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Changes in the alveolar connective tissue of the ageing lung

An immunohistochemical study

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Summary. The modifications of the extracellular matrix (ExM) components in the alveolar parenchyma of elderly subjects were investigated using a panel of polyclonal antibodies. The elastic fibers showed a notable decrease along the alveolar walls while type III collagen increased when compared with that of non-elderly controls. No variations of these components were detectable in the alveolar ducts or in the respiratory bronchioli. An increase in the thickness of the alveolar basement membranes was detected in some of the subjects when antibodies against type IV collagen and laminin were used, while antibodies to fibronectin and type V collagen did not reveal any modifications compared with the controls. The modifications revealed in the lungs of the elderly can be related to the alterations of the elastic recoil and pulmonary compliance observed in these subjects.

Key-words: Ageing lung – Extracellular matrix components – Immunohistochemistry

Introduction

Over the years, observations of changes in lung tissue in the elderly have encouraged a large number of studies and controversies between clinicians and pathologists (Hyeronymi 1961; Pierce and Ebert 1965; Turner et al. 1968; Hartung 1975). Important unresolved problems concern the existence of a true ageing emphysema (Giese 1959), and the qualitative or quantitative changes in the stromal network in the ageing lung. However, the fact that pneumologists still disagree on the functional role played by the stromal components of the normal lung obviously impedes interpretation of the functional significance of these changes. There is general agreement that, with unchanged total lung capacity a decrease of the elastic recoil, of the forced expiratory volume and of the vital capacity are typical of the ageing lung. Functional residual capacity, pulmonary compliance and the airway's liability to collapse during the maximum expiratory flow are all increased in the elderly (Turner et al. 1968). In old age the chest wall assumes a fixed inspiratory posture, showing a decrease in its compliance which is not compensated by the contemporary increase in the pulmonary compliance. These modifications induce the elastic tissue to account for much of the work (Hartung 1975; Thurlbeck and Angus 1975).

Mean decreases of more than 20% in the mass and the specific gravity of the lung tissue are observed within the age range from 60 to 90 years (Astrand et al. 1973; Andreotti et al. 1983). Moreover, a reduction of the respiratory surface and of the alveolar capillary bed (Hartung 1975; Thurlbeck et al. 1975), associated with a decrease of the maximum uptake of oxygen (Astrand et al. 1973) has also been described. These modifications correspond to a dilatation of the respiratory bronchioles and alveolar ducts associated with flattening of the alveoli and consequent increase of the radius of curvature (Giese 1959; Turner et al. 1968; Hartung 1975).

Hance and Crystal (1975) asserted that the elasticity of the lung is a consequence of the physical properties of the stromal fibers and of the alveolar surface and geometry. More recently, Yasuoka et al. (1977) and Shimura et al. (1986) have demonstrated that the quantitative and qualitative modifications of the surfactant in the ageing lung are both minimal. However, it must be remembered that the change in surface free energy is a product

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of surface tension and modifications in surface area (Brown 1957).

Therefore, the changes of the elastic properties in the ageing lung should be, at least, partially correlated with stromal changes. However, the relationships between stroma and elastic properties of the lung are unclear. A large majority of pneumologists consider the elastic fibers responsible for the physiological variation of the V/P rate, while the collagen fibers are thought to confer the necessary elasticity to the lung parenchyma at higher pressures (Hance et al. 1975). Other authors, such as Pierce et al. (1965), consider lung elasticity to be related to the quantity of collagen fibers undergoing stress rather than to elastic tissue, and suggest that the correct orientation of the collagen lattice of the lung parenchyma is maintained by virtue of the orderly pattern of the elastic fibers. Whichever of these models may be the most valid, it is certain that any qualitative or quantitative changes of the fibers will cause respiratory dysfunction.

The purpose of this paper is to study the stromal components of a series of non-pathological ageing lungs using immunohistochemistry. As far as we know, the changes of the stroma in the ageing lung have not yet been studied by these techniques.

