Simulation of Tissue Properties in Irreversible Diffuse Obstructive Pulmonary Syndromes

ENZYME DIGESTION

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ABSTRACT The length-tension properties of alveolar wall from normal cats were studied before and after exposure to enzymes naturally found in mammals (elastase, trypsin, collagenase, hyaluronidase). Hyaluronidase effected little change while all the proteolytic enzymes altered the mechanical properties of lung tissue. Collagenase removed the "mechanical stop" and the alveolar walls fractured at low forces. The properties of wall exposed to trypsin resembled those of elastase-treated tissue. Elastase increased the extension necessary to reach a given force and increased the maximum length (L_{max}) and resting length (L_o). Maximum extensibility (λ_{max}), the ratio of L_{max} to L_o, fell with both elastase and trypsin digestion. A reduction in λ_{max} simulates the changes in alveolar wall properties seen in the lungs of the aged and in those with an irreversible diffuse obstructive pulmonary syndrome (DOPS$_I$). Unlike these states, however, the energy loss in stretching alveolar wall increased with elastolysis. Furthermore, the changes in L_o necessary to effect a change in λ_{max} of alveolar wall comparable to that seen in DOPS$_I$ were excessive. The altered tissue properties that occur in man with obstructive pulmonary syndromes could not be produced with these proteolytic enzymes or with hyaluronidase.

INTRODUCTION

The lungs of patients with clinical emphysema or an irreversible diffuse obstructive pulmonary syndrome (DOPS$_I$)[1] commonly demonstrate a loss of elastic recoil (1, 2). Air and saline pressure-volume curves show most of the lung recoil to be a function of tissue forces at mid lung volumes. We have studied the properties of lung tissue and found the maximum extensibility ($\lambda_{max} = L_{max}/L_o$) of alveolar walls to diminish with age (3). A similar but greater change in extensibility was found in the lungs of those with DOPS$_I$. Maximum extensibility (λ_{max}) can decrease if the limit to extension (L_{max}) is reduced, or if the resting length (L_o) is increased. An increase in resting length is in keeping with the changes in lung volume and transpulmonary pressure found in DOPS$_I$. A reduction in L_{max} or the mechanical stop of the tissue is not. In other studies we found no age or DOPS$_I$ related change in the viscoelastic properties of alveolar wall (4).

Some authors have suspected that overinflation of the lung causes clinical emphysema (5). Recently, an animal model has used this mechanism (6). Metals or polymers extended beyond their "yield" point, show permanent deformation, or change in the resting length (7). Overextension of alveolar wall in vitro however, while producing a change in the extension curve resembling "yield," does not always result in a change in the resting length (4). An alternative means of simulating the tissue changes found in DOPS$_I$ might be through destruction of the tissue component(s) responsible for restoration of resting length. In these studies we have exposed normal alveolar wall to proteolytic enzymes (trypsin, elastin, collagenase) and to hyaluronidase.

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[1] *Abbreviations used in this paper:* DOPS$_I$, irreversible diffuse obstructive pulmonary syndrome; $F_{1,2,3,4}$, force at selected points on the length-tension curve; F_t, force at the first peak before tissue break; F_f, force at tissue break; F_t, force after treatment expressed as the percentage of the control; HR, hysteresis ratio, or the area within the length-tension loop divided by that beneath the extension curve; L_{max}, the extrapolated vertical asymptote of the length-tension curve; L_o, resting length; L-T, length-tension curve; λ, extension as a multiple of L_o; λ_{max}, L_{max}/L_o; λ_t, extension at first peak in force before tissue break; λ_f, extension at tissue break.

METHODS

Normal cat lung was used because the tissue: (*a*) was easily available; (*b*) easy to dissect; and (*c*) length-tension (L-T) characteristics are similar to those in normal man.

