Thurlbeck's Pathology of the Lung

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Henry D. Tazelaar
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3rd Edition

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Thieme
New York • Stuttgart

Thieme Medical Publishers, Inc.
333 Seventh Ave.
New York, NY 10001

Associate Editor: Owen Zurhellen
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Compositor: Compset Inc.
Printer: Maple-Vail Book Manufacturing Group

Library of Congress Cataloging-in-Publication Data

Thurlbeck's pathology of the lung.— [3rd ed.]/edited by Andrew M. Churg . . . [et al.].
p. ; cm.
Rev. ed. of: Pathology of the lung/edited by William M. Thurlbeck, Andrew M. Churg. 2nd ed. 1995.
Includes bibliographical references and index.
ISBN 1-58890-288-9 (TMP : alk. paper)—ISBN 3-13-665403-X (GTV : alk. paper)
1. Lungs—Diseases. 2. Lungs—Diseases—Diagnosis. 3. Diagnosis, Laboratory.
[DNLM: 1. Lung Diseases—pathology. 2. Lung—pathology. WF 600 T537 2005] I. Title: Pathology of the lung. II. Thurlbeck, William M. III. Churg, Andrew. IV. Pathology of the lung.
RC711.P38 2005
616.2′407—dc22 2004026296

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Printed in the United States of America

5 4 3 2 1

TMP ISBN 1-58890-288-9
GTV ISBN 3 13 665403 X

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Preface

This new third edition of *Pathology of the Lung* has now been christened *Thurlbeck's Pathology of the Lung* to commemorate the late William (Whitey) Thurlbeck, the senior editor of the first two editions and a personal friend as well as a colleague of many in the world of pulmonary pathology.

Much has changed in the ten years since the previous edition. The chapter on Lung Growth and Development now has a marked focus on molecular biology and the novel information these techniques have provided in understanding how the lung is formed. Infectious disease has continued to be an area of evolving knowledge and concern, and this edition has completely revised chapters on bacterial, mycobacterial, and viral infections. Chronic obstructive lung disease (COPD), long ignored by most pathologists, has become one of the leading causes of morbidity and mortality in the general population, and we have extensively revised the chapter on COPD to emphasize new information on the mechanisms behind the development of emphysema and the role of small airway remodeling in producing chronic airflow obstruction.

This third edition incorporates data from the 1999 and 2004 World Health Organization classifications of lung tumors and the International Society for Heart and Lung Transplantation Revised 1995 Working Formulation for Lung Rejection. Similarly, more attention is devoted to pulmonary vasculitis, including a contemporary approach to the classification of vasculitis based on the Chapel Hill Consensus Conference and American College of Rheumatology classifications. Perhaps the most dramatic change in classification has been in the area of the idiopathic interstitial pneumonias; the routine use of high resolution CT scanning combined with careful pathologic delineation of a variety of entities all formerly lumped under the term *apulmonary fibrosis* has allowed separation of these diseases into categories with clinically significant treatment and prognostic differences.

We have also attempted to simplify the organization of the book in this third edition, combining many of the previous chapters dealing with the handling of different specimens types or placing the discussion with the major descriptive chapters; for example the discussion on cytologic methods has been placed in Chapter 27, Diagnostic Lung Cytology. We believe that this leads to a more readable volume. We have, however, made

it a point to retain a chapter on special techniques, because many of these, such as morphometry and the preparation of Gough sections, are important to investigating lung disease, but this information on them is increasingly difficult to find.

Once again we wish to thank all the contributors to this book; it is never easy in a multi-authored text to convince everyone to get their chapters ready in time, but all the authors here proved an exemplary bunch.

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1

The Normal Lung

CHARLES KUHN III AND JOANNE L. WRIGHT

Functionally, the lung consists of the acini, which are units specialized for exchange of gas between air and blood, and the conducting tissues, airways, and blood vessels that distribute the air and blood to the gas-exchanging units. Disease processes may involve acini, airways, or vessels selectively or in combination. Differing as they do in structure, function, and cellular composition, these tissues react differently in disease. Thus an understanding of lung disease requires knowledge of the structure and function of the tissues that make up the normal lung.[1]

Gross Anatomy

General Features

The lungs are paired, asymmetrical organs roughly conical in shape. Their normal combined weight averages 850 g in men and 750 g in women.[2] The space that they occupy in the thoracic cavity is enclosed by the rib cage dorsally, laterally, and ventrally; the mediastinum medially; and the diaphragm inferiorly. The shape of the expanded lung, after removal from the body, corresponds with the cavities thus defined. The lung has three surfaces: a convex surface abutting the rib cage, more sharply curved posteriorly than anteriorly; a concave mediastinal surface; and a concave diaphragmatic surface conforming to the convexity of the diaphragm as it covers the domes of the liver and spleen. Toward the posterior portion of the mediastinal surface lies the hilum of the lung, the region in which the bronchi, blood vessels, lymphatics, and nerves enter (Fig. **1–1**). Distinct, sharp margins are formed anteriorly by the junction of the mediastinal and costal surfaces and inferiorly by the junction of the costal and diaphragmatic surfaces; the posterior transition from the costal to mediastinal surface and the junction of the mediastinal and diaphragmatic surfaces are rounded. The right lung is slightly larger than the left because of the space required to accommodate the heart on the left side of the mediastinum, but the vertical (cephalocaudal) dimension of the right lung is less than that of the left lung because of the higher position of the right hemidiaphragm where it covers the right lobe of the liver.

The lungs can move freely within the thoracic cavity, being attached only at the two hila. The free surfaces of the lung are covered by a serous membrane, the visceral pleura, which is reflected over the hilum to cover the mediastinum, chest wall, and diaphragm as the parietal pleura. The pleural reflection at the hilum continues inferiorly as the pulmonary ligament. In vivo, the parietal and visceral pleura are normally closely apposed, lubricated by a thin film of pleural fluid, the composition of which is similar to that of other interstitial fluids. The exact volume of pleural fluid in normal subjects is not known, but it is not more than a few milliliters.

The lungs are divided into their major subdivisions, the lobes, by clefts or fissures lined by visceral pleura (Fig. **1–1**). The right lung has three lobes. The major fissure follows an oblique course from a level above the hilum dorsally to the base of the lung anteriorly, dividing the inferior lobe from the remainder of the lung; a second (minor) fissure, nearly horizontal, separates the remainder into a superior and middle lobe. The left lung is divided into superior and inferior lobes by a single

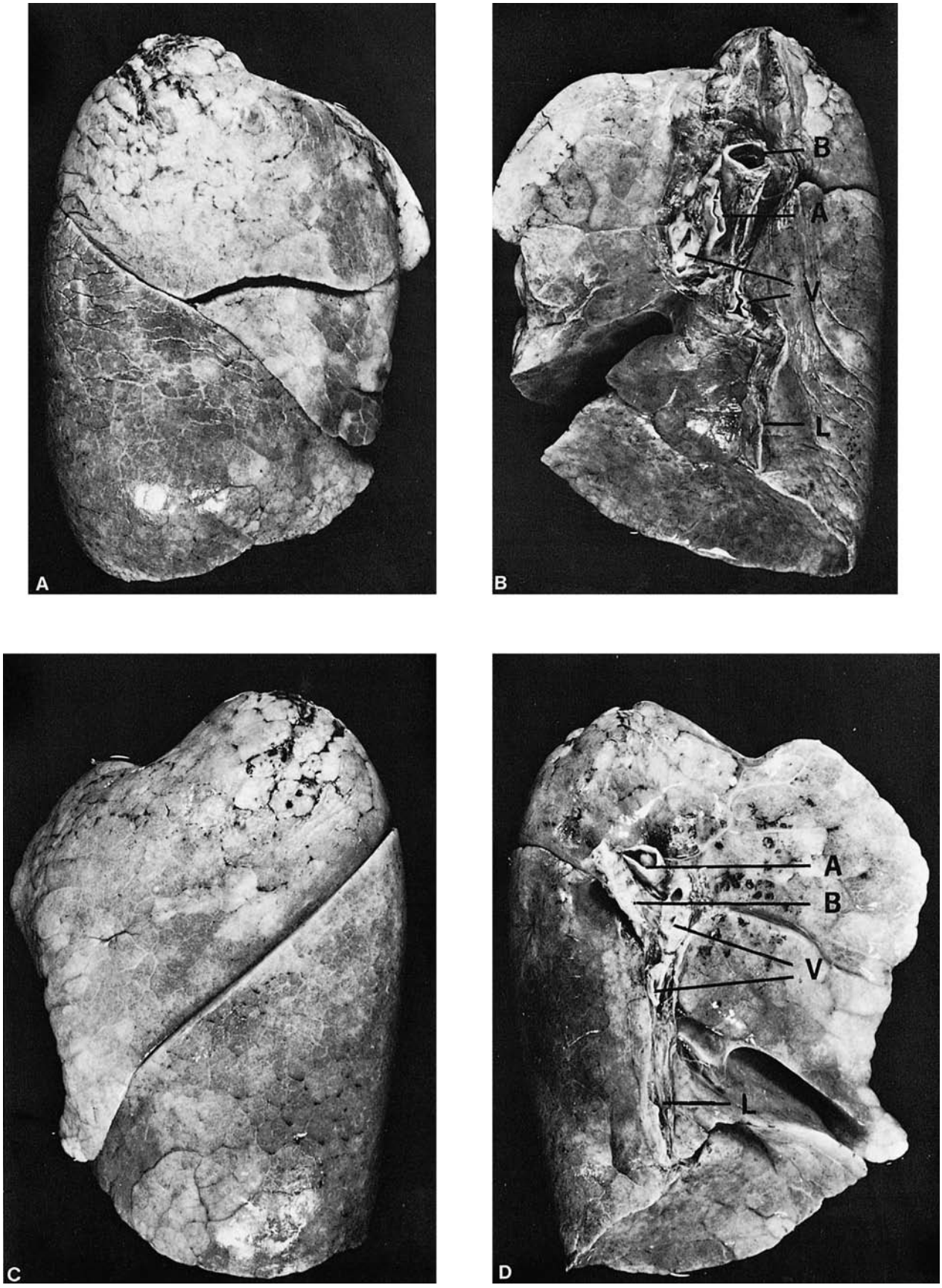


FIGURE 1–1 External appearance of fully inflated adult human lungs showing the normal lobe pattern. **(A)** Lateral surface, right lung. **(B)** Medial surface, right lung. **(C)** Lateral surface, left lung. **(D)** Medial surface, left lung. The hilar structures seen on the medial surfaces are the bronchi (labeled B), pulmonary arteries (labeled A), and pulmonary veins (labeled V). The pleural reflection at the hilus extends inferiorly as the pulmonary ligament (labeled L). Note that the right pulmonary artery enters the lung anterior to the bronchus, whereas the left pulmonary artery crosses over the bronchus.

oblique fissure; it has no middle lobe. The homologous region, the anterior and inferior portion of the superior lobe, is known as the lingula because of its tongue-like extension anteriorly into the costophrenic sulcus. It is frequently partially set off from the remainder of the lobe by an incomplete fissure.

The conducting airways, which distribute air to the gas-exchanging units, begin with the trachea, which originates at the larynx in the neck and descends into the mediastinum, where it branches at the level of T4–T5, giving rise to the left and right main bronchi. The branching is asymmetrical. The left main bronchus is narrower, longer, and given off at a greater angle than the right. The bronchus to the right upper lobe branches off the main bronchus just before they enter the lung at the hilum. Within the lung, the bronchi branch in a fashion classified as asymmetrical dichotomous in type, giving rise to progressively smaller airways. Branching is asymmetrical: the two daughters of a given branching may differ in diameter, length, and angle. The number of generations from the main bronchus to the acini varies from as few as 8 to as many as 25, depending on the region of the lung supplied.

Mixed venous blood is brought to the lung by the pulmonary artery, which leaves the right ventricle of the heart anterior and to the left of the ascending aorta and branches below the aortic arch. The right main pulmonary artery passes beneath the arch of the aorta to enter the lung anterior to the right main bronchus, with which it is closely associated. The left pulmonary artery passes above the main stem bronchus and lies above it in the hilus, where it passes over the superior lobar bronchus, coming to lie posterior to the bronchus. Within the lung, the pulmonary arteries accompany the bronchi, branching with them until they reach the acini. The pulmonary veins drain independently from the bronchi, with two trunks leaving each lung at the hilus to enter the left atrium separately.

The nutrient blood supply to the bronchi, associated lymph nodes, major pulmonary vessels, and part of the pleura comes from the systemic circulation via bronchial arteries, which vary in number and origin.[3] They arise either directly from the aorta or from the intercostal, internal mammary, or subclavian arteries passing along the esophagus and posterior wall of the main bronchi to enter the lung. Within the lung, they remain within the connective tissue sheath of the bronchi, where they extend peripherally to the level of the bronchioles. Bronchial vessels are not found within the acini. Apparently the venous blood provided by the pulmonary artery supplies all the metabolic substrates required by the lung parenchyma, and oxygen is supplied by diffusion from the airspaces. Venous drainage from the walls of the more peripheral bronchi enters the pulmonary veins. Bronchial veins drain only the central bronchi and nearby pleura and empty into the azygos and hemiazygos veins.

Subunits of Lung Structure

In addition to the lobes of the lung, various subunits of lung structure are recognized. The bronchopulmonary segments are important to thoracic surgery because they are subunits of a lobe that can be resected relatively conveniently with little hemorrhage or air leakage from the raw surfaces. Generally, they are the unit of lung supplied by the first generation of bronchi below the lobar bronchi, are roughly pyramidal in shape, and are pleural based. The nomenclature of the segments is given in Table **1–1**, and their pleural projections outlined in Fig. **1–2**. A detailed discussion of the many variations in segmental anatomy can be found in Boyden's monograph.[4]

When the pleural surface of the lung is viewed, connective tissue septa outline polygonal units ~1 cm in diameter. On the cut surface of the sliced lung, these septa extend a variable distance into the lung, in completely enclosing units known as pulmonary lobules or secondary lobules of Miller[5] (Fig. **1–3**).

The acinus is the basic unit of gas exchange. The unit is supplied by a single terminal bronchiole; it consists of respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. Individual acini cannot be recognized grossly. A lobule consists of three to five acini; although it is a less meaningful structure than an acinus in terms of lung function, the lobule provides a convenient reference for gross pathology. The terminal bronchioles branch near the center of the lobules, and their acini terminate in alveolar sacs abutting the connective tissue septa. Consequently, lesions affecting the proximal portions of acini predominate near the center of the lobules, and the lesions of the distal portions of the acini adjoin the lobular septa.

TABLE 1–1 Bronchopulmonary Segments

Right Lung	*Left Lung*
Upper lobe	Upper lobe
1. Apical	1, 2. Apical-posterior
2. Posterior	3. Anterior lingula
3. Anterior	
Middle lobe	Middle lobe
4. Lateral	4. Superior
5. Medial	5. Inferior
Lower lobe	Lower lobe
6. Superior	6. Superior
7. Medial basal (cardiac)	7. Anteromedial basal
8. Anterior basal	8. Lateral basal
9. Lateral basal	9. Posterior basal
10. Posterior basal	

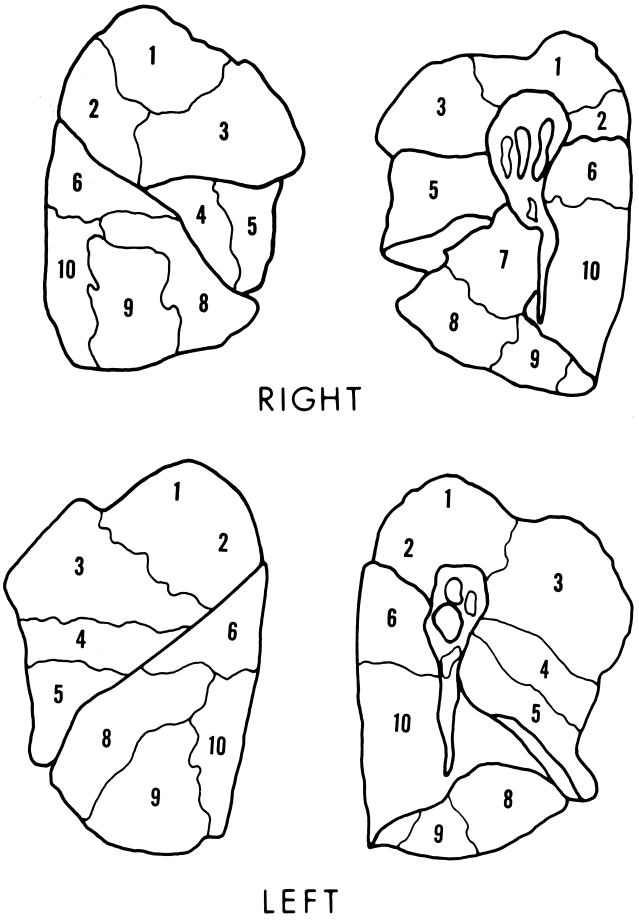


FIGURE 1–2 Pleural projections of the bronchopulmonary segments. Numbering is explained in Table **1–1**. There is no segment number 7 in the left lung.

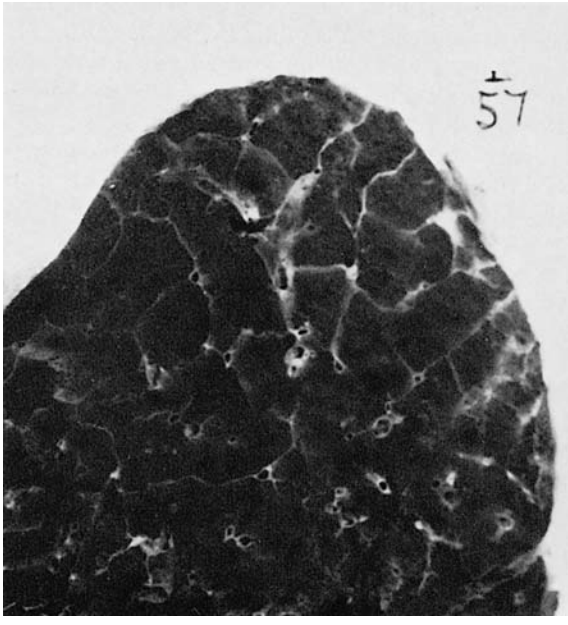


FIGURE 1–3 Sectioned surface of a lung showing lobules partially enclosed by connective tissue septa. The extent of development of the septa varies from lung to lung and from one region to another in the same lung.

■ Airways

Classification

Although the conducting airways share a common plan in that all are muscular tubes lined by a ciliated epithelium, they differ in detail depending on size.[6,7] The airways ~2 mm or more in diameter have walls reinforced by cartilage and are called bronchi. Conducting airways without cartilage are called bronchioles. In many species, rodents, for example, there is an abrupt change from the bronchioles to gas-exchanging tissue formed of alveoli. In humans and other primates, transitional airways called respiratory bronchioles have a bronchiolar structure over a portion of their circumference but also have alveoli over a portion. The term *terminal bronchiole* is used to identify the most distal generation of bronchioles completely free of alveoli, that is, the parent generation to the respiratory bronchioles.

Bronchi

The bronchi are lined by a pseudostratified ciliated columnar epithelium that rests on a homogeneous eosinophilic membrane 1 to 3 μm thick, often regarded as the basement membrane by light microscopists (Fig. **1–4**). As seen by electron microscopy, the bronchial epithelium rests on a typical slender basal lamina, but a layer of closely packed collagen fibers lies just beneath it; it is the combined structure that is recognized by light microscopists as basement membrane. Beneath the basement membrane is the lamina propria or subepithelial compartment (often mistakenly termed submucosa), which contains

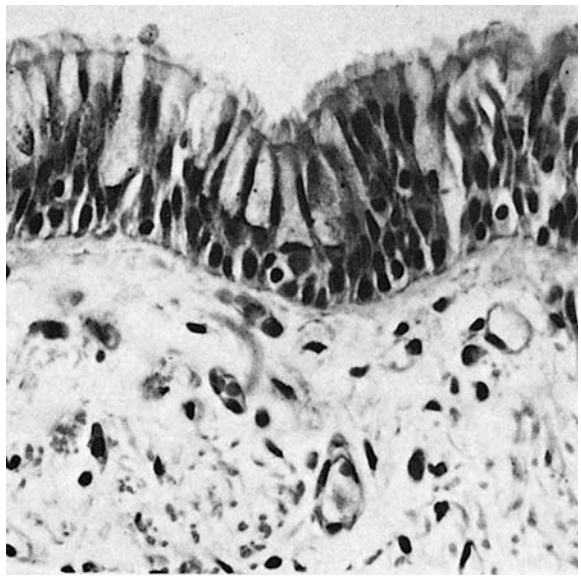


FIGURE 1–4 Mucosa of a major bronchus. Basal cells, goblet cells, and ciliated cells rest on a thin basement membrane (×400).

loose connective tissue with bundles of longitudinally arranged elastic fibers.

