the epithelial-mesenchymal interaction during branching (e.g., FGF-10, Gils, Sprouty4). In zone II the expression of hepatocyte nuclear factor/forkhead homologue 4 was observed.

Canalicular Stage (Weeks 16–26)
The canalicular stage comprises important steps in the development of the fetal lung. The lung morphology changes dramatically, owing primarily to: (1) the differentiation of the pulmonary epithelium and formation of the typical air-blood barrier; (2) the beginning of surfactant synthesis and secretion; and (3) the "canalization" of the lung parenchyma by capillaries. The latter process gave this stage its name. These alterations have most important functional consequences: At the end of the canalicular stage, the lung has reached a state of development in which gas exchange is possible in principle. Before these developmental steps, a prematurely born infant has no chance to survive. However, clinical experience unfortunately shows that survival is by no means assured at the end of the canalicular stage.

At the beginning of this stage, the future gas exchange region of the lung can be distinguished from the conducting tubules of the airway tree. Boyden has characterized this step as “birth of the acinus.” The early acini are composed of several very short generations of tubules arranged in clusters and taking origin from the actual last segment of the conducting airways, a prospective terminal bronchiole. The acinar borders can be recognized because of rarefaction of the mesenchyme (Figs. 5-6 and 5-8). In subsequent weeks, the distal segments of the airways grow in length and widen at the expense of the mesenchymal mass.

Epithelial Differentiation: Formation of the Air-Blood Barrier
To achieve an operational air-blood barrier, epithelial differentiation and capillary proliferation are needed during this
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The cuboidal glycogen-rich epithelium of the bronchial tree differentiates into two types of epithelial cells: (1) type II epithelial cells, which start to produce surfactant; and (2) type I epithelial cells, which reduce their height and develop sheet-like extensions that cover most of the internal surface of the parenchyma. In addition, the type I cells contribute to the formation of the first thin air-blood barriers (Figs. 5-7B, 5-7E, and 5-9). The lung parenchyma becomes “canalized” by capillaries, resulting in a dense capillarization of the primitive interstitium. Distal of the conductive zone the future airways not only grow in length, but also widen, causing a reduction of the mesenchymal volume (Fig. 5-7). In rats this reduction includes a peak of programmed cell death (apoptosis). The cellular death indicates that the condensation of the mesenchyme includes a reduction of the number of cells.

During the pseudoglandular stage the capillaries form a loose three-dimensional network inside the mesenchyme (Fig. 5-7A). During the canalicular stage a strong angiogenesis of the (micro-)vasculature takes place and the capillaries come into close contact to the epithelial tubules, forming a pericanalicular network (Fig. 5-7B). In the regions in which the epithelium has been thinned, the capillaries form the air-blood barrier by an intimate contact to the squamous cells (Figs. 5-7E and 5-9A). The epithelial and endothelial cells are only separated by one fused basement membrane that possesses one central lamina densa and two laminae lucidae. The mechanism by which the air-blood barrier forms is currently only poorly understood. It is likely that the interaction between the mesodermally derived endothelium and the endodermally derived epithelium takes part in this developmental step. This hypothesis is supported by a transgenic mouse, in which the sequence coding for the nidogen-binding site (γ1IIA4, within the laminin-γ1-chain) was selectively deleted by gene targeting. In these mice the basement membranes are disrupted or missing in large parts of the air-blood barriers. As a result epithelial and endothelial cells do not form close contacts. The mice die neonatally due to a failure of the gas exchange in the lungs.

During the canalicular stage the type II epithelial cells start to accumulate lamellar bodies containing components of surfactant. Soon afterward the type II cells actively secrete surface active material, which appears in the lung liquid. In contrast to most species, in which surfactant appears late in gestation (at about 80–85 percent of total duration of gestation), in the human fetus small amounts are already present at weeks 22 to 24 (approximately 60 percent of gestation). Before the lungs fully mature surfactant appears to be unevenly distributed. The surfactant appears to be more abundant in apical than basal regions. The clinical observation that in some premature born infants the development of hyaline membrane disease is more pronounced in basal than apical parts of the lung may be explained by this uneven appearance of surfactant.

Type II cells are also the progenitor cells of the type I cell population in adult lungs, as could be demonstrated during repair of the damaged alveolar epithelium. Therefore, it is not surprising that a few small lamellated bodies have been found in the cytoplasm of immature epithelial cells before they started to differentiate into type I and II cells. In cytokinetic experiments using tritiated thymidine, it has been shown that during lung development, early type II cells (or, more precisely, cells resembling type II cells) also represented the stem cells of the type I and II pulmonary epithelium. Recently, it has been shown in adult mice that circulating bone marrow cells may also be recruited to the lung. At the uterine periphery of the airspaces, the cuboidal cells of the epithelium remain undifferentiated until after birth (Fig. 5-9B).
Saccular (or Terminal Sac) Stage
(Weeks 24–38)
This stage lasts from about week 24 almost to term. At the beginning of this stage, the peripheral airways form typical clusters of widened airspaces termed saccules or terminal sacs. By widening and lengthening of all airspace generations distal to the terminal bronchioles, and probably also by the addition of the last generations of airspaces (Fig. 5–2), the future gas exchange region expands massively.

