Effects of a molecular change in collagen on lung structure and mechanical function.

N N Stanley, … , N S Cherniack, N A Kefalides

J Clin Invest. 1975;55(6):1195-1201. https://doi.org/10.1172/JCI108037.

Research Article

Semicarbazide, a lathyrogen, was given to growing rats to elucidate the consequences of altering the molecular structure of fibrous proteins within the lung. Static pressure-volume (P-V) measurements during deflation of saline-filled lungs showed normal recoil pressure and compliance values within the physiological range of lung volume. Quasi-static P-V measurements were also normal during slow reinflation, even beyond physiological limits to a recoil pressure of 20 cm H20. However, the lungs of experimental rats ruptured at much lower recoil pressures than controls. Histology was normal in lungs fixed at 20 cm H20. In contrast, lungs showed dilation of terminal air spaces, rupture of alveolar walls, and an increase in mean linear intercept in experimental compared with control specimens, when fixed at 30 cm H20. Biochemical analyses revealed reduced cross-linking of lung collagen without change in its total content. There were no detectable changes in the quantity or quality of lung elastin. It is concluded that semicarbazide may selectively impair the maturation of lung collagen and that immaturity of lung collagen is associated with a reduction in the tensile strength of lung tissue, without changes in elasticity within physiological volume limits.

Effects of a Molecular Change in Collagen on Lung Structure and Mechanical Function

N. N. STANLEY, R. ALPER, E. L. CUNNINGHAM, N. S. CHERNIACK, and N. A. KEFALIDES

From the Departments of Medicine and Biochemistry, University of Pennsylvania, and the Philadelphia General Hospital, Philadelphia, Pennsylvania 19104

ABSTRACT Semicarbazide, a lathyrogen, was given to growing rats to elucidate the consequences of altering the molecular structure of fibrous proteins within the lung. Static pressure-volume (P-V) measurements during deflation of saline-filled lungs showed normal recoil pressure and compliance values within the physiological range of lung volume. Quasi-static P-V measurements were also normal during slow reinflation, even beyond physiological limits to a recoil pressure of 20 cm H_2O. However, the lungs of experimental rats ruptured at much lower recoil pressures than controls. Histology was normal in lungs fixed at 20 cm H_2O. In contrast, lungs showed dilatation of terminal air spaces, rupture of alveolar walls, and an increase in mean linear intercept in experimental compared with control specimens, when fixed at 30 cm H_2O. Biochemical analyses revealed reduced cross-linking of lung collagen without change in its total content. There were no detectable changes in the quantity or quality of lung elastin. It is concluded that semicarbazide may selectively impair the maturation of lung collagen and that immaturity of lung collagen is associated with a reduction in the tensile strength of lung tissue, without changes in elasticity within physiological volume limits.

INTRODUCTION

The structural stability and elastic properties of lung tissue are largely determined by the collagen and elastin fibers in its connective tissue framework. The separate functions of these fibrous proteins have been deduced from histological studies of their arrangement in the lung parenchyma (1–3) and from the mechanical behavior of elastin and collagen fibers dissected out from other tissues (4, 5). Further knowledge has been gained by studying the effects of elastolytic and collagenolytic enzymes on lung structure and function (6–8). A different approach has been to give animals in a period of lung growth agents that inhibit the normal biosynthesis of collagen and elastin (9–11).

The mechanical properties of the fibrous proteins depend on the formation of intermolecular and intramolecular cross-linkages. These arise through the extracellular condensation of lysine or hydroxylysine residues on one polypeptide chain with lysine- or hydroxylysine-derived aldehydes on a different chain to form aldimine or aldol-type conjugates, as in collagen, or desmosine and isodesmosine, as in elastin (12). Semicarbazide (SC)[1] is one of several compounds, called lathyrogens, capable of interfering with the cross-linking process. Lathyrogens exert their effect through the inhibition of lysyl oxidase, the enzyme that converts lysine or hydroxylysine to the respective aldehyde, by reacting with preformed aldehydes and thus blocking cross-linkage formation, by disrupting preexisting aldimine cross-linkages, or though combinations of these (12–14). The net result is that the affected connective tissue is more friable, particularly in rapidly growing animals. The collagen may become more easily extracted and contain fewer cross-linked components and the elastin may have a lower content of desmosine and isodesmosine.