The Bronchial Wall

In the trachea and main bronchi, cartilage and smooth muscle are segregated in different parts of the airway wall, whereas in intrapulmonary airways they occur together. The anterior walls of the trachea and main bronchi are formed with V-shaped rings of hyaline cartilage, which open dorsally. The flat dorsal wall is composed of transversely disposed smooth muscle (the trachealis muscle). In the intrapulmonary bronchi, smooth muscle bundles lie deep to the zone of subepithelial elastic fibers and wind down the airway wall in a spiral with a shallow pitch. Consequently, the predominant effect of contraction of the smooth muscle is airway narrowing. Loose connective tissue and bronchial glands occupy the space between the muscle and the outermost layer of the bronchi, which consists of heavy circumferential bundles of collagen fibers and hyaline cartilage. In the intrapulmonary bronchi, the cartilage takes the form of variably shaped islands that diminish in size and number progressively with the decreasing caliber of the bronchi (Fig. **1–5**).

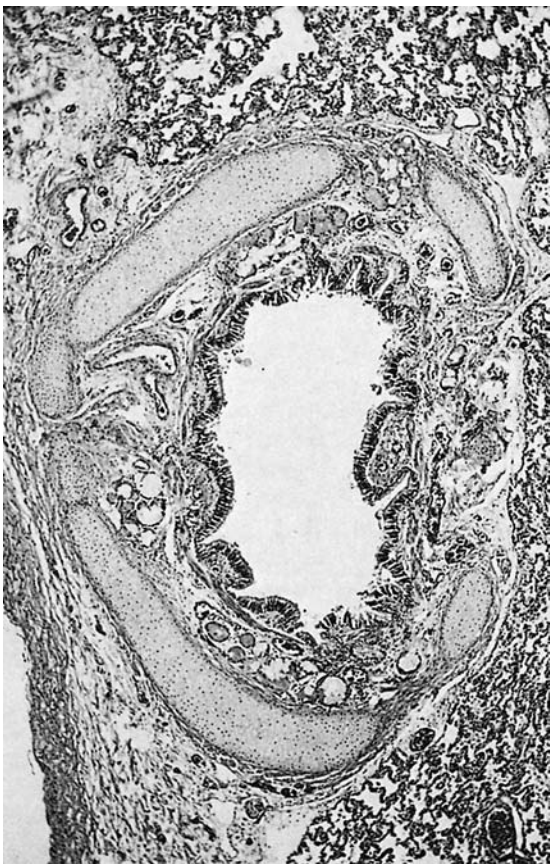


FIGURE 1–5 Large intrapulmonary bronchus in a child's lung. Multiple plates of cartilage completely enclose the bronchus, yet potentially permit it to vary in caliber. The pulmonary artery at the lower left occupies the same connective tissue sheath as the bronchus (×150).

Their distribution was investigated in detail by Hayward and Reid,[6] who found that, in the axial bronchial pathways, there was a high density of cartilage that effectively provided circumferential support for the first four to six generations. The axial bronchi had only scattered plates for another four to six generations. Laterally branching bronchi had circumferential cartilaginous support only at their orifices and occasional cartilage plates for another five to six generations.

Horsefield[7] pointed out that the pattern of cartilage organization is well adapted for an efficient cough mechanism. During coughing, forced expiration against a closed glottis generates marked positive intrathoracic pressure; simultaneously, the airways markedly narrow. When the glottis abruptly opens, the high pressure produces very rapid flows through the narrowed tubes to dislodge obstructing material. The extrapulmonary airways have no external support, and the cartilage rings enable them to remain open even when compressed by positive intrathoracic pressure. The intrapulmonary bronchi are resistant to collapse, being tethered by the surrounding lung tissue, and the incomplete cartilaginous plates provide additional buttressing while still permitting the bronchi to narrow.

The intrapulmonary bronchi are enclosed in a sheath of loose connective tissue containing bundles of collagen fibers and adipose tissue, together with the bronchial arteries, venous trunks, lymphatics, and nerves.

The Bronchial Mucosa

The epithelium lining the bronchi functions mainly in the production and propulsion of mucus.[8] If dust or organisms in the inspired air should impact on the bronchial wall, they deposit on a thin coating of mucus, produced by specialized secretory epithelial cells in the bronchial surface epithelium and glands located in the connective tissue of the bronchial wall.[9] The mucus streams in a generally cephalad direction to the oropharynx, propelled by the beating of cilia in the respiratory mucosa. The rate of movement of the mucus varies with the level of the bronchial tree, being slower in peripheral bronchi and fastest in the central bronchi and trachea, where particles are transported at a rate of 1 to 2 cm/min. The mucous blanket is considered to have two layers: a superficial gel layer rich in macromolecules, providing a viscoelastic barrier, and a watery serous layer beneath it in which the cilia beat. The mucous gel is composed of large polydispersed glycoproteins with molecular weights of several million daltons. They are composed of 75 to 80% O-linked carbohydrate side chains attached to a protein core. The mucin proteins are products of several genes on at least three different chromosomes. Mucin genes expressed in the respiratory tract are also expressed in other mucus-producing epithelia.[10,11] In addition to mucous glycoproteins, the bronchial secretions contain

antibacterial proteins such as lysozyme and lactoferrin,[12] immunoglobulins (Ig) (principally, IgA),[12] and locally produced proteinase inhibitors.[13]

In standard histologic sections, the bronchial surface epithelium consists of three principal cell types: basal, ciliated, and secretory cells (Fig. **1–4**). Round nuclei just above the basement membrane belong to the basal cells, small triangular cells that rest on the basal lamina and are excluded from the bronchial lumen by the neighboring columnar cells. They have a high nuclear-to-cytoplasmic ratio, tonofilament bundles, and numerous desmosomes and hemidesmosomes through which they anchor neighboring columnar cells to the basal lamina.[14] Although it is controversial whether basal cells are stem cells in the turnover of airway epithelium under normal conditions, they have the capacity to divide and differentiate into columnar cells[12] and doubtlessly serve as stem cells following injury to the columnar cells. Above the nuclei of the basal cells are elongated nuclei of columnar cells, ciliated and secretory. Ciliated cells are normally approximately three to five times more numerous than secretory cells.

Ciliated Cells

The ciliated cells are columnar cells attached to the basal lamina and reaching the bronchial lumen. They have a centrally placed oval nucleus, a large Golgi apparatus, and usually several large lysosomal residual bodies lying near the Golgi apparatus just above the nucleus. Mitochondria, which are found in all parts of the cell, are particularly concentrated in a zone near the apex of the cell close to the cilia, which they supply with adenosine triphosphate (ATP). Ciliated cells do not take up tritiated thymidine, and their replacement takes place by proliferation and differentiation of mucus-secreting and basal cells.[13]

The structure and cell biology of cilia have been reviewed by Satir.[15] The cilia are membrane-covered extensions of the apex of the cell, 5 to 8 μm in length and 0.3 μm in diameter. With a few exceptions, the basic structure of cilia is similar in most eukaryotic organisms. The basic machinery of the cilium resides in the axoneme, a complicated structure that arises as an outgrowth of basal bodies, centriole-like structures anchored in the apical cytoplasm. The axoneme consists of nine peripheral microtubule pairs arranged in a circle around two central single microtubules (Fig. **1–6**). Each of the peripheral doublet fibers consists of a complete 24 nm microtubule composed of 13 protofilaments (the A subfiber) fused to an incomplete tubule (B subfiber) composed of 10 protofilaments.[16] In conventional electron micrographs, there are paired asymmetrical arms at regular intervals of 24 nm along the A subfiber that project toward the B subfiber of the neighboring doublet (Fig. **1–6**). These arms contain the majority of the adenosine triphosphatase (ATPase) activity of the cilium.[17] Ciliary ATPases are known as dyneins; hence the arms are called dynein arms. Replicas of rapidly frozen and deeply etched cilia have shown a complex substructure to the dynein arms. The outer dynein arms are all similar and equally spaced at 24 nm intervals. Each has a globular head that interacts with two smaller globular units attached to the A subfiber and is connected by a thin stalk to the B subfiber of the neighboring doublet (Fig. **1–7**).[18] Inner arms occur in groups of three consisting of two arms with two heads each and one arm with three heads. The arms occur in sequence with a 24 to 32 to 40 nm spacing in a specific relationship with the radial spokes.[19] When studied biochemically, axonemes contain multiple dynein heavy chains with molecular weights greater than 300,000, each with specific binding sites for smaller associated proteins.

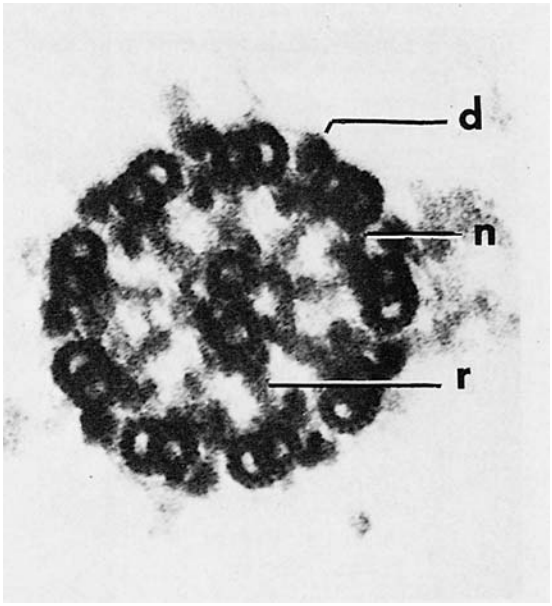


FIGURE 1–6 Cross section of an axoneme of a detergent-extracted nasal cilium viewed from the tip toward the cell. The plasma membrane was removed by the extraction. The axoneme consists of nine doublet microtubules in a ring surrounding two single microtubules (d, dynein arm; n, nexin link; r, radial spoke) (×250,000).

Each of the A subfibers is also joined on its inner aspect to the B subfiber of the neighboring doublet by a series of fine, highly extensible filaments known as nexin links. At regular intervals of 15 nm along both central singlet tubules, there are paired curved projections 18 nm in length, nearly perpendicular to the plane of the singlet tubules. These projections were known collectively as the central sheath before their structure was known in detail. Radially oriented spokes attached to the A subfibers of the peripheral doublet project inward to the central sheath, ending in a slight enlargement, the spoke head. The spokes are arranged in clusters of three, with an

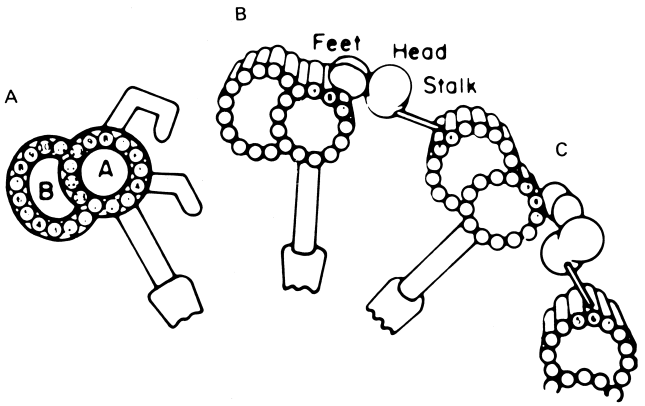


FIGURE 1–7 Diagram of the structure of the dynein arms based on conventional images and three-dimensional replicas of rapidly frozen cilia. (A) Conventional image as seen in thin sections. The A and B subfibers of the microtubular doublet are labeled. Two angulated dynein arms and a radial spoke with spoke head are shown attached to the A subfiber. (B,C) The components of the outer arm as seen in the replicas. (B) The configuration of the absence of adenosine triphosphate (ATP). (C) With ATP. (From Goodenough UW, Heuser JE. The substructure of the outer dynein arm. J Cell Biol 1982;95: 798–815. Used with permission.)

overall spacing of 90 nm, so that the spacing of each group of three radial spokes exactly matches six central sheath projections.[20]

The B subfibers, dynein arms, and radial spokes terminate 10 to 20 nm from the tip of the cilium, whereas the A subfibers continue into the tip to end in an electron-dense plaque that fills the 20 nm gap between the axoneme and the membrane over the tip. A cluster of five to eight bristles 25 to 30 nm in length anchored to the plaque penetrates the plasma membrane, forming the ciliary crown, a special glycocalyx on the tip of each cilium.[21,22] The ciliary crown is postulated to play a role in coupling the motile cilia to the overlying mucous blanket.

At the junction of the cilium and the apex of the ciliated cell, there is a specialized region known as the ciliary neck. In this region, the central singlet microtubules terminate, and radial spokes and dynein arms disappear. The doublet fibers are joined to one another and are connected to the plasma membrane by delicate Y-shaped linkers. Where the Y-shaped linkers join the membrane, freeze-fracture preparations show there are distinct circumferential rows of intramembranous particles, the ciliary necklace.[23]

The basal bodies, of which the cilia are an outgrowth, are cylindrical structures ~0.4 μm in length located in the apex of the cell. They are composed of nine triplet microtubules, two tubules continuous with the A and B subfibers of the axonemal doublet tubules, fused to a third (C) subfiber. The triplets are twisted to an angle of 30 to 40 degrees in a "pinwheel" arrangement. Among the accessory structures attached to the basal bodies are striated rootlets, which extend a few tenths of a micrometer into the cytoplasm, and a lateral triangular projection 0.1 μm in length, the basal foot, which is a nucleating site for cytoplasmic microtubules.[24]

The beating of the cilia is a complex movement; there is a rapid planar effective stroke in the direction of fluid movement in which the cilia engage the bottom of the mucous gel, followed by a slower recovery stroke in which the cilia recoil while swinging in a clockwise direction beneath the gel.[25] The plane of the effective stroke is perpendicular to the plane of the central singlet fibers, and the basal foot protrudes in the direction of the effective stroke.

The mechanisms of ciliary movement are poorly understood. The energy for ciliary beating is provided by ATP. Dynein is activated by Ca^{2+}, and also requires Mg^{2+} for optimal ATPase activity. The power for ciliary beating is generated by the sliding of groups of peripheral doublets relative to their neighbors driven by changes in the configuration of the globular heads of the dynein molecules. As the sliding is unidirectional, different groups of doublets may be activated during the effective stroke and during the recovery phase of the beat cycle.[15] The radial spokes and central pair-sheath complex are thought to participate actively in the conversion of the interdoublet sliding into the complex waveforms taken by cilia.[20]

The rate of respiratory ciliary beating is by intracellular calcium concentrations, and the coordination of neighboring cells by the passage of calcium-releasing second messengers (such as inositol triphosphate) through gap junctions between the cells.[26] Fields of beating cilia are then coordinated in metachronal waves by mechanical interaction of the cilia.

Another function of ciliated cells is control of the depth and composition of the periciliary fluid, which is critical for effective ciliary function. Airway epithelium actively transports ions (sodium away from the bronchial lumen and chloride toward it), while water follows the resultant osmotic gradient.[27,28] One of several proteins controlling this flux is the protein encoded by the gene that undergoes mutation in cystic fibrosis, *CFTR*, a tightly regulated chloride channel localized to the apical membrane of ciliated epithelial cells.

Mucus-Secreting Cells

The mucous gel is secreted by specific secretory cells in the tracheobronchial surface epithelium and in the glands of the subepithelial compartment. Two types of mucus-secreting cells have been identified in the surface epithelium by electron microscopy. Typical goblet cells are characterized by a bulging apex distended with coalescing secretory vacuoles.[29] As seen with the electron microscope, these vacuoles contain fibrillar electron-lucent material (Fig. **1–8**).

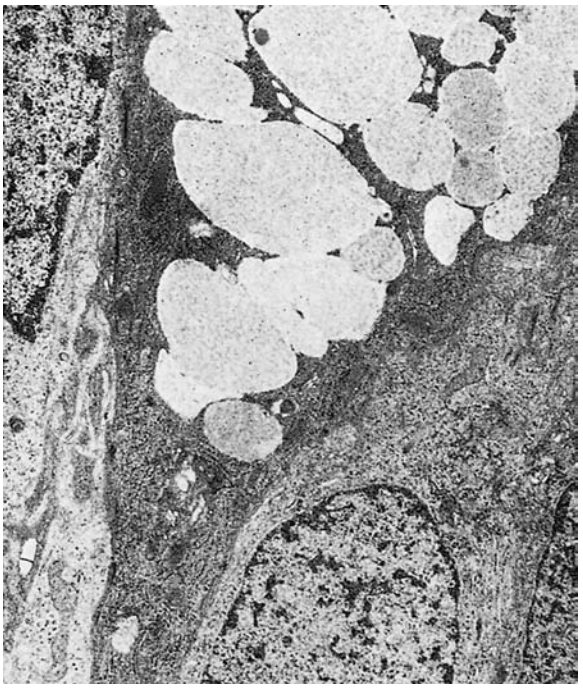


FIGURE 1–8 Goblet cell in the surface epithelium of a bronchus. The cytoplasm is relatively electron dense. The apical cytoplasm is filled with coalescent vacuoles containing electron-lucent mucus (×10,000).

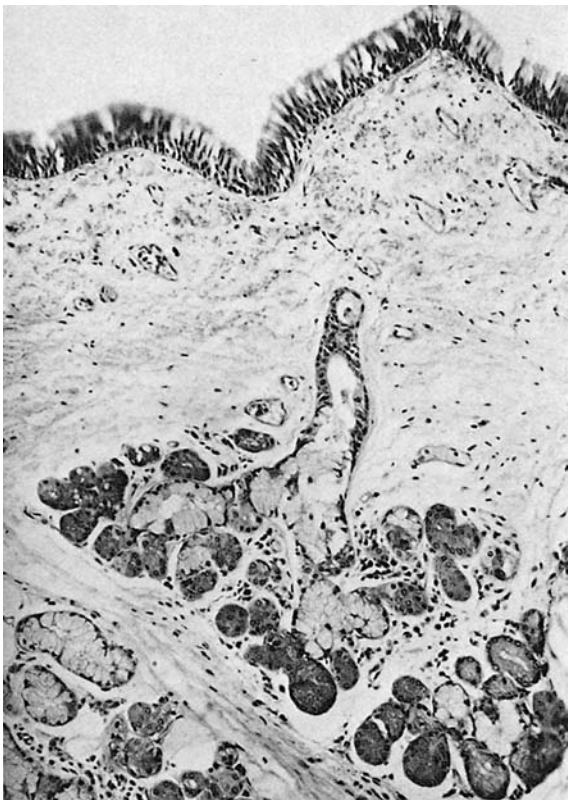


FIGURE 1–9 Bronchial mucous gland. The lumen of the bronchus is at the top. One duct branches, giving rise to secretory tubules lined by mucous cells in their proximal portions and serous cells in their terminal portions. The small dark cells in the interstitium between the tubules are mainly plasma cells (×100).

The apex of the goblet cells is covered by microvilli, and the cytoplasm is electron dense and contains a considerable amount of lamellar endoplasmic reticulum compressed into the basal and lateral portions of cytoplasm. Other mucus-secreting cells share the electron dense cytoplasm and other cytoplasmic features of the goblet cells but have only a few small mucus-filled secretory vesicles. These have been called "small mucous granule cells" (SMGC),[29] recognizing that these cells may not be a separate type of cell but only in a different phase of the secretory cycle than the goblet cells. A few bronchial columnar cells have neither cilia nor mucous granules. Such "indifferent" cells are infrequent and may represent a waystation between basal cells and one or both of the differentiated columnar cell types.

Mucous Glands

The mucous glands are compound tubular glands (Fig. **1–9**) that lie deep to the muscle, in the subepithelial compartment of the bronchi between the cartilage and surface epithelium. They often extend through the gaps between cartilages to occupy the connective tissue of the adventitia. Their three-dimensional organization has been described in detail by Meyrick and colleagues.[30] The secretory tubules drain into collecting ducts that discharge into the bronchial lumen at an estimated frequency of approximately one per square millimeter of bronchial surface in the central bronchi. The glands decrease in frequency distally, ultimately disappearing at the same level as the cartilage, at the 10th generation on the average.