Each new generation of this pathway is originally formed as a blind-ending saccule. As soon as it divides distally, it no longer a saccule, but an open-ended channel. As is discussed in the section on postnatal development, the morphology of all these channels and saccules undergoes change until the formation of the alveoli is completed in the postnatal period. Therefore, these structures have been designated as transitory ducts and transitory sacculae or, more generally, as transitory airways or airspaces, because their morphology changes further until alveoli are formed.

Also at this stage, within the mesenchyme, one or two populations of fibroblastic cells have differentiated. Not only are these cells responsible for the deposition of extracellular matrix and fibers but also, by way of interactions with the epithelial cover, they presumably play a role in epithelial differentiation and the control of surfactant secretion. Owing to the expansion of the lumina of the peripheral airspaces in the foregoing and the present stage, the proportion of interstitial matrix and fibers but also, by way of interactions with the septal walls of the parenchyma, from the peribronchial and perivascular sheaths to the pleural sac.

During the saccular stage the capillary network had formed a capillary layer around each future airway. Due to the expansion of the future airways, the surfaces of the airways approach each other and a capillary double layer is thus formed.

Comparing different species the time point of birth as determined relative to lung development correlates with the activity of the newborn. So far the marsupial quokka wallaby (*Setonix brachyurus*) represents the mammal possessing the most immature lung at birth. These animals are born during the saccular stage. Although human babies are born in the early alveolar stage, precocial mammals such as sheep are born during late alveolar stage (Table 5–1).

At birth the replacement of lung liquid by air and the onset of respiration represents a major caesura in pulmonary development. However, the change from the amniotic into the atmospheric environment is more functionally than a structurally relevant step. At this time, the complete set of airway generations seems to be present; however, the most peripheral ones are still relatively short. The pulmonary parenchyma consists of several generations of transitory ducts and, as the last generation on each pathway, the transitory saccules. At birth, these structures are on the way to being transformed into alveolar ducts and sacs, respectively, by the process of alveolarization. Although reports indicate that in humans the formation of alveoli starts during late intrauterine life, the alveolar stage of lung development is discussed in this section because most alveoli (more than 85 percent) are formed after birth.

**Arteries and Veins**
Keeping pace with the intense growth of the gas exchange region during this stage, the vascular tree grows in length and diameter and adds new generations. Measurements on arteriograms by Hislop and Reid have shown that arterial diameter is practically constant at a given distance from the end of the arterial pathway. This is true irrespective of age, either fetal or postnatal. Therefore, for example, a vessel of a given size will supply a large portion of a lobe in an early fetal lung, but only an acinus in a child's lung. In late fetal life, the wall structure of arteries is similar to that of adult lungs. Proximal arteries are elastic, with many elastic lamellae strung to each other by smooth-muscle cells that are arranged obliquely between the elastic sheets. Smaller arterial vessels show a transitional structure, the muscular component becoming increasingly prominent at the expense of the elastic component. Finally, the muscle layer of the media becomes irregular and assumes a spiral configuration. This configuration explains the "partly muscular" arteries seen in histological sections. Unfortunately, there are no strict relationships between vessel diameter, size of the region supplied, and character of the wall structure; these relationships may differ from one pathway to the other. Intrapulmonary veins are practically devoid of smooth-muscle cells until the end of the canalicular period. In the following weeks, however, a thin muscle layer is formed that, at birth, extends down to vessels of about 100 μm diameter.

**Time of Birth**
Comparing different species the time point of birth as determined relative to lung development correlates with the activity of the newborn. So far the marsupial quokka wallaby (*Setonix brachyurus*) represents the mammal possessing the most immature lung at birth. These animals are born during their saccular stage. Although human babies are born in the early alveolar stage, precocial mammals such as sheep are born during late alveolar stage (Table 5–1).
that the postnatal developmental processes do not differ in structural essentials; but, as expected, the timing is different between species.

At the end of the saccular stage the rat lung goes through a short phase of expansion without any alteration of the parenchymal complexity. The distal airways consist of smooth-walled channels and saccules corresponding to transitory ducts and definitive terminal saccules, respectively. The saccules of the saccular parenchyma are thick and contain a double capillary network. They have been called primary septa because they represent the basis for the formation of new septa, the secondary septa involved in alveolarization. In many species, among them mice, rats, and humans, alveolarization can be visualized nicely by light and scanning electron microscopy (Fig. 5-10). We have termed this fast and highly visible formation of alveoli during the alveolar stage "bulk alveolarization," to distinguish it from an eventual process of late alveolarization that is much more difficult to detect.