The present study is concerned with the effects on lung tissue produced by giving SC to growing rats. Their lungs were examined by mechanical, histological, and biochemical techniques. It is of special interest that SC was found to inhibit the cross-linking of collagen while producing no detectable abnormality in elastin maturation. This enabled us to characterize the struc-

Received for publication 6 November 1974 and in revised form 6 February 1975.

[1] *Abbreviations used in this paper:* Lm, mean linear intercept; P-V, pressure-volume; SC, semicarbazide.

tural and functional consequences of a selective defect at the molecular level in lung collagen.

METHODS

Male weanling rats (Sprague-Dawley), weighing 40–60 g, were divided into two groups. An experimental group was fed a diet containing 0.1% by weight of SC, and a control group was given the same diet without the drug. In preliminary experiments it was found that this was the maximum dose of SC compatible with normal growth in a reasonable proportion (> 50%) of treated animals. However, even at this low dosage there was evidence of a connective tissue disorder, since approximately 15% of the animals developed kyphoscoliosis. The rats in each group were sacrificed after 4–6 wk and experiments were limited to those without kyphoscoliosis whose body weight had risen to the range of 235–265 g. This policy was adopted to avoid the problem of comparing experimental data obtained in animals with different lung growth. From each group, 10 rats were used for mechanical and biochemical studies and another 20 were used for histology.

Experimental procedure. Each rat was anesthetized with sodium phenobarbital (40 mg/kg) and was given oxygen to breathe through a miniature face mask. 3 min later, the trachea was exposed and clamped in the neck, the chest was opened, and the lungs gradually collapsed due to adsorption atelectasis. After exsanguination via the abdominal aorta, the thoracic organs were carefully removed from the chest. The esophagus, cardiac ventricles, and aorta were dissected away, but to minimize damage due to handling, no further attempt was made to separate the lungs from the remaining vascular tissue for histology.

Physical characterization. During the initial period of anesthesia, the rats were examined for skeletal deformities and their body weights and nose-tail lengths were measured. The excised lungs were weighed together with the residual vascular attachments. Since the studies were restricted to animals without stunted growth or skeletal deformity, mechanical tests were performed on samples of skin and aorta to establish that SC administration had been effective in weakening the connective tissue of the experimental animals. Cylinders of thoracic aorta and of skin from the hind leg were cut to 5-mm lengths. These were looped around a pair of parallel rods, which were slowly pulled apart until the tissue ruptured. The load exerted at the breaking point was recorded with a force transducer (FT 03 B, Grass Instrument Co., Quincy, Mass.), placed in series with the system.

Lung mechanics. These were assessed by measuring the pressure-volume (P-V) relationship in saline-filled lungs. The excised lungs were immersed in a bath with a large surface area. The trachea was intubated with a short plastic cannula connected via a T-tube to a 30-ml syringe; the syringe and tubing had previously been filled with saline. The third limb of the T-tube was connected to a pressure transducer (P 23 Db, Statham Instruments, Inc., Los Angeles, Calif.). The syringe was placed in a constant infusion pump (Model 944, Harvard Apparatus Co., Inc., Millis, Mass.) set to run at 0.5 ml/min. The pressure signal was continuously displayed on a time basis with a strip chart recorder (DR-8, Electronics for Medicine Inc., White Plains, N. Y.). Lung volume changes during infusion or withdrawal of saline from the lungs were assumed to be equal to the volume change of the syringe.

Static P-V relationships were measured after inflation of the lungs from the atelectatic state with 12 ml of saline and then during deflation until the transpulmonary pressure had returned to zero. The pump was stopped at 3-ml volume steps during deflation. The pressure was measured at each of these volumes after 30 s without flow to allow the lungs to undergo complete stress relaxation or recovery. At the end of the deflation run, approximately 2 ml of saline remained trapped within the lungs and the absence of leaks was checked by reweighing the lungs. After this, the lungs were continuously reinflated with saline at 0.5 ml/min until their rupture terminated the rise in the pressure record. An extended quasi-static P-V curve was constructed by measuring the lung volume at 5-cm H_2O increments of transpulmonary pressure. The maximum pressure was also measured as an index of the pulmonary breaking stress. When the lung recoil pressure during continuous inflation was calculated, the resistive pressure gradient between the transducer and the tip of the tracheal cannula (usually 0.4 cm H_2O) was subtracted from the recorded pressure. After the mechanical studies, the lungs from the experimental and control rats were pooled separately and stored at −20°C for subsequent chemical analysis.