Materials and methods

A series of specimens of normal pulmonary parenchyma obtained at autopsy from 19 consecutive males were sampled. Subjects with chronic pulmonary diseases were excluded (Table 1). Twelve cases were used as probands (mean age 76 years, range 67 to 88 years), while 7 others were used as controls (mean age 45 years, range 28 to 58 years). Multiple samples (200 g of tissue) were obtained from the sub-pleural parenchyma on the bases of the upper lobes which had been previously inflated with 10% neutral formalin, using a technique described by Churg (1983), and maintaining a constant pressure. After adequate fixation, a series of blocks from each specimen were processed using routine histopathological techniques. The sections were stained with haematoxylin & eosin, Weigert-van Gieson, Hotchkiss-MacManus and with the silver stain technique for reticulin (Alessi and Eusebi 1969). A series of sections which had been deparaffinized and processed using a technique described by D'Errico et al. (1986) and D'Ardenne et al. (1983) were tested with the following sera:

Anti-laminin 1:100; Anti-fibronectin 1:200; Anti-type III collagen 1:200; Anti-type IV collagen 1:500; Anti-type V collagen 1:500; Anti-elastin 1:600

Rabbit antiserum to (mouse) laminin was purchased from Bethesda Research Laboratories Inc. USA (cat. No. 6265 LA) and was proved not to be cross-reactive with collagens (Type I, III, IV), fibronectin and heparan-sulphate proteoglycan (Bianchi et al. 1984). The antibody against human plasma fibronectin was prepared from immune rabbit antiserum. Specific IgG were isolated on a CNBr-Sepharose affinity column containing bound fibronectin from the total IgG fraction collected from a Sepharose-Staphylococcal protein A column specific IgG, eluted using 3M guanidinium-chloride, were then dyalized and lyophilized.

Rabbit antisera to unpepsinized EHS type IV collagen were prepared as previously described by Liotta et al. 1981 and purified through a 5 ml CNBr-activated Sepharose 4B affinity column containing bound type IV collagen (5 mg).

The antibody to human type V collagen from placenta was raised in NZW rabbits and purified on a human type V collagen affinity column using elution with glycine-HCl. In addition, antiserum was passed through type IV/III affinity columns. Specificity for this antibody was determined by ELISA (Barsky et al. 1982). Anti-type III collagen was provided by Dr. E. Chernes, Yale University School of Medicine, New Haven, Connecticut, USA; its specificity being tested by the ELISA method. Anti-elastin serum was obtained by immunizing New Zealand White Rabbits with both insoluble and soluble human elastin isolated from thoracic aorta. Insoluble elastin was purified by auto-claving (Partridge et al. 1955) followed by alkali treatment (Lansing et al. 1952). Soluble elastin was obtained by treating insoluble elastin either with 0.25 M oxalic acid (alfa-elastin) (Partridge et al. 1955) or with 1M KOH in ethanol (K-elastin) (Robert and Poullain 1963). By means of immunoelectronmicroscopy, the serum was found to be specific for elastic fibers (Daga-Gordini et al. 1984). The immunohistochemical technique used was Avidin-Biotin-Peroxidase (ABC) method (Hsu et al. 1981). The specificity of the sera were tested by substituting them with non-immune sera.

The immunohistochemical stains were performed simultaneously in a series of cases belonging to the proband and control groups. A double-blind technique was adopted to examine

Table 1

Probands	Controls
1) 76 years myocardial infarction	1) 28 years ulcerative colitis and peritonitis
2) 88 years prostatic carcinoma	2) 55 years septic shock
3) 78 years embolic occlusion of the pulmonary arteries	3) 47 years sudden cardiac death
4) 82 years cerebral infarction	4) 49 years cerebral infarction
5) 73 years hepatic cirrhosis	5) 40 years fulminant viral hepatitis
6) 84 years intestinal infarction	6) 32 years acute myelocytic leukemia
7) 71 years aortic dissecting aneurysm	7) 57 years traumatic injury
8) 73 years myocardial infarction after hemicolectomy	
9) 67 years myocardial infarction with heart rupture	
10) 67 years endometrial carcinoma with liver metastases	
11) 74 years peritonitis after surgical treatment	
12) 87 years sudden cardiac death	

the histopathological and immunohistochemical preparations. The morphological patterns observed in the control group were considered "normal", and any plus or minus variations with respect to these normal patterns were evaluated by comparing a series of microphotographs taken at standard magnifications. Only clearly evident modifications were taken into account.

