Simulation of Lung Tissue Properties

in Age and Irreversible Obstructive

Syndromes Using an Aldehyde

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ABSTRACT Weak solutions of CHOH alter tissue properties, probably by forming intermolecular cross-linkages. The maximum length (L_{max}) to which alveolar wall can be extended is reduced. If exposed to CHOH while extended, the resting length (L_0) of alveolar wall increases. Maximum extensibility ($\lambda_{max} = L_{max}/L_0$) decreases. Similar changes are found in the alveolar wall of man with aging and are significantly more marked in patients with irreversible obstructive pulmonary syndromes. A reduction in the energy loss of the length-tension cycle (hysteresis) was seen after exposure to CHOH, however, that does not occur with age or in obstructive syndromes. Because an exposure of alveolar wall to elastase increases L_0 and hysteresis, we used a staged exposure to CHOH followed by elastase. Tissue suitably prepared by exposure to CHOH followed by elastolysis better simulates the tissue changes of age and irreversible obstructive syndromes.

INTRODUCTION

The mechanical properties of mammalian tissue change with age, becoming less extensible and more rigid. This change varies from tissue to tissue depending upon the exposure to injurious agents, tissue susceptibility, chemistry of the scleroproteins, and their geometry. In the alveolar wall of man there is a progressive fall in maximum extensibility ($\lambda_{max} = L_{max}/L_0$)[1] with age (1), while the viscoelastic properties measured as the energy loss in stretching the alveolar wall are unaltered

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[1] *Abbreviations used in this paper:* DOPS$_1$, irreversible diffuse obstructive pulmonary syndromes; ϵ_1, ϵ_2, ϵ_3, ϵ_4, strains corresponding to specific forces on the control curve; HR, hysteresis ratio, the area of the L-T loop divided by total area beneath the extension curve; L_0, initial resting length, i.e., before treatment with CHOH; L_{max}, maximum length predicted; L_t, resting length of tissue after treatment with CHOH; L-T, length-tension; $\lambda_{max} = L_{max}/L_0$.

with age (2). Similar changes in the mechanical properties of lung parenchyma are found in irreversible obstructive pulmonary syndromes (DOPS$_1$) where they are significantly more marked. The decrease in λ_{max} is probably due to an increase in L_0 in these conditions. An increase in L_0 is compatible with the increased lung volume, loss of elastic recoil, fall in transpulmonary pressure, and reduced expiratory flow that accompany aging and are exaggerated in DOPS$_1$.

What changes L_0? Overextension of the lung has long been suspected of altering tissue properties. The attempts to produce clinical emphysema by using this mechanism have been reviewed by Strawbridge (3). Many materials, when overextended, demonstrate "yield" after which the tissue has a permanent set or an increased resting length. The lung, however, is not a homogeneous material and yield may take place without permanent set (2). A second possibility is to change L_0 by destroying the responsible tissue component, presumably elastin. The exposure of lung tissue to proteolytic enzymes that alter the properties of elastin should mimic the clinical condition in which there is an alpha$_1$-antitrypsin deficiency (4). Proteolysis of alveolar wall in vitro, however, does not quantitatively or qualitatively produce the tissue properties seen in the lungs of the aged or those with DOPS$_1$ (5).