### Septation: Alveolarization

The alveoli are formed by lifting off of new tissue ridges from the existing primary septa. In light microscopic sections this process produces a large number of small buds appearing along the primary septa (Figs. 5-10B and 5-11B). In three dimensions these buds correspond to low ridges representing newly forming septa (Figs. 5-10D and 5-12). Soon these low ridges increase in height and subdivide the airspaces into smaller units, the alveoli (Figs. 5-10D and 5-11C).

It has been proposed that the combination of the three components—myofibroblasts, elastic fibers, and collagen fibers—provides the critical driving force for septation (Fig. 5-11). Inside the preexisting septa (primary septa) PDGF–receptor-positive smooth muscle cell precursors proliferate and move to the locations in which the new septa will be formed. These cells produce a network of mainly elastic fibers, but also interstitial collagens. During lifting off of the new ridges the contractile cells as well as the fibrous network stay at the tip of the newly forming septa. As indicated by the mechanical stress sensitive expression of tenascin-C at the septal tip this network is supposed to take up mechanical forces.

The alveolar smooth muscle cells or myofibroblasts are required for septation. Alveolarization does not occur in PDGF–A-deficient mice, because these cells do not appear at their normal position and do not deposit the network of elastic fibers. However, it is not known whether these cells are only required for the production of elastic fibers, or whether they exert contractive forces in addition. This question could not be answered by the investigation of elastin null mice, because they do not enter the alveolar stage due to early death.

### Folding of Capillary Network

The described network consisting of the smooth muscle cells, elastic fibers, and collagen fibers is connected to the capillary layer underlying the septal surface. At the locations in which the new ridge will be formed, the capillary layer of the preexisting septum is pulled into the new septum. It gives rise to the double capillary networks within the secondary septa (Fig. 5-11). Wherever lifting off occurs, both types of septa, primary and secondary, now contain two capillary layers, one on each side of a central layer of interstitium. Such thick septa are called "immature" or "primitive" septa to distinguish them from the thin mature septa of adult lungs that contain only a single capillary network. Septation goes along with a tremendous increase in airspace surface area. It is evident that the capillary network has to keep pace with it. If not, septal budding could not occur or forming secondary septa would be devoid of capillaries. This hypothesis was tested by the application of antiangiogenic drugs during the alveolar stage. After a treatment of rats with fumagillin, thalidomide, or Su-5416, respectively, a decrease of the alveolarization by up to 22 percent was observed. In parallel, the pulmonary arterial density decreased by up to 36 percent. This finding emphasizes the importance of vascular growth in the process of alveolarization.

### Quantitative Aspects

Morphologic investigations supported by quantitative stereologic analyses have led to the following picture of alveolar formation. Due to the formation of the alveoli, the complexity and also the alveolar surface area are increasing. In simple isotropic growth (expansion of airspaces in proportion to the increase in lung volume), the surface area is expected to increase to the two-thirds power of lung volume. However, in rats the lung volume increases to the power of 1.6. Of interest is that the rapid increase in alveolar surface area is paralleled by changes at the subcellular level in type II cells. The total mass of lamellated bodies is augmented in proportion to the increase in surface area, so that lamellar body volume divided by total surface area remains almost unchanged. Interestingly, this means that, if secreted, the lamellar bodies could cover the entire respiratory surface with a film of surfactant that is constant in thickness.

### Cell Proliferation

Shortly before and also during the onset of septal formation, DNA synthesis is increased in cells located preferentially in the region of the forming crests. Autoradiographic studies with H3-thymidine showed that DNA synthesis was high first in the mesodermally derived cells, such as the interstitial and endothelial cells. A few days later (at the age of 1 week), type II cells exhibited the highest activity in DNA synthesis. Within 1 hour after the thymidine injection, not a single type I cell could be labeled, clearly indicating that type I cells are unable to divide. If type I cell labeling could be detected in some experiments, it was always with some delay, which allowed for the differentiation of labeled type II cells into type I cells. At the age of 2 weeks, all labeling indices were back to low levels. Nonetheless, alveolar surface area continued to increase at a high rate during the third week. The morphometric data indicate that this further gain in surface area was obtained by restructuring of the available tissue mass rather than by further proliferative activity.
Figure 5-10  Alveolarization of rat lung as seen by light microscopy (a + b) and scanning electron microscopy (c + d).
A. On postnatal day 1, the terminal bronchioles (tb) open into a smooth walled channel dividing into several sac-cules(s).
B. On postnatal day 21, the terminal bronchiole (tb) opens now into several generations of alveolar ducts (ad) surrounded by alveoli. Secondary septa have subdivided the channels and saccules (arrows).
C. As seen by scanning electron microscopy, the lung parenchyma is made of smoothly lined saccules at postnatal day 1. By the formation of the secondary septa (arrows) the smooth walled channels and saccules of panel a have been transformed into alveolar ducts (ad) and alveolar sacs, respectively (postnatal day 21). Both structures are lined with alveoli (a).
Light microscopical images, 32 × (a + b), scanning electron microscopical images 460 × (c + d).
Chapter 5  Development and Growth of the Lung