Results

Dilatation of the respiratory bronchioles and alveolar ducts as described by Turner et al. and Hartung was observed in 10 of the 12 probands but in none of the controls. In the affected patients, the alveolar spaces appeared to be flattened (Fig. 1). The severity of these changes was proportional to the age of the subjects. Furthermore, a thinning of the alveolar walls and a slight decrease in the number of capillaries was observed in the probands. The diameter of the capillary lumen was extremely irregular, while that of the controls was regular.

In the acini of the controls, large bundles of elastin were regularly observed encircling the respiratory bronchioles and ducts, as well as the openings of the alveoli; thinner fibers were located in

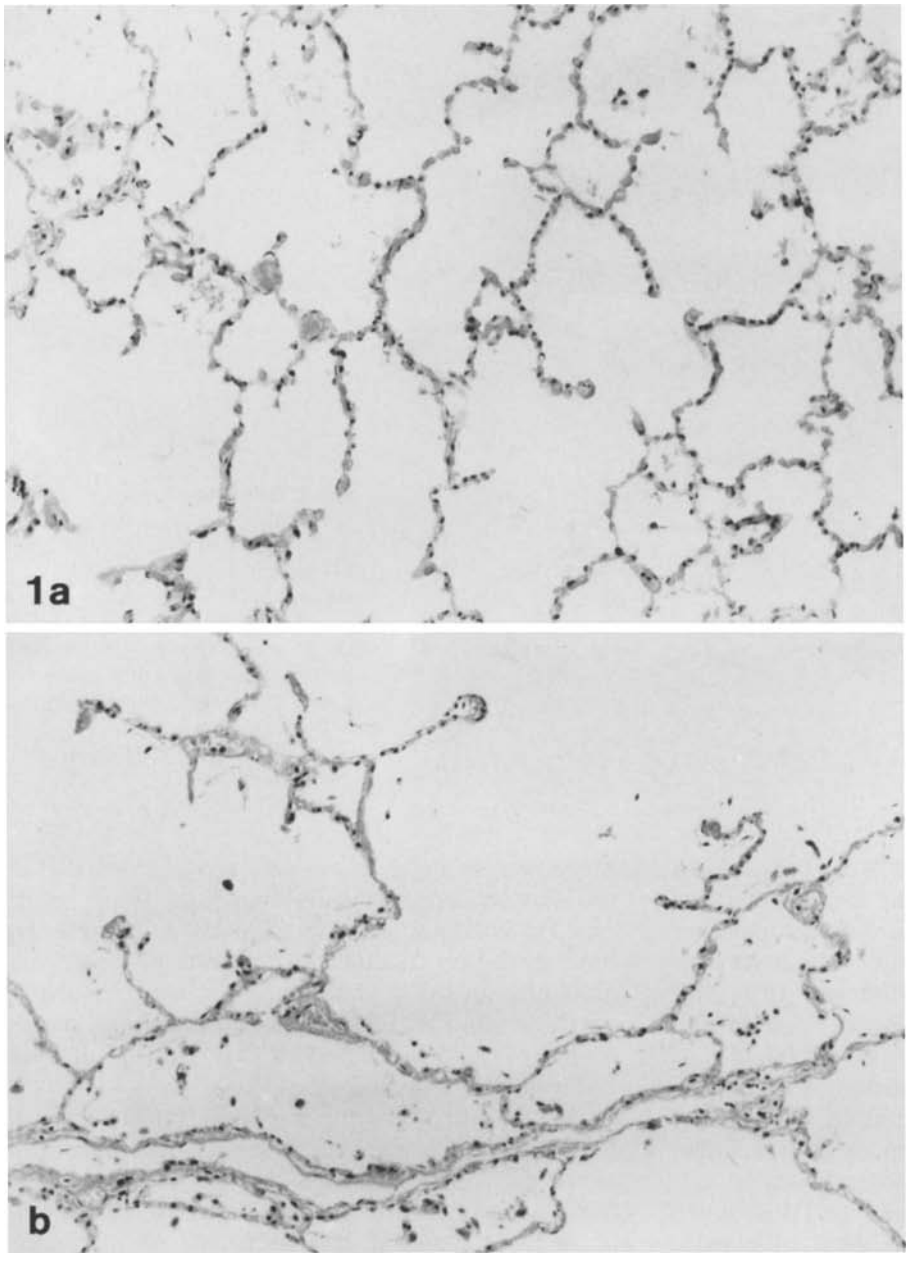


Fig. 1. Pulmonary parenchyma of a 40-year-old subject (**a**), compared with that of an 81-year-old subject (**b**). Note the remarkable dilatation of the alveolar ducts in the lung of the elderly subject. H&E. ×10

