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Three Dimensional Structures of Pulmonary Elastin; Airway VS Vascular Elastin

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Elastin is known to occur in the lung parenchyma and pleura as well as in the pulmonary vessels, but no detailed studies of this elastin's linkage between them have been done in three dimensions. For many years we have known that there is abundant elastin in the mammalian lungs, which may be associated with etiology of causing emphysema. We have developed selective casting methods to allow us to determine the location where elastin is found morphologically. The method involves casting either the vasculature via the right ventricle, or the airways via the trachea in the air sacs. Studies of the vasculature were done with the lung inflated to 80% of the vital capacity. The casted lungs were then put in 0.1 N NaOH at 75℃ for 48 hours, turning them frequently. This method removed all non-elastin tissues. The scanning electron microscopy (SEM) was used to reveal the three dimensional pictures of elastin structures from both lung parenchyma and pulmonary vessels. Elastin was seen as fenestrated sheets and some fibers in both the vessels and the airways. Elastin in the two different locations was often interconnected. Studies on 6 dogs, 8 rabbits, and 2 pigs showed no significant species difference at the level of resolution of the SEM, which was used to study the specimens after they had been freeze-dried.

Key Words: Pulmonary elastin, sheet and fibers, selective casting, vascular and alveolar elastin

Elastin is the most extensible biological material in mammals, and tends to occur in virtually all tissues and organs which undergo large changes in size. It can strain up to 200~300% of its initial length, and stores elastic energy very efficiently. Thus it is not surprising that elastin exists in both the blood vessels (Cox, 1984) and the lung parenchyma and the pleura (Mercer and Crapo, 1990, Mercer *et al.* 1987, Oldmixon and Hoppin, 1984, Sobin *et al.* 1988).

The lung contains two different systems for flow: the airways and the blood vessels. Flow in the airways is tidal and terminates in the alveolar sacs, while flow in the vessels goes through both large and small arteries and veins. Both systems must undergo changes in volume and so in diameter, although the strains (change in volume/initial volume) in the airways are larger, and the pressures lower, under normal physiological conditions. We (Song and Roach, 1983, 1984) have shown that aortic elastin can be studied by removing all non-elastin material with 0.1 N NaOH at 70~75℃ for 3~5 hrs. The elastin which remains appears as fenestrated sheets in the intima and media, but as closely interconnected fibers on the adventitial side. Here we used the same method to assess the pulmonary

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elastin, but needed longer digestion times as the lung floated. We found it impossible to separate pulmonary from vascular elastin without the aid of selective casting of each system. In this study it is aimed to reveal any differences or similarities between pulmonary and vascular elastin among various species of animals.

MATERIALS AND METHODS

All studies were done on lungs of healthy adult animals that had been euthenized with pentobarbital (100 mg/Kg) at the conclusion of acute experiments which should not alter the lung elastin. These experiments were carried out using 6 dogs, 8 rabbits and 2 pigs. Since morphologically there do not appear to be species differences, we have lumped the data from all these species.

(1) Vascular casts were made on 2 dogs, 2 pigs and 2 rabbits, by cannulating the pulmonary arteries through the right ventricle. Then approximately 20 to 100ml of Batson's methacrylate resin (Batson's monomer 2.5 gm, Catalyst 0.7 gm, promotor 0.05 gm and Serviton 1.2 gm) was injected at pressures less than 50 mmHg depending on the size of the animals, and allowed to set for 24 hrs after the lung had been inflated to about 80% of the vital capacity. Note that we used a high pressure for infusion as the material is very viscous. However, the material set at the pressure associated with the appropriate volume in the airway. Since Kratky and Roach (1984) showed that Batson's cast shrinks about 20%, the dimension of the cast may be incorrect, but the structure and shape filled should be reasonably correct and agreeable.