Bjorksten (6) described aging of connective tissue as in vivo tanning by cross-linking agents normally present in the tissue. An increase in intermolecular cross-linkages that limit the freedom of fiber motion might be expected to alter relative extension (λ_{max}). Reported here are studies on the properties of alveolar walls exposed to very low concentrations of formaldehyde (CHOH). This well-known tanning agent produces methylene cross-linkages in scleroproteins (7). Several authors have described similarities between aged collagen and collagen treated with CHOH (7, 8).
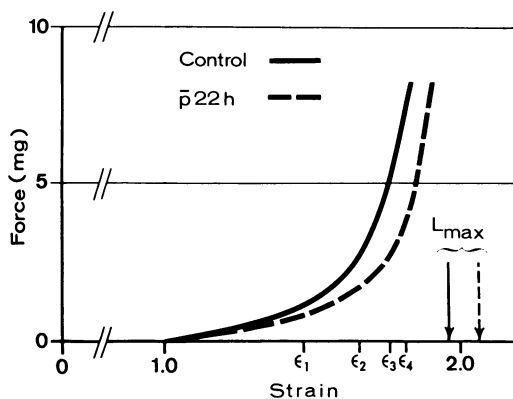
FIGURE 1 The L-T relationship (extension only) of alveolar wall after exposure to buffer solution (Bicine, pH 7.8) for 15 min and 22 h. Six tissues treated in this manner showed similar changes. ϵ_1, ϵ_2, ϵ_3, ϵ_4 represent the strain in this wall which produced a force of 1, 3, 5, and 7 mg, respectively (see text).

METHODS

The alveolar wall of cat lung was used in these studies because the mechanical properties of this tissue are similar to those in man. Alveolar wall was isolated ($\sim 200 \times 30 \times 30$ μm) and suspended between clamps in a measuring bath (0.1 liter) filled with buffer solution (0.2 M Bicine [Sigma Chemical Co., St. Louis, Mo.] at pH 7.8).

The upper tissue clamp was attached to a force transducer (Sanborn FTA 1-1, Hewlett-Packard Co., Palo Alto, Calif.) whose compliance (0.2 μm/mg) was neglected. The lower clamp was attached to a piston protruding through the bottom of the bath. The piston was driven hydraulically by a Wills manipulator. The movement of the piston and lower clamp 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 a small strain which was gradually increased to that strain produced by a force of 7 mg ($\pm$). Strain was expressed as multiples of the resting length. The length-tension (L-T) curves were repeated at this strain six times. Controls were selected from the third to sixth cycles.

CHOH was then added to the measuring chamber and L-T curves repeated at 0.5, 1, 2, 3, 5, and 22 h. Studies performed at room temperature and pH 7.8 were not different from those performed at 37°C and a pH of 7.4. CHOH concentrations ranged from 0.019 to 0.15%. The tissue strain during CHOH exposure varied. Some tissues were held at their resting length, while others were held at a strain equal to that produced on the control curve by applying 2 or 6 mg of force. In this manner each tissue determined the strain at which it was exposed to CHOH.

The rate of extension was constant in all studies (10 μm/s). The final strain for each tissue was the same in all L-T cycles to 22 h, after which the tissue was stretched to a complete break. All extensions started at the resting length (L_0) of the control cycle or that length at which force first increased with extension. Thus, if the tissue was exposed to CHOH at its resting length all L-T cycles began at this strain. If the tissue was held in a stretched state during exposure to CHOH it was released to its original resting length and L-T cycling followed without inter-

val. After each L-T measurement the tissue was returned to the same strain for further exposure.

Elastase (25 U [$\pm$], Worthington Biochemical Corp., Freehold, N. J.) was added to the measuring bath (0.1 liter Bicine at pH 7.8) 1-3 h after the CHOH exposure of several tissues. L-T cycles were repeated with calculations following the same format as that used on the CHOH-treated tissues.

Calculations. From the L-T curve we measured:

(a) Force at several fixed strains. We selected four strains from the control curve as those lengths corresponding to a force of 1, 3, 5, and 7 mg (Fig. 1, ϵ_1, ϵ_2, ϵ_3, ϵ_4). Force was measured at these strains on all subsequent curves.

(b) Resting length (L_0) was measured directly.

(c) With extension, force increases in a hyperbolic fashion. The vertical asymptote (L_{max}) expressed as a multiple of L_0 ($\lambda_{max} = L_{max}/L_0$) was predicted as previously reported (1).

(d) Hysteresis ratio (HR); the loading and unloading curves are not identical but show hysteresis. The ratio of the area within this loop to the area beneath the loading curve or the HR has been described elsewhere (2). HR has been shown to be independent of strain, age, and lung disease in man.

