I. Introduction

The mechanical properties of lung tissue are important determinants of the overall mechanical behavior of the respiratory system, contributing significantly to both elastic and dissipative (resistive) properties of the lung. In fact, the dissipative properties of lung tissue are the major determinants of lung resistance within the breathing frequency range (11, 14, 143). The biomechanical properties of connective tissues play fundamental roles in the functioning of almost every organ and are critical determinants of how mechanical forces acting on the body/organ produce physical changes at the cellular level (144). It is recognized that mechanical interactions between cells and the extracellular matrix (ECM) have regulatory effects on cellular physiology, leading to reorganization and remodeling of the ECM, which in turn influences lung function (19, 144).

The act of breathing entails the cyclic application of physical stresses at the pleural surface as well as the transmission of those stresses throughout the lung tissue and its adherent cells (40). The applied stresses result in length changes of parenchymal structures (strain), yielding volume variation. To support extreme strain variations, while maintaining the minimal tissue diffusional barrier necessary for adequate gas exchange, lung parenchyma presents important stress-bearing systems, including the gas-liquid interface, the connective tissue matrix, and the contractile apparatus (38). These stress-bearing elements can be significantly changed during pathological
resistance, \( V \) resistive pressure (Pres) refers to pressure dissipated to transpulmonary pressure at end expiration. Thus, the dissipative behavior in normal and pathological conditions.

II. TISSUE MECHANICS: ENERGY DISSIPATION AND STORAGE DURING VENTILATION

A. Elastance and Resistance

During quiet breathing, energy is used to overcome elastic and resistive forces. The classic equation of motion describes the relationship among pressure, volume, and flow as \( P(t) = EV(t) + RV'(t) + P_0 \), where at any instant \( t \), \( P \) is applied pressure, \( E \) is elastance, \( V \) is volume, \( R \) is resistance, \( V' \) is flow, and \( P_0 \) is pressure corresponding to transpulmonary pressure at end expiration. Thus, resistive pressure \( (P_{\text{res}}) \) refers to pressure dissipated to overcome resistance \( (R) \); whenever flow is absent, \( P_{\text{res}} \) equals zero (112). The representative model for this equation is the resistive-elastic model, characterized by a single resistive compartment connected to a single elastic compartment, and represented by a dashpot in parallel with a spring. To increase volume in this resistive-elastic model, part of the applied energy would be dissipated by the dashpot (resistance), whereas another fraction of the total energy would be stored in the spring (elastance), providing the driving pressure for expiration (Fig. 1). The resistive-elastic model, however, does not explain the pressure-time profile observed after interruption of a constant flow inflation, where a slow pressure decay can be found even after flow has ceased (stress relaxation).

It has been consistently observed that recoil pressures at the same lung volumes are always less during deflation than inflation (hysteresis), meaning that the mechanical energy (work) expended during inflation exceeds that recovered during deflation. With repeated cycling, loops enclose an area representing the energy lost per cycle (59). During quiet breathing, the area enclosed by the pressure-volume or stress-strain curves is nearly independent of frequency. Thus, under constant amplitude cycling, energy dissipation is nearly independent of frequency (1). However, the dissipation is proportional to the product of resistance and frequency. Taken together, these findings imply that resistance must be inversely proportional to frequency (6, 43). Tissue resistance sharply contrasts with airway resistance, which exhibits much less frequency dependence (59).

B. Viscoelasticity

The relationship between stress and strain, e.g., the constitutive equation, contains information on the underlying mechanisms and structure contributing to such a behavior (43). Closely related to the hysteresis seen during volume cycling of the lung are the phenomena of stress adaptation and creep. In this line, soft biological tissues are known to be highly viscoelastic in nature. In contrast to perfect elastic materials, viscoelastic substances do not maintain a constant stress under constant deformation; the stress, instead, slowly relaxes (stress relaxation). On the other hand, under constant stress, the material undergoes a continuous deformation in time, i.e., creep (15, 23, 97, 142) (Fig. 2). Indeed, viscoelasticity represents an important component of respiratory mechanics, being responsible, in some cases, for most of the pressure dissipated during breathing (4, 88, 129). The viscoelastic model adds a third component to the resistive-elastic one, the Maxwell body, in which a dashpot (viscous element) lies in series with a spring (elastic element) (55, 56) (Fig. 3). In this model, the spring can transfer energy to the dashpot that burns it as heat; hence, the energy of the system may diminish even when it is not moving. The slow decay in pressure observed after end-inflation occlusion can be perfectly superimposed to alveolar pressure measured by alveolar capsules, thus representing energy dissipation at the lung parenchyma level (119). The linear viscoelastic model predicts dynamic elastance and its frequency dependence quite well (26, 142), but it cannot account for about one-third of the energy loss (hysteresis), suggesting that additional nonviscous plastoelastic elements are required in the model.
They do not impart time and frequency dependence (stress relaxation and dynamic elastance) but do consume energy (59).

Lung parenchyma displays prominent viscoelastic behavior. However, resistive pressures across tissue, in contrast to airways, are non-Newtonian (55, 56). It is recognized that the major part of energy dissipation associated with cyclic lung expansion depends on the amount of expansion but not the rate of expansion, demonstrating that lung tissue shows a stress incompatible with the notion of a viscous stress (41). During cycling, the stress that develops in the viscoelastic body displays a component in phase with strain, which is the elastic stress contributing to the storage modulus (or dynamic elastance in the lung), and a component out of phase with strain, corresponding to the viscous dissipation and contributing to the loss modulus (or lung tissue resistance, or damping) (6, 55). Thus the relative contribution of tissue resistance to overall lung resistance can be magnified by large volume changes and by low flow rates, the opposite effect being observed in the airway component (83).

The viscoelastic properties of lung tissue cause the effective tissue resistance \( R_{ti} \) to be high at very low frequencies but then decrease quasi-hyperbolically to zero at higher frequencies (6, 12, 41, 51, 146). As a consequence, in many species, \( R_{ti} \) of the healthy lung represents the main contributor to total lung resistance during breathing (85). In this line, study using alveolar capsules disclosed that from 0.5 to 2.0 Hz and in the face of physiological tidal volumes, lung tissue resistance represents a major component of total pulmonary resistance (83). At breathing frequencies in the range of 0.2–0.4 Hz, tissue resistance can account for ~40% of lung resistance (6).