(2) Airway casts were made after degassing the lung in a vacuum dessicator. Then the trachea or bronchus was cannulated and 50 cc. of silicone elastomer (Dow Corning) injected to the airways (Song *et al.* 1985, 1992). This filled all of the large airways and many of the small ones, often down to and including the alveoli. This procedure was done on 2 dogs and 4 rabbits.

(3) Lungs with no casting material were filled with air to 80% of the vital capacity and the trachea was tied.

The lungs were removed and digested in 0.1 N NaOH at 70~75℃ for 48 hrs, turning them frequently to ensure uniform digestion. Usually rabbit lungs which were smaller needed only 24 hrs of digestion. If the digestion was too long or the temperature of the bath was too hot and/or too alkaline, all of the lung tissues except the casting material and cartilages were dissolved.

The lungs were then immersed in distilled water and the elastin, which appears to be neutrally buoyant, returned to approximately its normal configuration. Pieces of the lung were then processed for freeze-drying with a Virtis Freeze drier (Model No. 10-030) at −50° C for 24 hrs until completely dried. The dried specimens were then mounted on aluminum SEM studs (1.2 cm diameter, JB EM seies Inc.), sputter coated with Au-Pd in a Polaron Instruments Inc. coater, and studied with a Philips Model 501 SEM. The detailed method of observation had been presented in previous papers (Song and Roach 1983, 1984, Song *et al.* 1985, 1992).

OBSERVATIONS AND RESULTS

Lungs took much longer to digest than the aortas we had studied previously (Song and Roach, 1983, 1984), presumably because they tended to float even if turned over frequently and the air space that could hardly contact with chemicals. Initially the elastin, especially in the vascular walls, turned a greenish color, possibly due to denatured hemoglobin from the red blood cells. Eventually it became oyster-white and the texture looked like thin white paper when freeze-dried. Before being dried, it tended to adhere to any rough surface unless it was suspended in water.

Figs. 1 (a & b) show a dog's lung with both large and small vessels filled with casting material. It is worth to note the large amount of sheet-like elastin which presumably is both pleural and parenchymal. There are also a

