Chemical and Histochemical Studies of Human Alveolar Collagen Fibers

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Light and electron microscopic studies have established that the normal human alveolar argyrophilic (reticulum) fiber is collagen fiber. The silver impregnation method is highly sensitive and specific for histologic demonstration of the elaborate collagen fiber network of alveolar septa. The argyrophilic alveolar collagen fiber does not stain with the periodic acid–Schiff (PAS) or periodic acid–thiocarbohydrazide–osmium tetroxide (PTO) reaction. The materials positive for the PAS and PTO reactions in alveolar septa are epithelial and endothelial basal laminas, which are nonargyrophilic. Chemically, lung collagen fibers are composed of Type I and Type III collagens, which differ in amino acid composition, chain composition, and carbohydrate content. The chemical heterogeneity of lung collagen may have important biologic implications in the maintenance of normal structure and in the repair of lung injury. (Am J Pathol 86:81–98, 1977)

THE COLLAGEN FIBER is a major structural component of the lung, comprising 15 to 20% of the dry weight of the lung.[1-4] In reaction to injury, such as organizing pneumonia, sclerosing alveolitis, and a variety of pneumoconioses, increase in collagen fibers is the predominant pathologic process and may lead to obliteration of alveolar structure and profound alteration of the mechanical properties of the lung.[4]

Past attempts to describe or quantitate alteration of lung collagen fiber in order to gain insight into the underlying pathologic processes have encountered difficulty due to the lack of satisfactory histologic techniques and basic biochemical information concerning alveolar collagen fiber,[5] whereas the abundance of the reticulum fiber in normal alveolar wall and its alteration in reaction to injury have been documented.[6,7] However, the correlation of the histologic reticulum fiber with the ultrastructural components of extracellular materials in alveoli is not clear.[4] It has been variously correlated with collagen fiber,[8,9] microfibril,[9] and basal lamina.[10]

Current studies were undertaken to establish the structural and chemical identity of the lung reticulum fiber. As will be shown, the alveolar reticulum fiber is collagen fiber. The silver impregnation method is a sensitive and specific way of demonstrating the alveolar collagen fiber, compared with conventional staining techniques. Contrary to the conven-

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tional belief, the alveolar reticulum fiber does not stain with the periodic acid–Schiff reaction. Chemical analysis of lung collagen fibers has disclosed two types of collagen, Type I and III.

For clarity of presentation and discussion, certain terms used in this article will be defined here. The term *collagen* will be reserved for proteins of the collagen family in a strict chemical and molecular sense.[11] *Collagen fiber* is the smallest unit fiber discernable with a light microscope and is comprised of a bundle of smaller unit *collagen fibrils* with characteristic 64-nm periodicity under electron microscope.

Materials and Methods

Histochemical Studies

Six adult human lungs, from subjects ranging in age from 38 to 60 years old, were obtained from the autopsy and surgical pathology services of the University of Washington Hospital and Seattle Veterans Administration Hospital. Only those cases which had no clinical problems referable to diffuse respiratory illness and disclosed no histologic abnormality of lungs were included. The lung tissues were fixed immediately in 10% formalin solution buffered with calcium carbonate chips or half-strength Karnovsky's fixative.[12]

Histology

Tissue samples were processed for routine paraffin embedding. Sections were cut 4 and 20 μ thick, stained with hematoxylin and eosin, Gomori's trichrome, van Gieson's method for collagen fibers, and periodic acid–Schiff (PAS) reaction. Methods recommended in the *Manual of Histologic Staining Methods* of the Armed Forces Institute of Pathology [13] were employed. The silver impregnation technique of Slidders, Fraser, and Lendrum [14] was used because of better reproducibility.

Electron Microscopy

For electron microscopy of silver-impregnated sections, frozen sections of lung tissue with or without embedding in 4% gelatin solution were cut, in a cryostat, 20 μ thick. The sections were placed on gelatin-coated slides. After silver impregnation, one of the paired slides from each specimen was counterstained with hematoxylin for comparison with silver-impregnated paraffin sections. The other section was scraped from the slide glass with a razor blade in a long ribbon, immersed in a 2% agar solution at 45 C, diced into 1-mm cubes after solidification of agar at 20 C, dehydrated, and embedded in Epon.

For periodate–thiocarbohydrazide–osmium tetroxide (PTO) reaction, tissues after fixation in formalin or Karnovsky fixative were diced into 1-mm cubes, rinsed three times with distilled water, and placed in a 10-ml flask mounted on a rotary shaker, which provided shaking motion during periodate oxidation, rinsing, and thiocarbohydrazide reaction. The PTO reaction was performed according to the method of Seligman and associates.[15] Thin sections of Epon-embedded tissue, with or without uranyl acetate and Millonig lead stains, were examined with an AEI-801 electron microscope.

Extraction and Purification of Human Lung Collagen

Lungs were frozen immediately to −22 C until use; 1250 g (wet weight) of thawed lungs pooled from 2 patients, dissected free of major bronchi and blood vessels, were homogenized with a Polytron homogenizer. All procedures,including extraction and purification of collagen, were performed in a cold room at 4 C. Since it was found in a preliminary

experiment that the lung collagen was very insoluble in neutral 1.0 M NaCl or 0.5 N acetic acid, solubilization of collagen was accomplished by employing pepsin according to the method of Chung and Miller.[16] The lung homogenate, rinsed free of the blood with 0.85% NaCl solution, was sequentially extracted with ten times the volume of 1.0 M NaCl containing 0.01 M Tris at pH 7.4 and 0.5 N acetic acid for 3 days each. This was done primarily to remove the cellular debris.[16] After these preliminary extractions, the residue was then extracted with 1% pepsin (porcine stomach, Worthington) in 0.5 N acetic acid for 24 hours. After centrifugation at 48,000 *g* for 2 hours, collagen was precipitated by adding solid NaCl into clear supernatant to a final concentration of 5% (w/v).[16] The collagen precipitate obtained after centrifugation was redissolved in 0.5 N acetic acid overnight. Collagen was precipitated out again on the supernatant cleared by centrifugation. The cycle of purification was repeated three more times. The purified collagen was thoroughly dialyzed against 0.05 N acetic acid to remove NaCl and lyophilized.