During a physiological tidal excursion, there is little or no influence of airway inhomogeneities on measurements of lung mechanics in the healthy lung. On the other hand,

![Figure 2](http://physrev.physiology.org/) Viscoelastic materials, such as soft biological tissues, when held at a constant deformation (strain) show a progressive decrease in stress, called stress relaxation (A), and when held at a constant stress they show a progressive increase in deformation (strain), i.e., creep (B).

![Figure 3](http://physrev.physiology.org/) Viscoelastic model. A: anatomical representation with two resistive components (R1 and R2) and two elastic components (E1 and E2). B: mechanical representation characterized by a resistive component (R1) in parallel with a Kelvin body, which consists of an elastic component (E1), representing the static elastance, in parallel with a Maxwell body, a dashpot assembled in series with a spring, representing the viscoelastic behavior. The distance between the two horizontal bars is analog to lung volume (V), and the tension between them represents the airway opening pressure (P).
hand, true static lung elastance \( (E_s) \) is approached at very low frequencies, and, as frequency increases, \( E_s \) augments owing to either tissue viscoelasticity (i.e., a real tissue phenomenon) or airway inhomogeneities (i.e., an airway phenomenon) (85). In general, although \( R_n \) varies widely with tidal volume, lung volume, and lung size, tissue hysteresivity \( (\eta) \) remains remarkably constrained to a narrow range. Indeed, \( \eta \) exhibits small variability, frequency invariance, and numerical consistency across species (41).

Numerous studies have used models mainly in the context of describing oscillatory behavior of lung tissue where the hysteresis area of the P-V curve is nearly independent of frequency and the stress relaxation function follows a power law dependence on time (103, 142, 146). The constant-phase model by Hantos et al. (52), describing the viscoelastic properties of lung tissues, has been considered superior to the classic spring and dashpot representation. Although the electrical analog of viscoelastic processes as well as phenomenological and mechanical approaches yield good quantitative correspondence with data, they lack anatomic and mechanistic specificities (52).

Later models tried to deal with dynamic tissue behavior on a mechanistic basis (96). Some mechanisms have been proposed as contributors to the constant-phase tissue viscoelasticity, such as the structural disposition of fibers and their instantaneous configuration during motion (the interaction between fibers in close proximity), since elastic fibers dissipate energy as they slip with respect to each other (96). Additionally, lung tissue might exhibit molecular mechanisms similar to those proposed for polymer rheology (142). Maksym and Bates (86) suggested a role for the relative stress-bearing contributions of collagen and elastin fibers based on the differential elastic properties of these two types of fibers, in which collagen fibers were progressively recruited with strain. Bates (11) also proposed that the nonlinear elastic properties and linear elastic behavior of lung tissues arise from different physical processes, whereas elastic recoil is linked to geometry as fibers rearrange themselves; stress adaptation would reflect a process of diffusion due to the thermal motion of the fibers with respect to each other and to the ground substance (11).

Micromechanical studies have tried to determine tissue microrheological behavior, that is, the measurement of its complex elastic modulus \( G \). \( G \) represents the complex ratio between stress and strain in the frequency domain and can be split into an elastic component \( G' \) and a viscous or frictional component \( G'' \). Fredberg and Stamenovic (41) showed that dissipative and elastic processes are coupled in lung tissue, and hysteresivity \( (\eta) \) denotes the empirical variable that quantifies the dependence of dissipative processes on elastic processes, a tax that must be spent any time elastic energy is stored. These authors also postulated that dissipative and elastic processes are coupled at the level of the stress-bearing element, which represents a structure composed of anatomically distinct materials. Thus a modification in \( \eta \) reflects either a change in hysteretic behavior within one of those materials or an alteration in the mechanical interaction among them (41). However, the structural damping law is only descriptive; it does not specify the mechanisms through which the coupling of dissipative and elastic processes might come about, and thus the molecular basis of \( \eta \) remains obscure (62). Nevertheless, four processes are implicated in this phenomenon: the kinetics of cross-bridge attachment-detachment within the contractile element, the kinetics of the surface-active molecule adsorption-desorption to the surface film, the kinetics of fiber-fiber networking within the connective tissue matrix, and the kinetics of recruitment-derecruitment (41).

C. Stress-Strain Relationship and Lung Tissue Components

The elastic recoil of the lung at normal breathing frequencies is dominated by the nonlinear stress-strain characteristics of the lung tissue (139), whereas the main stress-bearing constituents are collagen and elastin fibers (43). In the lung parenchyma elastin provides elasticity, especially at lower stress levels, while the collagen fibers are loosely arranged and wavy, not becoming tight until the parenchyma is distended (43). In other words, elastin responds for load bearing at low strains; as strain increases, the collagen fibers progressively take up more load, thereby stiffening the tissue (86). These fibers significantly differ in their mechanical properties: elastin is highly stretchable before rupturing, while collagen is much stiffer and significantly less stretchable than elastin. Indeed, collagen represents the basic structural element for soft and hard tissues that provides mechanical integrity and strength to a variety of tissues and organs. On the other hand, elastin is the most “linear” elastic biosolid material. Both fiber types show a complex organization and are integrated with cells and intercellular substance in the tissues. This intercellular substance, called ground substance, contains proteoglycans, glycosaminoglycans, and tissue fluid. Depending on how the fibers, cells, and ground substance are organized into a structure, the mechanical properties of the tissue vary (43). The importance of fiber organization is especially evident for collagen that is organized into many different kinds of structures according to the organ/tissue function, varying from the simplest parallel fibered structure found in tendon and ligaments to the most complex structures of blood vessels (43).

Loading and unloading of animal tissues result in two different stress-strain curves (hysteresis loop), deviating
from the linear Hooke’s law and disclosing the existence of an energy dissipation mechanism (43). Furthermore, when held at constant strain, these tissues show stress relaxation and creep. Beyond physiological ranges, soft tissues usually have a large reserve of strength before they rupture and fail (43). These features of hysteresis, relaxation, and creep at lower stress ranges (“physiological”) indicate that biological tissues are inelastic, with their stress-strain relationship nonlinear. Soft tissues, however, can be treated as one elastic material in loading, and another elastic material in unloading (43).