Histology. The lungs of another 10 rats in each group were prepared for morphometric measurements by fixation with 10% neutral buffered formaldehyde solution at an inflation pressure of 20 cm H_2O. After fixation, the total volume of the inflated lungs was measured by water displacement. Midcoronal blocks of the left lung were cut and imbedded in paraffin. 5-μm sections were then prepared and stained with hematoxylin and eosin. The mean linear intercept (Lm), i.e., the distance between alveolar surfaces, was determined as an index of alveolar size by the technique described by Dunnill (15). To examine whether higher inflation pressures altered lung structure, the lungs of 10 other rats in each group were also examined by these techniques after fixation at a pressure of 30 cm H_2O.

Biochemistry. Before chemical analysis, the frozen lungs were thawed, the tracheas and upper bronchi were removed, and the lungs were homogenized in distilled water at 4°C in a VirTis 45 homogenizer (VirTis Co., Inc., Gardiner, N. Y.). They were then centrifuged at 10,000 *g* for 20 min and the supernate was discarded. The residual pellet was resuspended in distilled water and subjected to further homogenization and centrifugation. Blood was completely removed from the material by repeating this procedure until the supernate was colorless. The residue was then lyophilized and weighed. A 10-mg sample was hydrolyzed for 24 h in 6 N HCl at 100°C. The hydrolysate was dried in a rotary evaporator and analyzed in triplicate for hydroxyproline as an index of total lung collagen (16). The remaining lyophilized material was suspended in 300 ml of 0.5 M acetic acid and extracted at 4°C for 48 h. This suspension was centrifuged at 20,000 *g* for 30 min in a Sorvall RC 2B centrifuge (Sorvall-Dupont Instruments, Sorvall Operations, Newtown, Conn.). Sodium chloride crystals were added slowly to the supernate until the concentration reached 5%. The precipitate was removed by centrifugation, dissolved in 0.1 M acetic acid, dialyzed with three changes of distilled water, and lyophilized. Hydroxyproline analysis of portions of the material precipitated by sodium chloride provided an index of collagen solubility. Another portion of the precipitated material was dissolved in 0.06 M sodium acetate buffer at pH 4.8, and approximately equal weights from control and experimental groups were subjected to carboxymethylcellulose chromatography on 1 × 15-cm columns by the procedure of Piez, Eigner, and Lewis (17). Elastin was extracted from the residue remaining after acetic acid extraction by the method of

TABLE I

Body Weight, Nose-Tail Length, and Lung Weight of Experimental and Control Rats

	Control	SC
Number of rats	30	30
Body weight, *g*	250.9±1.2	249.1±1.2
Nose-tail length, *cm*	39.9±0.3	39.7±0.3
Lung weight, *g*	1.77±0.02	1.79±0.02

Lansing, Rosenthal, Alex, and Dempsey (18). 500-μg portions of the elastin preparation were hydrolyzed in 6 N HCl at 100°C for 72 h and amino acid analysis was performed by a JEOL Model 6 AH amino acid analyzer (JEOL Analytical Instruments, Cranford, N. J.). Desmosines and isodesmosines were eluted from the long column with a buffer change of 0.35 M sodium citrate at pH 5.21 after the elution of phenylalanine.

RESULTS

Physical characteristics. Table I shows that in addition to equality in body weight, there were no significant differences in nose-tail length or lung weight between the experimental and control groups. The similarity of the lung weights in the two groups indicated that lung growth was unimpaired in rats given SC in a dose that did not stunt body growth.

Aortas and skin. The mechanical tests with cylinders of aorta and skin demonstrated that both tissues were very weak in the experimental rats. The load at the breaking point (breaking load) in the aortic cylinders was only 168±13 g in experimental specimens, compared with 274±24 g in the controls, and in cylinders of skin was only 359±34 g in the experimentals, compared with 977±68 g in controls. Both differences were highly significant ($P < 0.001$). When viewed under the dissecting microscope, the specimens did not differ in length or wall thickness, which showed that they were similar in cross-sectional area. Thus, the low breaking loads in the experimental aortas and skin specimens were due to a reduction in their tensile strength.