For further fractionation of different types of collagen, 1.5 g of lyophilized purified collagen was dissolved in 0.5 N acetic acid followed by dialysis against 1.0 M NaCl buffered with 0.01 M Tris at pH 7.4. Collagens soluble in the buffer were salted out stepwise by increasing NaCl concentration to 2 M and 3 M.[16] The collagens precipitated out at 2 M and 3 M were further purified three more times by repeating the same salting out procedure and were then dialyzed against 0.05 N acetic acid and lyophilized.

Chemical Analysis of Collagens

Amino Acid Analysis

One milligram of material was hydrolyzed with 1 ml of 6 N HCl at 108 to 110 C for 24 hours under nitrogen. The hydrolysate was dried and analyzed for amino acids with a Beckman 120 C amino acid analyzer.

Carbohydrate Analysis

Samples were hydrolyzed with 2 N HCl at 100 C for 2 hours.[17] The dried samples were analyzed for glucose and galactose content with oxidase methods. The enzymes and reagent kits were purchased from Worthington Biochemical Corporation. A quantitative measurement of hexosamine was carried out with a modified Elson-Morgan reaction.[18] Samples were hydrolyzed with 2 N HCl at 100 C for 16 hours before analysis. Glucosamine was used as the standard.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The method of Weber and Osborn [19] was employed. The gel concentration was 5% in determining chain composition of collagen; 7.5% gel was used in electrophoresis of cyanogen bromide–cleaved peptides.[20] The samples, 70 to 100 μg, were preincubated at 65 C for 15 minutes in sample buffer containing 1% sodium dodecyl sulfate (SDS) and 1% dithiothreitol (reducing agent).

Carboxymethyl Cellulose Column Chromatography

Carboxymethyl cellulose (CMC) column chromatography of lung collagens was carried out with a 0.9 × 12 cm column with water jacket maintained at 42 C. The starting buffer was 0.02 M sodium acetate, pH 4.8, containing 1 M deionized urea. The salt gradient of eluting buffer was 0 to 0.1 M NaCl. The eluent from the column was continuously monitored with a Coleman 124 spectrophotometer at 230 mμ and recorded. The samples were denatured at 45 C for 30 minutes before loading.[21]

Cyanogen Bromide Cleavage of Collagen

Ten milligrams of collagens were dissolved in 2 ml of 70% formic acid in capped test tubes. After the tubes were flushed with nitrogen, 20 mg of CNBr were added. The tubes were capped tight and incubated at 30 C for 4 hours with gentle shaking.[22] At the end of the reaction, the reaction mixtures were diluted with 18 ml of distilled water and lyophilized to remove formic acid and cyanogen bromide. Two hundred micrograms of lyophilized cyanogen bromide peptides were used in SDS polyacrylamide gel electrophoresis.

Results

Light Microscopy

In the peribronchial and perivascular compartments, pleura, interlobular septa, and alveolar duct, abundant collagen fibers were observed as demonstrated by light green dye of Gomori's trichrome stain and acid fuchsin dye of van Gieson stain. These collagen fibers were stained only faintly with PAS reaction and light brown with silver impregnation method. In alveoli, Gomori's trichrome and van Gieson stains revealed only rare collagen fibers in 4-μ sections, which disclosed predominantly cross sections of alveolar septa. When the latter were examined *en face* in 20-μ sections, occasional randomly oriented undulating fibers were observed.

Both the silver impregnation method and PAS reaction disclosed, on cross section of alveolar septa, a similar reticular network, which encircled capillaries and underlay alveolar epithelium (Figures 1 and 3). When alveolar septa were observed *en face*, two staining methods revealed entirely different structures. Whereas the silver impregnation method disclosed a network of randomly oriented undulating argyrophilic fibers (Figure 2), 0.4 to 0.8 μ in width, the PAS reaction revealed predominently the profile of an anastomosing channel of 5 to 10 μ in width (Figure 4). The anastomosing channels are identical with the alveolar capillary network revealed by Gomori's trichrome stain, which stained red blood cells intensely red, thus marking the alveolar capillaries. When both silver impregnation and PAS reactions were performed in sequence on the same section, it became obvious that the argyrophilic fibers were distinct from the PAS-positive capillary network.

Electron Microscopy

The results of the silver impregnation method applied to paraffin-embedded sections and frozen sections with or without gelatin embedding showed no appreciable difference. Therefore, gelatin-embedded frozen sections were used for electron microscopic study because of better preservation of morphology.

Silver impregnation of lung resulted in heavy precipitation of silver granules in alveolar collagen fibrils (Figures 5 and 6). The size of silver granules ranged from 10 to 25 nm in diameter. They were always associated with collagen fibrils. On cross sections of collagen fibrils, the granules were within the fibrils, although extension beyond the profile of collagen fibrils was frequently observed. There was no relationship between distribution of silver granules and periodicity of collagen fibrils (Figure 6). The epithelial and endothelial basal lamina, elastic fibers, and microfibrils did not contain silver granules except for a fine background of electron-dense stippling of less than 4 nm in diameter (Figure 7). The background stippling was more pronounced in the cytoplasm as well as nuclei of cells.