Figure 5-11  Formation of secondary septa/alveolarization. A saccular (or primary) septum contains a double-layered capillary network, where every capillary layer appears as a perforated sheet in sections. Smooth muscle cell precursors, elastic fibers, and collagen fibrils (green spots) accumulate in immature septa at sites where new septa (or secondary septa) will be formed (blue arrows, A). Secondary septa are formed by up folding (green arrows) of one of the two capillary layers (red, B). As a result, newly formed secondary septa (black arrows) subdivide preexisting airspaces and new alveoli are born (C). At this stage, all septa present are immature, showing two capillary layers (= primitive septa). (Modified from Burri PH: Structural aspects of pre- and postnatal development and growth of the lung, in McDonald J (ed). Growth and development of the lung. New York, Dekker, 1997, pp 1–35.)

Figure 5-12  Visualization of alveolarization by x-ray tomographic microscopy. Mouse lungs were 3D-visualized by synchrotron radiation x-ray tomographic microscopy just before (postnatal day 4, A) and after the beginning of alveolarization (postnatal day 7, B). At the end of the saccular stage large terminal airspaces (saccules) were observed. B. Newly formed septa (arrow), which are surrounding newly forming alveoli (asterisk), are visible 3 days later during alveolarization.
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Microvascular Maturation Stage (Birth to 2–3 Years)
The essence of this stage is the restructuring of the double capillary networks in the parenchymal septa to the mature aspect with a single capillary system (Figs. 5-13 and 5-14). It is assumed that the latter structure bears functional advantages over the former. Mammalian species with an immature pulmonary microvascular system at birth are mostly of the altricial type, whereas the precocial species such as the sheep are born with mature septa.

The beginning and ending of microvascular maturation are very difficult to access. Septal restructuring is a complex mechanism closely related with growth. In addition, a large overlap with the stage of alveolarization exists. As shown in Fig. 5-15, reporting morphometric measurements of the state of maturity, the rat lung shows already some maturation of the alveolar septa at postnatal day 10, where only one-third of the bulk alveolarization is completed.

Capillary Fusion and Differential Growth
Ultrastructural and morphometric studies in the rat have revealed that the approximation of the two capillary layers was induced by a decrease in the absolute mass of the intercalated septal interstitium. In the third postnatal week the interstitial tissue volume decreased by 27 percent, despite a lung volume increase of 25 percent. The two capillary layers come to lie closer to each other, contact each other, and finally merge their lumen. This happens focally, but in numerous places. However, focal capillary fusions alone would not allow to transform rapidly and extensively the appearance of the interalveolar walls. We proposed that the merged areas were expanded by preferential growth, a process well known in embryology. In summary, microvascular maturation is the result of multiple focal fusions between the two capillary layers combined with preferential growth of the fused areas. By these means large areas of the lung parenchyma can gain the mature aspect within a short period of time.

Programmed Cell Death
In rats the maturation of the alveolar septa does not only lead to a reduction of the absolute mass of the interstitial tissue, but also to a decline of the absolute number of fibroblasts (10–20 percent) and of epithelial cells (greater than 10 percent). We asked how the number of the cells may be reduced and observed that the surplus of fibroblasts is eliminated by classical apoptosis toward the end of microvascular maturation (third postnatal week in rats). Apoptosis is morphologically defined by a typical pattern of structural changes of the dying cells, including the fragmentation of the cell into membrane-enclosed vesicles (apoptotic bodies). Although this happens in the septal myofibroblast population, programmed cell death eliminates the surplus of epithelial cells—mainly type II cells—without the appearance of apoptotic bodies. Most likely alveolar macrophages phagocytose the apoptotic epithelial cells in an early stage of programmed cell death before apoptotic bodies are formed. This peak of cell death disappeared in rats after a short neonatal treatment with glucocorticoids—showing the long-lasting effect of such a treatment.

During microvascular maturation the cell proliferation index stays low. The discrepancy between the rapid growth in surface area and lack of cell proliferation could be explained by an expansion of the type I epithelial cells, meaning that surface area covered by each individual type I epithelial cell enlarges. Because the type II epithelial cells cover only less than 10 percent of the alveolar surface, a reduction of these cells is only important for the production of surfactant and a reduction of the epithelial stem cells. Apparently, a surplus of type II cells exists toward the end of the stage of microvascular maturation. Most likely only type II cells which do not serve as stem cells for the forming of type I cells are removed.