Three types of cells are recognized in the secretory tubules: mucous, serous, and myoepithelial cells (Fig. **1–10**). Serous cells are most numerous at the ends of the secretory tubules. Their outline is roughly triangular with a basally placed nucleus and a polarized cytoplasm. The apical portion of the cell contains large eosinophilic granules, whereas the basal portion is strongly basophilic because of its high content of lamellar endoplasmic reticulum. The secretory granules contain asparagines-linked glycoproteins, including several specific proteins found in bronchial secretions [lysozyme (Fig. **1–11**), antileukoprotease, and lactoferrin].[31,32]

Mucous cells have basally located, rather pyknotic nuclei. The entire cell is filled with secretory vesicles with pale electron lucent fibrillar content, which compress the mitochondria and endoplasmic reticulum into a small intervening volume of electron dense cytoplasm. The Golgi apparatus is well developed and is found near the nucleus. The apical surface of the secretory cells is covered with short microvilli. The exposed surface is increased by the presence of intercellular canaliculi.[33]

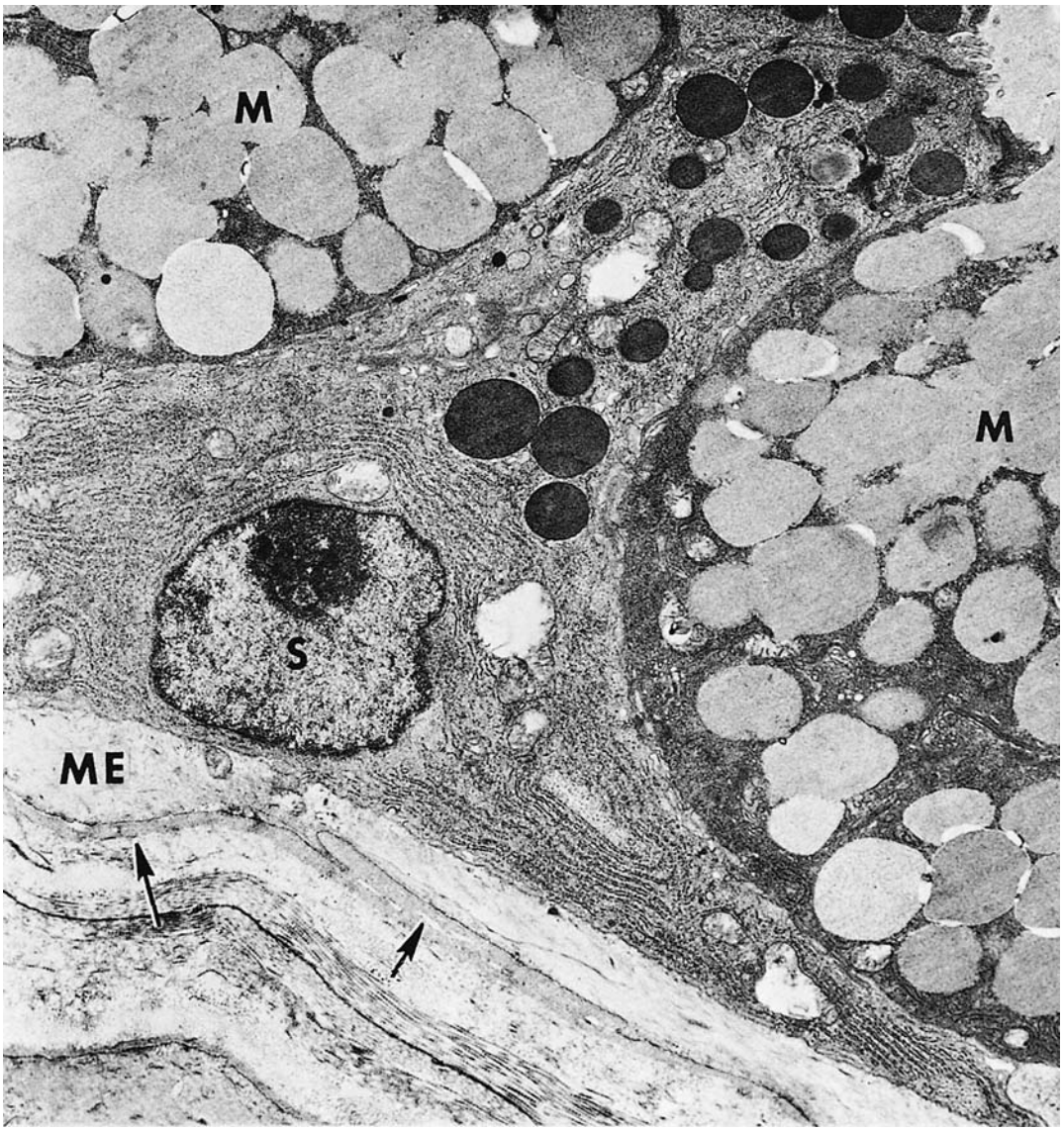


FIGURE 1–10 Mucous gland. The serous cell (S) has extensive endoplasmic reticulum in its basal portion and discrete apical granules. The cytoplasm of the mucous cell (M) is more dense, but the secretions are more lucent. A projection of the cytoplasm of a myoepithelial (ME) cell lies inside the basal lamina (arrows) (×6700).

The myoepithelial cells have the cytoplasmic features of smooth muscle. Their cytoplasm is filled with contractile microfilaments with focal, dense attachment bodies, and they express the smooth muscle isoform of α actin. However, like epithelium, they are enclosed within the basal lamina of the glands and are joined to the secretory cells by desmosomal junctions. Their appearance and location suggest that their function is to contract, milking the secretions toward the lumen. Cholinergic nerve endings have been identified among the various types of epithelial cells of the glands.[33]

Initially, both myoepithelial and mucous-secreting cells continue to line the secretory tubules as they coalesce to form the collecting duct. At variable distances along the duct, the lining epithelium changes to cuboidal or columnar cells, which are rather nondescript in the light microscope and are characterized ultrastructurally by the possession of a microvillous apical border and a large perinuclear Golgi apparatus, but no apparent secretory granules (Fig. **1–12**). Near the termination of the duct at the bronchial lumen, the epithelium again changes, becoming ciliated.

Plasma cells are often found in considerable numbers in the interstitial tissue around and between secretory tubules. There are nearly equal numbers of IgA- and IgG-containing plasma cells, but only a few cells containing IgM.[34] The immunoglobulin in mucus is principally 11S secretory IgA, consisting of two IgA molecules joined by J-protein and complexed to a "secretory piece." The IgA and J-piece are produced in plasma cells and

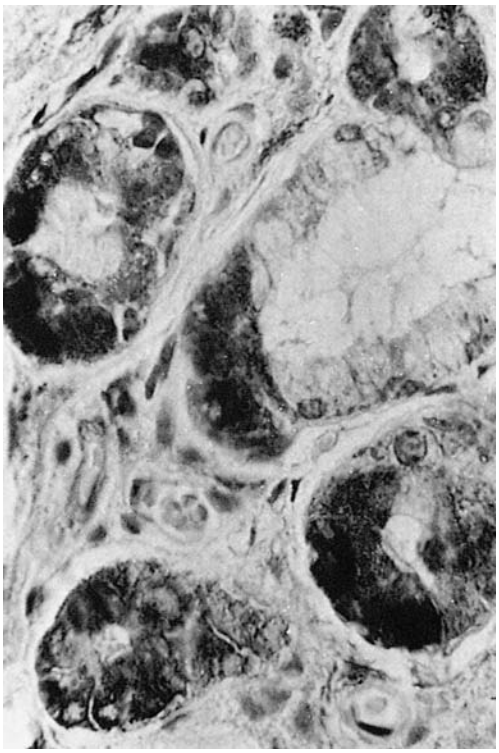


FIGURE 1–11 Mucous gland stained by the immunoperoxidase method using an antiserum to lysozyme. The serous demilunes show the dark reaction product (×400).

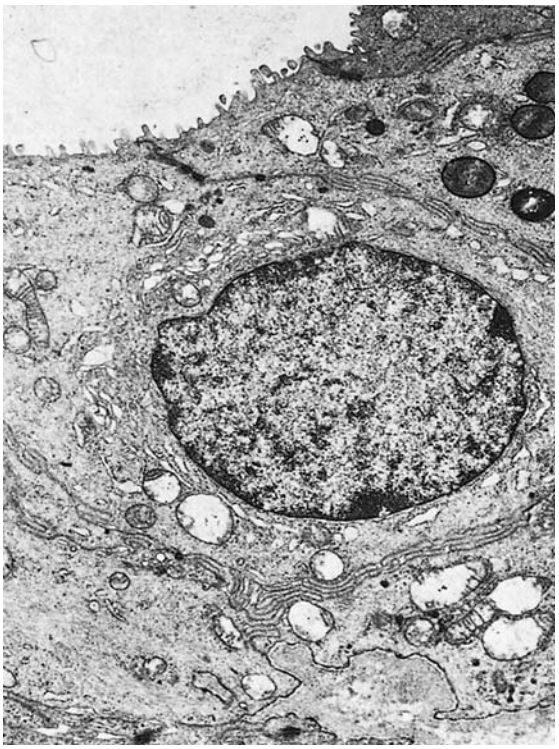


FIGURE 1–12 Epithelial cell lining the collecting duct of a mucous gland. The luminal surface has a few microvilli. The cell has a relatively large supranuclear Golgi apparatus but no secretory granules. The granules in the cell at the top right have the appearance of lysosomes (×8000).

complexed to the secretory piece at the membrane of the epithelial cells of the secretory tubules and transported across the epithelium to the tubular lumen.[35]

Neuroendocrine Cells

Argyrophilic stains and electron microscopy both demonstrate cells containing large numbers of cytoplasmic granules 0.1 to 0.2 μm in diameter, which have been variously named neuroendocrine cells, Feyrter cells, Kulchitsky (K) cells, amine precursor uptake and decarboxylation (APUD) cells, and "small-granule" cells.[36] These cells are more numerous in fetal than adult tissues and are most plentiful in small airways (bronchioles), although they also can be found in large airways and in ducts of mucous glands.[37–40] They occur in two anatomic forms (Fig. **1–13**). Solitary endocrine cells are found near the basal lamina between columnar cells. These endocrine cells are flask shaped and send out an apical extension between the columnar cells to the bronchial lumen, where they have a small exposed surface. Organized or corpuscular collections of neuroendocrine cells occur near bronchial branch points. The organized clusters, called neuroepithelial bodies by Lauweryns and Goddeoris,[41] are innervated by both afferent and efferent nerves and are associated with capillaries of the fenestrated type in the underlying lamina propria.

The histochemical properties of the solitary endocrine cells and the cells of neuroepithelial bodies are similar but not identical. Cells of both types are argyrophilic and show formaldehyde-induced fluorescence with a fluorescent spectrum characteristic of 5-hydroxytryptamine. They express neural markers by immunohistochemistry, including neuronspecific enolase, protein gene product 9.5, chromogranin A, leu 7, and synaptophysin.[42,43] Peptide hormones demonstrated in neuroendocrine cells include gastrin-releasing peptide, substance P, endothelin, calcitonin, and calcitonin gene–related peptide, a second peptide derived from the primary transcript of the calcitonin gene by alternative splicing. Leucine enkephalin can be identified in a few solitary neuroendocrine cells but not in neuroepithelial bodies.[44–46]

The functions of neuroendocrine cells are uncertain. Their morphology strongly suggests a secretory function, either paracrine or endocrine. Their greater abundance in the fetus suggests that they may have an important function in utero. One neuroendocrine cell product, gastrin-releasing peptide, has mitogenic activity for airway epithelium and enhances the growth and differentiation of fetal lung.[47] Various products of neuroendocrine cells influence ciliary beating, mucus secretion, and smooth muscle contraction. Lauweryns and coworkers[48,49] first

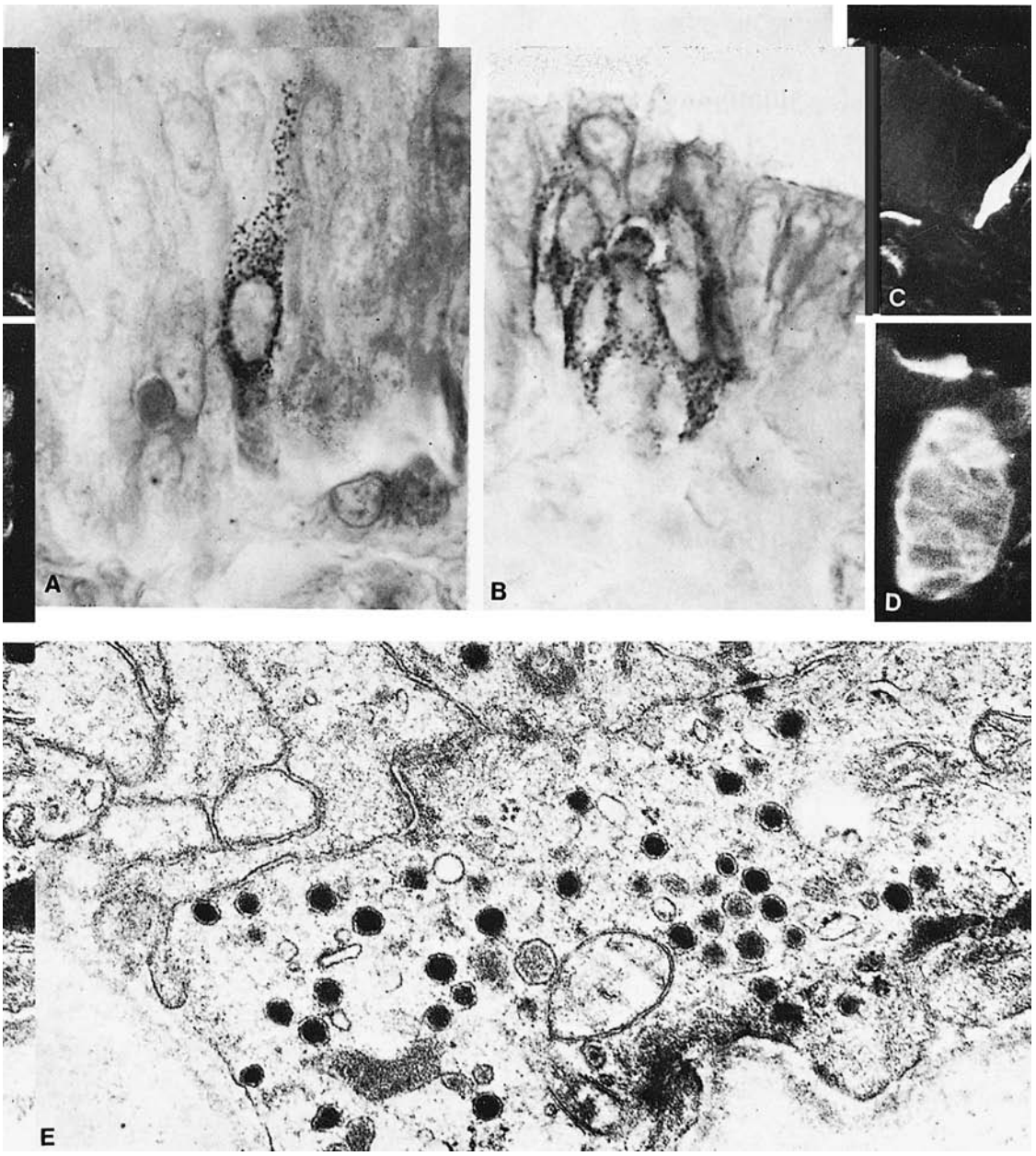


FIGURE 1–13 Bronchial cells. **(A)** Solitary endocrine cell in a human bronchus (Grimelius argyrophilic stain, ×1100). **(B)** Neuroepithelial body in a human bronchus (Grimelius stain, ×1100). **(C)** Solitary endocrine cell in a rabbit bronchiole (formaldehyde-induced fluorescence, ×400). **(D)** Neuroepithelial body in a rabbit lung (formaldehyde-induced fluorescence, ×400). **(E)** Granules in an endocrine cell of a human neuroepithelial body (×32,000). (**A**–**D** from Cutz E. Neuroendocrine cells of the lung: an overview of morphologic characteristics and development. Exp Lung Res 1982;3:185–208. Used with permission.)

proposed the hypothesis that neuroepithelial bodies are chemoreceptors sensitive to hypoxia and mediating hypoxic vasoconstriction. Supporting this suggestion are observations that hypoxia activates membrane potassium channel activity in the neuroepithelial bodies and that the activation is linked to the oxygen-binding protein reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,[50] properties that neuroepithelial bodies share with the chemoreceptor cells of the carotid body. Furthermore, the cells degranulate when exposed to acute hypoxia, but not when perfused with hypoxemic blood.[49]

Bronchioles

The walls of the airways less than 1 mm in diameter lack cartilage and consist of smooth muscle enclosed in a thin connective tissue space. The histologic appearance of the bronchioles varies considerably, depending on the degree

of expansion of the lung. In unexpanded lung, the mucosa is thrown into longitudinal ridges that appear papillary in cross section, whereas in the fully expanded lung the mucosa is thin with a smooth circular outline. The mucosa is of a simple columnar type. Basal cells are absent, and without the use of special stains for endocrine cells, only two types of cells can be identified: ciliated cells and nonciliated secretory cells, the so-called Clara cells.[51] Mucous cells are not normally found, but they may be present in a variety of diseases and are common in lung exposed to chronic respiratory irritants such as tobacco smoke.

The nonciliated cells of membranous bronchioles are columnar cells with a basal nucleus, a well-developed rough endoplasmic reticulum, Golgi apparatus, numerous mitochondria, and secretory granules in the apical cytoplasm (Fig. **1–14**). The apical cytoplasm often projects as a dome above the level of the apices of the more numerous ciliated cells and hence can be readily identified in the scanning electron microscope (Fig. **1–15**). The secretory granules of human nonciliated bronchiolar cells are 0.3 to 0.8 μm in diameter, membrane limited, and nonuniform in electron density with electron dense condensations against a more lucent background matrix. In respiratory bronchioles, the proportion of ciliated cells decreases, and in distal respiratory bronchioles ciliated cells often disappear completely (Fig. **1–16**). The

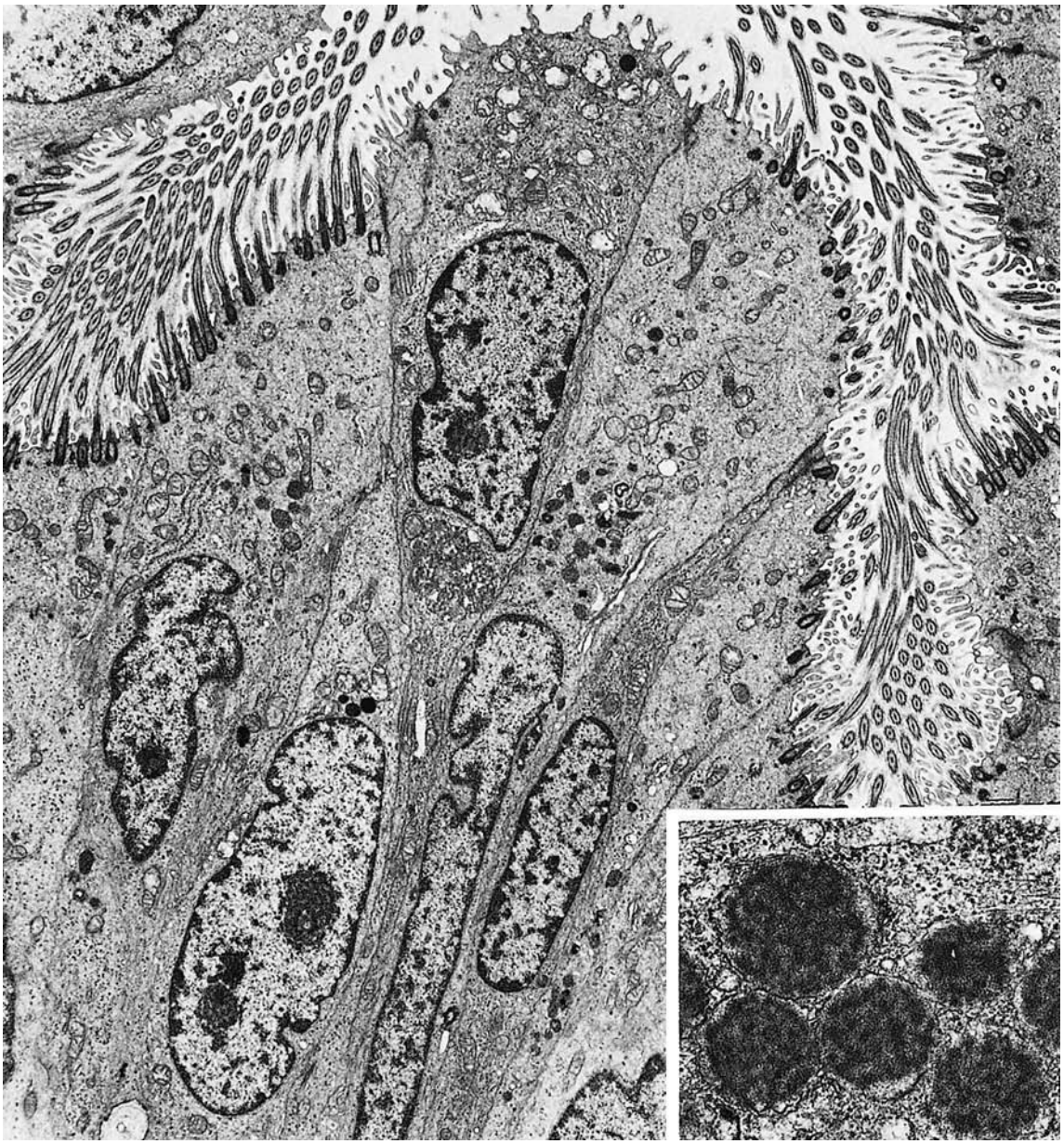


FIGURE 1–14 Epithelium of a membranous bronchiole. A Clara cell with a few secretory granules just beneath the apical membrane is sandwiched among several ciliated cells (×5000). Inset: The granules of a Clara cell have a flocculent texture (×31,000).