The results of the PTO reaction were the same with or without uranium acetate and lead stains. Both epithelial and endothelial basal laminas exhibited positive PTO reaction (Figures 8 and 9). The osmium staining was very consistent and uniform. It was absent when the periodic acid oxidation step was omitted, which indicated the staining is specific for aldehyde generated by periodate oxidation.[23] The collagen fibrils and amorphous component of elastic fibers did not stain with osmium. There were fine osmium precipitates in the interstices of collagen fibrils and cytoplasm of cells. Most of these were present even without periodate oxidation, indicating their nonspecific nature.

Chemical analysis

The lung collagen was very insoluble in 1 M NaCl, pH 7.4, or 0.5 N acetic acid. Only 0.1% of total hydroxyproline content of the lung could be solubilized by either method. This is consistent with the experience of others.[4,9] Pepsin was more effective [16] and solubilized 20% of the total hydroxyproline content of the lung. Out of 1250 g wet weight of the lung, 3.54 g of purified collagen were obtained after pepsin was added. Aliquots of 1.50 g of purified pepsin-solubilized lung collagen were further fractionated and purified for Type I and Type III collagens. There were 1.13 g of collagen recovered; of this, 0.423 g (37.5%) were Type I, and 0.704 g (62.5%), Type III. Collagens salted out at 2 M NaCl and 3 M NaCl were identified as Type III and Type I, respectively. The identification was accomplished by SDS polyacrylamide gel electrophoresis, CMC column chromatography, and amino acid analysis. Purified Type I collagen from human skin [24] and Type III collagen prepared from human aorta according to the method of Chung and Miller [16] were used as the standard reference. Type I lung collagen was composed of two α-chains, $\alpha 1$ and $\alpha 2$ at a ratio of 2 : 1 on both SDS polyacrylamide gel electrophoresis (Figure

10A) and CMC column chromatography, identical with Type I skin collagen. Type III lung collagen was composed of a single type of α-chain, which migrated to the $\alpha 1$ position on SDS polyacrylamide gel electrophoresis (Figure 10A) and eluted out as a single peak between the $\alpha 1$ and $\alpha 2$ positions of Type I collagen on CMC column chromatography.[16,25] These results were identical with the behavior of Type III collagen of human aorta and also established the purity of Type III collagen preparation.

Amino acid compositions of Type I and Type III lung collagens were similar to those of their respective skin and aorta collagens (Table 1). The Type III collagen had higher hydroxylysine content than Type I collagen, 16.9 to 7.7 residues per 1000 residues. The hydroxyproline content was also higher for Type III collagen, 121.2 versus 103 residues. The degree of hydroxylation of proline was 56.3% in Type III collagen as against 43.4% for Type I collagen. The presence of half cystine was unique for Type III collagen. The Type III collagen also had a higher carbohydrate content, 1.1% galactose and 0.8% glucose, compared to the 0.33% and 0.33% of Type I collagen. Glucose and galactose are the only carbohydrates known

Table 1—Amino Acid and Carbohydrate Composition of Human Type I and Type III Collagens

	Type I collagen		Type III collagen	
	Lung	Skin	Lung	Aorta
Amino acids (residues/1000 aa residues)				
Hyl	7.7	5.4	16.9	17.6
Lys	25.8	29.1	23.1	20.5
His	5.6	5.3	6.3	5.4
Arg	53.8	48.4	45.0	41.1
3-Hyp	0	1.4	0	Trace
4-Hyp	103.0	96.1	117.3	121.2
Asp	45.6	48.4	48.4	48.5
Thr	17.0	19.7	16.1	17.8
Ser	31.1	37.1	32.4	38.2
Glu	64.5	77.6	74.9	74.9
Pro	134.6	115.0	117.5	94.2
Gly	303.2	310.9	315.8	345.5
Ala	111.9	109.4	82.7	81.4
Val	28.3	26.4	20.6	17.0
Cys	0	0	3.3	1.9
Met	7.8	8.1	10.1	9.7
Ileu	13.8	12.1	18.7	17.0
Leu	27.7	29.8	30.0	25.9
Tyr	3.8	6.4	5.5	8.1
Phe	14.5	13.4	15.2	13.3
Carbohydrates (percent by weight)				
Galactose	0.33%	0.18%	1.1%	—
Glucose	0.33%	0.14%	0.8%	—
Hexosamine	0	0	0	—

to be covalently linked to collagen.[26] The absence of hexosamine is expected.

SDS polyacrylamide gel electrophoresis of cyanogen bromide–cleaved peptides of Type I and III collagen revealed peptide patterns different from each other (Figure 10B). The pattern of cyanogen bromide peptide bands of Type III collagen are identical with those of Type III collagen synthesized by WI-38 human lung fibroblasts in tissue culture as reported by Hance, Bradley, and Crystal, in terms of mobility, band width, and intensity.[20] The presence of peptide 6, which was referred to as CB-113 by Epstein [27] and CB-8 by Chung *et al.*,[29] is very characteristic of Type III collagen. The absence of this peptide in Type I collagen (Figure 10B) indicates the purity of the preparation. Additional slow moving bands near top of the gel are probably due to incomplete cyanogen bromide cleavage of methionine residues.[20]

Discussion

Electron microscopy has disclosed abundant collagen fibers in alveolar septa.[28,29] However, the light microscopy using conventional collagen fiber stains such as Gomori trichrome and van Gieson stains has failed to visualize the elaborate collagen fiber network, leading to the early conclusion that the alveolar septa are poor in collagen fibers.[10,30] Although, in my experience, more collagen fibers are visualized when the alveolar septa are observed *en face*, these fibers are small and the contrast imparted by stains is poor. The problem is further compounded by the inflammatory exudate in diseased tissues. The need for specific histologic stains which will bring a better contrast to alveolar collagen fibers is evident.