In the physiological state, lung tissues are not unstressed. In fact, the lung does not have a defined stress-free state; unlike most other organs, it is a pressure-supported structure (77). The chest wall and associated respiratory muscles provide the external distending stress to maintain the lung state of inflation, whereas the transpulmonary pressure may vary during breathing but always remains positive. Thus the mechanical behavior of the lung depends on the level of the prestress transpulmonary pressure, as well as on the mechanical behavior of its microstructural elements (138). The parenchymal microstructure functions as a tension-supported lattice (78, 91), and the transpulmonary pressure (distending stress) is transmitted throughout the parenchymal tissues, thus distending all intrapulmonary structures (91).

In the lung, airways and blood vessels are embedded in and attached to a surrounding tissue that consists of an interconnected network of thin walls (168). Intraparenchymal vessels and airways exhibit mechanical properties different from those of the parenchymal tissues in which they are embedded; therefore, parenchymal distortions arise during the act of breathing (40, 91). Transpulmonary pressure acts directly on the elastic surface of the lung, and this stress is transmitted to internal points of the organ by the elastic elements. Although the lung is exposed to transpulmonary pressure, its air spaces are distended solely by the force applied on them by surrounding tissues, as a result of mechanical interdependence. In a homogeneous lung, all distensible regions are exposed to transpulmonary pressure, while in nonuniformly expanded lungs, the effective distending pressure differs from transpulmonary pressure (91, 168) (Fig. 4).

Many efforts have been focused on establishing a mathematical relation, called the constitutive equation, which describes the mechanical behavior of the lung, i.e., its stress-strain relationship (138, 144). The simplest equation is the linear relationship (Hooke’s law), which contains only two independent coefficients: the bulk modulus, indicating the ability to resist a small uniform expansion, and the shear modulus, indicating the ability to resist a small isovolume shape distortion (138).

In the continuum elasticity model suggested for lung parenchyma (168), the shear modulus increases with augmenting distending stress. The effects of a local disturbance are spread out by the surrounding material if the shear modulus is low, whereas the effects of the surrounding material on the mechanics of a local element are greater if the shear modulus is high.

The microscopic properties of the parenchymal network influence both elastic and resistive properties of the lung. Hysteresis, which can be measured as the area enclosed by the pressure-volume loop of the lung, closely relates to tissue resistance (20, 41). Indeed, the potential sources of hysteresis in the normal lung include the air-liquid interface (92, 102, 169), parenchymal contractile cells (38), and the behavior of the collagen-elastin network (20, 96, 175), that is, the main stress-bearing systems (38).

**D. Stress-Bearing Elements: Mechanical Contributions of Elastin, Collagen, Fiber Network, and Interstitial Cells**

The principal stress-bearing elements of septal tissues have been commonly assumed to be the connective tissue fibers. Collagen, elastin, proteoglycans, and glycosaminoglycans constitute the major components of the connective tissue (93, 166). Although the precise contribution of each of these elements remains unknown, the
mechanical behavior of the collagen-elastin-proteoglycan matrix likely accounts for the main proportion of energy dissipation during breathing (3, 41, 95). It should be kept in mind that when fibers are arranged in a network, the latter can display hysteretic and viscoelastic properties (21, 22, 123). Furthermore, the elastic properties of collagen and elastic fibers differ to a great extent. Collagen, the most abundant component, admits only minimal stretching, showing great tensile strength but low compliance. Conversely, elastic fibers may be stretched by a factor of 2–2.3 in relation to their unloaded lengths, presenting lower tensile strength than collagen but higher compliance (59, 96, 166).

Different theories try to explain tissue viscoelastic behavior in terms of the movement of fibers within the connective matrix. The fiber-fiber interaction theory proposes that energy dissipation occurs not at the molecular level within collagen and elastin, but, instead, at the level of fiber-fiber contact and by the shearing of glycosaminoglycans that provide the lubricating film between adjacent fibers (96). A second theory suggests that the mechanical properties of the tissue matrix can be understood as a polymer, whereas viscoelastic behavior occurs because of conformational changes in the molecules, thus being secondary to the structural disposition of fibers and their instantaneous configuration during motion (142). This theory proposes that external stresses cause polymer reptation, a sort of snakelike unfolding of fibers that alters the configuration of molecules and creates distortions that dissipate energy (142).

Evidences of distinct tissue mechanical properties between mouse strains suggest a key role for specific extracellular fiber composition and the amount of contractile structures in the lung parenchyma. The total amount of collagen fibers in lung parenchyma strips has been positively correlated with tissue resistance and elastance, while elastic fiber content has been positively associated with tissue elastance (34). In addition, specific components of the elastic system may also play a role in the observed differences in tissue mechanics among species (35, 44, 94). Altogether these evidences suggest that collagen and elastic fibers directly contribute to lung tissue stiffness and viscosity. It is noteworthy, however, that not only the absolute amount of fibers constitutes an important factor affecting mechanical behavior, but rather the organization and/or interaction among these fibers (96, 174).

The better understanding of tissue resistance behavior requires the unfolding of the complexity of interactions between the different structures comprising this material. The anatomic elements responsible for tissue hysteresity, resistance, and elastance are thus far unknown (82). As underlined before, the potential factors contributing to tissue hysteresis include contractile elements in the lung parenchyma (73, 166), the collagen elastin matrix (96), the air-liquid interface or surfactant (92), and recruitment-derecruitment of atelectatic alveolar spaces (135). Schurch et al. (126) showed that after few consecutive cycles, the hysteresis of the surface film during small-amplitude cycling becomes negligible, suggesting that during tidal breathing the contributions of the surface film to lung hysteresis are small. In addition, dynamic properties of parenchymal tissue strips do not change after loss of cell viability (174). Taken together, these studies suggest that lung parenchyma mechanics are most likely dominated by the extracellular matrix (175). Furthermore, elastance of lung tissue strips is far less than that of collagen or elastic fibers alone, which represents a network effect where the total stiffness of a large network of interconnected springs can be smaller than the spring constant of individual springs (175). When matrix strains under an applied load, relative movement occurs among neighboring fibers, implying that strains at the macroscopic level may not follow strains at the microstructural level (96). Yuan et al. (175) showed that digesting both elastin and collagen resulted in a large decrease in lung tissue elastance and indicated that the actual topology of the network must play an important role in its macroscopic behavior. Tissue mechanics at the macroscopic level are dominated by the connective fiber network, whereas interstitial cells play a less significant role (174). Various network models have been proposed in the literature, including fiber recruitment type models (86, 87, 113), the line element model of the alveolar duct (170), or other geometric models (30).