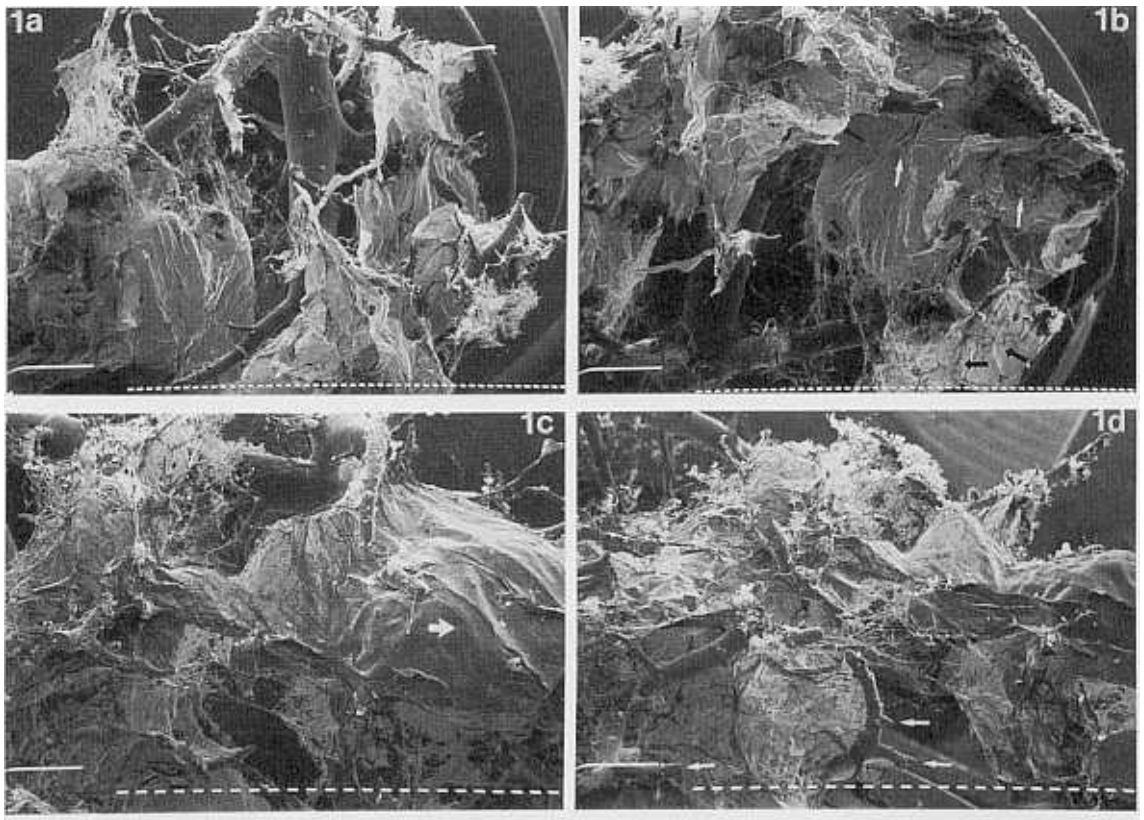

Fig. 1. *Structures of lung elastin after pulmonary vessels were filled with casting materials and then digested with hot alkaline solution for 48 hrs.*
(a) Dog's lung shows a large branch of pulmonary artery in the middle and small branches toward periphery. Mag. ×20. White bar=100um.
(b) Another dog's lung, small vessels (arrows) can be seen in the elastin sheet. Mag. ×20. White bar=100um.
(c) A pig's lung, though almost the right half covered with pleural elastin sheet, shows complicated vasculatures of pulmonary vessels on the left. Mag. ×40. White bar=100um.
(d) Another view of the pig's lung reveals various shapes of vascular endings due to incomplete fillings (arrows). Mag. ×40. White bar=100um.

few thread-like fibers connecting the sheet-like structures to the vessels. Fig. 1 (c & d) show similar structures from pig lungs. Since these sheet-like elastin structures are found further away from the vessels (arrows), they must be a part of interstitial or parenchymal connective tissues in the lung. In both Figs. 1 a, b and c, d, vascular systems are well delineated by the casting material and the pleural and parenchymal elastin appear separate, although there appear to be some connections between them.

Another series which also have casting material in the vessels are presented in Figs. 2 a, b, c, d and show closer relationships between vascular and airway elastin. The airways can be identified as hollow tube-like structures especially in Figs. 2 a, b, c and particularly at the upper left corner of Fig. 2a (black arrow). The tunnel-like structures seen on Figs. 2 b, c