Alveolar Reticulum Fibers Are Collagen Fibers

The electron-dense silver granules produced by silver impregnation provides a ready direct correlation of light and electron microscopic structures. Association of silver granules with fibrils of characteristic periodicity has established that the reticulum fiber is collagen fiber. Similar results have been obtained from studies of rat spleen [31] and rat skin.[32] The silver impregnation method is not only sensitive for the alveolar collagen fiber, but also very specific. Other extracellular materials, such as basal lamina, elastic fiber, and microfibril, are not stained. Therefore, the technique is recommended for the histologic demonstration of alveolar collagen fibers.

The histochemical basis of reticulum stains is unknown. The methods are purely empirical. This study refutes the notion of Glegg, Eindinger, and Leblond [33] that high carbohydrate content is responsible for argyro-

philia. The collagens from lung collagen fibers contain less than 1.9% carbohydrate compared with 10% in basal lamina,[34,35] which is PAS- and PTO-positive, but nonargyrophilic. The presence of fucose and mannose, which are present in basal lamina [34] but not in collagen,[36] indicates their preparations were mixtures of collagen fibers and basal lamina.[33] Irving and Tomlin [31] have suggested the argyrophilia depends on the complex of ground substance and collagen fibers and that the chemical milieu of collagen fibers is an important determinant. More studies are required to elucidate mechanisms of argyophilia of alveolar collagen fibers.

Are Alveolar Reticulum Fibers PAS Positive?

Based on histologic observation that the structure and distribution of argyrophilic and PAS-positive materials are similar, Lillie [37] and others [38] have concluded that the reticulum fiber is PAS-positive. This has been widely quoted in many histology textbooks [39-41] and the histology manual of the Armed Forces Institute of Pathology.[13] Since the alveolar collagen fiber is argyrophilic, PAS reaction may have potential use for its histologic demonstration.

This study has shown that the alveolar reticulum fiber is PAS negative, which is particularly evident when alveolar septa are viewed *en face*. The elaborate alveolar basal lamina scaffold undoubtedly casts the PAS-positive "fibrillar" network similar to reticulum fibers on cross-section of alveolar septa.[10] This conclusion is further supported by electron histochemical study.

The PTO reaction is an electron histochemical counterpart of PAS reaction for demonstrating carbohydrate.[15] It has a much higher degree of specificity than the periodic acid–methenamine silver method [23] used by others.[42,43] The collagen fibrils of alveolar septa, which are argyrophilic on silver impregnation, are PTO negative. Occasional osmium precipitates observed between fibrils are nonspecific products since they are present even without periodate oxidation.[23] The basal laminas, both epithelial and endothelial, are intensely PTO positive.

The Type I collagen of lung contains 0.66% by weight of carbohydrate; Type III, 1.9%. Although it is not known which types of collagen or in what proportion comprises the alveolar collagen fiber (vide infra), the carbohydrate content of either types of collagen is far less than 10% carbohydrate content of basal laminas.[34,35] The PAS reaction is not stoichiometric, and its histochemical sensitivity is unknown.[44] It is apparent from this study that the sensitivity of the PAS and PTO reactions is insufficient for distinctive histochemical demonstration of alveolar collagen fibers.

Chemical Heterogeneity of Lung Collagens

Collagen fibers are composed of polymerized monomeric units—tropocollagen. The long rod-shaped tropocollagen molecule consists of three peptide chains (α-chain) in a coiled coil triple helix conformation.[11] Although collagen fibrils are similar in periodicity and banding pattern under electron microscope, there is chemical heterogeneity of the building block—tropocollagen—in terms of amino acid and chain composition. Three types of tropocollagen—Types I, II, and III—are capable of copolymerizing into collagen fibrils of typical 64-nm periodicity. Type I collagen is present in bone, skin, and tendon and has a molecular composition of $[\alpha 1(I)]_2\alpha 2$. Type II collagen is found in cartilage exclusively, with molecular composition of $[\alpha 1(II)]_3$. Type III collagen is widely distributed in various human tissues, such as skin, blood vessels, uterus, and lung.[16,45] It has a molecular structure of $[\alpha 1(III)]_3$. There is another type of tropocollagen, Type IV collagen, which is present in basal lamina (basement membrane) and related structures.[17,46] Type IV tropocollagen does not form collagen fibril of typical periodicity.[47]

Because of the presence of collagen fibers, cartilage, and basal lamina, at least four types of collagen are expected in the lung.[4,9] The Type II collagen associated with cartilage is excluded by careful dissection of bronchi. Type IV collagen of basal lamina is not recovered in the purification process since only 5% NaCl in 0.5 N acetic acid is used instead of the 20 to 30% NaCl required for Type IV collagen precipitation.[17,47,48] The absence of 3-hydroxyproline indicates exclusion of basal lamina collagen from purified collagen preparation.[17] Therefore, the purified collagens are presumably derived from collagen fibers of the lung. Since lung collagens are highly cross-linked,[9] peptic solubilization is necessary for their isolation and characterization.[16,27] This is particularly true for Type III collagen, which is extremely insoluble even in the presence of lathyrogen.[20,27,49] Further fractionation and purification reveals two types of collagens, Type I and III. Their identity and purity are established by amino acid analysis, chain composition and cyanogen bromide peptides patterns on SDS-polyacrylamide gel electrophoresis, and CMC-column chromatography. Providing their solubility is similar in the presence of pepsin,[50] the Type III collagen is the predominant type of collagen, consisting of 62.5%. Type I collagen is only 37.5%.