Suki et al. (142) proposed a mechanistic basis for the constant-phase-type tissue viscoelastic behavior, i.e., the so-called “reptation” motion of the collagen-elastin fibers. The distribution of fiber width and length has been identified as a candidate mechanism at the level of the fiber network responsible for the macroscopic viscoelasticity (174). On the basis of the architecture of the microstructure of the lung, slow reptation of fibers could contribute to the viscoelastic properties of lung tissue (142).

Mijailovich et al. (95) also identified the connective tissue network as the primary source of lung macroscopic behavior, whereas a stick-and-slip motion transfers the load between fibers in close contact. On the other hand, distinct mechanical states can be observed owing to contractile cells in lung parenchyma contact. The other hand, distinct mechanical states can be observed owing to contractile cells in lung parenchyma (38). Experimental evidence supports that hysteresivity is directly associated with the cross-bridge cycling rate and the metabolic state of the cells (39). However, Yuan et al. (174) found nearly identical mechanical parameters in viable and nonviable tissue samples, implying that the extracellular matrix may also play an important role in the mechanical properties of intact lung tissues. Nevertheless, the authors concluded that this finding does not imply that the cellular components do not influence parenchymal mechanics, since tissue stiffness changes in response to different contractile agents owing to cell contraction (174).
How specific remodeling features affect lung tissue mechanical properties can be partially unshadowed by lung maturation studies. The maturing lung represents a naturally occurring model of altered extracellular matrix that changes rapidly during the postnatal stage. The alveolarization process, characterized by an increase in the number and size of alveolar walls and a decrease in alveolar thickness, is followed by a marked increase in the amount of collagen and elastic fibers during the first several weeks of life (101). Proteoglycans and glycoproteins also change, including a decrease in hyaluronic acid and chondroitin sulfate proteoglycan concentrations (148, 149).

Nardell and Brody (101) correlated morphological changes and extracellular appearance of collagen and elastin with lung mechanical properties in a rat model of lung growth. The potential influences of alveolar or airway surface forces and of chest wall were eliminated by performing saline volume-pressure curves on excised rat lungs from the perinatal period (day 4) to early adulthood (day 40). Postnatal lung growth in the rat can be divided in four stages: lung expansion (between birth and 4 days), alveolar accumulation (between 4 and 12 days), elastin concentration (between 12 and 20 days), and equilibrated growth (between 20 and 40 days). The authors showed that changes in lung elastin concentration are associated with an increase in lung recoil, while lung collagen is more closely associated with the structural integrity of the lung as measured by resistance to lung rupture. Furthermore, lung volume tends to follow the course of lung elastin concentration rather than collagen’s (101).

Another study, examining the viscoelastic behavior of isolated parenchymal strips from rat lungs at different ages, showed that tissue resistance and elastance decreases with maturation, while hysteresivity ($\eta$) increases (149). Since the amount of collagen and elastin rises with maturation (101), it may be concluded that fiber structure, orientation, and/or alveolar geometry, rather than the absolute amount of collagen and elastin per se may be fundamental in determining tissue viscoelastic behavior (149). Indeed, maturational changes in tissue viscoelastic behavior in rats are accompanied by changes in the composition and configuration of the alveolar wall, with increasing collagen and elastic fiber contents and diminishing hyaluronic acid and $\alpha$-actin smooth muscle-positive cells (148). These findings support the concept that the total amount of extracellular matrix fibers does not respond entirely for the mechanical behavior of the elastic elements. Indeed, changes in fiber subtype, topology, or cross-linking with maturation could contribute to it (148). In addition, proteoglycans and glycosaminoglycans may add to viscoelastic behavior because they coat the stress-bearing fibers and can affect tissue turgor. These molecules may act as “lubricant,” modulating the energy dissipation between fibers and thereby altering tissue mechanics (148). Indeed, the rise in $\eta$ with maturation could be possibly explained either by the increase in the absolute number of fibers interacting with each other or, alternatively, by changes in the ground substance (148, 149).

E. Mechanical Interactions Between Collagen and Proteoglycans

Proteoglycans consist of a protein core with large polysaccharide side chains, the glycosaminoglycans. Glycosaminoglycans interact with collagen, elastin, and cells probably modulating the formation and/or remodeling of the connective tissue, but their mechanical roles remain largely unknown (61). Decorin, a small leucine-rich proteoglycan present in tissues such as the lung with fibrillar collagen, plays a key role in regulating collagen fibril formation and the spatial arrangement of collagen fibers in the matrix (67). It may also take part in the remodeling associated with respiratory diseases such as pulmonary fibrosis and asthma, by means of decorin-regulated collagen fibrillogenesis (172). Decorin binds transforming growth factor-$\beta$, a cytokine implicated in remodeling (158). Additionally, decorin-deficient mice show altered lung tissue mechanics with increased compliance at higher pressures and stresses, and smaller airway resistance (45). The alterations in tissue properties observed in decorin-deficient mice are probably secondary to abnormal collagen fibril formation (28, 48).

Proteoglycans can also potentially alter tissue turgor and viscoelasticity (124). Since it has been proposed that tissue viscoelasticity reflects the mechanical friction generated as adjacent fibers slide across each another (96), the nature of this mechanical interaction could be modified by the lubricating film between them. Proteoglycans coat individual collagen and elastic fibers; hence, alterations in these molecules could possibly affect energy dissipation at the fiber interface (3). The hydrophilic nature of proteoglycans and glycosaminoglycans attracts ions and fluid into the matrix (124). Indeed, the swelling of charged proteoglycans influences the macroscopic mechanical properties of lung tissue strips (3, 25). Although it seems that the network organization of collagen and elastic fibers constitutes the dominant factor in the elastic behavior of lung tissue, interaction of the fibers with proteoglycans can affect fiber network stability, thus contributing to the tissue mechanical profile (3, 25, 34).

