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STUDIES ON THE COLLAGEN AND ELASTIN CONTENT OF THE HUMAN LUNG*†

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Connective tissues are important to the structure of all organs. Because ventilation imposes mechanical stress, the lungs are perhaps more dependent on connective tissue proteins for the maintenance of normal structure than are other vital organs. Surprisingly, the quantities of fibrous proteins in the lungs had not been measured until recently (1, 2). The purpose of this study was to determine the collagen and elastin content of human lungs. Variations in the age and sex of the subjects have been correlated with the findings.

MATERIAL AND METHODS

The right middle lobe was studied because it is an easily identified and complete unit of lung, and its size is convenient. The specimens were obtained at autopsy from subjects who did not die of diseases primarily involving the lungs. The right middle lobe bronchus was transected just proximal to its initial bifurcation leaving minimal hilar material attached to the specimen. Lobes were rejected if obvious parenchymal disease was present or if there was gross thickening of the pleura. The samples were stored at -4°C.

Measurements of collagen and elastin followed the technique of Lowry, Gilligan and Katersky (3). In brief, this procedure is gravimetric and depends upon the insoluble nature of the fibrous proteins. Finely divided tissue is extracted at room temperature in 0.1 N sodium hydroxide solution, alcohol-ether and ether. The constant dry weight of residue is obtained after separation in the centrifuge. Collagen is then converted to a soluble form with heat in the presence of water. The insoluble residue is again separated and dried to constant weight. Extraction is then carried out in 0.1 N sodium hydroxide solution in a boiling water bath. The residual material (elastin) is washed and its dry weight obtained.

It has been necessary to modify this method for use with lung tissue. Instead of being finely minced, the tissue was homogenized in water in a "Virtis 45" homogenizer. This permitted the accurate determination of dry solids on aliquots from the homogenate. One part of N sodium hydroxide solution was then added to nine parts of the homogenate. Lung collagen was converted to gelatin in the autoclave at 30 pounds of pressure for six hours. Studies of the material solubilized in this manner revealed a 17.5 per cent nitrogen and 10.5 per cent hydroxyproline content. These data characterize the conversion of collagen to gelatin and confirm the fact that no large amount of material other than collagen entered solution at this step. Reticulin would not be detected with the methods used and thus is a possible exception.

The final heated alkali extraction was continued for 45 instead of 20 minutes and the samples subsequently were washed at least twice. The material removed at this stage averaged 1.2 percent of the initial total dry solids.

Elastin for reference was prepared from bovine neck ligament by the methods of Richards and Gies (4) and Lowry and associates (3). Table I presents the results of nitrogen analyses on bovine elastin. The mean nitrogen content of purified bovine elastin was 16.34 percent on a dry, ash-free basis when prepared by the method of Richards and Gies (4). Although this nitrogen percentage is lower than measurements reported by others (4-7), it was determined with a procedure identical to that employed on the lung elastin residues. For this reason, it has been used for the calculation of lung elastin throughout this report. It is not inferred that human lung and bovine neck ligament elastins necessarily have identical amounts of nitrogen.

The final lung residues contained approximately 11 percent insoluble material which was not elastin. This undoubtedly included carbonaceous material, silica and perhaps insoluble calcium salts. In order to estimate elastin, the nitrogen content of each final residue was determined in duplicate with a semi-micro Kjeldahl method. Aliquots of each digestion product were analyzed in duplicate. The mean differences in duplicate analyses were 0.07 mg. for the same digestion product and 0.09 mg. of nitrogen per 50 mg. of residue for different digestions.

To determine that the material measured by the Lowry method was elastin, the residues were exposed to a commercial preparation of crystalline elastase.† Duplicate lung elastin samples (weighing 50 to 100 mg.) were incubated at 37°C. with 2 mg. of elastase in 10 ml. of carbo-

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‡ Investigator of the Arkansas Heart Association.

1 Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.
nate buffer at pH 8.8, ionic strength 0.1. This was continued for 24 to 48 hours or until the solution was homogeneous. The final residue was then separated by centrifugation, washed with water and dried to constant weight. The material solubilized was considered as elastin. Attempts to estimate elastin with elastase on fractions other than the final residue were unsuccessful.

Figure 1 compares the results obtained from nitrogen determinations to the results of elastase solubilization. In 30 of the present cases, the mean elastin purity of the final residues was 89 per cent by nitrogen analysis and 90 per cent by elastase solubility. The high degree of positive correlation ($r = +0.88$) between the results of the two methods offers an assurance that similar material was being measured in each instance.