The presence of Type III collagen in the lung has been alluded to by others,[20,45,51] but its abundance has not been appreciated. It is apparent from this study that Type III collagen is one of the major types of collagen in the human lung. There is circumstantial evidence to indicate that Type III collagen is present in the alveolar collagen fibers: a) Remberger and

associates [52] have shown with an immunohistochemical technique that the argyrophilic fibers of the normal and cirrhotic livers are of Type III collagen. b) Spiro [35,36,53] has pointed out that higher levels of carbohydrate in collagen are reflected in lower degrees of morphologic organization as seen under the electron microscope. When all related information is compiled, there is a remarkable inverse correlation between collagen fibril diameter and carbohydrate content of collagen.[47] The alveolar collagen fibril diameter is 25 nm,[28] one of the most delicate collagen fibrils in various tissues.[54] Type III collagen contains 1.9% glucose and galactose compared to 0.66% in Type I collagen (see Table 1). Therefore, the alveolar collagen fiber may contain carbohydrate-rich Type III collagen as its major constituent. c) Type III collagen has been shown to be closely associated with and may be synthesized by smooth muscle and smooth muscle–like cells such as in blood vessels and leimyoma of uterus.[16,45,50,55] In the lung, the alveolar collagen fibers are closely associated with contractile interstitial cells, which contain abundant actin filaments.[56]

Alveolar collagen fibers may also contain Type I collagen. In a preliminary study of lungs from a patient with hereditary Type III collagen deficiency (Ehlers–Danlos syndrome Type IV),[51] the alveolar argyrophilic fibers, composed exclusively of Type I collagen, are observed.[57] Cloned cat lung cells have been shown to synthesize both Type I and III collagens as WI-38 human lung fibroblast.[20]

In what proportion Type I and III collagens constitute alveolar collagen fibers, its alteration in pathologic state, and the biologic significance of collagen heterogeneity in reparative process of lung injury await further investigation. The biologic importance of Type III collagen is apparent from studies of hereditary Type III collagen deficiency. These patients exhibit a high propensity for rupture of large arteries and bowel and development of keloids and contracture of wound.[51,58] It is possible that some of the fibrotic diseases of the lung may be due to suppressed or imbalanced synthesis of Type III collagen, analogous to keloid formation in patients with Ehlers–Danlos syndrome Type IV. The presence of Type III collagen in wound repair in myocardium, skin, and tendon has been documented.[45]

Problems Related to Quantitation of Collagen Fibers and Collagen in Reaction to Injury

Chemical quantification of lung collagen has been based on analysis of certain "marker" amino acids of collagen-type proteins—hydroxyproline and hydroxylysine. Since both collagen fibers and basal laminas contain these amino acids, any changes in hydroxyproline or hydroxylysine content should not be attributed solely to changes in collagen fiber content.

Basal lamina has been shown to be increased in pulmonary reaction to injury such as in chronic congestion.[59] Analysis for 3-hydroxyproline, which is characteristic for basal lamina collagen,[17] may be helpful in assessing quantitative changes in basal lamina. Unfortunately, unlike glomerular basal lamina, the basic biochemical data of lung basal lamina are not available. The presence of Type I and Type III collagens further complicates the problem. Their hydroxylysine and hydroxyproline contents are different (Table 1). Unless the relative proportion of two types of collagen is known, the margin of error can be up to 100% based on hydroxylysine analysis and 14% based on hydroxyproline analysis.

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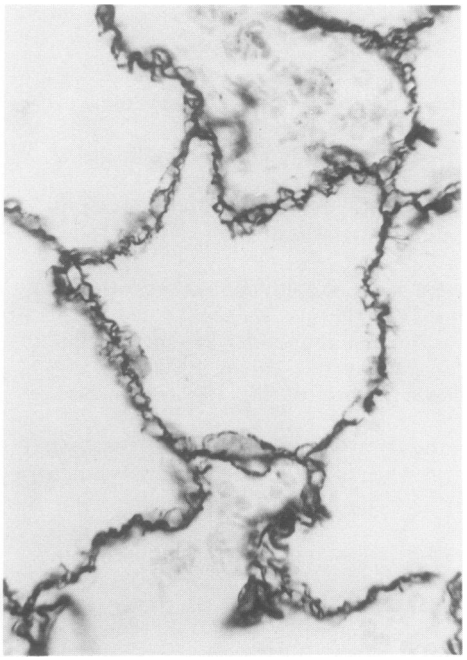
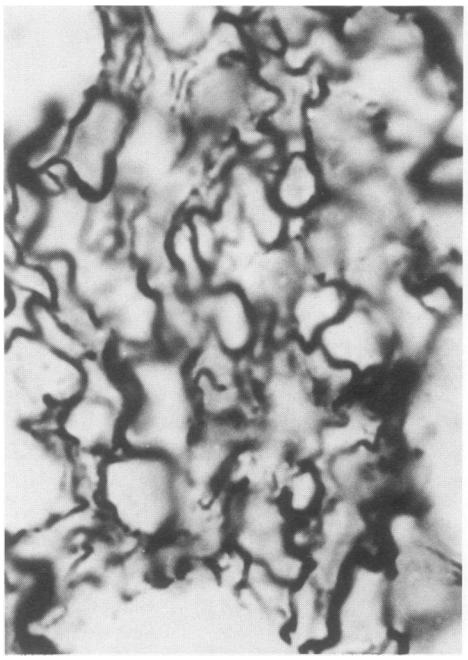
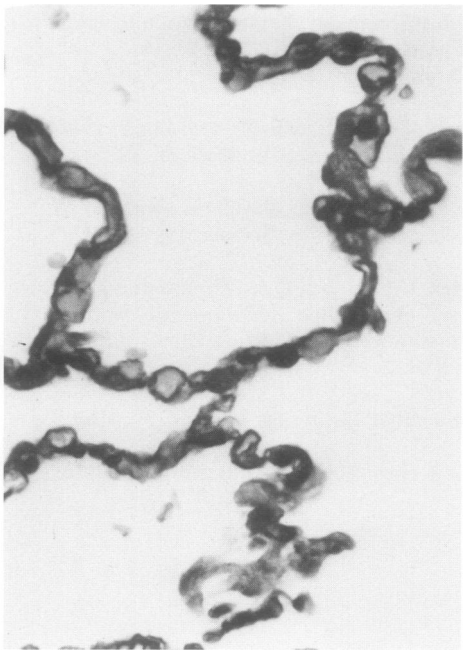