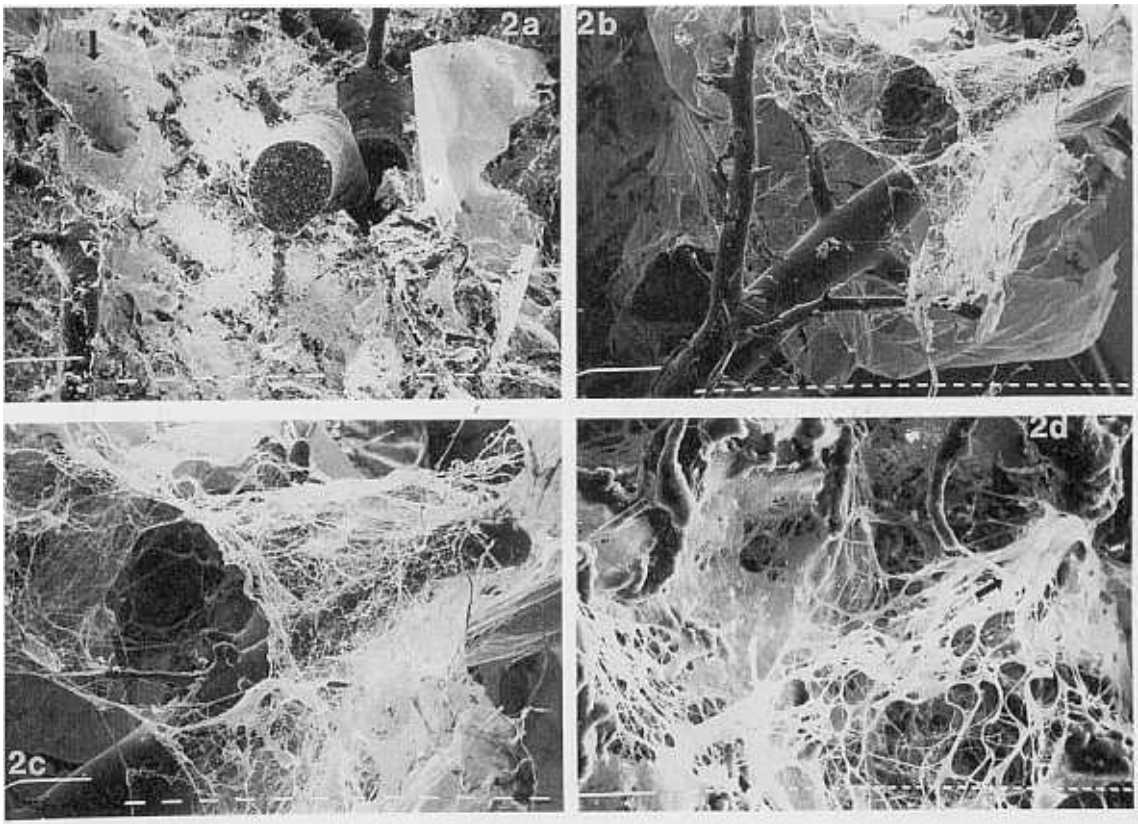

Fig. 2. Various forms of airway elastin in both pig and dog lungs after casting pulmonary vessels and hot alkaline treatment.

(a) A cut surface of vascular cast is showing in the center among airway elastin sheet especially on the right side of the pulmonary vessel. Tunnel-like shape at the left upper corner likens an airway structure (arrow). Mag. ×80. White bar=100um.

(b) One of dog's lungs shows a distinctive spider web-like elastin structure along the course of a pulmonary artery cast. Mag. ×40. White bar=100um.

(c) A magnified view of Fig. 2b is showing connections between pleural elastin sheet and fibrous airway elastin (dog). Mag. ×80. White bar=100 um.

(d) A pig's lung treated the same way as above shows a linkage between vascular elastin and airway elastin. Inside the lung the airway elastin is consisted of fibers and sheets with holes. Mag. ×160. White bar=10um.

are formed by fibrous mesh-like elastin walls and we believe these are part of the air duct system because they run parallel to the vascular tree. The interstitial elastin shown on Fig. 2c has fenestrations of various sizes as well as fibers. The next series of pictures (Figs. 3 a, b, c, d) show the airways of rabbit lung filled with casting materials. In Fig. 3a, a large bronchus (about 700 um in diameter) is shown in the center of the picture with smaller bronchioles filled also with casting material, and many alveoli around them. Some clusters formed by several alveoli are also clearly seen in Figs 3a, b, c. In these pictures hollow tubes such as at the bottom of the large bronchus in Fig. 3a, and the left lower corner