(e) Breaking force is the maximum force recorded, i.e., the force of the highest peak on the L-T curve.

(f) Breaking strain is the strain at which the tissue separates in two. The breaking force may not occur at the breaking strain if a peak force appears before the greatest strain. These strains were calculated from the L_0 measured in the control study.

Mean values and the standard error of the mean are reported. Correlations and regressions were calculated by using common statistical methods (9).

RESULTS

The effect upon the L-T relationship of an alveolar wall in buffer solution, with and without CHOH added, is shown in Figs. 1-3. Each figure is representative of the findings in six or more studies under the same conditions. Similarly, the data points in Figs. 4 and 5 repre-
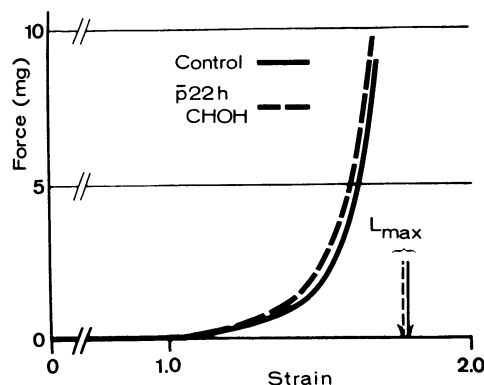

FIGURE 2 The L-T relationship of one alveolar wall before and after exposure to 0.019% CHOH for 22 h at resting length. These data are similar to six other studies using the same time and concentration factors.

sent the average of six or more studies under the same conditions.

(a) Changes in force with strain. The changes in force with increasing strain, with and without exposure to CHOH, are represented in Figs. 1–3.

Tissues immersed in buffer solution without CHOH show force to progressively decrease with time for all strains (Fig. 1). The length at which the tissue was held in the buffer solution affected the magnitude of this increase in tissue compliance. When held at the resting length there was a minimal increase in tissue compliance but this was more marked in tissues held at greater strains.

Tissues exposed to CHOH at their resting length required increased force to reach each strain (Fig. 2). The increase in force was proportional to the original value at that strain, i.e., a greater increase at higher forces. Tissues exposed to CHOH while being held in extension (strain at 2 and 6 mg) required less force to reach strains smaller than that at which they were exposed (Fig. 3), i.e., tissue held at the smaller strain (2 mg) required less force at strains less than this, but greater forces at higher strains (Fig. 3). Tissues exposed at a strain equal to 6 mg on the control curve required smaller forces to reach all strains below this (not shown). These L-T changes with exposure to CHOH took place within the first 3 h for the most part, with little change thereafter to 22 h.

(b) L_0 increased with CHOH exposure when the tissue was at strain (Fig. 3). The increase in L_0 was related to a decrease in maximum extensibility (λ_{max}).

(c) Changes in maximum extensibility

$$\left(\frac{\lambda_{max} - \lambda_{max_0}}{\lambda_{max_0} - 1} \times 100\right).$$
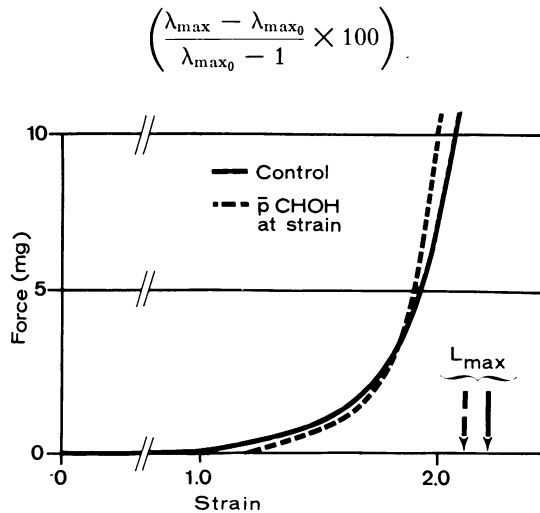