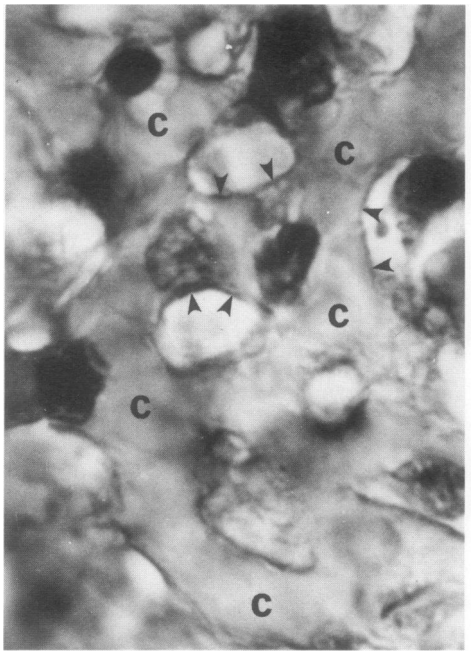
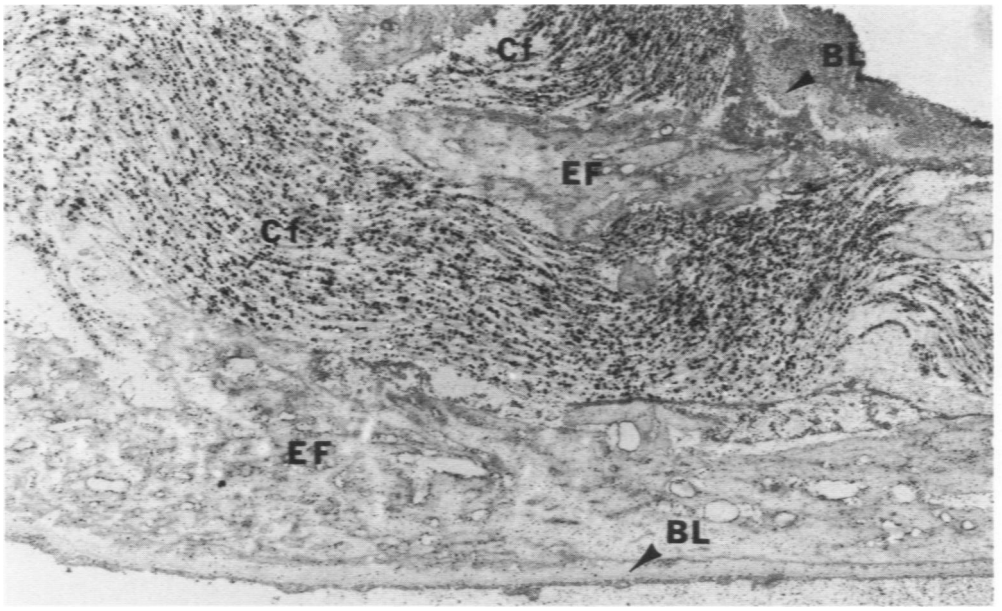
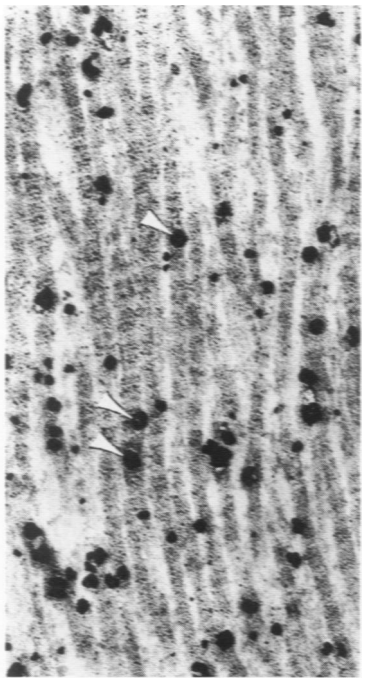


Figure 1—Reticulum fibers in alveolar septa, in cross-section (Reticulum stain, × 550). **Figure 2**—Reticulum fibers in an alveolar septum, *en face*; the elaborate network of undulating fibers, 0.4 to 0.8 μ in width, is apparent (Reticulum stain, × 1700). **Figure 3**—PAS-stained alveolar septa, in cross-section. There is a "fibrillar" reticular network similar to the reticulum fiber network shown in Figure 1. A PAS-positive membranous structure between the reticulum network is also evident. (PAS stain, × 560). **Figure 4**—PAS-stained alveolar septum, *en face*. The PAS-positive materials (*arrowhead*) mark the profile of an anastomosing capillary channel (*C*), distinct from the reticulum fiber network shown in Figure 2. (PAS stain, × 1800).