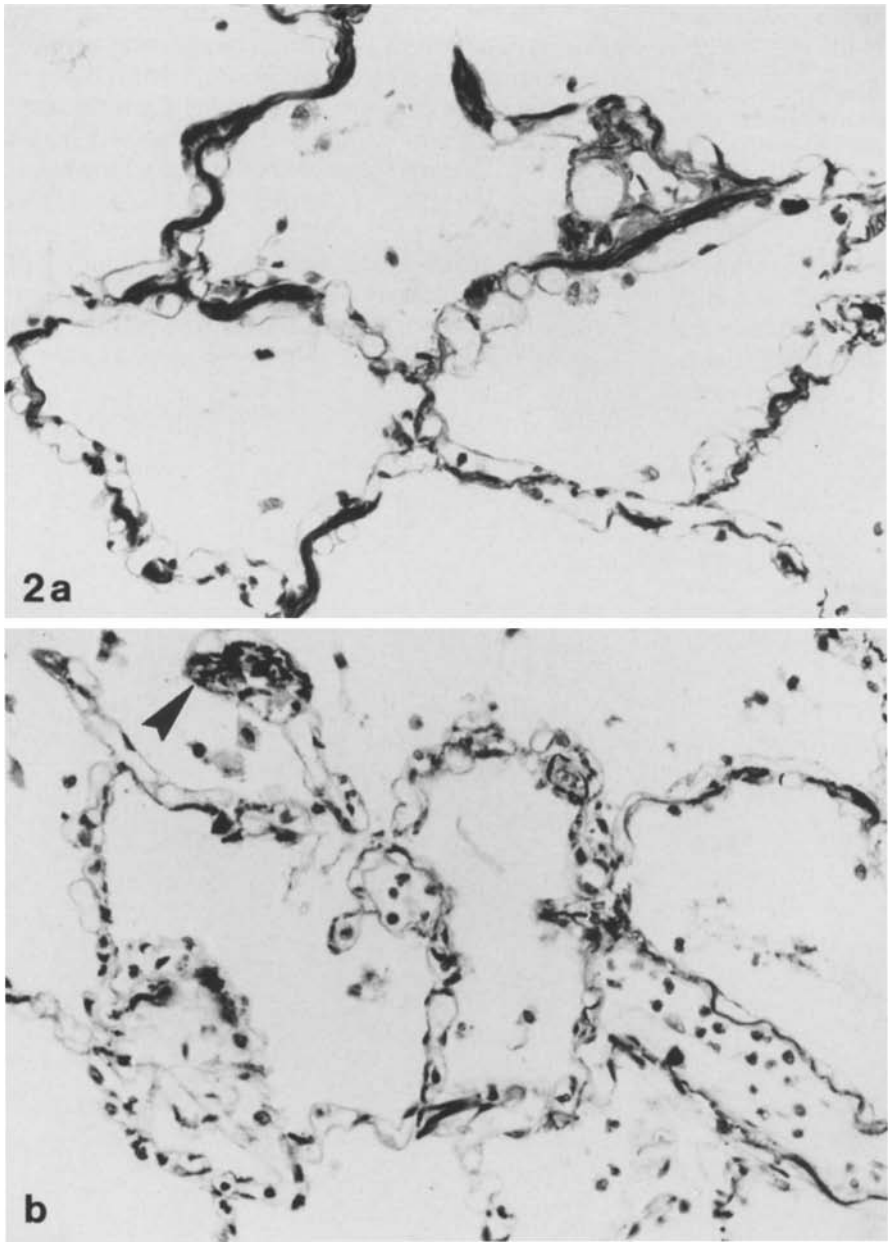


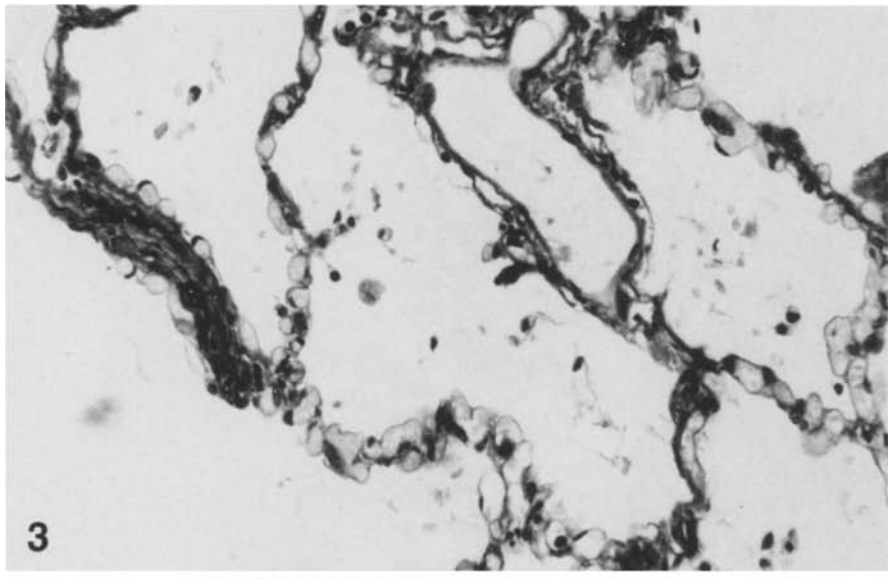
Fig. 2. Pulmonary alveoli of a 49-year-old subject (**a**), ABC method ×40, compared with those of an 87-year-old-subject (**b**), ABC method ×25. Elastic tissue, revealed by antibody anti-elastin, can be seen to be abundant in the control, while it is scarce in the axial stroma of the elderly subject, although it is still normally represented along the alveolar duct (see *arrows*)