F. Contractile Elements and Tissue Mechanics During Induced Constriction

Subpleural lung parenchymal strips are commonly used as a model for the study of mechanical and pharmacological properties of the lung periphery (34, 39, 115, 121,
The gas-free isolated parenchymal strip preparation abolishes the gas-liquid interface, thus eliminating an important stress-bearing system, as well as the effects of recruitment-derecruitment of atelectatic alveolar spaces. This preparation brings the experimenter a step closer to the attachment-detachment of cross bridges (38). The attachment-detachment of cross bridges during volume cycling (41) or the fiber network of the connective tissue (22, 123) could account for the hysteresis observed in the length-tension curve (41, 96).

The relevance of the parenchymal strip as a proxy for small airways has been questioned because the strip presents a complex morphology and contains a diversity of contractile cell types (33, 48). However, it can be considered as a proxy for the integrated contractile response of the parenchyma itself (38). Indeed, many authors have used the parenchymal strip as a means of investigating dynamic mechanical responses to constrictor challenge at the parenchymal level. Lung periphery constitutes a complex system comprising alveolar walls, bronchioles, and small vessels. The determination of the precise elements that respond to contractile stimulus is rather difficult, and it has been shown that the volume proportions of different anatomic structures, such as amounts of airway, vasculature, and alveolar wall, do not correlate with oscillatory mechanics in subpleural parenchymal lung strips under baseline conditions (82).

It is also noteworthy that in parenchymal strip preparations lung tissue undergoes uniaxial loading, and hence, microstructural elements are presumed to deform only unidimensionally, in contrast to the three-dimensional deformation present in the normally breathing lung. In the whole lung, macroscale hysteresivity is proportional but smaller than microscale hysteresivity, and microstructural elements respond both by reorienting and lengthening. On the other hand, during uniaxial deformation of lung strips, macroscale and microscale hysteresivity are similar, and microstructural components can respond only by lengthening; thus macroscale hysteresivity (η) is a direct reflection of hysteresivity of the microstructural element (38).

The pressure-volume behavior of the lungs can be changed by several contractile interventions (29, 66, 81). Many lung responses that have initially been ascribed to changes in airway caliber have lately been shown to reflect ongoing events at the lung tissue level (79, 83, 117, 159). Lung parenchyma can express diverse mechanical responses according to the specific agonist present in the cellular microenvironment, which reveal themselves mainly in the hysteretic nature of the tissue (38, 84). Indeed, it has been demonstrated that the hysteretic behavior of pulmonary tissues increases with activation of the contractile machinery (38). Three types of contractile elements can participate: smooth muscle of small airways, smooth muscle in alveolar ducts, and interstitial contractile cells (59, 73).

It is currently considered that connective matrix is passively driven by contractile cells along its functional kernel characteristics to a new operating level without intrinsic structural changes, thus without significant changes in the transfer of tissue mechanical properties (115). On the other hand, contractile cell systems show prominent hysteretic properties, presenting structural changes during constriction because of bridge dynamics (40) and plastic remodeling of the cytoskeleton (49). Authors agree that the connective tissue network dominates parenchymal mechanics, which can be influenced by the tone or contraction of interstitial cells (38, 82, 115). Recent findings suggest that mechanical properties of lung tissue in baseline conditions are actually dominated by the connective fiber network, whereas interstitial cells play a less significant role (174). Interestingly, constriction modifies intrinsic mechanical properties of connective matrix by mechanisms diverse from passive stretching (114, 115), suggesting that constriction of some element at the tissue level occurs (98, 99). The mechanical response to passive length changes obeys the constant-phase behavior in the frequency domain (44, 115), while the Newtonian component of resistance increases only during constrictor challenge, but not during passive stretch (115). The mechanical coupling of contractile cells and fibers depends on the amount of constriction or characteristics of the machinery involved. Contractile cells modulate the mechanical properties of the connective matrix (115). Thus energy dissipation at tissue level derives from a complex combination of changes in small airways, modifications in alveolar ducts, and distortion of parenchymal tissues secondary to activation of contractile elements (80, 98).

It has also been suggested that tissue response to bronchconstrictor agents can be secondary to increased inhomogeneities in peripheral airway resistance, which produce an artifactual increase in tissue resistance, suggesting the failure of the model to describe the lungs under highly heterogeneous constriction (13, 85). Although there is little or no influence of airway inhomogeneities on measurements of lung mechanics in healthy lungs during a physiological tidal excursion, airway inhomogeneities significantly increase tissue resistance during agonist-induced constriction (85). Lutchen et al. (85) also demonstrated that the frequency-dependent behavior of lung elastance (\(E'\)) is markedly influenced by inhomogeneities, rather than a strict consequence of altered tissue viscoelasticity. Indeed, methacholine challenge increases lung resistance at all frequencies and also the positive frequency dependence of \(E'\) with minute alterations in its low-frequency values, thus suggesting little changes in the true tissue elastance (85). These findings, however, can be
model dependent, since they were observed in a single-compartment homogeneous model used to characterize lung constriction.

III. TISSUE MECHANICS IN LUNG DISEASES

A. Lung Parenchyma Remodeling: Micromechanics of Injured Lungs

During disease states, small-scale heterogeneity in mechanical properties (such as local shear moduli) increases considerably, which contributes importantly to local stress distributions (64). The determinants of the lung parenchymal stress and strain distributions in the intact thorax depend critically on the lung resistance to a shape change. Thus the effects of injury on lung mechanical properties become of paramount importance. Several mechanisms have been suggested to explain the larger shear modulus of injured lungs, such as interfacial tensions associated with alveolar exudates, increased surface tension, and the consequently increased prestress of the axial elastin and collagen network, interstitial edema and matrix remodeling, and scar formation and fibrosis (64).

Damaged lungs present two attributes that account for their increased risk of deformation injury. First, the number of airspaces capable of expanding during inspiration diminishes ("baby lung"), increasing the risk of injury from regional overexpansion, since units that do not expand during breathing undergo a stronger deforming stress (47, 64). Second, local impedance to lung expansion is heterogeneous because of uneven distribution of liquid and surface tension in distal airspaces, which generates shear stress between neighboring, interdependent units that operate at different volumes (64, 91). Subpleural alveoli of edematous isolated rat lungs present wavy, flooded walls and contain air pockets of different sizes and shapes (63). The presence of differently sized air pockets with diverse radii of curvature implies a nonuniform alveolar gas pressure and/or uneven surface tensions (64). Bachofen and co-workers (8, 9) suggested that regional differences in the physicochemical properties of the surfactant could be the source of the nonuniform surface tension. A maintained nonuniform alveolar gas pressure may suggest that the air pockets are trapped by liquid and foam in conducting airways (64).