Interalveolar Pores (Pores of Kohn)
Interestingly, the formation of pores of Kohn is closely related to the process of septal thinning. Pores of Kohn represent rounded openings in the interalveolar walls with diameters of a few to several microns. Their frequency varies greatly among species. In the human lung they are not present in the newborn and they appear during postnatal development and growth. Local thinning of the alveolar septa facilitates trans-septal contact of the epithelial cells. The formation of junctional complexes is followed by the reorganization of the cell-cell contacts and retraction of epithelial cells. Finally, the latter leads to the formation of pores. The contacts may be formed by type I-type I and type II-type I cell contacts (for details, see Fig. 5-16). Due to the cuboidal shape of the type II cells the type II-type I contacts contribute more frequently to the formation of a pore than type I-type I cell contacts.

In mice a first peak of pore formation is manifest during the stage of microvascular maturation (third postnatal week). A second peak was observed during the 6th to 10th postnatal week. Because septal thinning continues with further aging of the lung, interalveolar pores may also be formed later, perhaps even up to an older age.

The pores may serve as interalveolar exchange of alveolar liquid, surfactant components, and macrophages. They are filled with surfactant under normal physiological conditions. Tubular myelin may be stored in the pores without increasing the gas diffusion pathway thickness of the active gas exchange surface itself. It is unlikely that interalveolar pores are used for collateral ventilation of alveoli during normal breathing.

In the human lung microvascular maturation starts very early, partly in parallel to alveolarization. At the age of 1 year, large parts of the lung possess already mature septa, but most likely the process goes on till the age of 2 to 3 years. Based on these morphological characteristics we can consider the lung of a 3-year-old child to represent a miniaturized version of the adult one.
Figure 5-13 Septal maturation during the phase of microvascular maturation. Immature septa contain a double-layered capillary network in which each alveolar surface is served by its own capillary layer. The two capillary layers are separated by a central sheet of interstitial tissue (A, electron micrograph of a human lung aged 26 days, 1540×; a’, schematic drawing, capillaries are drawn in red, interstitial tissue in green). Upon maturation the connective tissue layer (green) condenses and thins out so that the two capillary layers merge. The result is a septum where the connective tissue skeleton of the alveolar septum is interwoven with a now single layered capillary network (red/b, electron micrograph of an adult human lung, 1540×; b’, schematic drawing). Panels (C) and (D) show the same development in an overview (light micrographs of human lungs, aged 3½ weeks, 250× (C) and 5.8 months, 360× (D)). C. During alveolarization thick immature septa are present that contain a double-layered capillary network (arrowheads) and that are capable to form new secondary septa (open arrows). D. During the maturation of the alveolar septa the capillary layers fuse to a single-layered capillary network (closed arrows) that appears alternately on either side of the septum. In some places, immature septa containing a double capillary network are still present (arrowheads).
Figure 5-14  Microvascular maturation observed in vascular casts of rat lungs. Scanning electron micrographs of vascular casts (Mercox) of rat lungs are shown at postnatal days 4 (A) and 44 (B). The immature septa contain a double capillary network (open arrow, A). During maturation the capillaries rearrange and form in most parts of the septa one central single layered capillary network (closed arrows, B). The lung capillary networks grow mainly by intussusceptive growth. Slender transcapillary posts (holes less than 2 μm in diameter, open arrowhead, A) are introduced into the capillaries and grow out to capillary meshes (closed arrowhead, A). Bar, 50 μm. (From Burri PH: Lung development and pulmonary angiogenesis, in Gaultier C, Bourbon J, Post M (eds). Lung Disease. New York, Oxford University Press, 1999, pp 122–151.)

GROWTH OF THE LUNG

Transition from Development to Growth

Morphometric data obtained from the lungs of seven children aged between 26 days and 5 years and complemented with data from eight adult lungs allow us to distinguish two phases of lung growth. The first phase (from birth to about 18 months) corresponds to the period of ongoing lung development (alveolarization and microvascular maturation) and is therefore characterized by major shifts in the quantitative parameters of the parenchymal compartments. The parameters in close relationship with O2 transport, the airspace and capillary volumes, grow faster than lung volume, mainly at the expense of the parenchymal tissue mass. The fact that capillary blood volume increases massively during the phase of microvascular maturation is an indication that capillary restructuring is associated with intense capillary growth.

In the second phase (from 1 1/2 years until body growth stops), the lung grows in a more proportionate fashion. The lung volume increases to the power of 1 to body weight, and the pulmonary compartments augment linearly with lung volume. Most important, the surface area for gas exchange and the morphometrically determined pulmonary diffusion capacity increase both to the power of 1 to body mass.