TABLE II

Static Lung Recoil Pressures during Deflation of Saline-Filled Lungs

Lung volume	Recoil pressure	
	Control	SC
ml	*cm H_2O*	
12	7.96±0.48	8.07±0.46
9	4.95±0.30	5.02±0.28
6	3.25±0.19	3.30±0.21
3	1.19±0.16	1.22±0.16

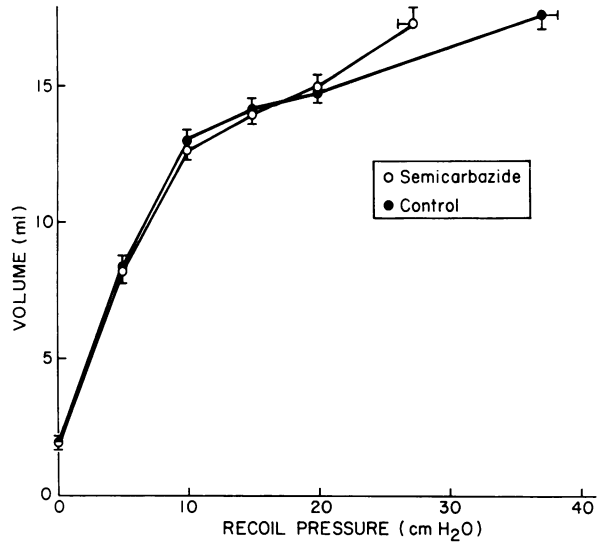

FIGURE 1 Extended quasi-static P-V curves during slow inflation with saline until the lungs ruptured. Data points for control and experimental lungs indicate mean volume at 5-cm H_2O pressure increments up to 20 cm H_2O, and mean volume and recoil pressure at time of rupture. Standard errors of all volume measurements and recoil pressures at time of rupture are indicated by vertical and horizontal bars, respectively. Note that the controls and experimentals are similar in volumes at zero pressure after the previous inflation-deflation run and at each pressure increment up to 20 cm H_2O. However, the experimental lungs are ruptured by a significantly lower recoil pressure than the controls.

Lung mechanics. The static P-V measurements are listed in Table II. The initial inflation with 12 ml of saline resulted in virtually identical recoil pressures of 8.07 and 7.96 cm H_2O in the experimental and control groups, respectively. At this volume the lungs were near their maximum physiological capacity, since preliminary experiments had shown that the lungs of 250-g rats could hold only 13–14 ml before the transpulmonary pressure exceeded 10 cm H_2O during saline inflation or 30 cm H_2O during air inflation. There were no significant differences between the two groups in the static recoil pressures during deflation to lower lung volumes.

TABLE III

Morphometric Data

	Control	SC	*P*
Fixed at 20 cm H_2O			
Total lung volume, *ml*	12.3±0.4	12.3±0.4	NS
Lm, mm^{-3}	55.5±2.2	56.2±2.5	NS
Fixed at 30 cm H_2O			
Total lung volume, *ml*	13.4±0.5	15.7±0.8	<0.05
Lm, mm^{-3}	58.7±2.4	71.4±4.4	<0.05

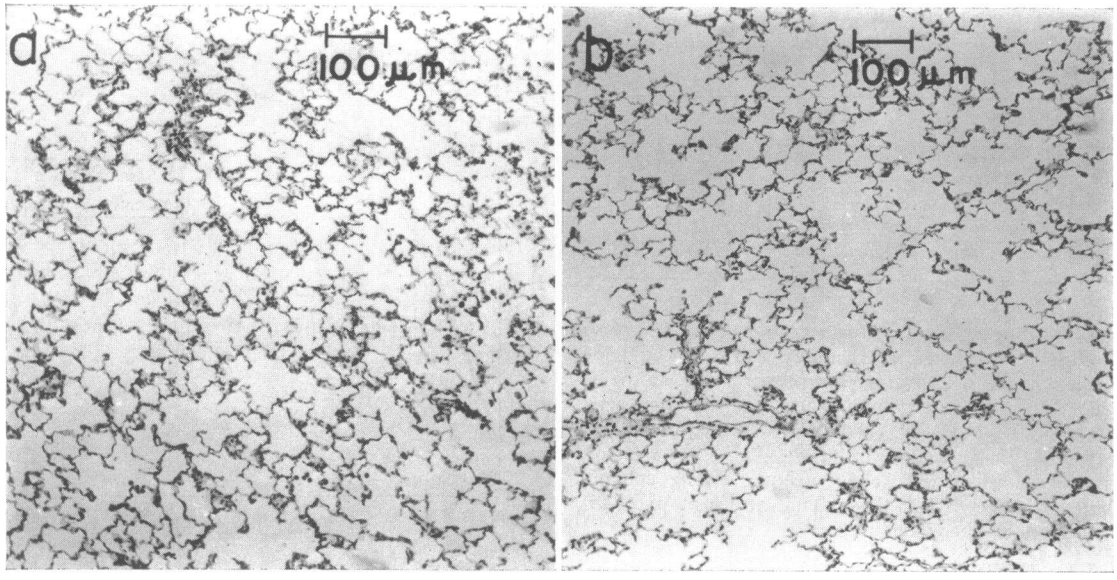

FIGURE 2 Light micrographs of lung parenchyma after formaldehyde fixation at 30 cm H_2O. Control on left (*a*), experimental lung on right (*b*). Note the irregular dilatation of terminal air spaces in the experimental section (hematoxylin and eosin stain).