FIGURE 3 The L-T relationship of one alveolar wall before and after exposure to 0.019% CHOH while at strain (see text) for 22 h. This is representative of the changes in eight walls exposed to this concentration for this time period.
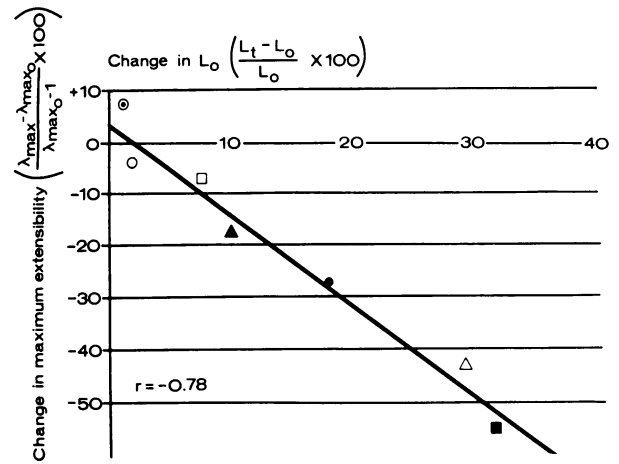

FIGURE 4 The change in resting length, the change in λ_{max}, the concentration of CHOH, and extension during exposure were related (see text). ⊙, control; ○, low concentration CHOH at L_0; □, high concentration CHOH at L_0; ▲, low CHOH at 2 mg extension; ●, low CHOH at 6 mg extension; △, high CHOH at 2 mg extension; ■, high CHOH at 6 mg extension.

were related to changes in resting length

$$\left(\frac{L_t - L_0}{L_0} \times 100\right)$$

where L_t and λ_{max} are the resting length and λ_{max} after treatment and L_0 and λ_{max_0} are resting length and λ_{max} before treatment (Fig. 4). A decrease in λ_{max} accompanied an increase in L_t ($r = -0.78$) with the intercept nearly at zero. The ordered relationships between L_0 and λ_{max} show both parameters to be a function of the strain during exposure and the concentration of CHOH (Fig. 4).

Three tissues out of six in the control studies showed an increase in resting length after 22 h immersion in
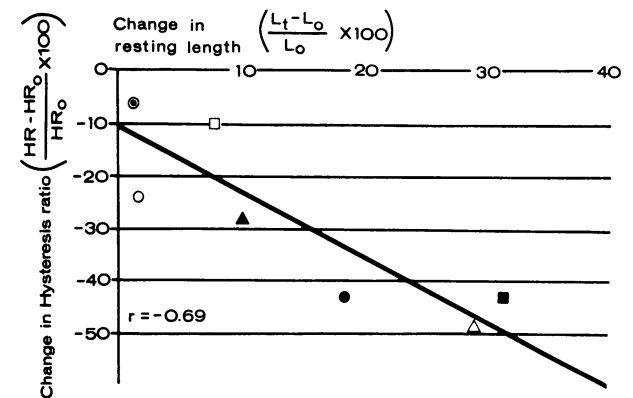

FIGURE 5 The increase in resting length with exposure to CHOH at strain resulted in a progressive decrease in the energy loss with cycling (HR). Symbols have the same meaning as in Fig. 4.

TABLE I

Breaking Strain, Expressed as the Multiple of Resting Length, and Breaking Force (mg) in CHOH-Exposed Tissues

	Force producing the strain	Breaking strain (±SE)	Breaking force (±SE)
	mg		
Control	0	5.12±0.55	44.6±12.7
CHOH	0	2.43±0.07	56±6.0
	2	3.16±0.56	43.4±8.2
	6	2.65±0.15	57.3±12.0

The strain at which the tissues were held during exposure was produced on the control curve by 0, 2, and 6 mg force.