One should not forget the important phenomenon of interdependence between elements of the lung parenchyma network, which promotes uniform expansion of individual units. Stress increases whenever the sum of forces of the surrounding tissue acts over a smaller surface area. Consequently, when an obstructed unit (e.g., an alveolus, a lobe, or a lung segment) resists expansion, the neighboring units exert a large inflationary stress on it. Moreover, the tension and strain of individual connective tissue elements onto the collapsed segment increase out of proportion in relation to those of more remote network structures (64).

Analysis by histochemistry demonstrated that lung collagen content augments in both acute and chronic interstitial diseases, suggesting that significant remodeling of alveolar tissue occurs even in acute situations (118). The extracellular matrix remodeling process occurs as a response to lung injury involving all of its components in a kind of chain reaction. Indeed, the elastic system, a major component of the extracellular matrix, that plays an important role in maintaining the patency of the airways and lung elastic recoil is also altered, with elastic fiber deposition, both in animal models of pulmonary fibrosis (42, 106, 111) and human lung fibrosis (104). On the other hand, increased elastin destruction occurs in certain pathological conditions owing to the release of powerful elastolytic proteases by inflammatory cells (130). Reactivation of elastin synthesis occurs in response to increased destruction, but in a disorderly manner. Thus elastosis could also partially respond for the loss of the normal architecture of the alveolar walls, contributing to the alveolar mechanical dysfunction and remodeling present in acute and chronic interstitial lung diseases (104).

The small leucine-rich proteoglycan decorin regulates collagen fibril formation and spatial arrangement in the matrix (67). Thus decorin may also play a role in the remodeling associated with respiratory diseases such as pulmonary fibrosis and asthma (16). In fact, decorin-deficient mice present decreased airway resistance and increased respiratory compliance in vivo, while in vitro length-stress curves similarly show increased compliance of lung strips. The alterations in tissue properties are likely the result of abnormal collagen fibril formation (45).

Mechanical forces applied to normal lung parenchyma, such as during mechanical ventilation, can also induce structural and functional changes (19, 105). Lung cells submitted to different types of force produce proinflammatory mediators (107, 162). In addition, mechanical forces induce expression of extracellular matrix proteins by lung tissue (19, 147). Evidences gathered from lung parenchymal strip oscillation disclosed that tissue elasticity and resistance augmented with increasing operating force and decreased with increasing amplitude. Additionally, a rise in force induced early tissue remodeling (46). In this model, force and amplitude variations simulate in vivo pressure and volume changes, respectively (65). Moreover, pathophysiological conditions of the lung may shift the balance of forces so as to chronically alter the amount of strain imposed on the airways (100, 164). Tepper et al. (150) demonstrated, in isolated rabbit intrapulmonary bronchial segments and lung parenchymal tissue slices, that the imposition of physiological levels of chronic mechanical strain resulted in significant...
changes in the passive and active physiological responses of these tissues. The absence of chronic strain on intraparenchymal airways resulted in smaller, stiffer airways that generated greater pressures and narrowed to a greater extent in response to contractile stimulation than airways that had been subjected to chronic strain. In addition, the effects of strain were most prominent in the smaller, more compliant airways (150).

B. Lung Fibrosis

Pulmonary fibrosis is characterized by abnormal lung physiology and by the excessive production of extracellular matrix molecules such as collagen, elastin, and proteoglycans (136, 140). Since $R_t$ accounts for a major proportion of energy dissipation during breathing, defining dynamic tissue behavior in fibrotic diseases represents an important goal (32). Since 1967 it is recognized that $R_t$ increases in patients with lung fibrosis (7). More recently, a bleomycin-induced rat model of pulmonary fibrosis disclosed that $R_t$ rises both in intact animals and in isolated lung parenchyma strips oscillated in an organ bath, with similar magnitude changes in vitro and in vivo (31). Besides the documented increases in collagen and elastin (136, 140), investigators have focused on changes in proteoglycans, the macromolecules that comprise the ground substance of the pulmonary matrix (32). Three major subclasses of proteoglycans have been described: hyalectins, which are large molecules such as versican that form aggregates with hyaluronic acid; basement membrane proteoglycans such as perlecan; and the small leucine-rich ones, which include lumican, biglycan, fibromodulin, and decorin (68). The proteoglycans present many different biological functions: they affect mechanical behavior, influence collagen fiber formation and assembly, modulate cell migration, and interact with various cytokines and growth factors (68). Indeed, a prominent deposition of these molecules is observed in both granulomatous and nongranulomatous forms of fibrotic lung disease (16, 17). Evidences suggest that the large proteoglycan versican (16, 17) or the small fibromodulin decorin and biglycan are altered in the early stages of fibrosis (167).

Links between changes in lung function and structure have been described in fibrotic disease. A modest correlation was observed between the degree of fibrosis in idiopathic pulmonary fibrosis and the shape of the lung pressure-volume curve (122). Moreover, positive correlations between volume proportion of collagen and parenchymal strip resistance and elastance were demonstrated in bleomycin-induced fibrosis in rats, with no correlation between collagen or elastin and hysteresivity (31). Ebihara et al. (32) described, in bleomycin-instilled rats, changes in oscillatory and quasi-static mechanics prior to measurable changes in volume fraction of either collagen or elastic fibers. Actually, those authors reported important positive associations between changes in structure and function, namely, biglycan and the three oscillatory parameters (resistance, elastance, and hysteresivity), suggesting that biglycan acts on lung mechanics by means of its influence on the matrix assembly (32, 125).

Oscillatory mechanics of lung strips in a murine model of silicosis showed time-dependent functional changes, with increased tissue resistance and elastance, followed by a later rise in hysteresivity. Tissue mechanical behavior was correlated with lung parenchyma inflammation and remodeling, characterized by a process of fibroelastosis, thus supporting a key role for the elastic system and its specific components on tissue mechanical properties (36).

C. Lung Inflammation: Asthma

Various lung diseases are characterized by underlying acute and/or chronic inflammation. Moreover, lung inflammation has been associated with structural changes collectively referred to as remodeling. This complex and variable mechanism occurs in conditions such as acute lung injury and fibrotic disorders, as described above, as well as in chronic obstructive pulmonary diseases.