The amount of enzyme used for each tissue in 100 cc of buffer solution and their sources include: (*a*) bovine pancreatic trypsin (Worthington TRL 12 × crystallized; Worthington Biochemical Corp., Freehold, N. J.), 20–30 U; (*b*) clostridium histolyticum collagenase (Worthington SLSPA), 70–80 U; (*c*) swine pancreatic elastase (Worthington ESFF chromatographically pure), 20–30 U; and (*d*) bovine testicular hyaluronidase (Worthington HSEP chromatographically pure), 30–300 U. Buffer solutions (0.2 M bicine pKa = 8.35, molecular weight 163.2) were pH adjusted for optimum enzyme activity (trypsin at pH 8.1, collagenase at pH 7.4, elastase at pH 8.8). A sodium acetate buffer (0.5 M) at pH 4.2 was used for hyaluronidase studies.

A piece of lung parenchyma just beneath the pleura was excised and reduced to approximately 30 × 30 × 200 μm in the saline bath. This tissue was suspended vertically between clamps in a small plastic chamber filled with 100 cc of buffer solution. This chamber was immersed in another bath filled with water at 37°C.

The upper tissue clamp was attached to a force transducer (Sanborn FTA 1-1; Sanborn Div., Hewlett-Packard Co., Waltham, Mass.) whose compliance of 0.2 μm/mg was neglected. The lower clamp was attached to a piston protruding through the bottom of the bath. The piston was hydraulically driven by a Wills micromanipulator. The movement of the piston was monitored by a length transducer (Sanborn 7DC DTO-50) which with the output of the force transducer was fed into an XY plotter.

Stretching of the tissue was initiated with small strains of 0.3–0.4. In a series of experiments the strain was gradually increased to get a control (L-T) curve with a peak force of 6–7 mg. After recording the control L-T curves an enzyme was added to the center chamber and further L-T curves were recorded at intervals of 30 min for a total of 3 h. At each extension, several L-T curves were recorded and the first two cycles discarded, i.e., adapted curves are reported here. All tissues were held at their resting length (control) during enzyme treatment with extensions equal to that of the control L-T curve during the 3 h of enzyme digestion. After this period, extension was usually increased to elicit irreversible tissue properties.

Definitions and calculations. A control L-T loop and the loading curve after 3 h of immersion in trypsin illustrate the measures used (Fig. 1). Since all tissues were stretched to a complete break, we could not measure the histologic cross sections. As a result, stress could not be calculated. Instead, changes in force as well as changes in the initial slope were measured in terms of the percentage of control value.

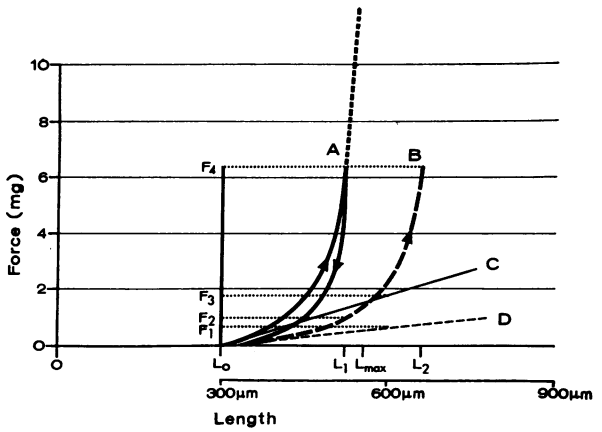

FIGURE 1 The L-T loop of alveolar wall (cat) before enzyme treatment (*A*) and the loading curve following trypsin digestion (*B*). Measured parameters are illustrated (see text).

On the length-tension curve after enzyme treatment (*B*, Fig. 1) the force (F_2) was measured at the greatest extension (L_1) achieved by the tissue strip during control measurements (curve A) and maximal force (F_4). The force (F_2) was expressed as a percentage of the maximal force ($F_t = F_2/F_4 \times 100$). F_t represents the change in peak force after digestion for a given extension.

The slope of the loading curve for each tissue was defined as the force measured on a tangent drawn through L_o at an extension twice the resting length (*C*, *D*, Fig. 1). The change in slope before and after enzyme exposure was expressed in percent ($F_1/F_3 \times 100$).

At the end of 3 h of enzyme exposure, the tissue was stretched to a force equal to peak force on the control curve (F_4). The tissue length at this point was measured (L_2, Fig. 1) and expressed as the multiple of the resting length ($\lambda_t = L_2/L_o$, Table I). Here L_o is the resting length of the control curve.