Bicine. The average increment was 1.3% (Fig. 4), and in no instance was the change in resting length more than 10 μm.

(d) HR decreased significantly with time in tissues exposed to CHOH. The decrease in HR was progressively greater and more rapid in tissues held at increased strain between measurements (Fig. 5). Control studies showed little change with time. This decline was significantly related to the decrease in λ_{max} ($r = 0.77$) and increase in resting length ($r = -0.69$).

(e) Breaking force and (f) breaking strain. Breaking force often was greater in tissues exposed to CHOH than in control tissues but the difference was insignificant (Table I). There was, however, a significant reduction in breaking strain after such exposure. The large variance in breaking strain was reduced with exposure to CHOH. The larger breaking strain and greater standard error seen in those tissues held at a strain equal to 2 mg in CHOH was greatly influenced by one sample with a breaking strain of 5.29. Excluding this tissue the mean was 2.63±0.19 in keeping with the other data. Control studies showed several peaks in force before complete tissue rupture with the first peak about equal to the breaking strain of the CHOH-treated tissue (2.53±0.13).

(g) Effect of elastolysis. After 22 h of treatment (0.019% CHOH) the tissue bath was diluted with 4 vol (±) of Bicine and elastase (Worthington ESFF, 23–27 U) was added in some experiments. This produced quantitative changes in the measured properties. If the tissue was held at its resting length during CHOH treatment followed by 1 h of elastase digestion, there was a large decrease (40–70%) in the force measured at ϵ_4. This was similar to the fall in force (78%) after elastolysis in tissues that had no CHOH exposure. Those tissues that were held at some strain during CHOH exposure showed more resistance to elastolysis. For example, alveolar wall held at a strain equal to 6 mg of force during exposure to CHOH had a reduction in force of only 6% at ϵ_4 after elastolysis for 1 h.

Additional tissues after control L-T studies of the alveolar wall, were exposed to 0.15% CHOH for 60 min while extended approximately 1.7 times L_0. The CHOH was flushed from the bath by dilution and elastase (25–30 U, Worthington ESFF) was added. As reported above, tissues exposed to CHOH while extended showed an increase in L_0 and a fall in λ_{max} and the HR (Fig. 6). Peak force at similar extensions rose. After elastase exposure, there was a further increase in L_0, a fall in λ_{max}, an increase in HR, and a decrease in peak force.

DISCUSSION

In this study we explore a means of simulating the L-T changes in the alveolar wall of man that accompany age and DOPS₁. Increasing the cross-linkage of scleroproteins in the tissue should limit their freedom of motion and alter the properties of the tissue. Several tissue changes have been reported to accompany increased cross-linkage, increased thermal shrinkage (10), decreased swelling in acids (11), increased tensile strength (12), resistance to proteolysis (13), and altered solubility in weak acid (14). Such changes have been observed in collagen from aged individuals as well as youthful collagen in which cross-linkage has been induced or stabilized with sodium borohydride (12, 15, 16) or CHOH (8, 10).

The mechanical properties of lung and other tissues have been pictured structurally as relatively inextensible, curled collagen fibers, with the curls maintained by elastin fibers (17, 18). Collagen provides the mechanical stop for tissues, the tensile strength, the thermal shrinkage, etc. Elastin is responsible for L_0 (5, 17) and the early more linear portion of the L-T curve. Age- and DOPS₁-related changes in L_0 of alveolar wall would be
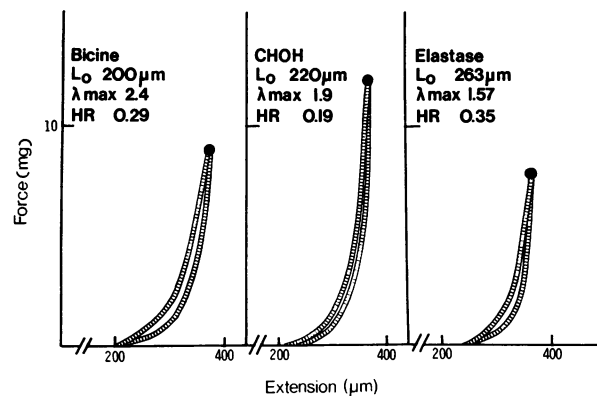