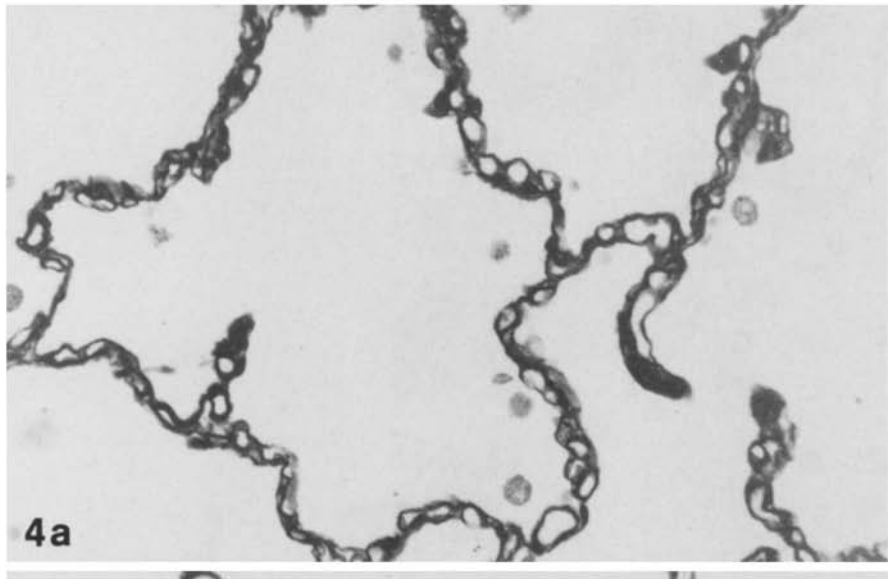
the axial region of the alveolar wall (Fig. 2a). In the probands, elastin showed the same pattern as that of the controls in the respiratory bronchioles and ducts, but along the alveolar walls it was revealed as thin and fragmented filaments (Fig. 2b). No relationship appeared to exist between the age of the probands and the changes described.