Hydrolysates of the final lung elastin residues were prepared in 6 N hydrochloric acid by heating for four hours at 135° C. in sealed tubes. Hydroxyproline was then measured by the method of Stegemann (8). This measurement was to detect contamination of the final residue by collagen. The mean hydroxyproline content of lung elastin in 31 of the present cases was 1.73 per cent. This is similar to the values reported for purified elastin. Had these preparations contained any significant amount of collagen, a higher concentration of hydroxyproline would have occurred.

Hydrolysates of the final residues were also subjected to two-dimensional paper chromatography. The solvents were phenol-water (80:20) and sec.-butanol-formic acid-water (75:15:10). A solution of ninhydrin and isatin (9) was used to develop color. The patterns of amino acids for human lung elastins were closely similar to those obtained with bovine neck ligament elastins.

When purified bovine neck ligament elastin was analyzed with the Lowry method (3), approximately 90 per cent of the initial dry weight was recovered in the final elastin residue. Moreover, the analysis of three dog aortas with the present technique revealed a mean of 22.8 per cent of the initial dry solids each for collagen and elastin. These results agree well with the values of 25.5 per cent collagen and 25.2 per cent elastin for corresponding parts of the aorta as calculated from the data of Harkness, Harkness and McDonald (10).

Table II presents data on the right middle lobes from 48 subjects. Figure 2 presents the frequency distribution for age, total middle lobe collagen, total elastin and the ratio of collagen to elastin. Figure 3 (A and B) shows collagen plotted against age. These graphs fail to reveal any significant change in lung collagen with advancing age. There was no significant difference in total middle lobe collagen between men and women. A discrepancy existed in the percentage of collagen which was related to the smaller weight of the middle lobe in women. The mean wet and dry weights, respectively, of the right middle lobe were 48 and 8.2 Gm. for women, and 76 and 13.5 Gm. for men.

Elastin, shown in Figure 4 (A and B), increased with age whether considered as a percentage of initial dry weight or as total amount. The women had a higher percentage of elastin than the men, but the total quantity of middle lobe elastin did not differ between the sexes.

The various correlations with age are shown in Table III. The three small children in the series were excluded from the calculation of the correlation coefficients in order to avoid the undue influence of these extreme values. The ratio of lung
Collagen and elastin measurements on the right middle lobes of 48 subjects

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* Right middle lobe weight.
† Calculated from analysis for nitrogen.
‡ Per cent of total initial dry solids.
§ Collagen to elastin.

Collagen to elastin is plotted against age for all of the subjects in Figure 5. This ratio is useful since it excludes such variables as the weight of the lungs at the time of death and the sample size.

It was interesting that when the wet weight of the right middle lobe was 60 Gm. or less, a linear correlation was found with the dry weight which averaged 16.7 per cent of wet. Samples that weighed more than 60 Gm. wet had a smaller dry weight percentage. No relationship was evident...
between the age of the subject and the weight of the right middle lobe. In the whole series, the right middle lobe averaged 6.5 per cent of the total weight of both lungs as measured by the pathologist. This percentage did not vary with age.

**DISCUSSION**

A question of basic importance concerns the precise nature of the substances measured with the present method. The initial alkaline extractions make it unlikely that protein complexes other than collagen, elastin and reticulin deserve serious consideration. Generally accepted terminology includes as collagen the material insoluble in dilute sodium hydroxide at room temperature but converted to a soluble form with heat in the presence of water. Elastin is the residual insoluble protein after these procedures and an additional heated alkali extraction.

It must be emphasized that both collagen and elastin are complex proteins. Collagen fibers contain one or more mucoprotein fractions while elastin fibers contain mucoprotein and probably lipid fractions. A small amount of collagen also exists in a soluble form. This has been termed procollagen, and would not be measured by the present method. The fate of reticulin in this method is uncertain. Although it resembles collagen more closely than elastin, at least a part of reticulin may remain insoluble in boiling water (11). Care was taken to insure the purity of the

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**TABLE III**

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<th>No.</th>
<th>Elastin %</th>
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<td>Women</td>
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* Calculated from dry weight of initial solids.
† Total right middle lobe elastin in dry grams.
‡ Collagen to elastin.
material reported here as elastin. Thus the lung elastin residues were found to enter solution on exposure to elastase. They contained less than 2 per cent of hydroxyproline, and had an amino acid composition similar to that found in purified bovine elastin. It may be safely concluded therefore that human lungs contain no less elastin than the amount measured by the method used in this study.