FIGURE 6 Cat, female, 6 mo. The same alveolar wall was successively exposed to buffered Bicine at pH 7.8; 0.15% CHOH for 60 min; 30 U elastase for 30 min. Note the increase in L_0 with CHOH and a further increase with elastase exposure while the HR decreased in CHOH and increased with elastolysis.

a function of elastin in this model. Age changes in other tissues also suggest altered elastin properties, i.e., an increase in resting length. As in lung, the L-T curve for skin is parabolic (19) so that an increase of L_0 along this curve causes the tissue to sag, become furrowed, and less extensible.

The number of cross-linkages in elastin increases with age. Partridge, Elsden, and Thomas (20) described the amino acids, desmosine and isodesmosine, as cross-links in bovine elastin. The content of these amino acids increase during childhood in the aorta and lung (21, 22) although they change little during the adult years where the change in properties of alveolar wall have been described (1). Hall (23) has discussed several other bonds that may also exist in elastin: a calcium complex bond, a lipid-polysaccharide, and a peptide-sugar link. These have not been identified further. Any intermolecular bond that may be formed within the elastin fibers should change the recoil properties of the tissue.

In tissue metabolism, aldehydes form Schiff bases that involve the ϵ-amino group of lysine in the cross-linkage of collagen (24, 25). An aldehyde has been described in the synthesis of the desmosine cross-linkages found in elastin which also incorporates lysine (26, 27). Relatively high concentrations of aldehydes are present in cigarette smoke, an agent often incriminated in the production of DOPS$_1$. We have used a weak solution of an aldehyde (CHOH) as a synthetic cross-linking agent in these studies. CHOH reacts with the ϵ-amino group of lysine to produce a methylol group that can react with another amino, primary amide, or guanidyl group (7, 28).

CHOH is commonly used in the fixation of tissues to markedly alter their properties. Such fixation requires a time-dose relationship far in excess of those used in these experiments. The weak solution (0.019%) of CHOH is 1/500th the usual concentration. When this solution is used the tissue response is complete within 3 h rather than the 5-6 days used for routine tissue fixation. Fixation of the tissue results in a shrinkage which can be predicted (29) and this was not seen in alveolar walls exposed to CHOH at their resting length for 22 h (Fig. 2). The extended tissue exposed to CHOH actually showed an increase in resting length rather than a shrinkage (Fig. 3). This increase might occur if the viscosity of the ground substance were altered by the formation of a gel that limited the motion of collagen and elastin fibers. Such a viscous ground substance, however, should increase hysteresis or the energy loss in L-T cycling. Hysteresis actually decreased after exposure to CHOH (Fig. 5). The tissue changes described here, therefore, would seem to be those of cross-linkages formed in the scleroprotein, particularly the elastin.