TABLE I

*The Peak Force (Mean±SE) on the L-T Curve before and after Exposure to Enzyme Digestion**

Enzyme	Control	Collagenase	Trypsin	Elastase	Hyaluronidase
Peak force (mg)	7.2±0.3	5.7±0.5	6.6±0.6	7.1±0.6	7.4
λ	1.95±0.08	1.96±0.12	1.76±0.07	1.89±0.09	1.89
F_t (0.5 h) (%)	98±3.6	41±6.9	33±7.4	27±5.0	105
F_t (1 h)	99±4.2	30±8.2	26±5.6	22±5.0	91
F_t (2 h)	95±7.0	15±6.0	19±3.8	14±3.6	92
F_t (3 h)	71±5.8	12±5.8	13±2.9	10±2.9	87
λ_t	2.04±0.08		2.31±0.24	2.54±0.21	1.95

* Proteolytic enzyme digestion caused a fall in peak force at a fixed extension with time. The increase in strain (λ_t) necessary to reach the peak force of control tissue is shown for trypsin and elastase.

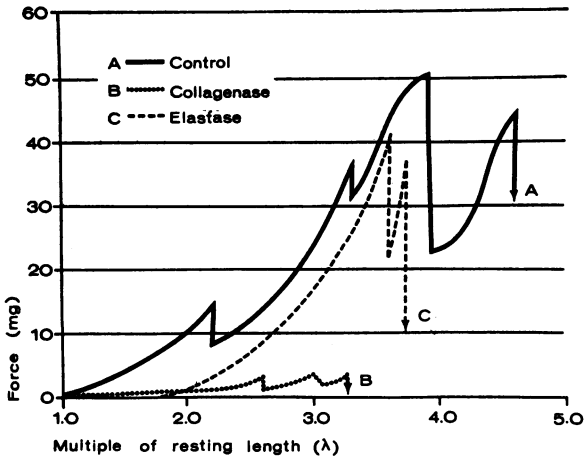

FIGURE 2 The loading curve of control and enzyme treated (elastase, trypsin, collagenase) tissues. All curves start with the original resting length and end with the breaking point (tissue separation).

Maximum extensibility ($\lambda_{max}=L_{max}/L_o$) is the vertical asymptote of the L-T curve expressed in terms of extension (λ). The method of predicting maximum length (L_{max}) has been reported (3). L_o is the greatest extension of tissue requiring no force for its maintenance. In calculation of λ_{max} if any change in L_o occurred following exposure to an enzyme, the new L_o was used. Any increase in L_o was expressed as a percent of the original L_o.

The hysteresis ratio (HR) is a parameter reflecting viscoelasticity (4) calculated as the ratio of the area within the L-T loop to the area beneath the loading curve. These areas are measured by planimetry. The hysteresis ratio is an expression of energy expended and not recovered during relaxation.

Stretched from resting length to a complete break, tissues showed several peaks of force before parting (Fig. 2). Each peak represented partial tearing or fraying of tissue components (collagen, elastin, reticulum, etc.). We defined the extension at which the first peak occurred as λ_i and the force at this point as F_i. The extension at the final peak was termed λF and the force at the final peak as F_f. The force of the highest peak was defined as F_{max}.

RESULTS

Control studies. Immersion of alveolar wall in the buffer solutions without enzymes present caused no significant change in any of the measured parameters for the first 2 h. At the 3rd h a decrease in the peak force (F_i) was observed that averaged 30% (Table I). This change, however, did not significantly alter the maximum extensibility (λ_{max}) nor hysteresis ratio (Table II). Changes in pH in the range of 7.4 to 8.8 had no significant influence on the measured parameters.

Three to five peaks in force occurred before normal tissues broke (Fig. 2). Breaking studies without enzyme digestion were performed in two groups: (*a*) a breaking experiment was performed immediately after immersion in buffer solution at pH 8.1 (I, Table III), and (*b*) a breaking experiment was performed after 3 h immersion in buffer solution (II, Table III). The initial breaking force (F_i) was significantly lower after 3 h immersion in buffer, while other parameters showed insignificant differences.