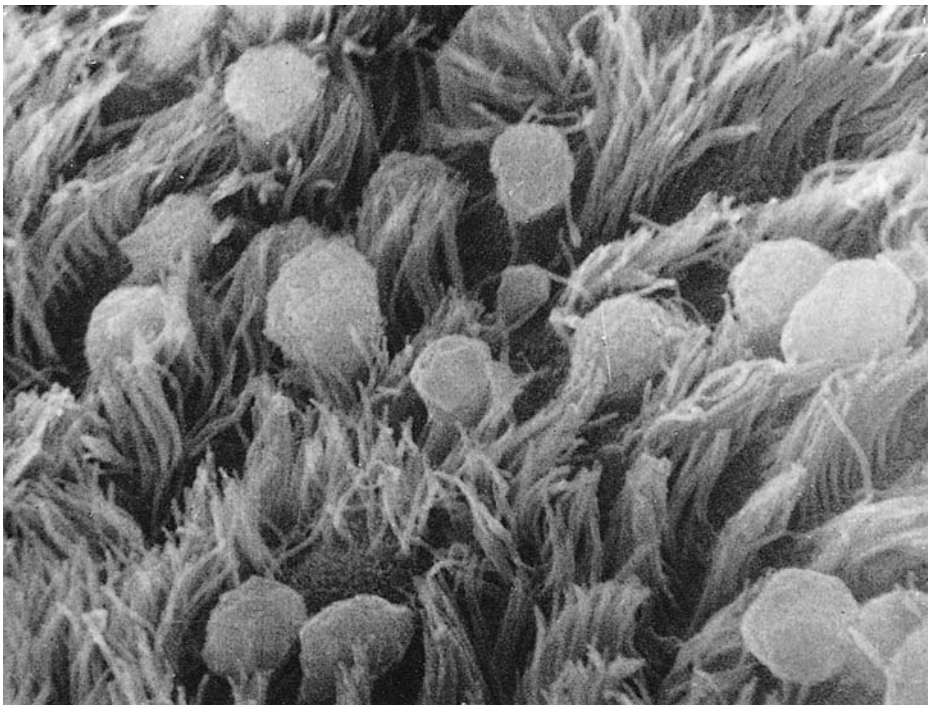


FIGURE 1–15 Mucosal lining of a bronchiole from a child's lung. The Clara cells protrude above the level of the cilia (scanning electron micrograph, ×4000).

nonciliated cells become cuboidal, and their granules have a homogeneous electron dense matrix.[52]

The bronchioles are lined by fluid that is covered by a surfactant film.[53–55] Its lipid components and some proteins probably are transported from the alveoli, but additional proteins in the hypophase are contributed by Clara cells. Clara cells synthesize several secretory proteins, including surfactant apoproteins A, B, and D,[56,57] and the low molecular weight inhibitor of leukocytic proteinases, antileukoproteinase. They also produce a unique l0 kd homodimeric protein, which has substantial homology to the rabbit protein uteroglobin.[58,59] Its function is unknown, but it binds environmental pollutants, such as polychlorinated biphenyls. Because the cuboidal nonciliated cells of the distal respiratory bronchioles lack the 10 kd specific protein, they are distinct from the columnar Clara cells of more proximal bronchioles.[58]

Nonciliated bronchiolar cells also serve as stem cells in the repair of bronchiolar injury and can proliferate and differentiate into ciliated cells following necrosis of bronchiolar epithelium.[60] Nonciliated bronchiolar cells in rodents are a rich source of mixed-function oxidases and are important in the metabolism of a variety of simple organic chemicals, some of which are transformed to toxic intermediates that selectively damage the nonciliated cells.[61,62] Rodent nonciliated bronchiolar cells possess an extensive smooth endoplasmic reticulum, the presumed site of mixed function oxidases, whereas human nonciliated bronchiolar cells have mainly rough endoplasmic reticulum. Because of this difference, it may not be valid to extrapolate the rodent experiments to humans.

■ The Acini

General Organization

Acini are units of lung supplied by a single terminal bronchiole. All the air passages constituting an acinus partake to some extent in gas exchange and all have alveoli. The components are respiratory bronchioles, alveolar ducts, and alveolar sacs (Figs. **1–17** and **1–18**). Respiratory bronchioles are air passages composed in part of muscular bronchiolar wall covered with cuboidal epithelium and in part of alveoli. Alveolar ducts are conducting structures lined entirely by alveoli and lead to a final generation of alveolus-lined spaces that end blindly, the alveolar sacs. Three-dimensional reconstruction of acinar structure is exceedingly time-consuming, and only a few acini have been analyzed completely. Boyden[63] spent 3 years reconstructing a single acinus from the lung of a 6-year-old child by serial sectioning. Others have carefully analyzed and dissected casts (Fig. **1–19**),[64–66] although in casts of airspace lumina it is often impossible to distinguish respiratory bronchioles from alveolar ducts.

Acini vary considerably in their detailed structure, depending on the space to be filled. Some abut the pleura or perilobular septa, some occupy the niches between bifurcations of bronchi or vessels, and some are entirely enclosed by lung parenchyma. The number of generations of respiratory air passages from terminal bronchiole to alveolar sac is variable even within a given acinus, ranging from 4 to 10. The first-generation respiratory bronchiole is usually mainly bronchiolar in structure, with an average of

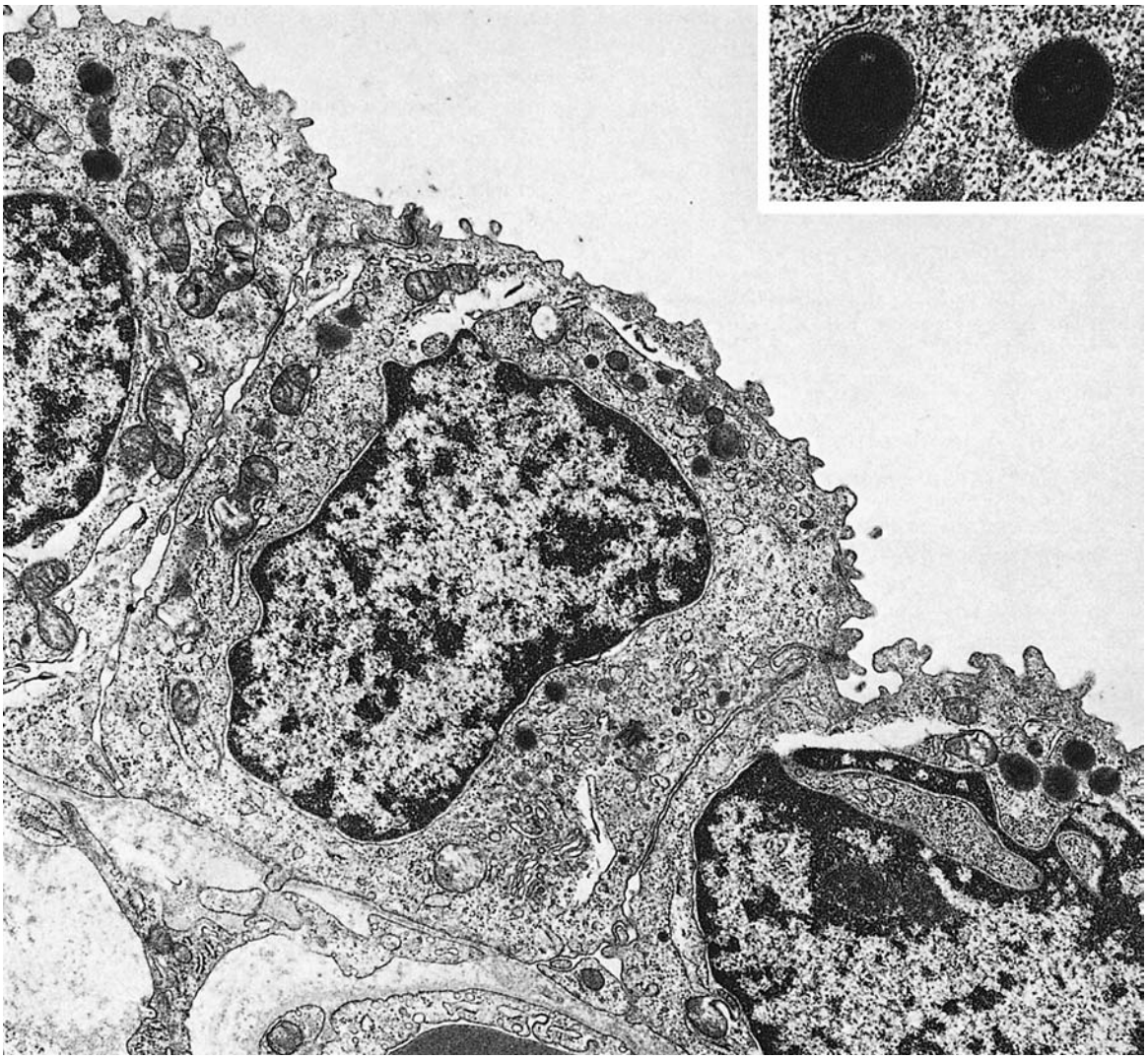


FIGURE 1–16 Epithelium of a respiratory bronchiole. The epithelial cells are cuboidal and the secretory granules are solid, differing in texture from those in the more proximal bronchiole in Fig. **1–15** (×9000). Inset: Granules at greater magnification (×28,000).

only three alveoli; subsequent generations of respiratory bronchioles consist mainly of alveoli with a cuboidal bronchiolar type of lining limited to one side where the accompanying artery runs. Usually after two or three generations of respiratory bronchioles, the passages become fully alveolated alveolar ducts. There are from two to six generations of alveolar ducts, the final generation in a given pathway giving rise to as many as six alveolar sacs. Individual respiratory bronchioles or alveolar ducts vary in length, number of alveoli, and angle and pattern of branching. Although dichotomous branching predominates, trichotomy and greater degrees of branching are often encountered.

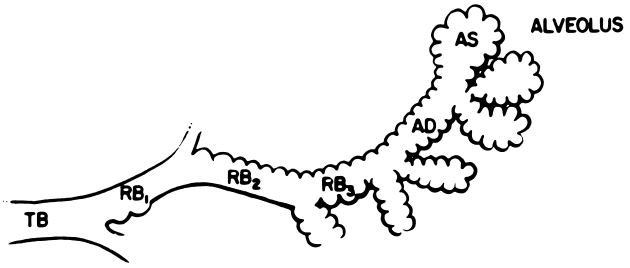


FIGURE 1–17 Schematic diagram of the components of an acinus. AD, alveolar duct; AS, alveolar sac; RB, respiratory bronchiole; TB, terminal membranous bronchiole. From Thurlbeck WM. Chronic obstructive lung disease. In: Somers SC (ed). Pathology Annual. New York: Appleton-Century-Crofts; 1968:377.

The Blood Supply to the Acinus

The pulmonary arteries accompany the bronchi and bronchioles, usually sharing a common connective tissue sheath. The muscular arteries are only slightly smaller than the bronchioles they accompany. Within the acinus, the diameter of the arteries decreases more rapidly than the air passages, so that in the periphery of the lung, the diameters of the arterial branches are much smaller than

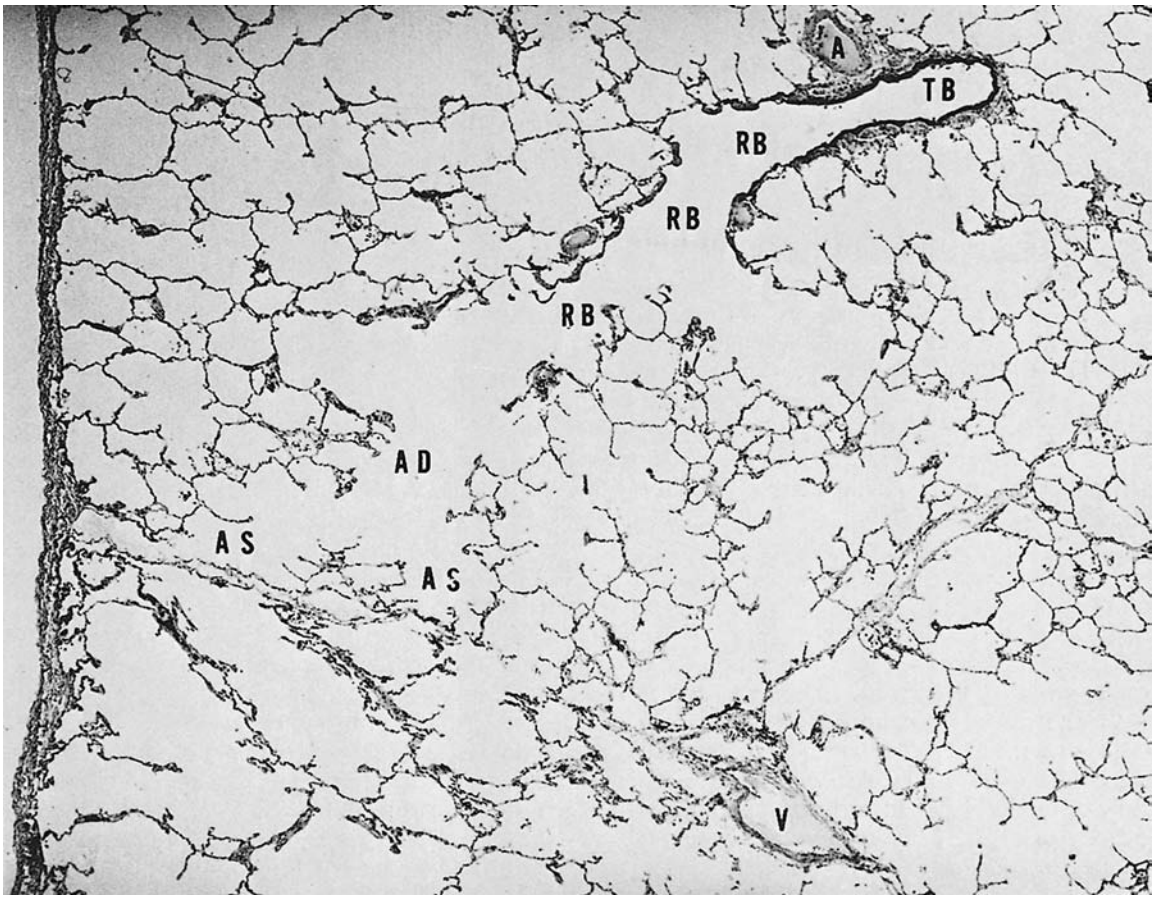


FIGURE 1–18 Histologic section showing the components of an acinus. A, muscular pulmonary artery; V, vein. The remaining abbreviations are listed in Fig. **1–17**.

those of respiratory bronchioles or alveolar ducts they accompany. Some muscular pulmonary arteries do not branch with the bronchial tree but arise as side branches; these vessels have been termed supernumerary.

The muscular arteries give rise to precapillary vessels, which in turn feed the rich capillary network that forms the major part of the alveolar walls. The capillaries are collected into venules that drain into veins at the periphery of the acini, receiving blood from two or three contiguous acini. Where the interlobular septa are well formed, the veins lie within the septa (Fig. **1–18**). Thus the blood enters the acini along the bronchioles, is distributed through the alveolar walls, and is collected into veins located at the periphery.[5]

Alveolar Ducts

The organization of the alveolar ducts has been the subject of several studies because it is critical to the process of ventilation.[67–69] The alveoli, which evaginate from the alveolar ducts, vary in size and shape but are on the average hexagonal in outline and 150 to 200 μm in diameter in the fully distended lung. In ordinary histologic sections, the free tips of the interalveolar septa are enlarged and contain smooth muscle and connective tissue fibers. These enlargements are the wall of the alveolar duct proper. Studies using thick or serial sections show that the smooth muscle and accompanying bundles of collagen and elastic fibers spiral down the alveolar duct like a spring. Alveoli are arranged in tiers between the thick spiral fiber bundle, with thinner rings of collagen and elastic fibers at their mouths (Fig. **1–20**). The elongation and enlargement of the alveolar ducts during inspiration take place in part by axial elongation of the connective tissue fiber bundles but also in part by uncoiling of the spring.[67,68]

Alveoli evaginating from adjacent alveolar ducts or sacs are packed in an interdigitating manner similar to the packing of soap bubbles so that the junctions of alveoli are always formed of three alveolar walls.[69] The walls separating adjacent alveoli are composed of interwoven networks of connective tissue and capillaries covered by a thin layer of epithelium. The alveolar capillaries are arranged in a net or grid-like pattern (Fig. **1–21**), the mesh of which is tightest where the wall separates two airspaces and somewhat more open where an airspace abuts the pleura, interlobular septum, or bronchovascular sheath.[5] An analysis of the organization of the capillary net by Sobin and associates[70] indicates that the blood flow

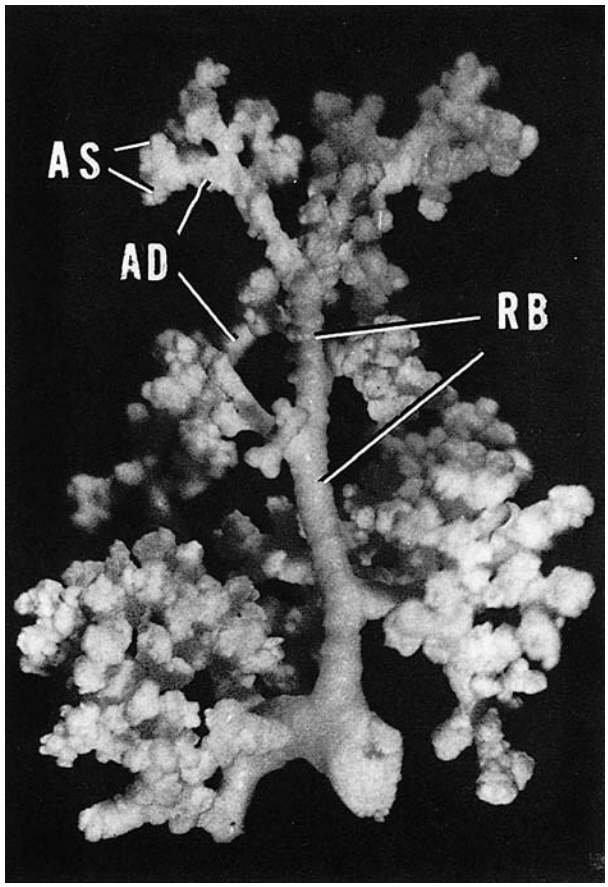


FIGURE 1–19 Partially filled cast of an acinus to demonstrate the variability and complexity of branching. Abbreviations are listed in Fig. **1–17**.

through the lung is approximated by a laminar flow model in which the blood is spread into a thin film percolating between "posts" (the spaces between capillaries) rather than the more usual model of flow through tubes.