Lung Parenchyma: Late Alveolarization

As discussed, the stage of alveolarization represents a very distinctive and visible period of lung development with its ongoing bulk alveolarization. It corresponds to a period of intense septation accompanied by a marked increase in gas exchange surface area. The question arises whether alveoli are formed once and for ever or further alveoli are added later at a slower pace and in a much less obtrusive manner during the period of so-called normal growth between the age of three and young adult age. This latter assumption implies that growth of existing structures is complemented by the formation of new interalveolar walls. The question can be important, because of some clinical implications. Acute lung injuries and acute respiratory distress syndromes are common causes of morbidity and mortality in intensive care units. Irrespective of the initial cause of the lung injury, both diseases are characterized by a diffuse damage of the lung parenchyma, which includes a reduction of the diffusion capacity and may lead to a loss of alveolar septa. If the lung were able to produce new alveoli all along childhood, this could favor a late recovery from various forms of structural damage. Furthermore, steroids are widely used during the treatment of lung diseases such as asthma and wheezing illnesses or other diseases such as inflammatory bowel diseases. Retinoids are used for the treatment of psoriasis and severe acne. Both drugs are known to alter the lung structure when given neonatally or during the phase of bulk alveolarization. It is evident that these drugs could have negative side effects on the lung in children and adolescents if alveolar formation is still active.

The reports about the end point of alveolarization in humans are conflicting and have been much debated in the past. So far the limitations of alveolar counting techniques,
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Figure 5-15  Maturation of the alveolar microvasculature. Intersection counting was used to determine the percentage of double- (immature) and single- (mature) layered capillary networks of the inter-airway septa of rat lungs. A basic level of single-layered networks was observed at the end of the saccular stage (day 4). Some maturation of the capillary network is already seen during the phase of alveolarization (day 10), which demonstrates the large overlap between alveolarization and microvascular maturation. However, at the end of the phase of microvascular maturation 20 percent of the capillary networks are still immature. Standard deviations are given as error bars. (From Roth-Kleiner M, Berger TM, Tarek MR, et al: Neonatal dexamethasone induces premature microvascular maturation of the alveolar capillary network. Dev Dyn 233:1261–1271, 2005.)

Figure 5-16  Formation of interalveolar pores of Kohn. Interalveolar pores start to form after maturation of the septum (a → b, f). Pores may be formed with (f–i) or without (b–d) the involvement of type II epithelial cells.

A. Immature interalveolar septum with double capillary network.
B. Cross section of a part of an interalveolar septum covered by type I epithelial cells (ep I) and containing two capillaries.
C. Due to a thinning of the septum in the region of a capillary mesh, the connective tissue disappears in a spot such as area and permits a contact between the type I cells resting on both surfaces of the septum. After rupture (arrowhead) the cell margins withdraw (arrows) and only one leaflet of one of the type I cells remains. D. The same process happens again (arrowhead and arrows) resulting in the formation of an interalveolar pore (i). E. Type II epithelial cells may also be involved in the formation of interalveolar pores. In a first step a type II cell makes a contact to a type I cell resting on the opposite side of the septum. F. The type II cell becomes also integrated into the epithelium opposite of its original side. G. In parallel to the retraction of the type II cell the two leaflets of the type I cells move toward each other. H. While forming the type I cell-cell junction the type II cell retracts completely and gives rise to a new pore. I. Finally, the type II cell is shifted along the septum and thus leaves the immediate vicinity of the pore. Alternatively, it may stay there or differentiate into a type I cell. Cellular junction running all around the pore (open arrowhead). c, capillary; ep I, type I epithelial cells; ep II, type II epithelial cells; ic, interstitial cell; p, pore. (Based on Weiss M, Burri PH: Formation of interalveolar pores in the rat lung. Anat Rec 244:481–489, 1996.)

With the following considerations we would like to shed some light on these questions.

1. Disseminated interalveolar septa with an immature aspect can be found even in adult lungs. They could represent sites of focal alveolar formation (Fig. 5-13D).
2. In a study in which rats were treated neonatally with high doses of glucocorticoids early, the authors observed a focal premature microvascular maturation...
Part II  Scientific Basis of Lung Function in Health and Disease

POSSIBLE MECHANISMS FOR LATE ALVEOLARIZATION

(27 percent mature single-layered capillary networks in treated rats versus 16 percent ones in controls), transient septal thinning, and transient inhibition of alveolarization. Following the withdrawal of the drug, the induced structural changes were readily compensated. By postnatal days 36 and 60 the lungs had almost completely recovered from the early insult. This suggests a high plasticity of alveolarization.

3. In the lung periphery, i.e., underneath the pleura and around the adventitial layer of the bronchi and blood vessels, the capillary network involved in gas exchange rests on a sheet of connective tissue. In these regions the capillary layer can be folded up in a manner resembling closely the classical mode of alveolarization (Figs. 5-7 C, 5-17, and 5-18). For geometrical reasons the lung periphery makes up a high proportion of the total lung volume, especially in smaller lungs. In an adult rat lung a subpleural tissue mantle of 2-mm thickness represents almost 50 percent of the parenchymal lung volume. Therefore, the subdivision of peripheral airspaces can be very effective in increasing alveolar number.