Although a wide range of values was encountered, there was no significant change in lung collagen with advancing age. Elastin, however, showed a highly significant and striking increase with age whether expressed as per cent of initial dry weight or as the total elastin of the right middle lobe. There was a consistent decrease of the lung collagen to elastin ratio with advancing age. This ratio is independent of such factors at death as vascular congestion, pulmonary edema and bronchial mucus.

The lungs of women were found to have a higher content of collagen and elastin when expressed as a per cent of initial dry weight of the sample. There was, however, no significant difference between men and women in the total quantities of collagen and of elastin in the right middle lobe. The mean weight of middle lobes obtained from women was 63 per cent of that from men. Physiological studies (12, 13) indicate that

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**Fig. 3. Scattergrams of Collagen with Age**

A. Collagen expressed as per cent of the initial dry weight of the right middle lobe. B. Total middle lobe collagen in grams dry weight. The open circles refer to women; the closed circles refer to men.

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**Fig. 4. Scattergrams of Elastin with Age**

A. Elastin expressed as per cent of the initial dry weight of the right middle lobe. B. Total middle lobe elastin in grams dry weight. The open circles refer to women; the closed circles refer to men.
women have approximately 70 per cent of the total lung capacity found in men. Thus, it appears that women have more lung scleroprotein per unit of lung volume. Whether this observation bears any relation to the known preponderance of pulmonary emphysema in men is uncertain.

A preliminary report (1) presented analyses of the middle lobes on 20 subjects not included in this paper. The procedure was less elaborate but the results of that study agree with the present findings. Briscoe and Loring (2) measured lung elastin in 29 subjects of various ages with a modification of the Lowry method which was different from the one used in this study. These authors found an increase with age in elastin expressed as per cent of the dry weight of the sample. The values reported are somewhat higher than those reported here. The discrepancy may be related to differences in methods. Briscoe and Loring (2) found a difference in the percentage elastin between males and females which was confirmed in the present study.

Lansing, Rosenthal and Alex (14) studied the elastin of the defatted media of the pulmonary artery in 106 subjects. Those authors reported a slight increase with advancing age in elastin expressed as per cent of the defatted media. Because of the marked differences in technique, these results are difficult to compare with the present findings. Hence the influence of the vasculature on the observed changes in total middle lobe elastin cannot be assessed at the present time.

The present interest in lung collagen and elastin stemmed from a demonstration that the physical properties of the lungs were markedly different in old and young men (15). The question arose whether these changes might have resulted from alterations in the connective tissues of the lungs, especially a decrease in lung elastin. The results of the present study suggest that whatever morphologic alterations occur in the lungs of the aged, the quantity of lung scleroproteins is not decreased.

Why should elastin in the lungs increase with age? The answer is not known, but one may speculate. Bunting (16) proposed that the development of elastic fibers might be stimulated by rhythmic stress of tissue. He observed few elastic fibers in immobile scar tissues but found numerous elastic fibers in scars involving the myocardium, pericardium or pleura. He noted that, in adhesions, the elastic fibers were found parallel to the lines of tension. Dyson and Decker (17) also commented on the characteristic association in tissues of elastosis with fluctuating or rhythmic stress. One difficulty encountered in the interpretation of such data is the uncertainty whether stained elastic tissue is identical with the protein elastin as characterized chemically. Further, it has been proposed that collagen may be transformed into elastin. Although this would involve drastic alterations in the amino acid composition of protein molecules in the fibers, some tendency toward such a transformation has been observed in vitro (18, 19). Unfortunately, however, the morphologic observations (20) are not entirely specific and the chemical observations (21) are not sufficiently complete to be conclusive. Finally, it seems remotely possible that an unknown protein may exist which has an amino acid composition closely similar to but not identical with elastin. The accumulation of such a protein could account for the present findings. The available information does not permit the correct possibility to be selected at the present time.

**Summary**

1. Collagen and elastin were measured on the right middle lobes of 48 people with a modification of the Lowry, Gilligan and Katersky alkaline extraction method (3).
2. An increase in elastin occurred with advancing age.
3. The total collagen and elastin content of the
right middle lobes was similar in men and women, although the weight of the lobes was smaller in women.

ACKNOWLEDGMENTS

We are indebted to Dr. Richard V. Ebert for valuable advice and constant encouragement. The expert assistance of Miss Mary Ann Templeton is gratefully acknowledged.

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