The interstitial connective tissue of the airspace walls contains types I, III, and V collagen and elastic fibers.[71] Generally, the collagen and elastic fibers run together in bundles that are interwoven through the capillary net (Fig. **1–22**) so that in most planes of section, the connective tissue and capillary appear side by side. Thus, in cross section, the capillary has a thin blood–air barrier on one side, where there is no connective tissue bundle, and a thick blood–air barrier on the opposite side, where the connective tissue bundle is found (Fig. **1–23**). This organization provides support for the alveolar wall while minimizing the barrier to diffusion.[72,73]

Alveolar Walls

The principal cells composing the walls of the interalveolar septa are the alveolar epithelium, endothelium, and interstitial cells.[74] There are nearly equal numbers of endothelial cells and interstitial cells, and both types are more plentiful than epithelial cells (Fig. **1–24**). The epithelial cells, which cover the alveolar septa, are of two types. One, commonly called the type I epithelial cell or membranous pneumonocyte,[75] is a simple squamous, epithelial cell that is thickened in the region of the nucleus with an extensive thin sheet of cytoplasm ~0.1 to 0.2 μm thick that extends out to cover most of the airspace walls. These cytoplasmic extensions of the type I cells are too thin to be consistently resolved by light microscopy, which accounts for the longstanding controversy as to whether the alveolar capillaries are naked or covered by

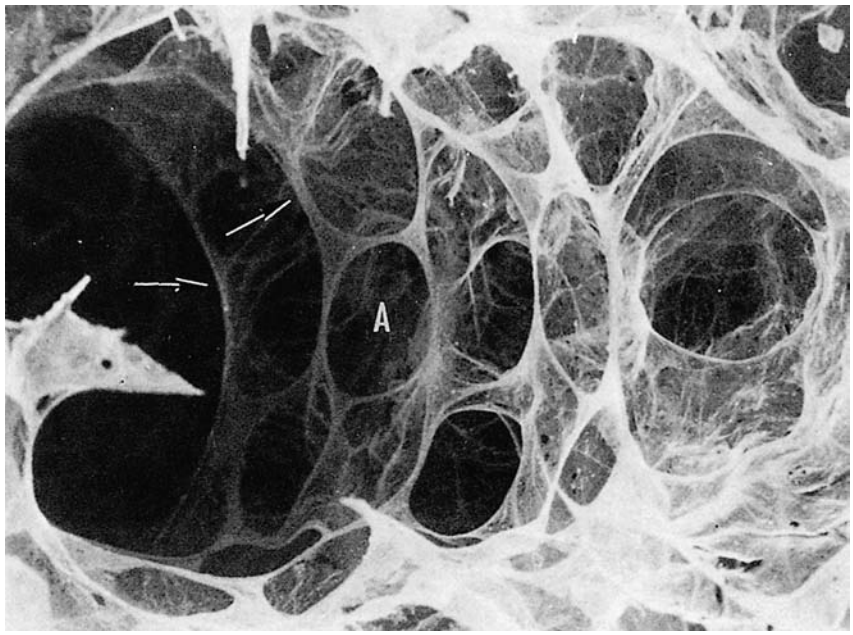
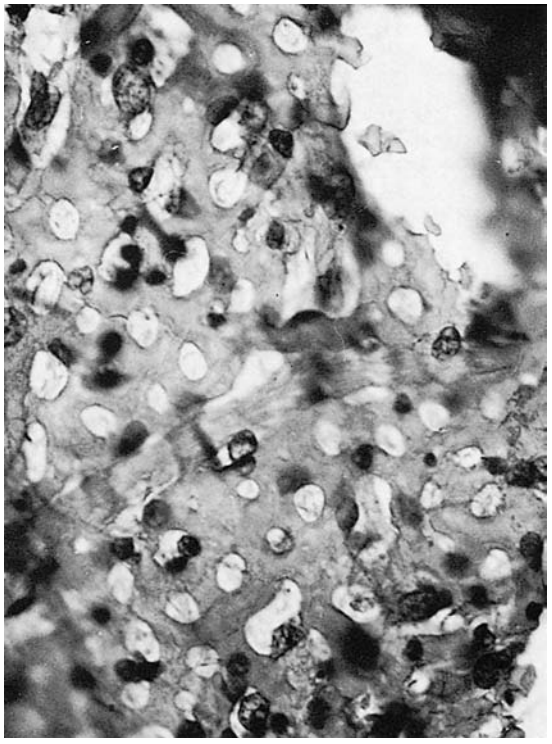
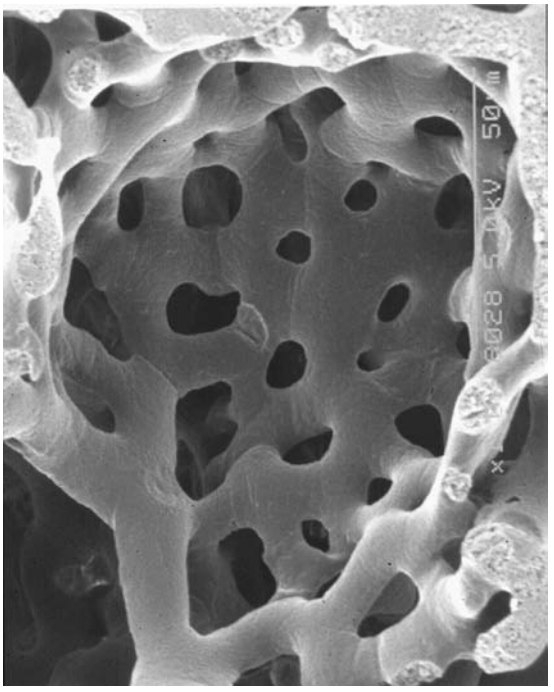


FIGURE 1–20 Connective tissue skeleton of a lung prepared by extracting the cellular protein with NaOH. The lung was then dried fully inflated and prepared for scanning electron microscopy. Heavy ridges of connective tissue wind down the alveolar duct (arrows). Rings of connective tissue outline the mouths of alveoli (A). Fine fiber bundles can be seen crossing alveolar walls (×120).



A



B

FIGURE 1–21 **(A)** En face view of an alveolar wall in a 50-μm thick histologic section showing that the capillaries form a grid-like network rather than a series of tubes (×1500). **(B)** Scanning electron micrograph of a capillary vascular cast demonstrating the grid-like network in a single alveolus (bar = 50 μm).

epithelium. This was finally resolved when electron microscopy was applied to the lung.[76] The type I cell cytoplasm contains some mitochondria and endoplasmic reticulum, mainly but not exclusively confined to the perinuclear region. Organelles are sparse in the thin cytoplasmic extensions except for variable numbers of pinocytic vesicles.

The second type of epithelial cell, the type II cell or granular pneumonocyte, is cuboidal with microvilli on its apical surface. In many mammals, the type II cells are recessed in niches in the alveolar septa, but human type II cells often protrude into the alveolar space. In the light microscope they can be recognized by their content of cytoplasmic vacuoles 1 to 2 μm in diameter. In the electron microscope, type II cells have a fairly large Golgi apparatus, conspicuous mitochondria, and characteristic secretory granules known as lamellar bodies, which are composed of closely packed whorled lamellae of osmiophilic membrane-like material, the alveolar surfactant (Fig. **1-25**). These inclusions are extracted by lipid solvents, leaving the empty vacuoles that characterize type II cells in paraffin sections. Type II epithelial cells are more numerous than type I cells but cover only a tiny fraction of the alveolar surface (2.5% in the rat), the majority being covered by the attenuated cytoplasmic extensions of type I cells.[77] Often the cytoplasm of the type I cell even extends as a flap covering part of the type II cell, leaving only a portion exposed to the alveolus

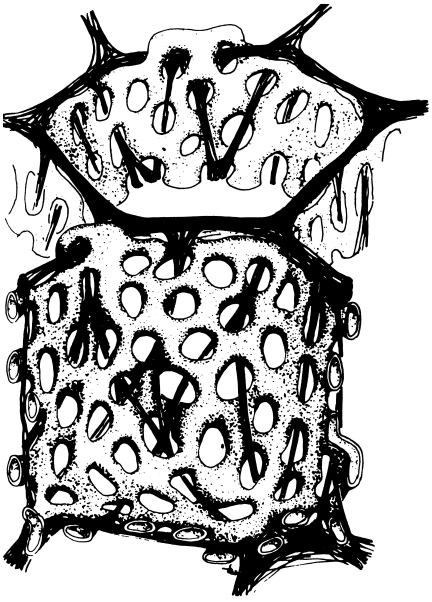


FIGURE 1–22 Diagram of the interweaving of the capillary network and bundles of connective tissue fibers in alveolar walls. (From Weibel ER, Gil J. Structure-function relationships at the alveolar level. In: West JB, ed. Bioengineering Aspects of the Lung. New York: Marcel Dekker, 1977:181. Used with permission.)

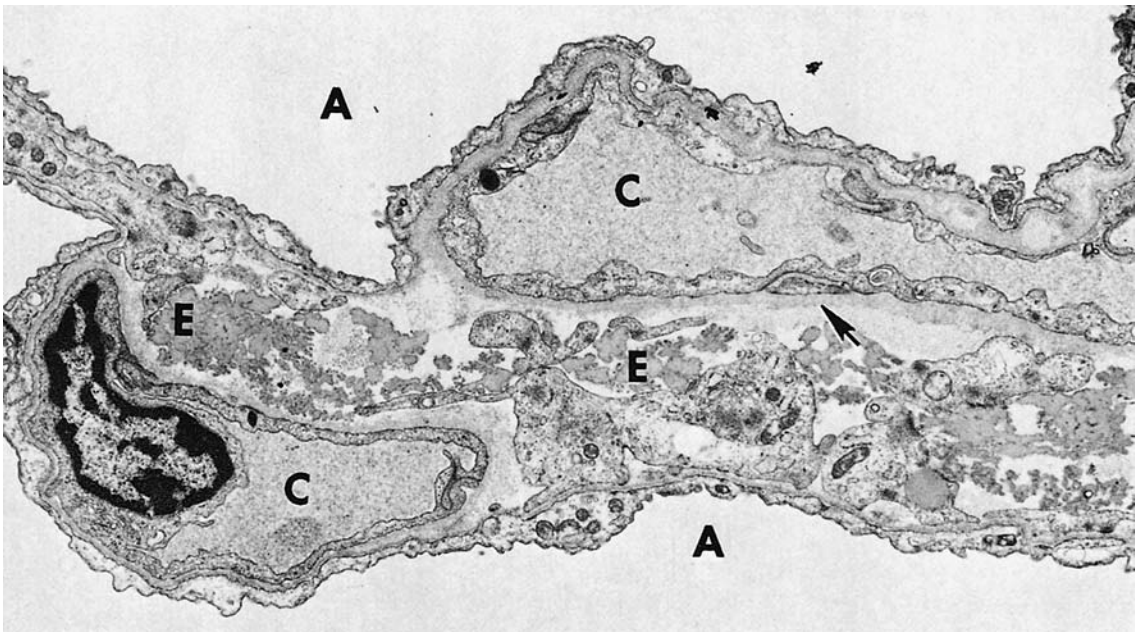


FIGURE 1–23 Cross section through an alveolar septum. A connective tissue bundle containing elastic fibers (E) passes through a gap between two capillaries (C). The capillaries and connective tissue space appear side by side, so that on one side each capillary is separated from the alveolar space (A) only by a thin barrier consisting of epithelium, endothelium, and a shared basal lamina, whereas on the opposite side, the barrier is thick because of the presence of the connective tissue between epithelial and endothelial basal laminae. The arrow indicates the cytoplasm of a pericyte associated with the capillary (×8000).

(Fig. **1–25**). The intercellular junctions between type I and II epithelial cells are low permeability tight junctions with three to five junctional strands in electron micrographs of freeze fracture replicas.

Type II cells have three established functions: secretion of surfactant,[78–80] the absorption of ions and fluid from the alveolar space, and repair in alveolar injury.[81,82] The airspaces of the lung are lined by a layer of fluid that was first detected by Terry[83] using the technique of micropuncture. Having a fluid lining, the alveoli are subject to the forces that act at the air–fluid interface. These forces produce a fine modeling of the alveolar walls in the air-filled lung and also are responsible for a major part of the elastic recoil of the lung.[84]

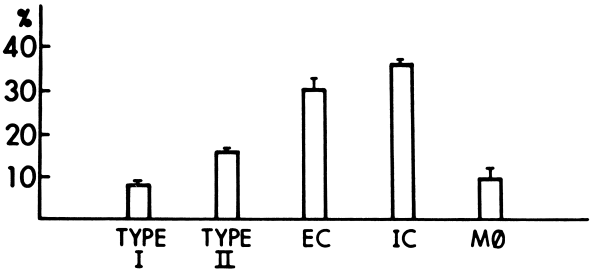


FIGURE 1–24 The relative numbers of different types of cells in the alveolar walls as a percentage of the total, from data by Crapo and associates.[74] Type I and type II, alveolar epithelial cells; EC, endothelial cells; IC, interstitial cells; Mϕ, macrophages.

The alveoli of the air-filled lung are not polygonal but have smoothly curved surfaces. With the resolution of the electron microscope, the smoothing of the alveolar contour can be seen to be produced in part by pleating of the alveolar capillary membrane, with folds projecting into the capillaries (Fig. **1–26**) and in part by the filling of irregular depressions in the alveolar wall by the fluid of the lining layer.[85] The changes in alveolar volume that occur with ventilation are accompanied by an unfolding of the pleats in the alveolar wall, which project more deeply into the capillaries at low lung volume and become shallow as the lung is expanded.[86]

One suggested function of the so-called alveolar surfactant is to facilitate the pulling apart of the folds.[87] The other function is to stabilize lung volume. This function of surfactant can be understood if one assumes the airspaces to be spherical. LaPlace's law relates the pressure in a gas bubble to its radius:

$$P = 2\gamma/R$$

in which P is the pressure, γ is the surface tension, and R is the radius. If surface tension in the lung lining fluid were constant, the pressure in spherical airspaces of smallest radius (alveoli) would be higher than that in large airspaces (alveolar ducts) and, as a result, the alveoli would tend to collapse and the alveolar ducts tend to dilate. The alveolar surfactant has the property of lowering surface tension γ as the area decreases. Hence, as alveoli decrease in volume and R falls, γ also falls, which

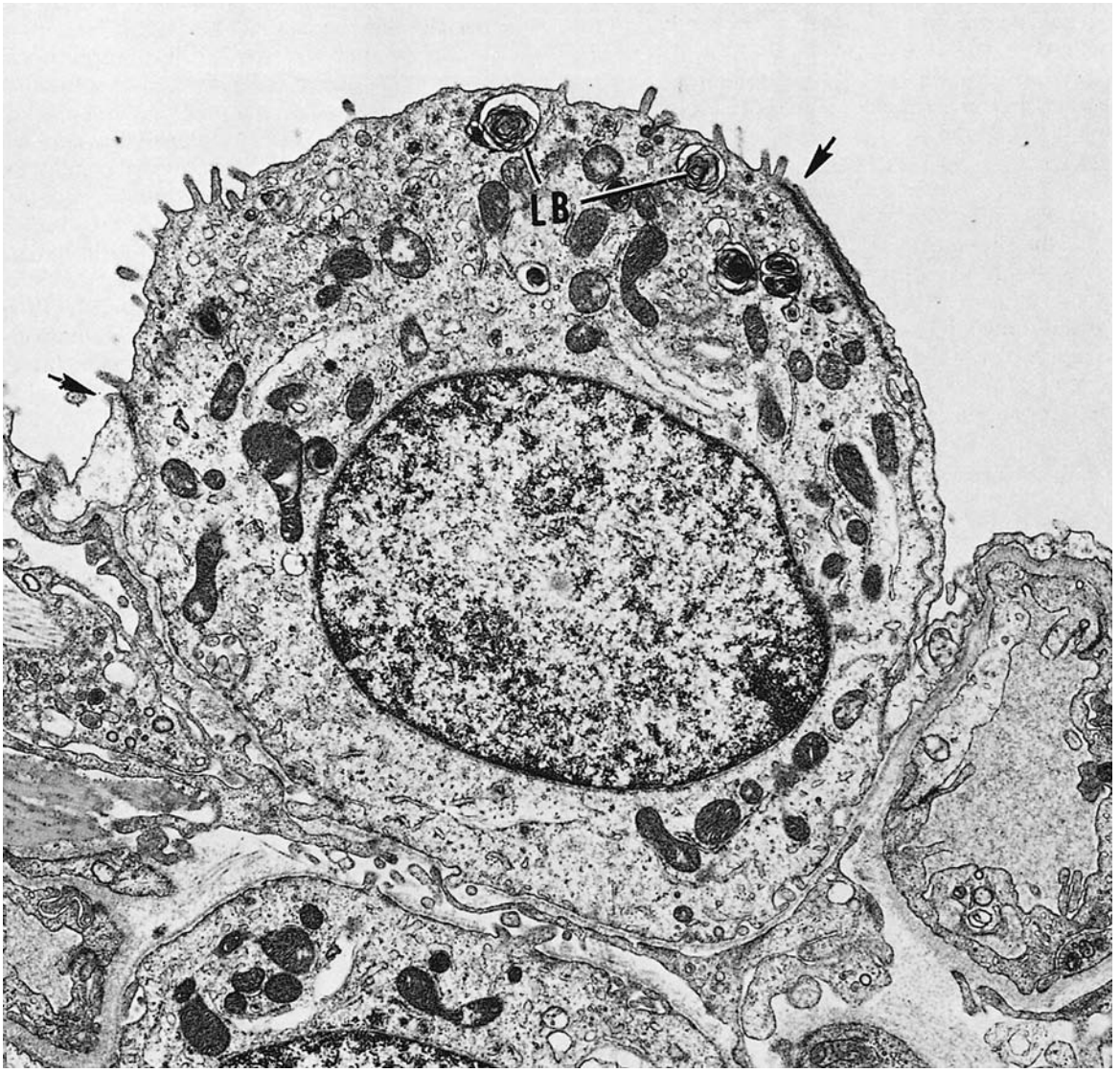


FIGURE 1–25 Type II epithelial cell from a human lung. The alveolar surface is partially covered by flaps of cytoplasm from type I epithelial cells up to the intercellular junctions at the arrows. There are several lamellar bodies (LB) in the apical cytoplasm. Microvilli are present on the exposed apical surface (×19,000).

tends to prevent alveolar collapse. In reality, of course, airspaces are not spherical and the actual mathematical relationships are more complicated.[73]

The alveolar surfactant is a complex mixture of lipids and proteins that are synthesized by type II cells and packaged together in lamellar bodies. The most abundant lipid is saturated phosphatidylcholine, but several other lipids are present in lesser amounts, including phosphatidylglycerol, a relatively rare phospholipid in mammalian cells.[88] Protein makes up 15% of the surfactant (Table **1–2**)[89] and is involved in surfactant function.[90,91] Surfactant proteins A, B, and D are synthesized in Clara cells and stores in lipid-poor granules; all four of the proteins secreted by type II cells are packaged in lamellar bodies. Surfactant proteins A and D are members of a family of innate immune molecules termed collectins because of their collagen-like and calcium-dependent pectin domains.[92] Surfactant protein A (SPA) also functions in receptor-mediated reuptake of surfactant and in the regulation of surfactant synthesis.[93] Both proteins have important roles in the immune response to microbial challenge, in addition to participating in other inflammatory regulation processes within the lung,[92,94] and in this sense are often termed "defensins."

Like many epithelia, type II cells transport ions. The net effect is the conduction of sodium from the luminal side to the interstitium, via a cyclic adenosine monophosphate (AMP)-regulated apical sodium channel and powered by a sodium potassium ATPase and other transporters

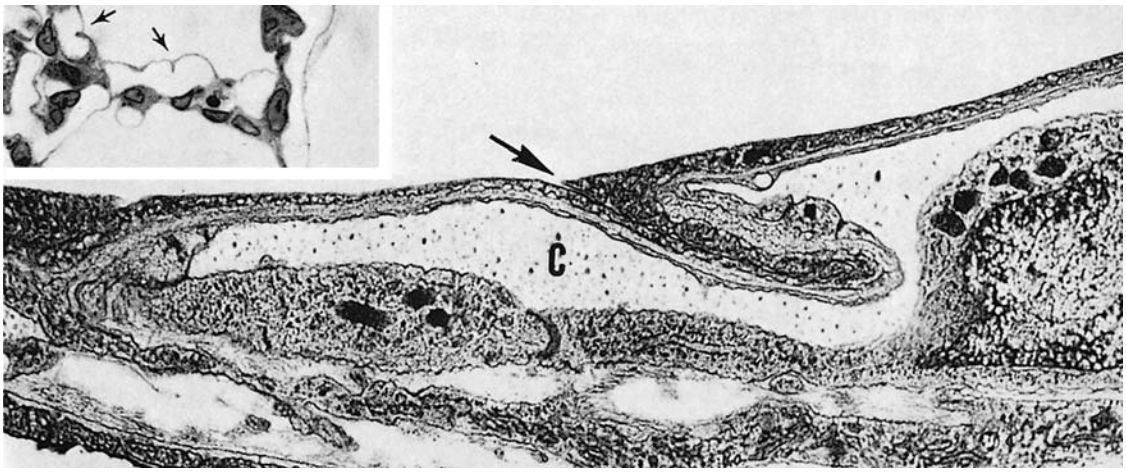


FIGURE 1–26 Electron micrograph of a hamster lung prepared by rapid freezing in situ to fix the tissue without disrupting the various physical forces that act in vivo. The alveolar surface is smoothly curved; the alveolar capillary membrane is folded on itself at the arrow, projecting into the capillary lumen (C). The depth of the folds decreases and increases with inflation and deflation of the lung (×8000). Inset: Lung fixed by perfusion through the arterial system. The capillaries are empty as a result of perfusion. This technique also achieves fixation while surface forces at the alveolar surface are maintained. Again, the alveolar capillary membrane folds into the capillaries (arrows) (×1400).

located in the basolateral plasma membrane.[95] Because water accompanies the sodium flux, this activity helps to keep the alveoli dry and to clear edema.

The reparative function of type II cells is manifest in a variety of forms of lung injury. The type I cells, with 50 times as much exposed surface as type II cells, are easily damaged, and their thin cytoplasmic extensions disintegrate. Type I cells appear unable to divide, but Evans and associates[82] have shown that type II cells proliferate and can differentiate into type I cells.