Trypsin digestion. The peak force required for a given extension diminished progressively with enzyme exposure (Fig. 1, Table I) and the extension required to reach the original peak force (λ_i, Table I) was increased appreciably, i.e., the maximum extension (L_{max}) increased. The variation in breaking force (F_{max}) and breaking strain (λ_F) was very large (Table III) presumably because the tissue cross section varied. Indeed, no significant change in breaking force or breaking strain was found in trypsin digested tissue over that of the control study. The initial slope of the loading curve decreased significantly (Table IV) while maximum extensibility (λ_{max}) varied with enzyme concentration. Using 20–30 units there was an insignificant increase in λ_{max}. The hysteresis ratio was essentially unchanged before and after trypsin digestion with this low concentration of enzyme. The effect upon λ_{max} and the initial slope were related to resting length changes. The alveolar walls from cats of various ages and either sex, exposed to the same enzyme concentrations for 3 h, showed a wide range

TABLE II

Maximum Extensibility (λ_{max}) and Hysteresis Ratio (HR) in Control Tissues and Those Exposed to Enzyme Digestion (Mean±SE)

		Control	Trypsin	Elastase	Hyaluronidase
λ_{max}	Before	2.18±0.08	1.96±0.09	2.11±0.08	2.05
	3 h	2.25±0.09*	2.11±0.21	1.68±0.10‡	2.18
HR	Before	0.26±0.02	0.30±0.03	0.26±0.02	0.29
	3 h	0.25±0.02	0.33±0.03	0.35±0.05	0.27

* Two out of eight tissues were measured at 2 h immersion.
‡ Significant ($P < 0.05$).

TABLE III

Force and Strain Measured in Breaking Studies Immediately (I) and 3 h (II) after Immersion in Buffer Solution, and after 3 h of Enzyme Digestion (Mean±SE)

Enzyme	I	II	Collagenase	Trypsin	Elastase	Hyaluronidase
λ_i	2.89±0.33	2.39±0.14	2.61±0.15	3.03±0.38	3.61±0.89	2.21
F_i (mg)	51±12.4	14±3.7	2.7±0.73	31±12.3	40±13.1	31
λ_F	3.51±0.21	4.52±0.58	3.08±0.29	4.18±0.67	3.77±0.35	3.29
F_f	59±15.5	44±11.6	2.9±0.78	33±11.2	37±12.0	37
F_{max} (mg)	65±12.2	51±11.0	3.5±1.20	48±13.5	41±13.0	50

of effects on L_o. Tissues from three lungs showed L_o to increase from 18 to 48% over initial length, while the remainder had no change. In those tissues with L_o changes, λ_{max} was reduced while in the others it tended to increase. Similarly, HR was higher in those with L_o changes with no net effect considering all tissues. A change in the resting length of alveolar wall was obtained consistently by exposure to a greater enzyme concentration (80 + units). These tissues with a change in L_o showed a fall in λ_{max} and an increase in HR.

Collagenase digestion. Peak force diminished following exposure to collagenase (Fig. 2, Table I) and unlike trypsin digested alveolar walls, the breaking force was markedly reduced (Table III). No λ_{max} could be estimated because tissues parted at such low forces. The initial slope decreased markedly (Table IV). Resting length did not change with the exception of one tissue in which L_o increased by 38% with 3 h of digestion.

Elastase digestion. The peak force diminished and the extension necessary to reach the initial force increased in elastase digested tissue, i.e. L_{max} increased (Table I, Fig. 2). The breaking forces and strains were not significantly different from control values (Table III). Elastase treated tissue breaks with a single peak in most instances so that λ_i and λ_f are similar (Fig. 2). Only two elastase-treated tissues had peaks, which explains the slight difference between the average λ_i and λ_f (Table III). The λ_{max} diminished significantly. The initial slope of the length-tension curve was zero because resting length increased appreciably in all tissues (Fig. 3). The fall in λ_{max} was significantly related to an increase in L_o ($r = -0.65$). HR was appreciably greater in those tissues having the larger L_o changes when the original strain was not exceeded (Table II). Following any given period of elastase digestion, there was a marked increase in stress relaxation, i.e. peak force fell more rapidly than seen on the control curve so that the length-tension cycle changed its shape. Although the change in L_o varied considerably between different lungs, a linear increase occurred in the average L_o with duration of digestion from 17±4.5% at 30 min to 44±9.8% after 3 h digestion. These tissues were stretched to a fixed length each time. If tissue was stretched to a fixed force which increased the strain, the increment in L_o increased from 17±4.7% at 30 min to 90±27.5% in 2 h.