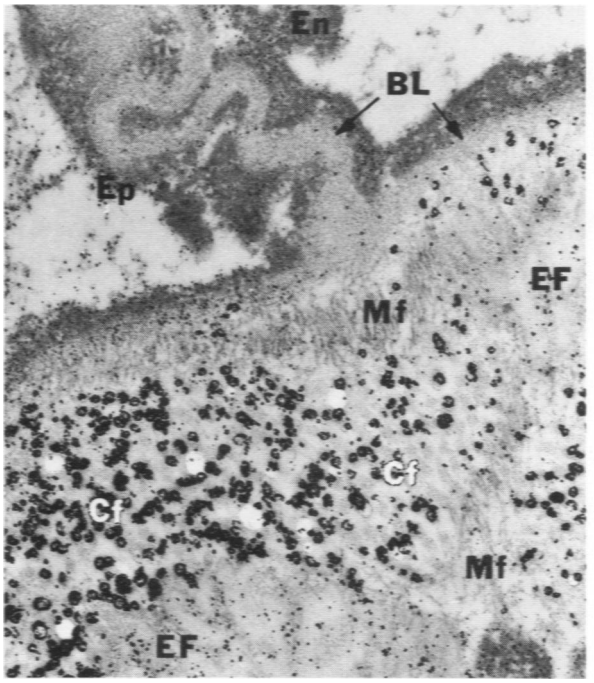
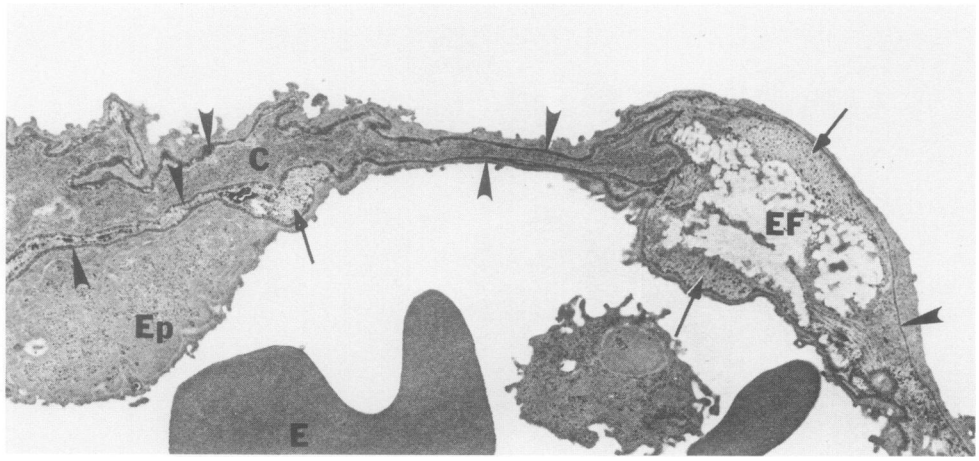


Figure 5—Electron micrograph of a silver-impregnated alveolar septum. The electron-dense silver granules are associated with collagen fibrils (*Cf*). The elastic fiber (*EF*) and basal lamina (*BL*) are not stained. (Uranyl acetate and lead stain, × 10,000) **Figure 6**—Alveolar collagen fibrils after silver impregnation. Silver granules (*arrowheads*), 10 to 25 nm in diameter, are seen in close association with collagen fibrils, which exhibit characteristic 64-nm periodicity. (Uranyl acetate and lead stain, × 73,500) **Figure 7**—Silver-impregnated alveolar septum. The epithelial and endothelial basal lamina (*BL*), microfibrils (*Mf*), and elastic fiber (*EF*) are free of characteristic silver granules, which are specifically deposited in collagen fibrils (*Cf*). Fine metal precipitates, less than 4 nm in diameter, appear as a nonspecific background stippling. They are more pronounced in cytoplasm and nuclei of epithelial (*Ep*) and endothelial (*En*) cells. (× 35,000)