Both the alveolar epithelium and the capillary endothelium rest on basal laminae, which they probably secrete. The capillary and alveolar basal laminae remain distinct and separate on the thick side of the blood–air barrier, but they fuse on the thin side, so the blood–air barrier on the thin side of the septum consists only of alveolar lining fluid, epithelium, shared basal lamina, and endothelium, a total thickness of ~0.4 μm (Fig. **1–23**). Biochemically, the alveolar and capillary basal laminae differ somewhat. Both contain type IV collagens, laminin, and fibronectin, but the proteoglycan of the alveolar basal lamina is heparan sulfate, whereas the capillary basal lamina has sparser proteoglycan particles containing chondroitin sulfate.[96]

The space between the alveolar and capillary basal laminae is the interstitial compartment of the septum. The principal cell of the interstitium is a mesenchymal cell that resembles a fibroblast, particularly a cultured fibroblast with stress fibers (bundles of contractile filaments that insert at points of attachment to its substrate). The interstitial cells are highly irregular in outline, with long slender projections of cytoplasm that extend into the interstitial space. They are commonly closely associated with collagen and elastin fibers, of which they are almost certainly the source. As Kapanci and associates[97,98] noted in the rat, these cells have distinct bundles of contractile filaments, which suggests that they have a contractile function. The cytoplasmic processes of different interstitial cells make contact via gap or nexus-type junctions,[99] which function in the electrophysiologic coupling of cells; so if indeed the interstitial cells are contractile, several cells may contract as a functional syncytium. Kapanci and colleagues have suggested that these cells may help in the matching of ventilation to perfusion, but further work is needed to establish the validity of this intriguing suggestion.

The interstitial compartment of the alveolar walls also contains dendritic cells, cells of the immune system

TABLE 1–2 Surfactant Proteins

Name	*Precursor Protein*	*Alveolar Protein*	*Properties*	*Functions*
SPA	28kD	28–35 kd and multimers up to 1.6×10^6	Collagenous; lectin-like; Ca binding; glycosylated	Spreading; recycling; feedback regulation of synthesis
SPB	42kD	18-kd dimer	Hydrophobic	Spreading; recycling
SPC	22kD	8-kd dimer	Hydrophobic	Spreading; recycling
SPD	43kD	129-kd trimer	Collagenous; lectin-like; homology to conglutinin	Spreading; recycling; antibacterial

specialized for accessory cell function and antigen presentation. They are not recognized by light or even electron microscopy but can be selectively stained by immunologic markers. They are characterized by expression of the class II major histocompatibility complex (MHC) human leukocyte antigens (HLA)-DR and -DQ coupled with the absence of receptors for the Fc portion of immunoglobulin.[100,101]

The capillary endothelial cells are nonfenestrated and form a continuous lining of the alveolar capillaries. The individual cells are joined by tight junctions, which physiologic studies have shown to be more permeable to macromolecules than are the junctions between epithelial cells. In freeze-fracture replicas, endothelial junctions have from one to three junctional strands and occasional discontinuities in the strands, which contrast with the three to five strands in the junctions between epithelial cells.[102] (The morphology and metabolic functions of endothelial cells are discussed in greater detail in the section on pulmonary circulation.)

Pericytes are uncommon in the normal pulmonary capillaries. They lie within the capillary basal lamina and send long, slender processes along the capillary. Their cytoplasm contains relatively few organelles but has bundles of contractile microfilaments containing smooth muscle isoforms of actin. Pericytes increase in number in fibrotic lungs.

Interalveolar Pores (of Kohn)

In all mammalian species so far examined, there are openings called pores of Kohn penetrating the interalveolar septa, the number varying considerably among species.[103] Pores are generally absent at birth, but they appear early in life and are established in human lungs by 1 year of age. They are round to oval, varying in diameter with the degree of lung inflation. In humans at total lung volume, they range from 2.5 μm to an upper limit of normal taken somewhat arbitrarily at 12 to 15 μm. Takaro and associates[104] found 5.3 μm to be the average diameter. Electron microscopy has invariably shown pores of Kohn to be lined by alveolar epithelium, thus confirming that they are not an artifact.[104–106]

The presence of openings between alveoli offers an obvious pathway for collateral ventilation. The actual importance of the pores of Kohn for collateral ventilation remains uncertain. As discussed by Macklem,[103] the diameter of the pores in vivo at tidal lung volumes depends on surface forces influenced by their detailed geometry and relationship to the alveolar lining fluid. Takaro[104] demonstrated that only a minority of pores are patent. The majority of pores are filled with alveolar lining fluid, and surface tension acts across the diameter to constrict them. The minority that are open are larger, suggesting that surface tension acting on the margins of open pores tends to stretch them. This hypothesis has considerable mathematical support.[107]

Whether the pores of Kohn are to be regarded as anatomic structures or a pathologic condition has been a controversial issue for more than 100 years (see Chapter 22 on emphysema). In adult animals, they do not appear to increase with age, but Pump[108] reported that in humans they do increase in number with age. He considered them acquired lesions, the earliest form of emphysema. Certainly in a statistical sense the presence of pores is normal. In this regard, it is notable that resistance to collateral ventilation in adults does not demonstrably decrease with age.[109]

■ Macrophages

Under normal conditions, less than 10% of the cells in the acinar zone of the lung are alveolar macrophages, mononuclear phagocytes that are found spread on the surface of the alveolar epithelial cells partially immersed in the alveolar lining fluid. In routine histologic preparations, the alveolar macrophages are floated off their moorings on the alveolar wall and appear free in the airspaces. Other macrophages are normally spread on the bronchial epithelium or located in various interstitial sites, including small numbers in the alveolar interstitium, in the mucosa of airways, and in the loose connective tissue around blood vessels. Still other pulmonary macrophages are found in the organized pulmonary lymphoid deposits.

The macrophages of the alveolar spaces and airways are easily obtained from humans or animals by bronchoalveolar lavage, and far more is known about them than about the interstitial macrophages.[110,111] The alveolar macrophages are relatively large cells, averaging 25 μm in diameter, with an eccentric oval or reniform nucleus. They have a prominent cytocenter containing a centriole and large Golgi apparatus surrounded by a zone of large lysosomal granules of varied size, shape, and content (Fig. **1–27**). Intermediate filaments are often prominent in the central zone of the cytoplasm. The periphery of the cell is thrown into leaflike folds and pseudopods, which usually contain few organelles except for a cortical gel of fine contractile filaments.

Metabolically, the alveolar macrophages differ from other mononuclear phagocytes. They are larger and have many features, suggesting that they are more highly activated than other resident macrophages, including a more abundant cytoplasm containing high levels of lysosomal enzyme activity, high levels of secretion of certain neutral proteases, and high levels of oxygen consumption.[112,113] They are specifically adapted to function in the high oxygen tensions that exist in

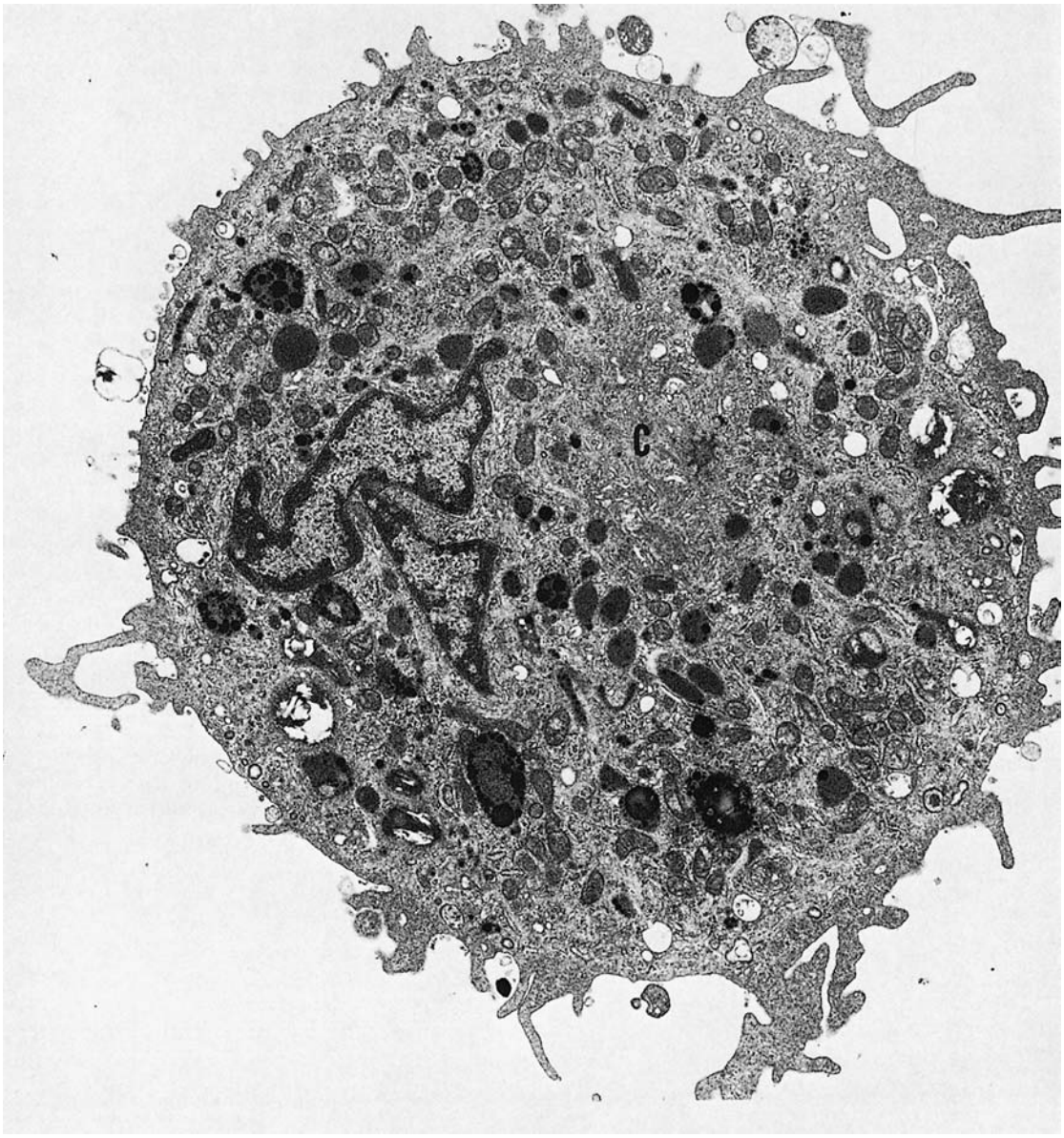


FIGURE 1–27 Alveolar macrophage. The cytocenter (C) contains a centriole and Golgi apparatus. The cytoplasm contains many mitochondria and lysosomes of varied content. The periphery of the cell projects as irregularly shaped pseudopodia (×10,000).

the alveoli by having higher levels of superoxide dismutase and higher levels of the enzymes involved in the electron transport chain and lower levels of glycolytic enzymes than peritoneal macrophages or monocytes.[114–116]

Macrophages are continuously removed from the alveoli and transported to the oropharynx by mucociliary clearance. Masse and colleagues[117] have estimated the rate of removal at 3.5% of the resident population daily. This rate of removal requires continuous replacement. In addition, in response to irritants, infectious agents, or even relatively inert dusts, the macrophage population can expand rapidly. Much controversy has been generated concerning the immediate source of alveolar macrophages. The three suggested sources are (1) the bone marrow via the circulating monocytes, (2) the interstitial macrophage pool in the lung, and (3) proliferation of macrophages within the alveolar spaces. If total body irradiation is given to eliminate endogenous sources and syngeneic bone marrow transplanted, replacement of alveolar macrophages comes from the donor marrow.[118–120] In patients with aplastic anemia or leukemia or in animals treated with corticosteroids, the blood monocytes can disappear from the circulation, but only a minor if any reduction of alveolar macrophages occurs.[121,122] Under these conditions, local sources in the lung can maintain the population. Thymidine labeling indicates that, under some experimental conditions,

interstitial macrophages are an important local source.[123,124] The alveolar macrophages have receptors for macrophage growth factor and can proliferate extensively in vitro,[125,126] and some are in cycle in the resting lung.[123,127] Thus, under specific experimental conditions, each of the three sites can provide replacement of macrophages. Thymidine labeling under normal steady-state conditions indicates that between one third and two thirds of the replacement of macrophages is from local pulmonary sources.[123,127,128]

The function of alveolar macrophages as professional phagocytes is well known. They are a major defense against inhaled bacteria and dust.[129] Compared with other macrophages and monocytes, alveolar macrophages are poor antigen-presenting or accessory cells.[130,131] In addition, there are few lymphocytes present in normal alveolar spaces. These properties may explain why the lung is not perpetually inflamed despite nearly continuous exposure to potential antigens. Macrophages also produce a wide variety of secretory products (reviewed elsewhere[110,111]). These products include enzymes, protease inhibitors, complement components, nonimmunologic opsonins such as fibronectin, and a variety of factors that influence inflammation and tissue repair (Table **1–3**).

■ The Circulatory System

The lung receives blood through both the bronchial and pulmonary arteries. The former carry blood at systemic pressure and have thicker, more muscular walls than the pulmonary arteries, which carry blood under one sixth as much pressure. The microscopic appearance of the pulmonary blood vessels is strongly influenced by the technique of fixation. The tunica media appears thinner when the lung is fully distended than when it is collapsed. Elastic stains are a great aid in the study of vascular structure, and Brenner's classification of pulmonary arteries (used in the following section) is based on the pattern of the elastic tissue.[132]

TABLE 1–3 Some Secretory Products of Macrophages

Enzymes
Lysosomal enzymes
Lysozyme
Urokinase plasminogen activator
Collagenase
Other matrix metalloproteinases
Protease inhibitors
$Alpha_1$-protease inhibitor
$Alpha_1$-antichymotrypsin
$Alpha_2$-macroglobulin
Tissue inhibitor of metalloproteinases
Plasminogen activator inhibitors
Modulators of cell activity
Chemotactic factors
Growth factors
Interleukins
Colony-stimulating factors
Prostaglandins
Leukotrienes
Interferons
Other
Oxidants (H_2 O_2, O_2^-, HO, HO)
Fibronectin
Complement components

Histology

Elastic Pulmonary Arteries

The pulmonary trunk and pulmonary arteries larger than 500 or 1000 μm in diameter are designated elastic pulmonary arteries. Their media consists of multiple concentric elastic laminae separated by smooth muscle, collagen, and ground substance containing proteoglycan (Fig. **1–28**). At birth, the pulmonary trunk is equal in thickness to the aorta, and both have a similar configuration of elastic fibers. The laminae in the pulmonary trunk are somewhat fewer in number than those in the aorta but tend to be thicker and more variable. During the first few months of life, the laminae become fragmented and relatively reduced in density. By the age of 2 years, the adult pattern is achieved. By then the thickness of the pulmonary trunk is only 40 to 70% of that

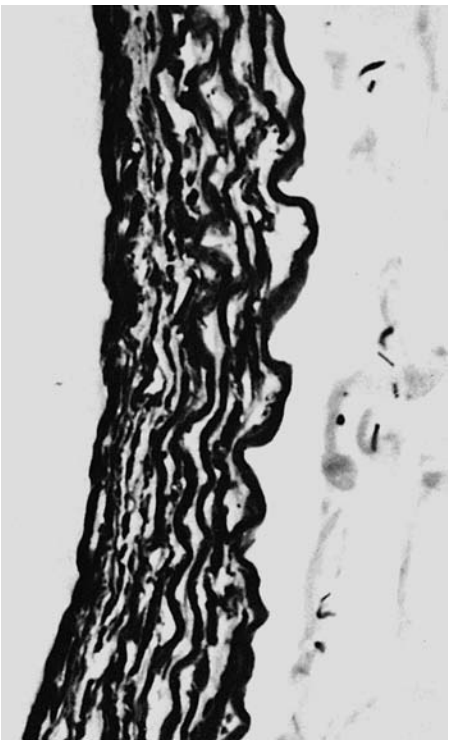


FIGURE 1–28 Part of an intrapulmonary elastic pulmonary artery showing the parallel arrangement of elastic laminae alternating with bands of smooth muscle (elastin-van Gieson, ×600).

of the aorta, and the elastic tissue is sparser, consisting of short fibrils separated by numerous slender wisps of elastin that branch in all directions.

The elastic tissue of the intrapulmonary arteries does not show the widely separated irregular elastic fibrils of the pulmonary trunk but instead takes the form of concentric laminae. The number of laminae varies from 16 or 20 in the central arteries to three to four in arteries of 1000 μm in diameter or less. A thin intima containing collagenous fibers separates the endothelium from the innermost elastic lamina.

Muscular Pulmonary Arteries

The arteries that accompany the distal membranous and respiratory bronchioles range from 500 to 100 μm and are muscular in structure. They have a tunica media of circularly oriented smooth muscle sandwiched between distinct internal and external elastic laminae of nearly equal thickness (Fig. **1–29**). The media is much thinner than that of typical systemic arteries. Medial thickness of muscular pulmonary arteries expressed as a percentage of the external diameter of the vessel ranges from 2.8 to 6.8% in lungs fully distended with fixative[133] and averages 4.4%.[134] In young adults, no intima is discernible in muscular arteries, and the endothelial basal lamina abuts the internal elastic lamina.[135]

Muscular pulmonary arteries lie close to the bronchioles, respiratory bronchioles, and alveolar ducts, and they branch with the bronchial tree. The diameter of the arteries decreases more rapidly than the air passages they accompany, so that the diameters of the arterial branches in the periphery of the lung are much smaller than those of adjacent bronchioles. Some muscular pulmonary arteries do not branch with the bronchial tree but arise as side branches. These vessels have been termed supernumerary.[136]

Bronchial Arteries

The lung receives a dual blood supply for the bronchial tree that is supplied by blood via the bronchial arteries. On leaving the aorta, the bronchial arteries have a coat of circularly oriented smooth muscle and a thick internal elastic lamina. Once they enter the lung, the bronchial arteries lie in the walls of bronchi, where they are subjected to the stimulus of repeated longitudinal stress. In response, they sometimes develop a characteristic layer of longitudinally oriented smooth muscle. The muscle fibers in this layer are often separated by elastic fibrils (Fig. **1–30**). The internal elastic lamina is well developed, but the external elastic lamina is thin or even absent. The bronchial arterioles are typical of the systemic vasculature, with a thick media of circular smooth muscle. Bronchopulmonary anastomoses are found in the normal lung of the neonate and infant and probably are of functional significance.[137] On the other hand, the presence of such anastomoses in the adult lung is doubtful. Bronchial arteries show age changes in the form of deposition of collagen in the intimal layer, and they may appear to be occluded by such fibrotic changes.

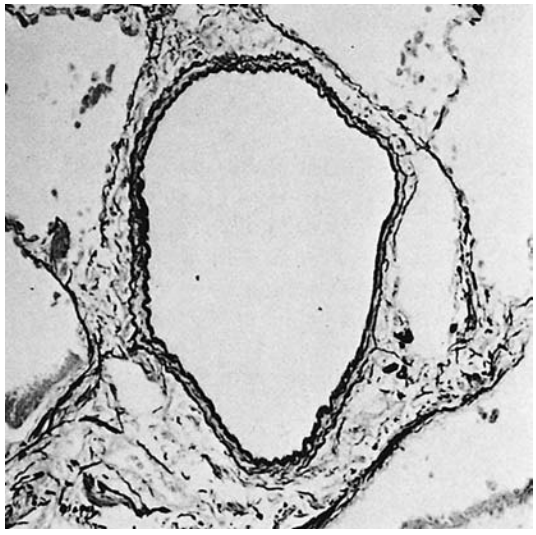


FIGURE 1–29 Transverse section of a muscular pulmonary artery consisting of a thin media of circularly oriented smooth muscle sandwiched between internal and external elastic laminae (elastin-van Gieson, ×165).

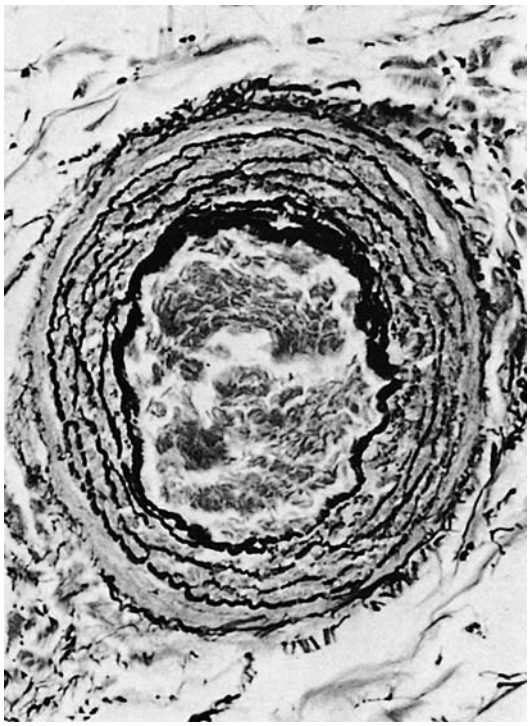


FIGURE 1–30 Transverse section of a pulmonary arteriole near its origin from a muscular pulmonary artery. Half of the circumference of the vessel has a muscular media, but the remainder consists merely of a single elastic lamina (elastin-van Gieson, ×420).