4. There is recent evidence that capillary up-foldings can even be formed in mature rat septa thanks to local duplications of the capillary network at the base of the fold. This has been demonstrated in casts of rat lungs using synchrotron radiation x-ray tomographic microscopy allowing a 360-degree inspection of the critical sites (Schittny, unpublished data).

Conducting Airways

Unlike the lung parenchyma, the structure of the conducting airways is largely mature at birth—except perhaps for the terminal bronchioles, part of which may transform into respiratory bronchioles, as described by Boyd. Whereas the branching pattern does not change with age, it is not clear whether the bronchial tree grows proportionately after birth. In one study, the relationship between diameter and relative distance from the hilum was found to remain almost constant with age. In another analysis, this was true only after the age of 1 year, whereas during the first year of life, the larger bronchi showed a faster growth rate than the smaller conducting airways. Detailed studies of the airway epithelium of hamsters and rhesus monkeys indicate that the airway lining is largely mature at birth. Although there is some postnatal functional maturation, most developmental changes occur before birth.

Arteries and Veins

During fetal life, blood flow through the lung is limited to between 10 and 15 percent of the cardiac output. Clearly, the most important vascular event accompanying the onset of air breathing is the closure of the ductus arteriosus and the shunting of the entire cardiac output through the lung.
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The ductus arteriosus, first obstructed by muscular contraction, is anatomically closed within a few weeks by the fibrotic organization of an intravascular clot. The ligamentum arteriosum represents the tombstone of this important prenatal structure.

After birth, the wall thickness of pulmonary arteries decreases relative to their diameter. In small vessels (up to 200 μm in diameter), this decrease occurs very rapidly. It was assumed that it was due to a fall in smooth muscle tone. A study performed in pigs, however, related the vascular dilatation more to a concurrent extensive rearrangement and shape change of the vascular smooth muscle cells than a change in muscle tone. A similar adaptation has been noted in the small arteries of the human lung, with the structural remodeling being most rapid during the first month of life. In the larger arteries in humans, the thinning of the wall occurs less abruptly: The transformation is achieved by structural adaptations and therefore takes several months. After 1 year of age, the central pulmonary arteries no longer change their relative wall thickness appreciably; instead, they grow more or less proportionally.

Although the central vessels that accompany the conductive airways do not multiply after birth, the situation is completely different for the peripheral vessels. During the first 1 or 2 years of life, intra-acinar arteries undergo intense development and growth as they follow the extension of the peripheral airspaces. The number of small vessels therefore increases both absolutely and relatively. The relative increase

Figure 5-19  Intussusceptive capillary growth. A–C. During intussusceptive capillary growth the number of segments, the surface area, and the volume of an existing capillary network increases by the insertion of new transluminal tissue pillars. D. After formation, the pillar enlarges to form a new mesh. E. A Mercox cast of the alveolar microvasculature. Pillars appear as tiny holes, often in the range of 1.5 μm in diameter (scanning electron micrograph, ×5850, bar 2 μm). F. A longitudinal section of a transcapillary tissue pillar. The pillar structure exhibits a central axis formed by the cytoplasmic extension of a myofibroblast (mf) with actin filaments, and some collagen fibrils (cf), c, capillary; ec, erythrocyte; ej, endothelial cell junctions; en, endothelial cell; epI, type I cell (electron micrograph, ×9400, bar 2 μm). (Panels A–D are based on Kurz H, Burri PH, Djonov VG: Angiogenesis and vascular remodeling by intussusception. From form to function. News Physiol Sci 18:65–70, 2003.)
implies that their number augments per unit area of lung section. From the age of 5 years on, the relative number decreases again, reflecting the enlargement of the alveoli. The newly formed vessels are thin walled and are partly muscular or non-muscular, because muscle formation lags behind the increase in diameter. Gradually, muscularization then proceeds toward the periphery, a process that continues into adulthood.

Veins have a smaller amount of smooth muscle than do arteries. However, in principle, the same observations apply to veins as well as arterial development and growth.

Growth of the Lung Microvasculature

Interestingly, both in rats and humans, lung volume was found to increase about 23 times between birth and young adulthood. In both species lung microvasculature grows even more: The capillary volume increases by a factor of 35, whereas the capillary surface area augments about 20 times. Furthermore, scanning electron microscopy of microvascular casts reveals that the capillary meshwork in the interalveolar walls remains at the same high density, which means that all along growth the capillary system expands through the addition of new capillary segments, i.e. by angiogenesis. Angiogenesis is defined as the formation of new vascular segments from preexisting vessels. The classical concept for the expansion of the capillary networks in growing organs was vascular sprouting. Sprouting angiogenesis is characterized by a number of typical steps: local vasodilatation, increased vascular permeability, proteolytic degradation of basal lamina, proliferation and migration of endothelial cells, and formation of a solid sprout, which then is reorganized into a tubule. Finally, the new tubule connects to a neighboring vessel.