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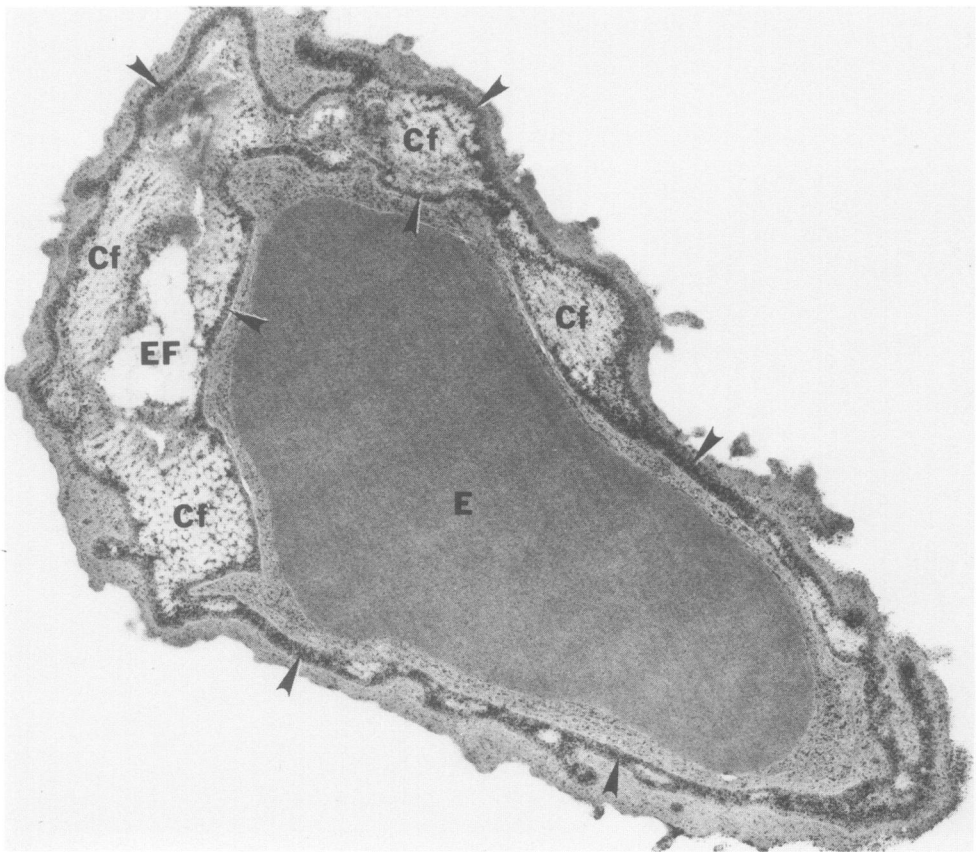

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Figure 8—Alveolar septum after PTO reaction, without other heavy metal stains. The epithelial and endothelial basal lamina (*arrowheads*) is intensely stained. Collagen fibrils (*arrows*) and elastic fiber (*EF*) are not stained. *Ep* = epithelial cell, Type I pneumocytes; *C* = capillary; *E* = erythrocyte. (× 6800) **Figure 9**—Higher magnification of alveolar septum after PTO reaction, without other heavy metal stains. The PTO-positive material is epithelial and endothelial basal lamina (*arrowheads*). A capillary is filled with an erythrocyte (*E*). Collagen fibrils (*Cf*) and elastic fiber (*EF*) do not stain with PTO reaction. (× 17,800)

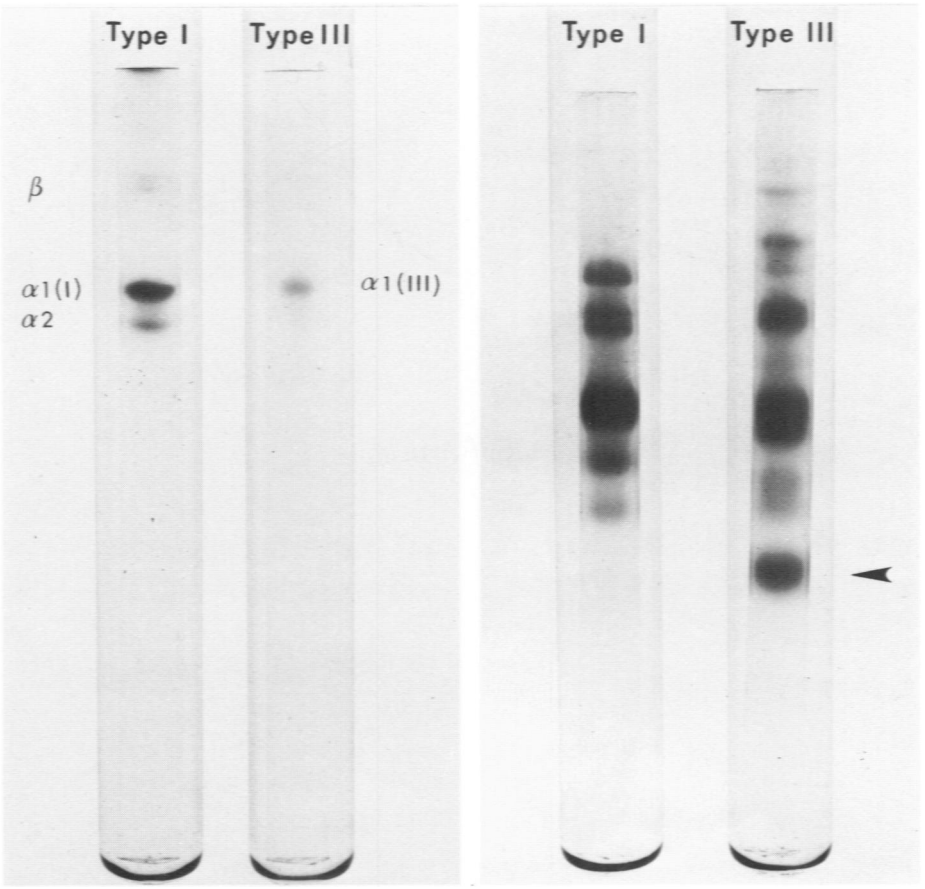

Figure 10—SDS polyacrylamide gel electrophoresis of Type I and Type III collagens from human lung. **A**—Chain composition. **B**—Cyanogen bromide–cleaved peptides. The presence of cyanogen bromide peptide 6 (*arrowhead*) is very characteristic of Type III collagen. The length of gels is 11.5 cm.

[End of Article]