Asthma is characterized by reversible airway obstruction, airway hyperresponsiveness, and airway inflammation. As in many chronic inflammatory disorders, asthmatic airway inflammation is also believed to cause tissue injury and subsequent structural changes (58, 133). These changes have attracted a great deal of interest because they may account for pathophysiological aspects in asthma that are poorly addressed with current anti-inflammatory strategies (58).

There are evidences that asthmatic inflammation extends to distal airways and lung parenchyma (76, 155, 156, 171). Prolonged inflammation may lead to thickening of the airway wall, which can be found throughout the bronchial tree including airways <2 mm in diameter (116). In nonfatal asthma, where airways are only slightly thickened, changes are most pronounced in the small airways (24, 57). Another characteristic structural change, the reticular basement membrane thickening with collagen deposition in the subepithelial space, constitutes an almost pathogenomic feature of asthma, although its clinical significance has not yet been established (133). Moreover, distal airway and lung parenchyma remodeling features have been described in a murine model of chronic allergic sensitization displaying increased tissue elastance and resistance (127, 128, 171).

Other elements of the extracellular matrix, such as proteoglycans and glycosaminoglycans, are altered by remodeling and have been related to changes in tissue
mechanics, cellular activity, cytokine function, and fluid balance. Deposition of the proteoglycans versican, biglycan, and lumican, as well as of the glycosaminoglycan hyaluronan has been described in asthma (62, 110, 132, 161). The finding of increased amounts of proteoglycans in the airways of asthmatics and ovalbumin-sensitized/challenged mice is of particular interest. Proteoglycans bind with fibrillar collagen and influence the interaction of collagen fibrils and their assembly (53). They also bind to other components of the extracellular matrix and growth factors, affecting cell adhesion and extracellular matrix deposition (53, 108). The importance of increased airway extracellular matrix deposition in the pathogenesis of asthma remains uncertain. However, asthma severity and decline in FEV1 have been correlated with subepithelial fibrosis (60). Furthermore, a significant association was observed between enhanced proteoglycan deposition and increased airway responsiveness (61).

Abnormal elastic fiber network also occurs in the asthmatic airway, perhaps caused by changes in the elastolytic process (57, 160). Fragmentation of central airway fibers and marked elastolysis are described in fatal asthma (57). Furthermore, increased levels of elastase were found in sputum of asthmatic patients (160). Elastic fiber fragmentation has also been observed in an animal model of chronic allergic inflammation (171). Airway geometry, function, and dynamics are subject to these remodeling processes (133).

Data exist suggesting that epithelial damage participates in the proinflammatory process by releasing cytokines, growth factors, and mediators (57, 133). Epithelial injury can result in a self-sustaining phenotype of wound repair modulation, by activation/reactivation of the epithelial-mesenchymal trophic unit (18, 70, 151). Collagen, reticular and elastic fibers, proteoglycans and glycoproteins, all of which contribute to lung remodeling, can be produced by mesenchymal cells influenced by epithelial cells. Thus epithelial-mesenchymal interactions play an important role in extracellular matrix production and deposition (18, 57, 133). The presence of remodeling features in bronchial biopsy specimens of young children, in the absence of eosinophilic infiltrates, suggests an early role for epithelial-mesenchymal trophic interactions, secondary to epithelial injury and stress (37, 105).

Tomioka et al. (152) studied airway and tissue mechanics at baseline and after methacholine challenge in an allergic murine model that simulates some features of asthma. They observed that allergic inflammation significantly increased the responsiveness of the lung periphery. In their model, the rheological properties of lung parenchyma were not altered to any significant extent by inflammation. In contrast, the inflammation caused a sizeable increase in the responsiveness of tissue elastance and resistance to methacholine measured by the alveolar capsule, as well as of tissue dissipative ($G''_H$) and conservative ($H''_H$) parameters determined by forced oscillations (152). The effects of allergen itself on lung parenchyma have also been investigated (50). Indeed, the notion that mechanical abnormalities of the lung parenchyma are also important in asthma has progressively attracted more and more attention (127, 128), as suggested by the finding that parenchymal lung resistance may be significantly elevated even in patients with spirometric data within normal limits (71, 72). Lung biopsies also provide evidence that asthmatic inflammatory processes involve the lung parenchyma (24, 75).

Finally, it is noteworthy that the mechanical stress developed during asthmatic bronchoconstriction may also induce remodeling. Epithelial cells and fibroblasts respond to mechanical stress by increasing collagen synthesis, as well as the ratio between matrix metalloproteinases and tissue inhibitors of metalloproteinases (147).

D. Emphysema

Pulmonary emphysema has been defined by pathological criteria as an irreversible destruction of lung parenchyma distal to the terminal bronchioles with airway wall destruction and airspace enlargement, not associated with significant fibrosis (137). The most common cause of emphysema is cigarette smoking, and the central elements in its definition are the concepts of destruction and irreversibility of the pathological process (173). Although environmental toxins affect both proximal airways and alveolar lung tissue, the two anatomic compartments respond differently: airways present cell proliferation, while alveolar lung tissue disappears (173). The major mechanisms thought to be responsible for the development and progression of emphysema include the protease-antiprotease hypothesis, inflammation, oxidative stress, alveolar cell apoptosis, and matrix remodeling (10, 145, 173). Probably, these are not distinct processes and each can contribute to disease complexity (145).

The most widely accepted hypothesis for tissue destruction in emphysema rests on the imbalance of protease and antiprotease activities, leading to enzymatic degradation of elastin (10). The concept that tissue destruction results from protease burden, mainly neutrophil elastase, has relied on the findings in patients deficient in $\alpha_1$-antitrypsin, the major inhibitor of neutrophil elastase (141, 173), in concert with the use of intrapulmonary instillation of elastolitic enzymes to develop animal models of emphysema (131). However, experimental findings suggest that neutrophil elastase may not be the principal protease regulating tissue destruction in emphysema (145). Macrophage elastase was demonstrated to be sufficient to cause emphysema after chronic inhalation of cigarette smoke (54), while experimental emphysema can also be induced by overexpression of collagenase (27) or impaired synthesis of specific proteoglycans (157).
Inflammatory cells, including macrophages, T lymphocytes, B lymphocytes, and neutrophils represent the best-known source of proteases in the lungs. In addition, lung tissue destruction can be further enhanced by inflammatory cells recruited by elastin fragments liberated during elastic fibers degradation (131). Cigarette smoke inhalation induces neutrophils, lymphocytes, and macrophages infiltration in the lung, but the specific pathological role of each inflammatory cell in emphysema remains to be determined. Lung infiltration of inflammatory cells in emphysema has been attributed to either cigarette smoke/pollutants or oxidative stress as the result of exposure to environmental hazards (173). Recent findings suggest, however, that inflammation could be the result of an autoimmune attack to lung tissue (2).