Hyaluronidase digestion. Alveolar walls exposed to hyaluronidase showed no change in peak force or the strain necessary to reach peak force (Table I). The initial slope was unchanged (Table IV) as was the hysteresis ratio and maximum extensibility (Table II). Breaking force and resting length were similar to those in control tissues (Table III).

DISCUSSION

One segment of the population lacks protease inhibitors in the serum and often develops emphysema with DOPS[1] (8). This has prompted the suggestion that protease digestion may be one mechanism producing obstructive pulmonary syndromes. Animal models have often had anatomic emphysema as their goal (9–11) without consideration of the functional change that brings the patient to the clinic. Recently, emphysema has been pro-

TABLE IV

Initial Slope of the L-T Curve (Mean±SE) before and after 0.5–3 h of Enzyme Digestion Expressed as the Percent of Control Value

	Control	Collagenase	Trypsin	Hyaluronidase
Slope* (g/cm²/ϵ)	134±15.7	84±17.9	117±9.6	108
½ h	102±5.5	79±7.2	56±10.5	100
1 h	105±4.5	73±10.2	45±12.0	104
2 h	107±3.1	49±16.2	40±10.9	97
3 h	96±1.1	33±18.3	36±10.1	95

* Slope is calculated from dissecting scope dimensions of the tissue (~1,000 μm²).

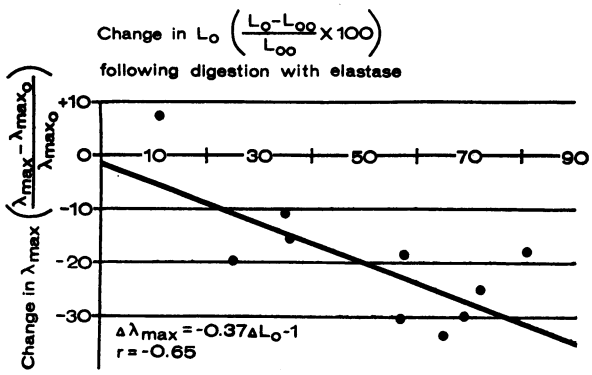

FIGURE 3 The relationship between λ_{max} and resting tissue length (L_o) with elastolysis of alveolar wall. Those tissues stretched to a constant strain for 3 h and also those stretched to a constant force are included (L_o is the resting length following elastin digestion, L_{oo} that before digestion).

duced with considerable lung destruction accompanied by rather mild changes in the mechanical properties of these lungs (12, 13). Lung tissue studies suggest that changes in the properties of alveolar wall are responsible for the functional abnormalities so that DOPS1 may be present with normal gross pulmonary architecture (3). The studies described here are aimed at producing change in the tissue properties found in DOPS1, i.e. a decrease in λ_{max} through an increase in L_o without changing the viscoelastic properties as represented by the hysteresis ratio (3, 4). In vitro, L_o can be increased and λ_{max} decreased by elastase and trypsin digestion when the duration of exposure and the enzyme concentration are suitably balanced. These changes are not accompanied by any change in the structure of the alveolar wall as observed in the tissue bath through the dissecting microscope (40 ×). Elastase produces changes in L_o more consistently, and in lower concentrations, than does trypsin. The response to either enzyme varies, however, from lung to lung, even though time and concentration factors are held constant.