In all cases, the stroma of the acinus was intensely positive to the anti-fibronectin sera. No meaningful differences were documented between probands and controls or between single cases.

In the controls the pattern of type III collagen distribution was very similar to that of elastin. Moreover, in no case was this antigen revealed in the alveolar capillary walls (Fig. 3). In 10 probands type III collagen was detected as thick bundles both in the centriacinar structures and, more significantly, in the alveolar capillary walls, whilst in the remaining two instances the distribution was similar to that of the controls. No relationship with age was demonstrated (Fig. 4).

All basement membranes were sharply marked by antisera to type IV collagen and laminin both in the probands and controls (Fig. 5a). In 4 probands, an evident thickening of the basement membranes was detected, similar to the positivity for





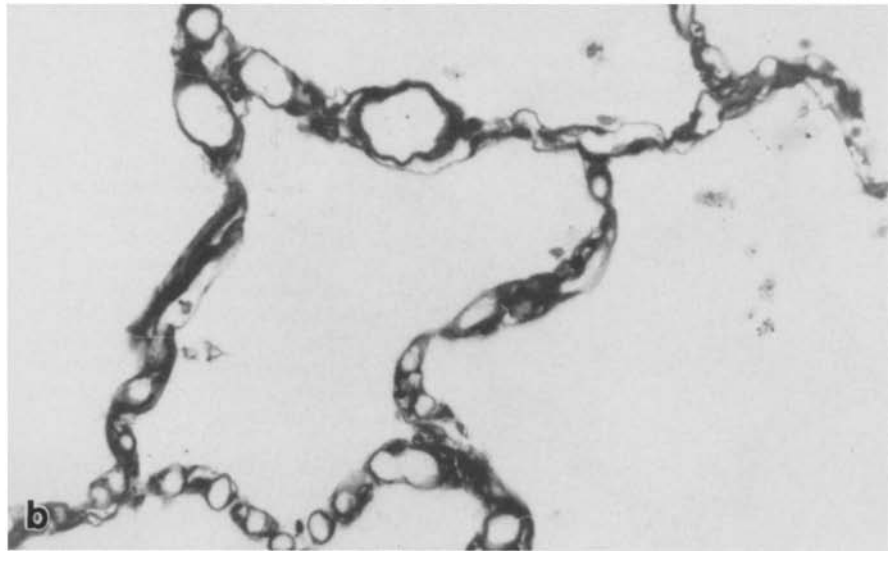
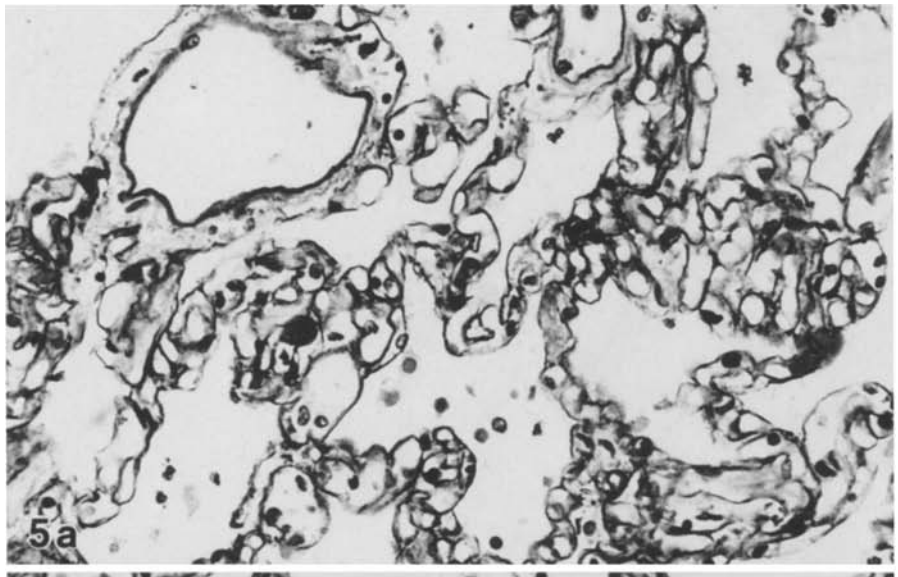
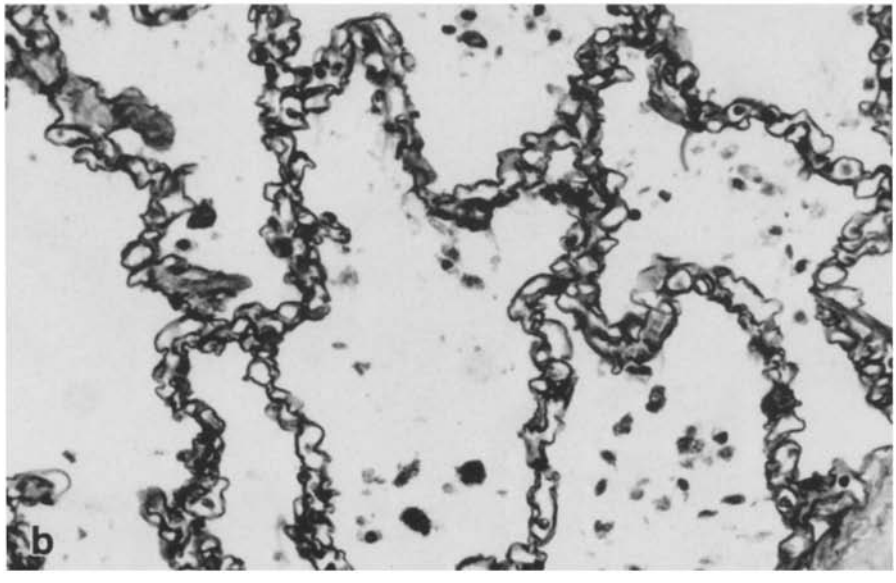