Also, the volumes of saline trapped at the end of the deflation run in the experimental group (1.91±0.16 ml) and in controls (1.98±0.17 ml) were very similar. Thus, SC administration had caused no discernible change in tissue recoil over a large proportion of the physiological lung volume range.

The quasi-static P-V curves obtained during the second inflation period are shown in Fig. 1. Lung volumes measured at pressure intervals of 5 cm H_2O revealed no significant differences between the two groups, even when the lungs had been overinflated to a recoil pressure of 20 cm H_2O. The P-V curves also indicated that lung compliance was normal until the lungs had been expanded beyond physiological limits, since their slopes were identical until the recoil pressure exceeded 15 cm H_2O. At higher pressures, the P-V curves were steeper in the experimental group, and the lungs ruptured at a pressure of only 26.3 cm H_2O compared with 37.2 cm H_2O in the controls ($P < 0.001$).

TABLE IV
Effects of SC on Lung Collagen and Elastin

	Control	SC
Collagen data		
Total hydroxyproline*	0.225	0.210
Solubilized hydroxyproline	2.7	22.7
Elastin data		
Elastin*	5.3	5.7
Desmosine‡/lysine ratio	0.27	0.26
Residues desmosines§/1,000 residues	1.6	1.5

* Percent (wt/wt) dry weight.
‡ Calculated as lysine equivalents ÷4.
§ Desmosine + isodesmosine.

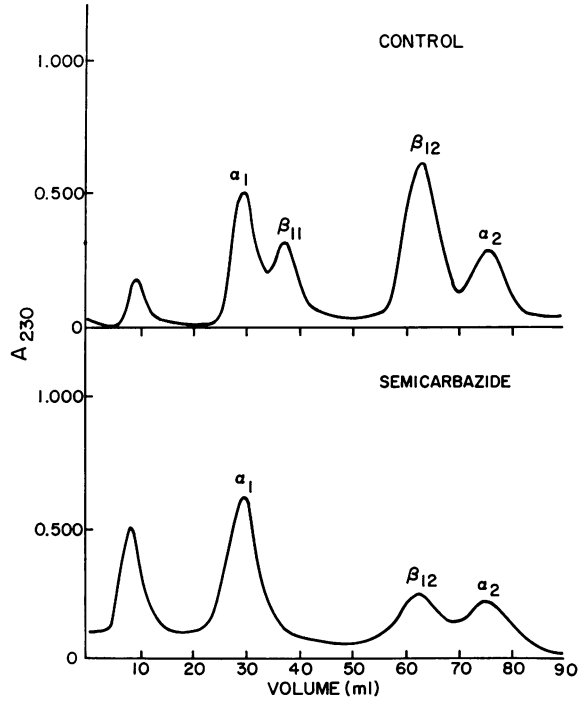

FIGURE 3 Carboxymethyl cellulose chromatography of acid soluble lung collagen from control (upper) and experimental (lower) animals. Note dimunition of β components in the collagen from experimental animals.

Histology. The morphometric measurements are given in Table III. The lung volumes after fixation at 20 cm H_2O were identical in the experimental and control specimens. There were also no differences in their appearance under light microscopy or in the measurements of the mean linear intercept (Lm). This suggested that SC administration had not altered lung structure in vivo. However, when the fixation pressure had been 30 cm H_2O, the volume and Lm values were significantly higher in the experimentals than the controls. Often there were obvious microscopic abnormalities in sections of experimental lungs fixed at the higher pressure. A typical example is shown in Fig. *2b*, in which there is an irregular dilatation of the terminal airspaces and rupture of alveolar walls; these changes were not seen in any control specimens fixed at 30 cm H_2O.