Factors responsible for this variable susceptibility of tissue to enzymatic digestion are suggested in the work of others. Trypsin, for instance, has been reported as having no effect upon native collagen or pure elastin (14). Balo and Banga (15) report no effect of this enzyme on arterial wall. The exposure of alveolar wall to low concentrations of trypsin in this study, however, resulted in a progressive loss of peak force for a given extension and an increase in L_{max} (Table I). Trypsin digestion of lung parenchyma caused a significant decrease in the initial slope of the loading curve (Table IV) with little change in λ_{max} (Table II). This warranted examination of other tissues and pericardium was selected because it is structurally well organized and has a high scleroprotein content. The collagen content of whole lung is reported as being 5.9 to 27.5% by weight (16) and parenchyma should have considerably less than the bronchi, vessels, and connective tissue structures. Pericardium is principally collagen and elastin with little cellular material. Collagen fibers in pericardium are oriented in layers and so curled that L_{max} can be predicted from tissue sections cut in the right plane by calculating the maximum length of the straightened fibers.[2] In alveolar wall on the other hand, collagen and elastin bundles are randomly distributed. Several fragments of pericardium were exposed to different amounts of trypsin (< 100 U $\times$ 3 h) in a tissue bath and no change was found in L_{max} at a given force. The initial slope did show a change. Increased enzyme concentrations (100–500 U) produced changes in L_{max} as well as slope. Quantitative differences remained in pericardium exposed to trypsin however, as an increase in extension of only 8% was required to reach a given force on the control curve while lung had to be extended 55% over the original extension to reach a similar level (Fig. 2). It is apparent that trypsin does affect those properties of tissue usually ascribed to collagen and elastin. Trypsin acts on the peptide bond between the amino acid and the carboxy group of lysine or arginine, both of which are present in elastin and collagen. Biochemical evidence, suggests that the main structural features of these scleroproteins are not hydrolyzed by trypsin (14, 15). Extrahelix peptide appendages, or telopeptides, are susceptible to digestion however, and these may form many of the intra- and intermolecular cross-linkages of collagen (17). The changes in L_o and the early slope of the length-tension curve suggest elastin is altered as well.

The variation in tissue response to enzyme digestion may be a function of fiber chemistry, the penetration of enzyme, or the total amount of material to be digested. We used pieces of pericardium similar in size to those of alveolar wall, but the total amount of material that digested in the former is considerably greater because of fiber density. Lung parenchyma with little scleroprotein and more ground substance was more susceptible to trypsin digestion than pericardium that has a preponderance of extracellular protein. That there is a difference in protease digestion because of the number and type of inter- and intra-molecular cross-linkages has been suggested by the work of others (18–20).

Another difference in the effect of enzymes on alveolar walls when compared with other tissues was found when they were exposed to "hyaluronidase." The group of enzymes marketed under this name deplymerize the mucopolysaccharides (hyaluronic and chondroitin sulfuric acids) of ground substance. When applied to ligamentum nuchi, hyaluronidase reduced the work of extension (21). Others report hyaluronic acid acting as a lubricant for connective tissue (22). No such effect occurred on alveolar wall, however, and in fact, the hysteresis ratio

[2] C. J. Martin. Unpublished observations.

tended to decrease when it should have increased if the lubricant were diminished and more internal friction developed. Even with very high hyaluronidase concentrations, however, we were not able to show that the acidic mucopolysaccharides were important to the mechanical properties of lung.

Evidence presented here shows that exposure to elastase or trypsin alone cannot be responsible for the tissue changes seen in DOPS$_I$. The larger changes in L_o following elastase digestion are invariably accompanied by an increased loss of energy in length-tension cycling, i.e. an increase in HR (Table II). The HR of alveolar walls is not increased, however, in the lungs of the aged or those with DOPS$_I$ (4). L_o can be changed without altering HR measurably by balancing time and enzyme concentration. A 20% increase in L_o may be obtained without an increase in HR. A 20% increase in L_o with elastase digestion, however, does not cause a significant decrease in λ_{max} in these tissues (Fig. 3) because L_{max} increases as well. That is, it will not reduce a normal λ_{max} of more than 2.2 below 1.6 as found in DOPS$_I$.