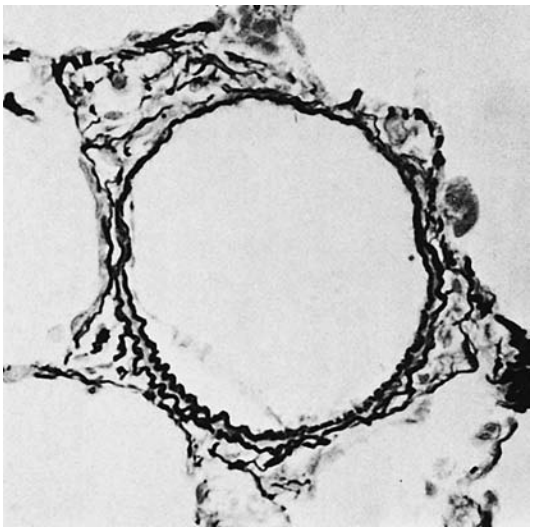


FIGURE 1–31 Transverse section of bronchial artery. The vessel consists of a clearly defined internal elastic lamina, a media of circularly oriented smooth muscle, and a discontinuous, poorly defined external elastic lamina. The intima contains numerous small fasciculi of longitudinally oriented smooth muscle separated by anastomosing elastic fibers (elastin-van Gieson, ×425).

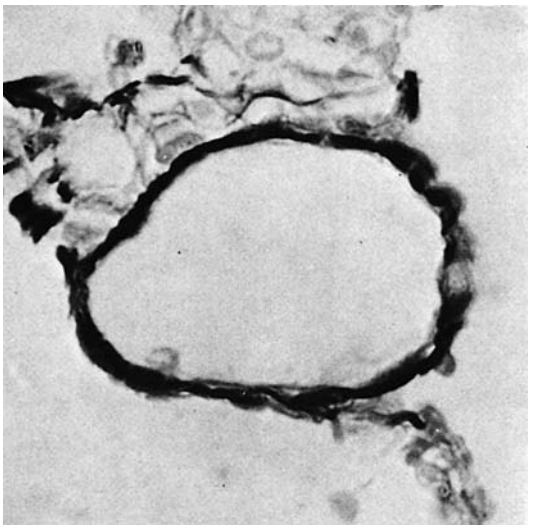


FIGURE 1–32 Transverse section of a pulmonary arteriole consisting of a single elastic lamina lined by endothelium. Except for position within the lobule, such vessels are indistinguishable from pulmonary venules, and serial sections may be required to demonstrate their origin from muscular pulmonary arteries (elastin-van Gieson, ×600).

Pulmonary Arterioles

Pulmonary arterioles arise as terminations or side branches of parent muscular pulmonary arteries. The muscle coat of the parent vessel extends for a short distance in the wall of the arteriole. The diameter at which the arteriole loses its coat is not 100 μm, as originally defined,[132] but ~70 μm.[138] The muscle coat of the parent muscular pulmonary artery is lost gradually, running as a spiral in the wall, so there is a transitional zone of the arteriole for a variable length before the muscle completely disappears. In the spiral area, cross sections through the arteriole show muscularized segments alternating with segments devoid of muscle (Fig. **1–31**). Thus, the histologic appearance of a pulmonary arteriole varies depending on the level of section. The proximal part has a complete media, the intermediate area has an interrupted media, and the distal portion is devoid of muscle (Fig. **1–32**). Arterioles have a lining of endothelial cells resting on a basement membrane and are enclosed by a single elastic lamina. Pericytes and so-called intermediate cells can be identified by electron microscopy in the space between the endothelium and elastic lamina.

Pulmonary Capillaries

The pulmonary arterioles give rise to precapillaries, which then branch to form a rich capillary network in the walls of the alveolar ducts and alveoli. Electron microscopy shows that the pulmonary capillaries are lined with endothelial cells, which lack fenestrae but do have numerous caveolae intracellularis.

Pulmonary Venules and Veins

The smallest tributaries of pulmonary veins are indistinguishable histologically from the pulmonary arterioles except by tracing their origin and drainage. Thus, the venules have a wall consisting of a single elastic lamina, gradually acquiring a muscular media downstream. Formed near bronchioles, they pass through successive generations to drain into muscular veins in the connective tissue septa between secondary lobules. In young people, the intima of the veins is thin, composed mainly of collagen and a few myofibroblasts, but it gradually thickens with age (Fig. **1–33**). The tunica media is slightly irregular in thickness, consisting of bundles of obliquely and circularly arranged smooth muscle fibers and collagen. Irregular elastic fibrils occur in both the media and the adventitia, and the boundary between these two coats is frequently ill defined (Fig. **1–34**). The adventitia includes mainly longitudinally oriented elastic fibers and bundles of muscle. In humans, there is some extension of cardiac muscle from the left atrium along the walls of the major pulmonary veins at the hilus, but this is not as prominent as in the lungs of rodents. There are no valves in pulmonary veins.

Endothelial Specialization

Using techniques with greater resolving power than routine light microscopy, one can see differences in structure between the endothelium of the capillaries and larger vessels (Table **1–4**) and indeed between different segments of the arterial and venous systems. These differences include size and shape of the cells, their surface

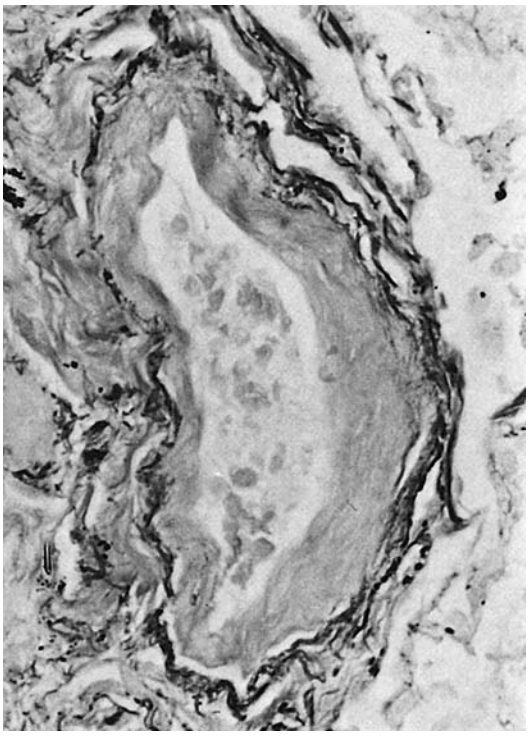


FIGURE 1–33 Oblique section of a small pulmonary vein from a 55-year-old man in which the intima shows a proliferation of acellular collagenous tissue. This is typical of age-change intimal fibrosis (elastin-van Gieson, ×412).

configuration, intercellular junctions, and organelle content. Undoubtedly, these differences reflect differences in endothelial function.

The endothelial surface can be studied by scanning electron microscopy. The endothelium of the pulmonary arteries is covered with microvilli, whereas capillary endothelium is flat.[139] The intercellular junctions between arterial endothelial cells are complex structures with tight junctions formed of two to six interconnecting rows of particles and large gap junctions between the rows of tight junction particles (Fig. **1–35**). In the capillaries, there are fewer rows of tight junction particles. At the arterial end of the capillary bed, there are two to six rows, but at the venous end there are only one to three rows, often with discontinuities in the rows. Only a few small gap junctions are present at the arterial end of the capillary bed, and none occurs at the venous end. In the pulmonary veins, the junctions consist of several rows of sparsely interconnecting tight junction particles. Gap junctions are fewer than in the arteries and tend to be more numerous in small veins than in large veins.[140] The pattern of tight junctions suggests that large vessel endothelium is less permeable than capillary endothelium and that the arterial end of the capillary is less permeable than the venous end.

Arterial and venous endothelia also contain organized bundles of contractile filaments.[141] The presence of filament bundles along with the gap junctions, a specialization commonly associated with electrophysiologic or metabolic coupling of cells, suggests that these endothelia may contract as a functional unit, contributing to the maintenance of vascular tone. In the capillaries, in contrast, microfilaments are few (mainly associated with the junctions) and are not organized into distinct bundles.

Weibel-Palade granules (Fig. **1–36**) are elongated, electron-dense granules containing von Willebrand's factor that are found only in endothelium.[142] They vary in number from cell to cell but can be found in both arterial and venous endothelium. They are lacking in capillaries.

TABLE 1–4 Morphologic Features of Endothelium from Different Vessels

Type of Vessel	*Surface Microvilli*	*Contractile Filaments*	*Gap Junctions*	*Weibel-Palade Granules*
Artery	Many	Yes	Many	Yes
Capillary	None	Few	Rare	No
Vein	Few	Yes	Moderate	Yes

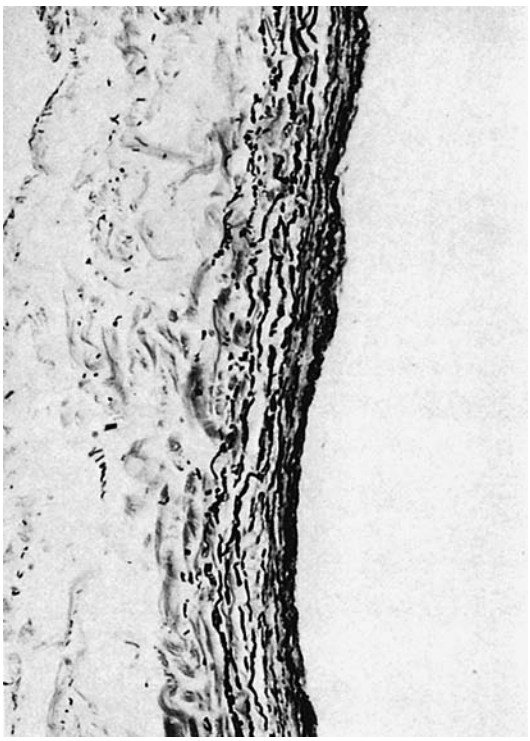


FIGURE 1–34 Part of a transverse section of a large pulmonary vein. The media consists of circularly oriented smooth muscle cells interspersed with short, irregular elastic fibers. The internal elastic lamina is broad and continuous, but the distinction between the media and the adventitia is poorly defined (elastin-van Gieson, ×165).

FIGURE 1–35 Freeze-fracture replica of an intercellular junction from a pulmonary artery in a rat lung. On the extracellular face, rows of tight junction particles are situated in shallow grooves (arrowhead). On the protoplasmic face, the tight junction is a low ridge with few attached particles (arrow). The tightly packed arrays of particles (*) are gap junctions between the meshes of tight junction (×75,000). (From Schneeberger EE. Segmental differentiation of endothelial intercellular junctions in the intraacinar arteries and veins of the rat lung. Circ Res 1981;49:1102–1111. Used with permission.)

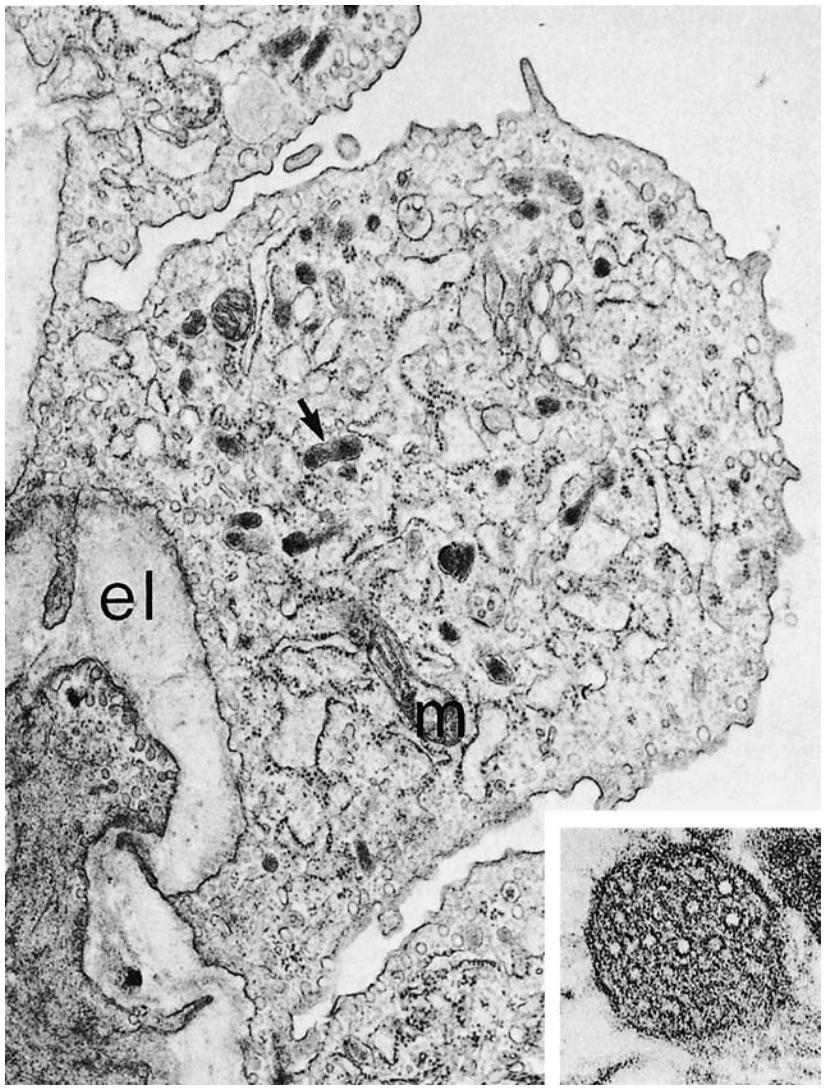


FIGURE 1–36 An endothelial cell from a muscular pulmonary artery of a rat. Except for a thin basal lamina, the cell rests directly on the internal elastic lamina (el). The cell contains mitochondria (m), rough and smooth endoplasmic reticulum, and the characteristic Weibel-Palade bodies (arrow) (electron micrograph ×25,000). Inset: Weibel-Palade granule showing characteristic tubular content in cross section (×140,000).

Biochemical Functions of Endothelium

Once considered only a passive barrier to gas exchange and fluid filtration, endothelium is now recognized to have important biochemical activities. These biochemical activities are shared by systemic vessels, but their impact on the host is greater in the pulmonary circulation because it receives the entire cardiac output. Pulmonary endothelium efficiently and selectively degrades or inactivates many vasoactive mediators, preventing them from reaching the systemic circulation in active form. Other compounds, some of similar structure to the ones that are destroyed, pass through the pulmonary capillaries unchanged to exert hormonal effects on distant targets; one, angiotensin, is even activated to greater physiologic potency (Table **1–5**). Some peptides and nucleotides are metabolized in the vascular lumen by ectoenzymes on the endothelial plasma membrane; other mediators require uptake by the endothelial cells.[143]

Endothelium actively contributes to the maintenance of vascular patency by the synthesis of prostacylin and tissue plasminogen activator. Pulmonary endothelium is also a rich source of thrombomodulin,[144] a cell surface protein that enhances the anticoagulant properties of endothelium by binding thrombin, which then catalyzes the activation of protein C. Activated protein C then inhibits clotting by proteolytically cleaving factors Va and VIIIa.[145] The discovery of the endothelial-derived relaxing factor nitric oxide[146] and the potent vasoconstrictor peptides, the endothelins,[147] established a role for endothelium in the paracrine regulation of vasomotor tone. The capacity of endothelium to respond to stimuli by the expression of specific surface-binding proteins for circulating phagocytes and lymphocytes makes it an active participant in inflammation.[148–150]

It is now known that endothelial cells have the ability to modulate their own function through the synthesis and release of several bioactive substances that act on the underlying smooth muscle cells, and also act on the endothelial cells in a feedback mechanism (reviewed elsewhere[151]). Two of these substances are endothelin, which acts primarily as a vasoconstrictor, and nitric oxide, which produces vasodilatation. Nitric oxide is liberated by conversion of L-arginine to citrulline through the action of nitric oxide synthase (NOS). There are three separate isoforms of NOS: c(n)[constitutive (neuronal)] NOS, i(inducible)NOS, and e(endothelial cell)NOS, also known as NOS 1 to 3. Nitric oxide is highly diffusible, but has a short half-life, and is readily inactivated by binding to heme.

Endothelin is secreted toward the abluminal side of the endothelial cell, and binds to a receptor site on underlying smooth muscle cells (Et-A), increasing intracellular calcium concentration, and causing vasoconstriction even after endothelin is removed from the receptor.[152] Et-B receptors are primarily on endothelial cells, and appear to stimulate nitric oxide release.[153] Endothelin production is itself stimulated by vascular stress and by cytokine growth factors, including transforming growth factor-β (TGF-β),[152] and oxidants and airway inflammation also appear to induce endothelin release.[154]

The importance of endothelin and nitric oxide in the regulation and physiologic reaction of smooth muscle cells has become increasingly apparent,[151,152,155] and it appears that there is an intimate association between these two mediators, with each modulating the production of the other in an autocrine fashion, and the actions of the other in a paracrine fashion referred to as a "yin-yang" relationship.[151] Endothelin stimulates production and release of nitric oxide, and activation of nitric oxide production dissociates endothelin from its receptors, and interferes with the pathway for calcium mobilization, thus inhibiting end responses to endothelin.[156] Nitric oxide itself also has a feedback mechanism that reduces the activity of NOS.[157]

Vascular endothelial growth factor (VEGF) also appears to be important in vascular remodeling and has a known relationship with nitric oxide synthase (discussed later in the chapter). VEGF is a glycoprotein that can be translated into multiple forms by alternative splicing. It is secreted in a biologically active form and binds to two endothelial cell tyrosine kinase receptors, Flt-1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2). VEGF is the most specific endothelial growth factor so far discovered; in addition to its mitogenic effects, VEGF promotes endothelial cell growth, differentiation and survival, and accelerated restitution of endothelial integrity and function.[158] Through this role, it appears to have an important role in the development of emphysema (see Chapter 22). However, it also induces expression of interstitial collagenase in endothelial cells, thus potentially affecting smooth muscle cell proliferation or matrix reorganization. There are high levels of VEGF in lung, with messenger RNA

TABLE 1–5 Metabolic Specificity of Endothelium

Class of Compound	*Metabolized*	*Not Metabolized*
Adenine nucleotides	Adenosine monophosphate Adenosine diphosphate Adenosine triphosphate	
Vasoactive amines	Serotonin	Histamine
	Norepinephrine	Epinephrine
Prostaglandins (PG)	PGE_2	PGA
	$PGF_{2\alpha}$	PGI_2
Vasoactive peptides	Angiotensin I	Vasopressin
	Bradykinin	Vasoactive intestinal peptide
Lipoproteins	Very-low-density lipoprotein	Low-density lipoprotein

(mRNA) expressed in the alveolar cells as well as in endothelial cells, and in stimulated macrophages.[159] VEGF expression is affected in a paracrine fashion by other protein mediators. Nitric oxide decreases expression of both VEGF and VEGFR, similar to its effect on endothelin.[160] Conversely, VEGF appears to augment nitric oxide release from the endothelium.[161] TGF-β upregulates VEGF and inhibits NOS.[162]

■ The Lymphoid System

The lung's lymphatic drainage is commonly divided into two systems: the superficial or pleural lymphatics and the deep lymphatics, consisting of the septal perivenous lymphatic plexuses and the bronchoarterial plexuses.[5,163–165] The pleural lymphatic plexus consists of lymphatic trunks located mainly at the junctions of the interlobular septa with the pleura, fed by fine lymphatic capillaries crossing the polygonal spaces enclosed by the larger lymphatic trunks. Lateral channels off the smaller lymphatics end blindly. The pleural lymphatics are larger in the lower zones of the lung than in the upper zones,[163,164] presumably because the vertical gradient in hydrostatic pressure favors greater lymph formation at the bases. The deep lymphatics run in the loose connective tissue of the interlobular septa and surrounding the vessels and conducting airways. The major lymphatic trunks course longitudinally along the major vessels and airways with an extensive plexus of tributaries surrounding the vessels and airways. In the interlobular septa, the septal and perivenous lymphatics anastomose, forming a common plexus. Because the bronchi and arteries run together and often share a common connective tissue sheath, their lymphatics also anastomose extensively. The bronchoarterial lymphatics begin at the level of respiratory bronchioles; there are no lymphatic capillaries in the interalveolar septa or in the walls of alveolar ducts. However, when tracers such as labeled albumin, horseradish peroxidase, or ferritin are injected into the airspaces, they are cleared in part by the lymphatic system.[166] Lauweryns and Baert[164–166] have pointed out that many of the lymphatic capillaries have a close relationship to the alveolar spaces, although they occupy adventitial connective tissue spaces (Fig. **1–37**); they call them juxta-alveolar lymphatics and have suggested that they contribute to the removal of fluid from the alveoli.