Neutrophil elastase and matrix metalloproteinases (MMPs) are the most studied candidates for protease/antiprotease imbalance in emphysema. Evidences support a significant role for the macrophage metalloelastase (MMP-12) and the collagenase MMP-1 in emphysema, while a role for an imbalance between MMP and tissue inhibitor of metalloproteinases (TIMP) has also been suggested (173). The targets of proteolitic enzymes and free radicals liberated by polymorphonuclear cells and monocytes are collagen, elastin, proteoglycans, and α1-antitrypsin (163). In addition to proteases, other factors have been implicated in tissue destruction leading to emphysema, such as oxidative stress and apoptosis of lung structural cells (131). However, one interesting question emerges: do the inflammatory elements target directly components of cigarette smoke or is inflammation, rather, a secondary response to cellular and molecular alterations induced by cigarette smoke? Each puff of cigarette smoke contains numerous toxic compounds, free radicals, and potent oxidants such as hydrogen peroxide, hydroxyl anion, and organic radicals (154). Tuder et al. (154) underlined the important similarities between the pathophysiological responses to aging and emphysema owing to chronic cigarette smoking, both presenting oxidative stress and apoptosis as common molecular mechanisms. The authors suggested that emphysema may recapitulate events underlying accelerated lung aging processes resulting from failure of lung maintenance and repair (154). In this context, inflammation might represent the response to unpaired organ injury, rather than the first line of response to environmental stresses (153, 154).

The finding that decreased vascular endothelial growth factor (VEGF) or VEGF signaling, an obligatory lung maintenance factor throughout lung development, can induce emphysema suggests that alveolar maintenance is required for the structural preservation of the lung (74, 154, 173). Thus alveolar cell destruction in emphysematous lungs might be caused by apoptosis, possibly unrelated to preceding matrix protease degradation. Indeed, apoptotic cells retrieved by bronchoalveolar lavage after the induction of acute apoptosis and alveolar enlargement were able to degrade elastin (5, 173). On the other hand, oxidative stress decreases the expression of VEGF coreceptor and an accessory molecule that potentiates VEGF binding (89). In emphysema, free radical species can be generated by exogenous sources, such as cigarette smoke, and by endogenous origins, particularly pulmonary inflammatory cells. In addition, data from chronic obstructive pulmonary disease (COPD) patients support the occurrence of an overwhelming oxidative burden from reactive oxygen species in moderate or severe disease, while healthy smokers and stable patients show adaptive responses against oxidative stress, probably mediated by glutamate cysteiny ligase, the main regulator of the most important antioxidant glutathione (173). In fact, the interactions among apoptosis, matrix proteolysis, and oxidative stress have been proposed as the final conduit of alveolar destruction in emphysema (153).

Although most studies suggest that elastin constitutes the major target of destruction in emphysema, pathophysiologiocal changes can also result from abnormalities in the collagen matrix (69). Indeed, collagen presents higher stiffness and failure threshold than elastin, protecting the lung from rupture at high volumes. Thus alveolar wall failure suggests that the collagen network in the emphysematous lung cannot be normal. Several MMPs, including MMP-1, -8, and -13, are capable of degrading collagen (145). In addition, cigarette smoke can undermine the repair function of fibroblasts, epithelial cells, and mesenchymal cells, yielding persistent abnormalities in tissue structure (109). Elastin and collagen seem to be the major constituents of the extracellular matrix, accounting for lung tissue viscoelastic mechanical properties, and, interestingly, elastic fibers behave more linearly than collagen fibers (69). In a mouse model of elastase-induced emphysema, Ito et al. (69) observed increased total collagen content and collagen-related dynamic nonlinearity, while failure stress of isolated parenchymal tissues decreased. These findings suggest that, despite the increased collagen content, the alveolar walls and the collagen fibers were likely to be weaker in the emphysematous lung as a consequence of the process of degrading and remodeling. In this model, emphysematous mice also showed increased hysteresivity and lower elastance. Although in normal lung tissue a positive association between hysteresivity and elastin-to-collagen ratio can be found, emphysematous mice show increased hysteresivity, whereas collagen content also increased. Based on these results, the authors suggested that remodeling in emphysema produces weak and viscous alveolar walls that also fail at lower stress. Thus abnormal collagen remodeling may play a significant role in lung functional changes in emphysema (69). Indeed, according to Brewer et al. (20), normal lungs show a fall in hysteresivity with
increasing transpulmonary pressure (P_L), while emphysematous rat lungs display constant hysteresivity at all P_L levels. Lung elastance also showed a significantly reduced dependence on P_L in the treated animals. In normal lungs, the volume-dependent nature of hysteresivity can be attributed to the progressive contribution of collagen at higher lung volumes (134), since the hysteresivity of collagen is less than that of elastin (95, 175). Thus, taken together, the results suggest that the P_L dependence of elastance and hysteresivity can signal the early remodeling of elastin and collagen fibers in the alveolar walls during the development of emphysema (20).

IV. CONCLUSION

In this review we summarized the basic concepts involved in energy dissipation during breathing, focusing on those pertaining to lung tissue behavior. The potential participating mechanisms, the different models available for the determination of tissue properties, as well as the participation of the stress-bearing elements were reviewed. The mechanical properties of lung tissue are important determinants of lung physiological functions, being affected in diverse diseases. More recently, models have tried to deal with dynamic tissue behavior on a mechanistic basis, and evidences suggest that events at the microstructural level may not follow those at the macroscopic level. However, the precise molecular basis for an integrative biological phenomenon remains unknown.

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Address for reprint requests and other correspondence: W. A. Zin, Laboratório de Fisiologia da Respiração, Instituto de Biofísica Carlos Chagas Filho-C.C.S., Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-902 Rio de Janeiro, Brazil (e-mail: wazin@biof.ufrj.br).

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