Biochemistry. The data related to the content and extractability of lung collagen are given in Table IV. As a percentage of dry lung weight, the hydroxyproline contents were similar in the experimental and control groups. Since there had been very little difference in lung weight, these results show that total lung collagen content was virtually equal in the two groups. However, 22.4% of the hydroxyproline in the experimental lungs could be solubilized in acetic acid, compared with only 2.7% in the controls. This suggested that the lung collagen in the experimental rats was deficient in cross-linkages. This was confirmed by carboxymethylcellulose chromatography, which revealed a paucity of β components in the lung collagen obtained from the experimental group (Fig. 3). The α_1 and α_2 components were present in the ratio of 2:1, which suggested that the collagen solubilized by acetic acid was a type I form of collagen.

The results of the elastin analysis are also given in Table IV. The content of lung elastin was similar in the two groups. Also, the amino acid analysis of the lung elastin preparations showed that the desmosine and isodesmosine fractions were present to an equal extent in both groups (Table IV). Thus lung elastin, unlike collagen, appeared to be normal with respect to the degree of cross-linking in the experimental animals.

DISCUSSION

The object of the present study was to evaluate the mechanical and structural changes produced by altering the molecular composition of the fibrous proteins in the lung. It was found that administering SC to growing rats caused a reduction in the tensile strength of lung tissue without altering its elastic recoil and compliance in the physiological range for lung volume. Lung histology and morphology were normal in specimens fixed with an inflation pressure of 20 cm H_2O. However, when fixed at 30 cm H_2O, there was dilatation of terminal air spaces with rupture of alveolar septa in the experimental group, and the Lm was greater than in the controls. Associated with these changes, there was a reduction in the cross-linking of lung collagen, but there was no detectable change in the biochemical composition of lung elastin.

Since the lungs of the experimental rats were normal in weight and structure, except when overinflated, their rupture at low inflation pressures was presumably due to a reduction in the tensile strength of lung tissue, as was also demonstrable in specimens of aorta and skin. This finding is in accord with the reduction in the cross-linking of lung collagen, since the tensile strength of most tissues depends on the presence of collagen fibers normal in quality as well as quantity. Immature collagen is relatively weak, in spite of a normal microscopic appearance, and acquires tensile strength by the formation of intermolecular and intramolecular cross-linkages. This involves the enzymatic synthesis of aldehydes at the site of lysyl and hydroxylysyl residues, which are then used to form chemical bonds of various types between adjacent tropocollagen molecules or between the α chains of an individual molecule (19, 20). Several agents, including SC, interfere with the synthesis or the utilization of these aldehydes and thereby inhibit the biosynthesis of cross-linkages (12, 14). Administering these agents in sufficient dosage to growing animals produces the physical condition known as lathyrism, characterized by skeletal deformities, stunted growth, and aortic aneurysms (12, 20). Reduced tensile strength has previously been demonstrated in aorta, skin, intestine, and tendon of lathyritic rats (21). The collagen content of the weakened tissues was normal, but was deficient in cross-linkages, like the lung tissue of our experimental group.

In spite of the biochemical defect of lung collagen produced by SC administration, compliance and elastic recoil were normal except when the lungs were inflated beyond physiological limits. However, the elastic properties of lung tissue may depend more on its elastin than on its collagen fibers, and the biochemical composition of elastin seemed normal in the experimental rats. Consistent with this, the extensibility of elastin is much greater than collagen (22). Also, even though the uncoiling of these fibrous elements may affect lung elasticity more than their linear extension (3), lung compliance is increased after exposure to elastase but not collagenase (8). Nonetheless, Setnikar (4) proposed that collagen fibers lying in parallel with the elastin fibers might limit distensibility of the lungs at high volumes, and Carton, Clark, Dainaukas, and Barron (5) calculated that elastin fibers alone cannot account for all the tissue elasticity of the lungs near full expansion. In keeping with this concept, the quasi-static

P-V curves were steeper in the experimental lungs when the inflation pressure exceeded 15 cm H_2O. However, small pleural leaks, due to reduced tensile strength rather than to altered elasticity of lung tissue, may also be the basis of this observation, since in this high pressure range, several of the experimental lungs were close to the point of rupture.