Fig. 3. Pulmonary alveoli of a 49-year-old-subject, stained with antibody anti-type III collagen. This is found abundant along the alveolar ducts, while it is scarce in the alveolar walls. ABC method $\times 40$

Fig. 4. Antibody anti-type III collagen shows a strong positivity along the stroma of the alveolar walls both in a 88 year-old-subject **(a)** ABC method $\times 40$, and in a 87 year-old-subject **(b)** ABC method $\times 40$





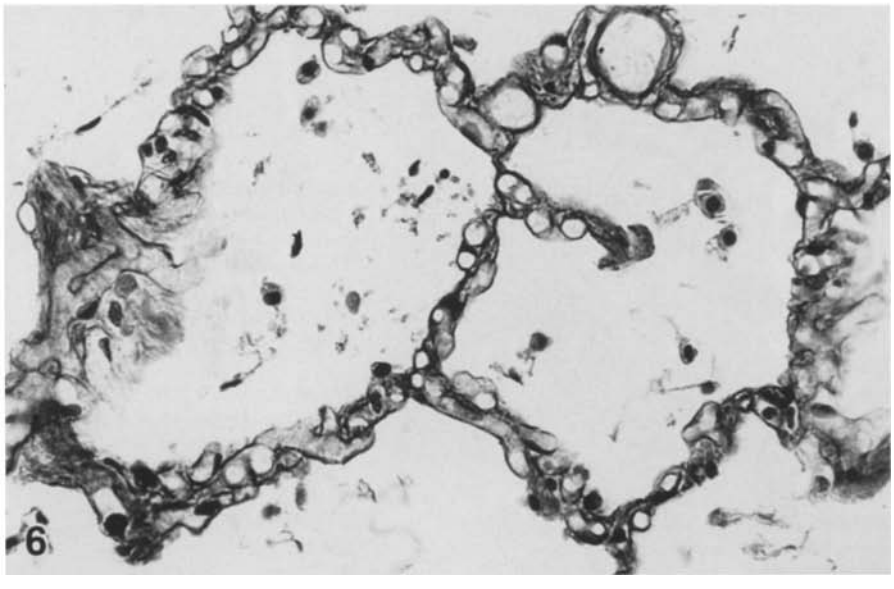


Fig. 5. Immunohistochemical staining with antibody anti-type IV collagen. The basement membranes are evident and continuous in the alveolar walls of a 57 year-old-subject (**a**). ABC method ×40. In a 81 year-old-subject, basement membranes showed a more evident thickening (**b**). ABC method ×40