When alveolar wall in extension is exposed to CHOH there is an increase in resting length, a reduction in maximum extensibility (λ_{max}), and a decrease in the early slope of the L-T curve (Figs. 2 and 3). The change in L_0 when the tissue is exposed under strain is in keeping with lung that is held in extension after the first breath and exposed to a lifetime of cross-linking agents. The changes in L_0 and λ_{max} are similar to those seen in the alveolar wall from aged persons and those with DOPS$_1$. λ_{max} ($\lambda_{max} = L_{max}/L_0$) falls in these conditions and this change is most likely due to an increase in L_0. Quantitatively these changes are reasonable. The alveolar wall of a 70-inch 20-yr-old healthy man can be extended ~ 2.2 times the resting length. As this individual ages and develops DOPS$_1$ we expect a decrease in extensibility to 1.6 times L_0 or an increase in resting length of 40%. Lung volume is proportional to the cube of the linear dimension. Alveolar wall tension approaches zero at 8-10% of the total lung capacity (30, 31). The 20-yr-old with a total lung capacity of 7 liters would have a 0.7-liter lung volume at L_0 ($\pm$). With age and the development of DOPS$_1$, a 40% increase in L_0 would make this volume 1.92 liters, while reducing λ_{max} to 1.6. A loss of elastic recoil at low lung volumes would result. The residual volume increase would be well within that seen in DOPS$_1$. The volume of lung required for the extension during exposure to cross-linking agents is also reasonable. The force of 2 mg used in extending the tissue during exposure to the aldehyde resulted in an extension of about 60% over L_0 (Fig. 1), or it is comparable to holding the lung described above at a volume of 2.87 liters, which is well below the expected functional residual capacity of the young man. Qualitatively, therefore, these volumes, exposures, and tissue lengths can be reasonably attained during life.

The exposure of alveolar wall to CHOH, however, was invariably associated with a fall in HR while increasing L_0 (Fig. 5). The 40% increase in L_0 reduced the energy loss in L-T cycling by more than 50% which was not found in aging tissue or in patients with DOPS$_1$. Qualitatively, the CHOH exposure does not simulate the tissue properties of alveolar wall seen in the aged or DOPS$_1$.

Elastase or trypsin digestion of alveolar walls cause an increase in HR, an appreciable increase in resting length, and a minimal increase in the maximum length (5). On exposure to CHOH there is a decrease in hysteresis, and L_0 increases while the maximum length changes but little. Neither proteolysis nor the probable formation of cross-linkages completely simulate the change in tissue properties seen in aging and DOPS$_1$. A better model may be a staged exposure of alveolar wall to CHOH, followed by proteolysis with elastase (Fig. 6). The decrease in hysteresis upon exposure to

CHOH is counteracted by the increase in HR with proteolysis. Both increase L_0. A lung suitably prepared by age with cross-linking that is exposed to proteolytic action appears to fit the clinical state better.

The effect of altering the cross-linkage of tissue upon lung properties has been reported by others (32, 33). Inhibition of cross-linkage (BAPN, penicillamine) in young rodents has resulted in reduced lung elastic recoil and greater total lung volume in experimental animals when compared with untreated controls. Inhibition of cross-linkages may be more easily accomplished in fetal and early extrauterine life (21, 25) than in mature tissues where the turnover of the scleroproteins is less rapid (21, 34).

Exposure of alveolar walls to CHOH alters those properties of tissue usually attributed to collagen. We found no increase in tensile strength or breaking force (Table I) as has been reported in rat tail tendon exposed to CHOH (8). There was, however, a significant reduction in breaking strain. The mechanical stop was shortened. The failure to alter tensile strength may be due to the geometry of these fibers in alveolar wall, i.e., the fibers are small with few molecules involved and randomly distributed within the wall. The reduction in breaking strain was more marked with greater orientation of the fibers, i.e., in tissues held at strain during exposure (Figs. 2 and 3). The reduction in the mechanical stop, however, was less effective in reducing λ_{max} than the change in L_0 (Fig. 4). The changes in the mechanical properties usually ascribed to elastin were altered after CHOH exposure, namely the resting length, and the compliance of the early part of the L-T curve. Ciferri and Ragjagh (35) have pointed out that in a well organized fiber system of cross-linked, oriented chain molecules (collagen or elastin) the force of retraction should be independent of the degree of cross-linking. A progressive increase in L_0 with the extent of cross-linkage has been observed in such oriented polymer systems. We did not appreciably affect the force of retraction and did not change L_0 in alveolar wall exposed at resting length to CHOH in keeping with a random distribution of molecules within the elastin fibers of the alveolar wall. That L_0 increased when extended tissue was exposed to CHOH may be due to greater orientation of these fibers with extension.

ACKNOWLEDGMENTS

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