In contrast with the effects of SC, the administration of penicillamine to rats has been found to inhibit the cross-linking of lung elastin rather than collagen (9). This was accompanied by a reduction of lung elastic recoil and an increase of lung compliance, which was most pronounced at low lung volumes (10). The difference in the biochemical effects of SC and penicillamine is intriguing. Since the penicillamine studies also used weanling rats (10, 11), age and species differences in the susceptibility of lung collagen and elastin cannot explain the present findings with SC. However, differences in the physical manifestations of lathyrism produced by different agents have previously suggested that SC may have less effect on elastin than on collagen biosynthesis in other tissues. Thus, dissecting aortic aneurysms, which have been attributed to a deficiency of elastin cross-linkages (23), rarely occur in lathyrism due to SC administration although lathyrism frequently causes skeletal deformities due to impaired cross-linking of collagen (24). The explanation may possibly be related to the different modes of action by which different lathyrogens prevent the formation of cross-linkages. Unlike SC, penicillamine does not inhibit the synthesis of aldehydes at the lysyl and hydroxylysyl residues. However, it does combine with these aldehydes so that they are unavailable for cross-linking (14, 25). It is also conceivable that SC exerts a greater effect upon the aldimine type of cross-linkage than upon the formation of the desmosines. Nonetheless, whatever the molecular basis for these differences in the effect of SC and penicillamine, it appears that the cross-linking of lung collagen and elastin may be selectively blocked by different lathyrogens. This may be useful in further studies to separate the roles of these fibrous proteins in lung structures and function.

The present observation that the collagen extracted from the lathyritic lungs was the type I form of collagen is consistent with the findings of Bradley, McConnell, and Crystal (26), using the lungs of lathyritic rabbits. In both studies it was also found that only a small fraction of the total lung collagen was extractable even after the induction of lathyrism. Chung and Miller (27) have recently isolated a type III collagen from vascular tissue, which was extremely insoluble. It is therefore possible that the relative insolubility of lung collagen may be related to a high content of type III collagen, solubilized only after proteolysis with pepsin.

The main purpose of studying the pathophysiology of the lungs in experimental lathyrism has been to determine whether a cross-linking deficiency in its fibrous proteins might be relevant in the pathogenesis of human lung disease. Emphysema was not produced by SC administration alone, since the mechanical and histological findings were normal unless the lung inflation pressure was very high. However, the fragility of lung tissue in the experimental group suggests that impaired collagen cross-linking could provide a pathway to emphysema by predisposing the lung to rupture under conditions of physical stress. It is therefore interesting that bullous emphysema and spontaneous pneumothorax have frequently occurred in the Marfan syndrome (28); possibly the underlying cause is a deficiency of collagen cross-linkages, since increased quantities of soluble collagen are present in the skin (29). Whether a cross-linking defect may occur in the more usual cases of emphysema is still unknown. The contents of lung collagen and elastin are normal (30, 31), but the possibility that a qualitative abnormality of the fibrous proteins may exist merits further investigation.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Jacqueline Zavodnick for her technical assistance.

This work was supported by National Institutes of Health Grants HL-15061, HL-08805, AM-14526, and AM-14805.

REFERENCES

1. Carton, R. W., J. Dainauskas, B. Tews, and G. M. Hass. 1960. Isolation and study of the elastic tissue network of the lung in three dimensions. *Am. Rev. Respir. Dis.* **82**: 186–194.
2. Wright, R. R. 1961. Elastic tissue of normal and emphysematous lung. A tridimensional histologic study. *Am. J. Pathol.* **39**: 355–367.
3. Pierce, J. A., and R. V. Ebert. 1965. Fibrous network of the lung and its change with age. *Thorax.* **20**: 469–476.
4. Setnikar, I. 1955. Origine e significate delle proprieta mecchaniche del polmone. *Arch. Fisiol.* **55**: 349–374.
5. Carton, R. W., J. W. Clark, J. Dainauskas, and A. Barron. 1964. Estimation of tissue elasticity of the lung. *J. Appl. Physiol.* **19**: 236–242.
6. Pierce, J. A., J. B. Hocott, and B. F. Hefley. 1961. Elastic properties and the geometry of the lungs. *J. Clin. Invest.* **40**: 1515–1525.
7. Turino, G. M., R. V. Lourenço, and G. H. McCracken. 1968. Role of connective tissues in large pulmonary airways. *J. Appl. Physiol.* **25**: 645–653.
8. Johanson, W. G., Jr., and A. K. Pierce. 1972. Effects of elastase, collagenase, and papain on structure and function of rat lungs in vitro. *J. Clin. Invest.* **51**: 288–293.
9. Hoffman, L., O. O. Blumenfeld, R. B. Mondshine, and S. S. Park. 1972. Effect of DL-penicillamine on fibrous proteins of rat lung. *J. Appl. Physiol.* **33**: 42–46.
10. Hoffman, L., R. B. Mondshine, and S. S. Park. 1971. Effect of DL-penicillamine on elastic properties of rat lung. *J. Appl. Physiol.* **30**: 508–511.