Even more important to the enzymatic mechanism however, is the relationship of L_o to λ_{max} (Fig. 3). It is necessary to increase L_o by 80% to decrease λ_{max} to the level seen in parenchyma from patients with DOPS$_I$ ($\lambda_{max} < 1.6$). This large change in the linear dimension would increase volume by almost six times. The lung tissue is principally responsible for elastic recoil on the deflation curve at mid-lung volumes and below. Elastic recoil becomes zero at the resting tissue length which is presumably the minimal lung volume. If we assume this to be near residual volume (1.5 l) elastic recoil would be near zero at six times this volume (9 l) when this lung has DOPS$_I$. These are clearly excessive volumes so that with these naturally occurring enzymes we cannot produce an appropriate change in λ_{max} without L_o becoming too large. This occurs because of a concomitant increase in L_{max} with enzymatic digestion.

Several reasons exist for believing that enzyme digestion is not the sole factor producing the change in lung properties occurring with age or in DOPS$_I$. If the alteration in properties follows a loss of tissue (emphysema), a loss of elastin or collagen content would be expected. Yet the elastin and collagen content as well as the ratio of collagen to elastin is normal in lungs with anatomical emphysema (23). Elastin may be decreased, however, in the panacinar emphysema accompanying the homozygous alpha$_1$-antitrypsin deficiency (24). In vitro, elastolysis does not produce emphysema. Johanson and Pierce (25) have reported that elastase administered via the airways to excised rat lungs caused enlargement of centrilobular spaces without apparent destruction of tissue. In the studies reported here, proteolysis with elastase or trypsin did not destroy alveolar walls. One might expect to evoke changes in these studies of excised tissue when the amount of serum and sputum inhibitors are reduced, that would not occur in vivo. Such generous amounts of these inhibitors are available in normal man that to envision a source of proteases in sufficient quantity to reduce elastin content is difficult. Furthermore, unlike in vitro experiments this digestion must continue or some interference with repair be present to produce and maintain the tissue damage (26). Such repair might in itself produce a change in tissue properties.

The objection to elastase (trypsin) digestion as a mechanism producing DOPS$_I$ based on scleroprotein content is obviated if qualitative changes occur. Lungs with emphysema are reported to show a reduction in the ratio of nonpolar amino acids in elastin (27), although the functional state of these lungs is unknown. Elastolysis of ligamentum nuchae has been shown to alter the modulus of elasticity with very small loss in total protein (28). A similar change in lung elastin with proteolysis, however, would not remove the objections to this model based on the tissue properties reported here.

This poses the question, "Which scleroprotein is responsible for the changes in L_o and λ_{max}?" Several years ago, Burton (29) proposed a model of tissue properties to explain the elastic behavior of blood vessels. He pictured curled collagen fibers interlaced with elastic fibers, the early linear part of the length-tension curve reflecting the properties of elastin and the steep rise in force at greater extensions reflecting the properties of collagen. This model fit length-tension properties of skin (30) and blood vessels (31) and the change in these properties with enzyme digestion. Setnikar (32) and Mead (33) applied this concept to normal lung. In DOPS$_I$ this would see the increase in L_o without a change in L_{max} as abnormal elastin.

The major effect of the enzymes used fit the model well. In lung, collagenase effectively removes the "mechanical stop" and leaves a weak and easily fractured elastin (Fig. 2). Elastase moves L_o and changes the slope of the early part of the loading curve.

The parabolic extension curve, the effect of collagen upon the early extension and the change in "yield" of the loading curve when overextended with and without enzyme digestion (Fig. 2), suggests that collagen and elastin are not independent fiber systems. On the control parabolic extension curve some fibers approach a limit to extension and yield, or a fall in force occurs, presumably due to fracture. Beyond this, a second "yield" is suggested with even a third and fourth before the tissue finally parts. Possibly, elastin is involved in

these "yield" points, because after exposure to elastase the curve is smooth and few tissues show more than one "yield" at extensions up to the breaking point. Furthermore, the elastin material that remains after purified collagenase digestion, does not generate the force seen on control curves up to extensions of 200% (Fig. 1). This part of the curve may be explained better by a network of fibers with intermolecular bonds. Such bonds may be between like molecules or may anchor elastin and collagen. Since the specificity of these enzymes is poor, this must remain speculation.

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