The structure of the pulmonary lymphatics is similar to that of lymphatics in other tissues. The smallest lymphatic channels (lymphatic capillaries) are essentially tubes of endothelial cells that rest on the connective tissue with only a few discontinuous patches of basement membrane material as support. The basal surface of the endothelial cells dips into the connective tissue in a series of projections, each projection attached to or enmeshed

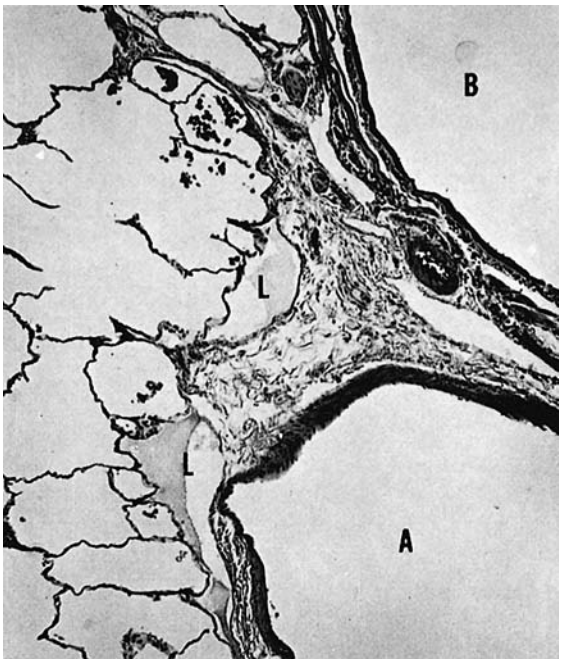


FIGURE 1–37 Two juxta-alveolar lymphatics (L) in the connective tissue sheath enclosing a bronchiole (B) and the pulmonary artery (A). The lymphatics are closely associated with the alveolar walls and may drain alveolar as well as interstitial fluid.

in bundles of 10 nm filaments, the so-called lymphatic anchoring filaments. The contacts between neighboring endothelial cells are often patent, overlapping without forming specialized attachment structures. The endothelial cytoplasm contains both intermediate and actin filaments and pinocytic vesicles and occasionally lysosomes.[165,167] Lauweryns and Baert's[166] tracer studies indicate that the main route of uptake into the lymphatics is through the intercellular junctions. The predominant direction of pinocytosis seemed to be back from lumen into the cytoplasm.

The lymphatic collecting vessels have a less open structure. The endothelial cells are joined by tight junctions and rest on a basal lamina. There is a muscular media one to two cells thick.

The valves present in the lymphatic trunks consist of a delicate core of connective tissue covered by endothelial cells that are joined by tight junctions.[165] Most observers have considered that the valves are bicuspid, although Lauweryns and Baert[165] concluded that the majority of those that they studied by serial sections had only a single conical cusp. The few lymphatic valves that we have observed in the scanning electron microscope have been bicuspid.

Organized collections of lymphoid cells are associated with the lymphatic vessels at several points.[5,168] Nodules of lymphocytes are often found at the bifurcations of small bronchioles or respiratory bronchioles, where they lie in the connective tissue between the bronchioles and

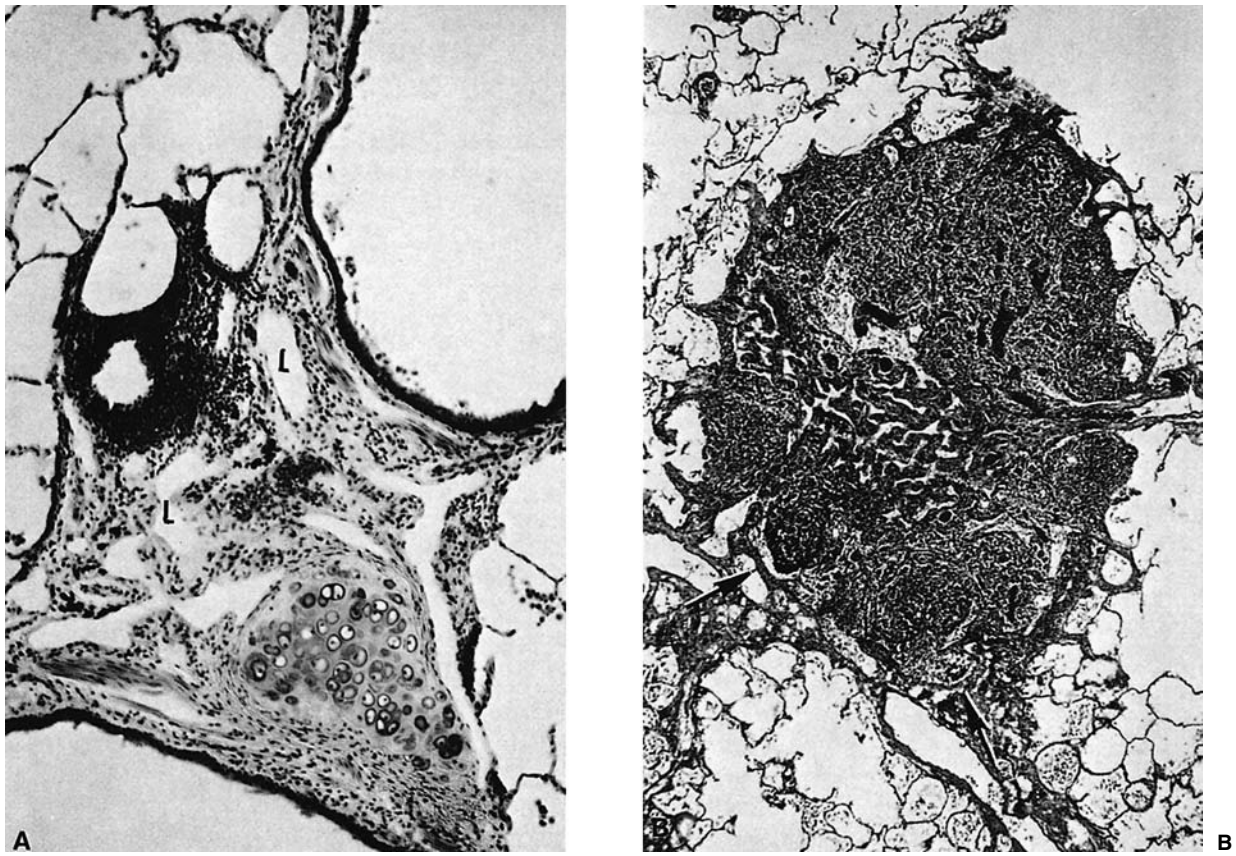


FIGURE 1–38 Lymphoid tissue in the lung. **(A)** Lymphoid nodule in the tissue surrounding a small bronchus. The nodule has no nodal structure, but there are lymphatics (labeled L) adjacent to it (×200). **(B)** Lymphoid tissue associated with a perilobular septum. The nodule has partial nodal architecture. Sinusoids are present in the center of the nodule, and a subcapsular sinus is present alongside the septum (arrow), but not where the nodule abuts the alveoli (×75).

artery. They are usually closely associated with an efferent lymphatic but have neither a true capsule nor a subcapsular sinus (Fig. **1–38**). Nodules of lymphocytes are also found associated with the lymphatics at the periphery of the acini, both in the pleura and in the interlobular septa. They vary in extent of development from nodules to structures partially enclosed by a subcapsular sinus (Fig. **1–38**). Trapnell[169] found fully formed lymph nodes with a complete subcapsular sinus in the periphery of 7% of lungs.

Miller[5] inferred from the direction of the valves that the drainage of the pleural lymphatics was through the superficial plexus over the surface of the lung to the lymph nodes of the hilus, whereas the deep lymphatics drained centripetally along the airways and vessels. Injection studies[163,164] indicate, however, that some of the drainage of the costal pleura passes directly via the septal and venous plexuses and even occasionally through anastomoses to the bronchoarterial portion of the deep lymphatic system to the hilar lymph nodes.

The hilar lymph nodes, which are encountered within the lung at the bifurcations of large bronchi, are complete nodes with a subcapsular sinus and follicles. From the hilar nodes, the lymph drains via extensively anastomosing channels through tracheobronchial lymph nodes clustered alongside the main stem bronchi, beneath the carina, and along the course of the trachea. In the mediastinum, the pulmonary lymph is joined by lymph from the trachea, heart, esophagus, diaphragm, and chest wall before emptying into the venous system. On the right side, the thoracic lymphatics enter the subclavian vein at its confluence with the jugular vein, either via a separate duct or after joining the lymphatic trunks from the arm, head, and neck to form a common duct. On the left, the mediastinal lymphatics may join the thoracic duct or empty into the subclavian vein independently.

■ The Nervous System

General Organization

The control of nerve growth and its functional plasticity are areas of interest, because of the evidence that dysfunction of the nervous system within the lung contributes to the bronchoconstriction found in a variety of chronic inflammatory conditions.[170] The lung receives its major sensory and motor innervation from

the 10th cranial (vagus) nerve. Postganglionic fibers from the thoracic sympathetic plexus mingle with the vagal fibers as they enter the lung at the hilus, and the combined nerves break up into plexuses, which accompany the ramifications of the arteries, bronchi, and veins.[5,171] According to Larsell,[172] the nerves to the bronchi form two plexuses: a large plexus external to the cartilages and a smaller plexus deep to the cartilage. Beyond the termination of the cartilages, the two plexuses merge and continue distally to the respiratory bronchioles. Ganglia are present within the larger nerves of the extrachondral plexus along the proximal three bronchial generations, becoming rare farther distally.[172]

The periarterial plexus lies in the adventitia of the arteries, and its bundles anastomose with those of the extrachondral bronchial plexus. Fibers from the arterial plexus enter the media with the vasa vasorum and supply its outermost portion. The plexus continues distally as far as the arterioles.

The perivenous plexus accompanies the veins into the perilobular septa and even reaches the visceral pleura to supply the subpleural alveolar walls. Spencer and Leof[173] have described twigs of the venous plexus reaching and ramifying in the subendothelial space of the veins. A few ganglia are present in the perivenous plexus near the hilus.

Tiny unmyelinated nerve fibers have been identified by electron microscopy in the walls of alveoli in animals (Fig. **1–39**) and also in humans.[174] They are distinctly rare; according to one estimate,[175] there are only 10^4 fibers for the 300×10^6 alveoli per lung. It is not clear whether the source of these fibers is the bronchial or perivascular plexuses or both.

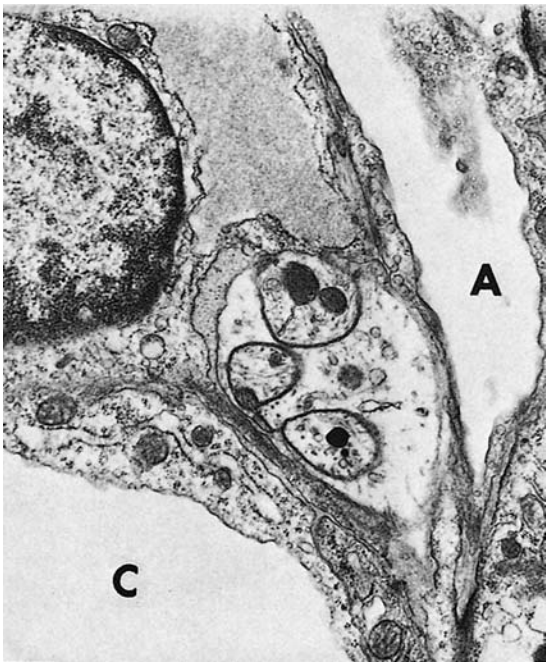


FIGURE 1–39 Unmyelinated nerve in an alveolar wall of a rat lung. A Schwann cell containing three axones (A, alveolar space; C, capillary). The cell with its nucleus included in the micrograph is an interstitial cell (×17,000).

Most sensory (afferent) fibers originate from neurons located in the nodose ganglia and travel down the vagus nerves to reach the lung. Other sensory fibers may travel through the thoracic sympathetic plexus. Physiologists have identified receptors of several types in the walls of airways.[176,177] In the main bronchi are the receptors that when stimulated initiate the cough reflex. In smaller airways, irritant receptors respond to inert dusts, mechanical stimuli, and irritant gases with reflex bronchoconstriction, hyperventilation, and a sensation of chest discomfort.

There are many methods available to examine the neuronal network in the lung.[178] These include electron microscopy, light microscopy with associated specifically directed special stains or immunohistochemical stains, fluorescence immunohistochemistry, confocal fluorescence immunohistochemistry, and multiphoton fluorescence microscopy. These techniques have demonstrated delicate unmyelinated nerves in relation to the cholinergic ganglion cells, bronchial smooth muscle, and blood vessels.[179–181] Branches corresponding to the irritant receptors penetrate the epithelial basement membrane and ramify between the columnar bronchial epithelial cells. These fibers, termed the C fibers because of their sensitivity to capsaicin, contain several neuropeptides, which they release via axon reflexes to mediate local responses when the irritant receptors are activated by physiologic or nonphysiologic stimuli (Table **1–6**).

The receptors responsible for the Hering-Breuer reflex are mechanoreceptors located deep in the airway walls. They fire off with increasing frequency as the lung is expanded and progressively inhibit the inspiratory center. Nerve endings associated with the smooth muscle of the

TABLE 1–6 Major Neuropeptides of the Human Airways

Nerves	*Peptides*	*Actions*
Sensory	Substance P	Secretogogue
	Neuropeptide K	Bronchoconstriction
	Neurokinin A	Increase bronchial blood flow
	Calcitonin gene-related peptide	Increase cholinergic secretion
Cholinergic	Vasoactive intestinal peptide	Secretogogue
	Peptide histidine methionine	Bronchodilation
		Vasodilation
Sympathetic	Neuropeptide Y	Constrict bronchial and pulmonary vessels
		Decrease cholinergic secretion

Based on Lundberg JM, Saria A. Polypeptide containing neurons in airways smooth muscle. Annu Rev Physiol 1987;49:557–572; Uddman R, Sundler F. Neuropeptides in the airways: A review. Am Rev Respir Dis 1987;136:S3–S8; and Barnes PJ, Baraniuk IN, Belvisi MG. Neuropeptides in the respiratory tract. Am Rev Respir Dis 1991;144:1187–1198, 1391–1399.

bronchial wall are believed to account for the mechanoreception, although ultrastructural detail is still scant.[177]

The unmyelinated fibers identified in the alveolar walls may be the J-(juxtacapillary) receptors. In animals, these receptors respond to interstitial fluid pressure as well as certain chemicals; they cause transitory reflex apnea followed by rapid shallow respiration.[176]

Chemoreceptor tissue similar in structure to the carotid and aortic bodies is found constantly in the adventitia of the posterior wall of the pulmonary trunk, just caudal to its bifurcation.[182] Other microscopic glomera have been described in the soft tissue surrounding the major pulmonary arteries near the hilus and within the lung.[183]

The physiologic role of these structures is unclear, because there is little physiologic evidence that the pH or partial pressure of oxygen or carbon dioxide in pulmonary blood plays a role in the regulation of ventilation or perfusion.[184] It is controversial whether the pulmonary glomus receives its blood from the pulmonary or bronchial circulation.[182,184]

Motor Innervation

The main motor innervation of the lung is cholinergic. Stimulation of the nerves to the lung produces bronchoconstriction and secretion of mucus, which can be blocked by atropine. The parasympathetic fibers carried in the vagus nerve synapse with the ganglion cells in the bronchial wall.[185] Unmyelinated postganglionic cholinergic fibers enter the subchondral plexus to end in close association with the mucous glands and bronchial smooth muscle.[186]

Inhibitory neural control of bronchial muscle can only be demonstrated in the presence of atropine blockade of cholinergic efferents and when end-organ tone is stimulated by noncholinergic means, for example by histamine. Under these conditions, field stimulation of pulmonary nerves relaxes bronchoconstriction.[187,188]

The main inhibitory modulator of cholinergic bronchoconstriction is vasoactive intestinal peptide, which is stored and released together with acetylcholine by the parasympathetic neurons.

Despite a great deal of interest among physiologists in the control of blood flow through the lung, the role of neural factors in regulating human pulmonary circulation is poorly understood. Anatomic studies have shown that there are cholinergic, catecholamine-containing, and neuropeptide-containing endings on vascular smooth muscle of both arteries and veins.[188–190]

Pleura

The pleural investment of the lung is a serous membrane that continues over the lung root and pulmonary ligament to cover the mediastinal, diaphragmatic, and costal surfaces of the thoracic cavity. It consists of connective tissue covered by simple squamous epithelium known as the mesothelium, which is composed of polygonal cells 15 to 30 μm in thickness in the region of the nucleus and thinning toward the periphery. These cells are joined by terminal bar complexes with tight and gap junctions and desmosomes. The nucleus lies in the center of the cell surrounded by mitochondria, short lamellae of endoplasmic reticulum, and a well-developed Golgi apparatus (Fig. **1–40**). The thinner peripheral portions of the cell have fewer mitochondria, but have cisterns of endoplasmic reticulum and small vesicles, both free in the cytoplasm and in contact with apical and basal plasma membranes. The most conspicuous feature of the mesothelium is the presence of long, sinuous microvilli 0.1 μm in diameter and up to 3 μm in length covered by a thick glycocalyx. The density of the microvilli varies in different regions of the pleura but is generally higher on the visceral pleura and over the inferior portions of the mediastinal pleura than on the costal and phrenic surfaces of the parietal pleura.[191] Occasionally, a mesothelial

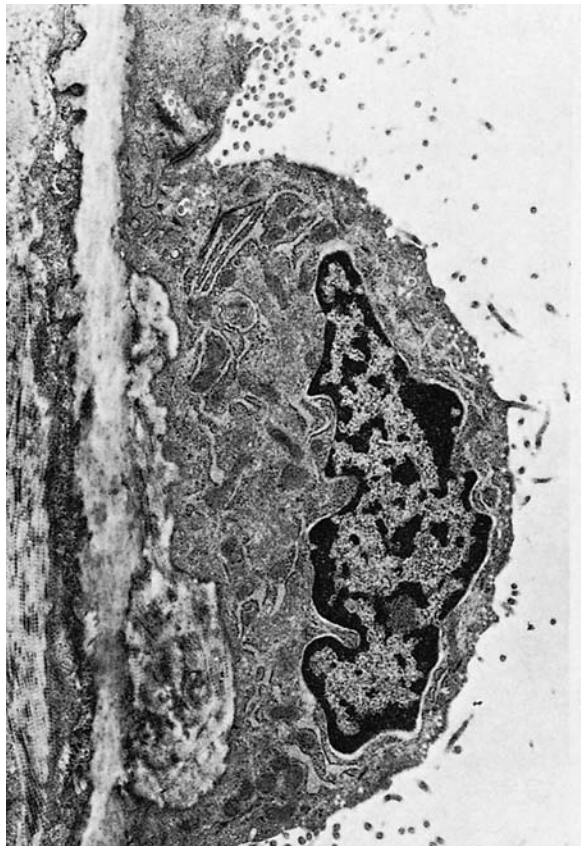


FIGURE 1–40 Mesothelial cell of visceral pleura of a rat lung. The characteristic long microvilli are seen here mostly in cross section. The well-developed endoplasmic reticulum suggests that this cell has a major biosynthetic function and is not merely a passive lining cell (×9000).

cell thrusts a single cilium into the pleural space. These are probably nonfunctional cilia arising from the centriole and lacking a central pair complex.

The mesothelium rests on a basal lamina covering a superficial layer of coarse connective tissue fibers. In the visceral pleura, the connective tissue includes one or two layers of thick elastic fibers beneath the mesothelium. Below this layer is a zone consisting mainly of collagenous fibers with a few fine elastic fibers, blood vessels, and lymphatics. Where the pleura abuts the alveoli, there is an additional layer of elastic fibers, which is properly part of the elastic network of the alveoli. The deep connective tissue layers of the pleura extend into the parenchyma as the interlobular septa containing collagen, a lymphatic plexus, and the pulmonary veins. The elastic network of the alveoli delimits the outer edge of the interlobular septa.[5]

The structure of the parietal pleura is generally similar to that of the visceral pleura. The parietal mesothelium rests on a connective tissue layer, the fibers of which merge with those of the endothoracic fascia. The capillary supply of the parietal pleura is superficially located immediately beneath the mesothelium. The parietal pleura possesses a rich lymphatic plexus, which communicates directly with the pleural cavity through special stomata up to 5 μm in diameter.[192,193] At the stomata, the lymphatic endothelium is continuous with the pleural mesothelium. Pinchon and associates[193] have suggested that flaps of endothelium behave as valves directing flow away from the pleural cavity. These stomata are of sufficient size to permit the uptake of particles the size of erythrocytes from the pleural cavity. The parietal pleural lymphatics drain to the internal mammary, para-aortic, and diaphragmatic lymph node groups.

Pleural fluid forms as a filtrate of plasma derived mainly from the parietal pleural microvessels, driven by Starling forces. The fluid is efficiently cleared by way of the parietal pleural lymphatic stomata, accounting for the small volume of pleural fluid under normal conditions. Visceral pleural capillaries and lymphatics play only a minor role in pleural fluid dynamics.[194,195]

■ Acknowledgments

Portions of the section on the pulmonary circulation were adapted from a manuscript kindly provided by Professor Donald Heath and Dr. Paul Smith, who also provided Figs. **1–28** through **1–34** and **1–36**. The authors are also indebted to Dr. Ernest Cutz and Dr. Eveline Schneeberger, who generously provided illustrations from their work.

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