11. Caldwell, E. J., and J. H. Bland. 1972. The effect of penicillamine on the rabbit lung. *Am. Rev. Respir. Dis.* **105**: 75–84.
12. Barrow, M. V., C. F. Simpson, and E. J. Miller. 1974. Lathyrism: a review. *Quart. Rev. Biol.* **49**: 101–128.
13. Bornstein, P. 1970. The cross-linking of collagen and elastin and its inhibition in osteolathyrism. Is there a relation to the aging process? *Am. J. Med.* **49**: 429–435.
14. Vančíková, O., and Z. Deyl. 1973. Aging of connective tissue. Solubilization of collagen from animals varying in age and species by reagents capable of splitting aldimine bonds. *Exp. Gerontol.* **8**: 297–306.
15. Dunnill, M. S. 1962. Quantitative methods in the study of pulmonary pathology. *Thorax.* **17**: 320–328.
16. Juva, K., and D. J. Prockop. 1966. Modified procedure for the assay of H^3- or C^{14}-labeled hydroxyproline. *Anal. Biochem.* **15**: 77–83.
17. Piez, K. A., E. A. Eigner, and M. S. Lewis. 1963. The chromatographic separation and amino acid composition of the subunits of several collagens. *Biochemistry.* **2**: 58–66.
18. Lansing, A. I., T. B. Rosenthal, M. Alex, and E. W. Dempsey. 1952. The structure and chemical characterization of elastic fibers as revealed by elastase and by electron microscopy. *Anat. Rec.* **114**: 555–570.
19. Page, R. C., and E. P. Benditt. 1968. Molecular diseases of connective and vascular tissues. III. The aldehyde content of normal and lathyritic soluble collagen. *Lab. Invest.* **18**: 124–130.
20. Tanzer, M. L. 1965. Experimental lathyrism. *Int. Rev. Connect. Tissue Res.* **3**: 91–112.
21. Fry, P., M. L. R. Harkness, R. D. Harkness, and M. Nightingale. 1962. Mechanical properties of tissues of lathyritic animals. *J. Physiol. (Lond.).* **164**: 77–89.
22. Carton, R. W., J. Dainauskas, and J. W. Clark. 1962. Elastic propertes of single elastic fibers. *J. Appl. Physiol.* **17**: 547–551.
23. Grant, M. E., and D. J. Prockop. 1972. The biosynthesis of collagen (third of three parts). *N. Engl. J. Med.* **286**: 291–300.
24. Alper, R., J. T. Prior, and W. R. Ruegamer. 1968. Histological and biochemical studies on the ground substance of the aortas of lathyritic rats. *J. Atheroscler. Res.* **8**: 787–801.
25. Deshmukh, K., and M. E. Nimni. 1969. A defect in the intramolecular and intermolecular cross-linking of collagen caused by penicillamine. II. Functional groups involved in the interaction process. *J. Biol. Chem.* **244**: 1787–1795.
26. Bradley, K. H., S. D. McConnell, and R. C. Crystal. 1974. Lung collagen composition and synthesis. Characterization and changes with age. *J. Biol. Chem.* **249**: 2674–2683.
27. Chung, E., and E. J. Miller. 1974. Collagen polymorphism. Characterization of molecules with the chain composition $[\alpha_1 (III)]_3$ in human tissues. *Science (Wash. D. C.).* **183**: 1200–1201.
28. Dwyer, E. M., Jr., and F. Troncale. 1965. Spontaneous pneumothorax and pulmonary disease in the Marfan syndrome. Report of two cases and review of the literature. *Ann. Intern. Med.* **62**: 1285–1292.
29. Laitinen, O., J. Uitto, M. Iivainen, M. Hannuksela, and K. I. Kivirikko. 1968. Collagen metabolism of the skin in Marfan's syndrome. *Clin. Chim. Acta.* **21**: 321–326.
30. Pierce, J. A., J. B. Hocott, and R. V. Ebert. 1961. The collagen and elastin content of the lung in emphysema. *Ann. Intern. Med.* **55**: 210–222.
31. Johnson, J. R., and F. A. Andrews. 1970. Lung of scleroproteins in age and emphysema. *Chest.* **57**: 239–244.