Fig. 6. Antibody anti-laminin shows a clear thickening of the alveolar basement membranes in a 87 year-old-subject. ABC method ×40

type III collagen in the wall of the alveolar capillaries (Figs. 5b and 6).

In both controls and probands the anti-type V collagen antiserum marked the alveolar basement membranes of the acinus and some fibrils present both in the alveolar walls and ducts, and in the respiratory bronchioles.

Discussion

This study confirms the changes usually described in the lungs of persons over 65 years old, with the typical pattern of dilatation of the alveolar ducts and respiratory bronchioles. The classical findings are generally accompained by evident changes of the stromal components of the lung parenchyma. These can be summarized as follows: in 75% of the cases the elastic fibers were observed as thin and fragmented bundles in the alveolar wall, while in all cases thick strands of type III collagen were revealed along the axial zone of the alveolar wall; in half the cases, type III collagen was also observed at the alveolar-capillary membrane. In a quarter of these cases the alveolar-capillary membranes showed an evident thickening using the anti-type IV collagen and anti-laminin sera. These data throw some light on the apparent disagreement in the literature about the quantitative and qualitative changes in the stromal components of the pulmonary acinus observed in the ageing lung.

As far as the elastic tissue is concerned, some authors have described a decrease of this tissue in the ageing lung (Hance et al. 1975; Pump 1976; Ranga et al. 1979), while others have reported an increase of the elastic tissue proportional to age (Briscoe et al. 1959).

Turner et al. (1968) and Andreotti et al. (1983) demonstrate an increase in stability of elastin proportional to ageing; this is probably due to an increase of the cross-links among elastin proteins and is not associated with quantitative variations of elastic tissue. Thus elastin increases only with respect to the weight of the organ (Andreotti et al. 1983). In fact, the latter always decreases with age, sometimes by more than 20% and this would explain the discrepancies concerning elastin usually found in the literature. There is no proportional decrease when the quantity of elastin is compared with the volume of the lung. However, Hyeronymi (1961) describes an increase of the elastic tissue in the centriacinar zones when the alveolar walls are unaffected. Our findings demonstrate a regular distribution of elastin in the ducts and bronchioles and a decrease in the alveolar walls, where elastin normally consists of thinner fibrils than those found in centriacinar zones (Pierce et al. 1975).

Briscoe et al. (1959) and Hance et al. (1975) have demonstrated an increase of collagen in the senile lung with respect to the dry weight of the organ and a decrease in the soluble fraction of collagen. However, Turner et al. (1968) and Andreotti et al. (1983) have simply described changes in the quality of the lung collagen, due to a decrease in its insoluble fraction.

In these studies the whole lung is considered, not just the acinus, and no attention is paid to the different distribution and prevalence of the single types of collagen within the different sites of the acinus. Our study confirms the presence of dynamic matrix changes in ageing lungs, mainly manifest along the alveolar walls. In particular, the presence of thin and fragmented elastic fibrils intermingled with thick strands of type III collagen suggest the prevalence of this last component, often accompained by a remarkable thickening of the basement membranes. The changes in the ageing lung are mainly limited to the alveolar wall, as established by Giese (1959) and Pump (1976).

Our findings explain the decrease in the elastic recoil and the increase of the pulmonary compliance with age. The latter is partially compensated by the decrease of the compliance of the chest wall. The changes described are also accompained by a reduction of the alveolar capillary network and, in some cases, by a thickening of the capillary wall. These modifications may explain the reduction of the maximal uptake of oxygen reported in the literature (Astrand et al. 1973; Hartung 1975; Thurlbeck et al. 1975).

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