While we were investigating the capillary maturation in lung development by studying vascular casts in the scanning electron microscope, one of us (Burri) observed numerous tiny holes sometimes less than 1.5 μm in diameter (Fig. 5-19E). We hypothesized that these holes could represent newly formed intercapillary meshes (or “baby meshes”). By repetitive insertion of such tiny meshes, which then would grow and expand to normal-sized capillary meshes, the network would increase in surface area and gain in complexity (Fig. 5-20). Although this concept was first based on observations of casts alone, it was clear that matching structures had to be found in interalveolar walls at the ultrastructural level. The holes in the cast should correspond to some form of tissue pillars traversing the capillary lumina. By electron microscopic investigation of serial sections through the interalveolar septa, we demonstrated indeed the presence of tissue pillars of adequate diameters (Fig. 5-19F). The analysis of their ultrastructure allowed us to derive a plausible mechanism for their formation. As depicted in Fig. 19A–D, the classical formation of a transcapillary tissue pillar is a four-step process: (1) formation of symmetrical or asymmetrical protrusions from opposite parts of the capillary (Fig. 5-19B), which join and create a zone of inter-endothelial cell contact; (2) following reorganization of the inter-endothelial cell junctions the contact zone is centrally perforated: a transcapillary pillar of tissue is formed (Fig. 5-19C); (3) invasion of the pillar core by cell processes of pericytes and myofibroblasts, thus stabilizing the pillar structure (Fig. 5-19F); (4) the

\[ A \]  

\[ B \]  

Figure 5-20 Drawing of intussusceptive microvascular growth of a capillary layer. While the alveolar capillary networks are growing, the size of the capillary meshes stays quite constant. To achieve this, pillars are inserted into the capillaries (arrows) by intussusceptive angiogenesis. A–B: Two schematic drawings of consecutive stages of intussusceptive capillary growth. While newly formed pillars are growing in size (arrowheads in A and B), additional pillars are inserted into existing capillaries (arrows in B). Notice the larger surface covered by the capillary network in (B) than in (A). The schematic drawing is based on scanning electron micrographs of vascular casts. (From Burri PH, Hlushchuk R, Djonov V: Intussusceptive angiogenesis: Its emergence, its characteristics, and its significance. Dev Dyn 231:474–488, 2004.)
piller grows in diameter (Fig. 5-19 D) and finally becomes a normal-sized intercapillary tissue mesh.

We termed this new process of capillary growth intussusceptive microvascular growth (IMG). In histology, the term is commonly used to describe the growth of cartilage, which also grows within itself. Intussusception means “addition of new, but similar elements of formative material among those already present.” Since its discovery in 1986, IMG has been well documented in numerous species and various organ systems. In tumor growth, IMG appears to play an important role besides sprouting angiogenesis and may be responsible for the failure of some antiangiogenic treatments targeting sprouting vessels.

This new “non-sprouting” intussusceptive angiogenesis has some advantages over the sprouting form; it is fast (hours versus days); it is not based on cell divisions in a first step; it is less leaky than sprouting; and it is efficient, because it happens while the blood is circulating through the vessel. However, it cannot bridge vascular gaps, such as cuts of the skin or scars.

Recent work has emphasized the significance of the intussusception process in demonstrating that besides capillary growth it was involved in the de novo formation of vascular trees and optimizing vascular branching geometry, and vascular pruning (for review, see Burri and Djonov).

### Dimensions of the Adult Lung

Table 5-2 summarizes the relevant data for a “standard” adult human lung. In a direct comparison of quantitative data of adult and newborn lungs, it is manifest that lung structure is far from representing a tissue framework of stable composition. So, with age, the volume density (volume per lung volume) of interalveolar septa decreases, the volume density of the airspace increases, and, within the interalveolar walls, the volume fraction of the blood vessels increases at the expense of the tissue volume. Although the arithmetic mean thickness of the air-blood tissue interface (calculated by dividing total tissue mass by the alveolar surface area) falls from around 5 μm at birth to 2.5 μm in the adult, the functionally more relevant harmonic mean thickness of the air-blood tissue barrier, a measure of the average effective diffusion distance, remains largely unaffected by the structural alterations of postnatal development.

Despite the lung undergoing extensive structural remodeling during development and growth, the gas exchange surface areas of airspaces and capillaries increase linearly with body weight. This fact illustrates that the implemented structural alterations are always well balanced to constantly meet the O2 needs of the organism throughout lung development.

#### Table 5-2

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<th>Dimensions of the Human Lung Based on Morphometry</th>
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