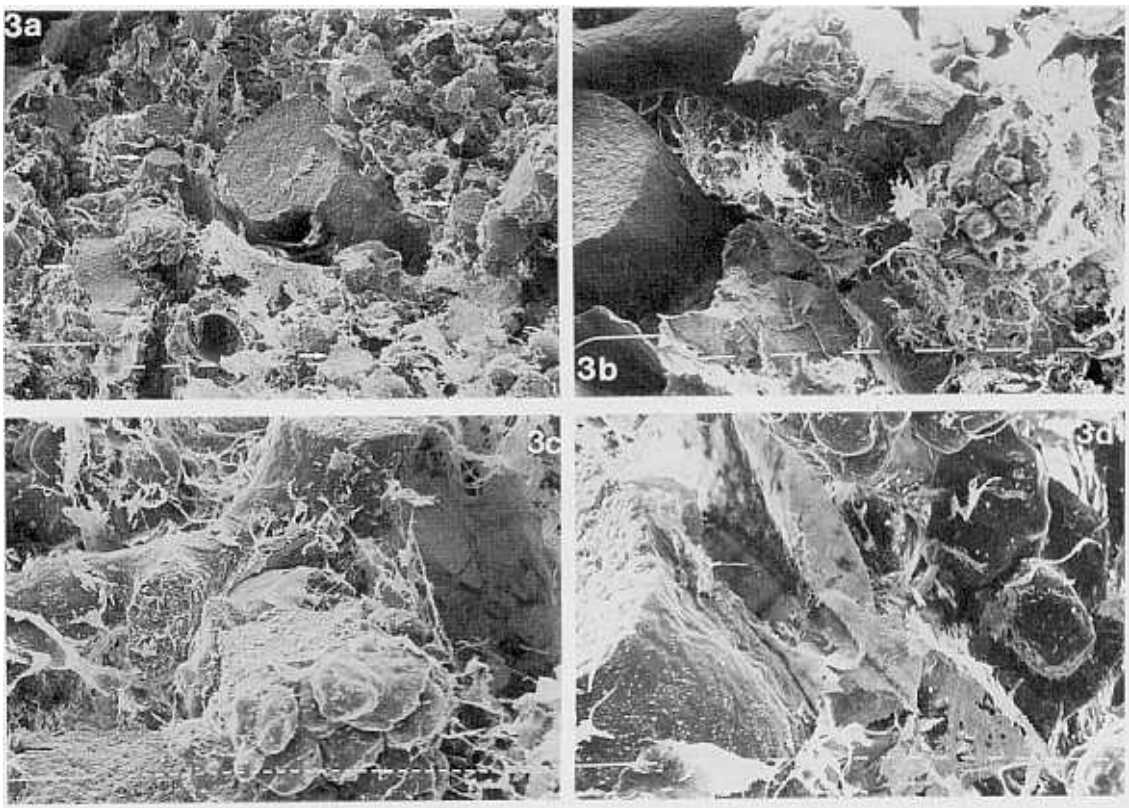

Fig. 3. *A series of rabbits' lung pictures after airway was filled with casting material and then digested with hot alkaline solution for 24 hrs.*

(a) A large bronchus and several small bronchi surrounding the large one at the center are shown as being cut after filling the airway (Arrows). Patches of alveoli clusters can be seen. Mag. ×40. White bar=100um.

(b) An empty tube of vascular elastin is seen at the lower left corner of the picture next to a bronchus which is filled with casting material. Various forms of non-airway elastins can be seen. Mag. ×80. White bar=100um.

(c) A cluster of alveoli elastin is shown at the lower center and these alveoli are connected with casting material to a bronchus showing a cut edge at the upper center of the picture. Mag. ×160. White bar=10um.

(d) Between a cast of bronchus and alveoli clusters, there is a tube-like elastin sheet with several fenestrations. Mag. ×320. White bar=10um.

of Fig. 3b must be vascular elastin in contrast to the hollow tubes seen in Figs. 1 and 2 which are from the airways.

The fenestrated sheet of vascular elastin is more apparent in Fig. 3d near the lower central part of the picture. A few fibers seen in Fig. 3c could be a part of vascular elastin or small vessels embedded in elastin fibers. At this level of magnification it is difficult to judge between arteriolar elastin and capillaries surrounded by elastin fibers.

Figure 4 shows a dog's lung prepared in the same way as the rabbit lungs shown in Figs. 3. In this photograph the casting material can

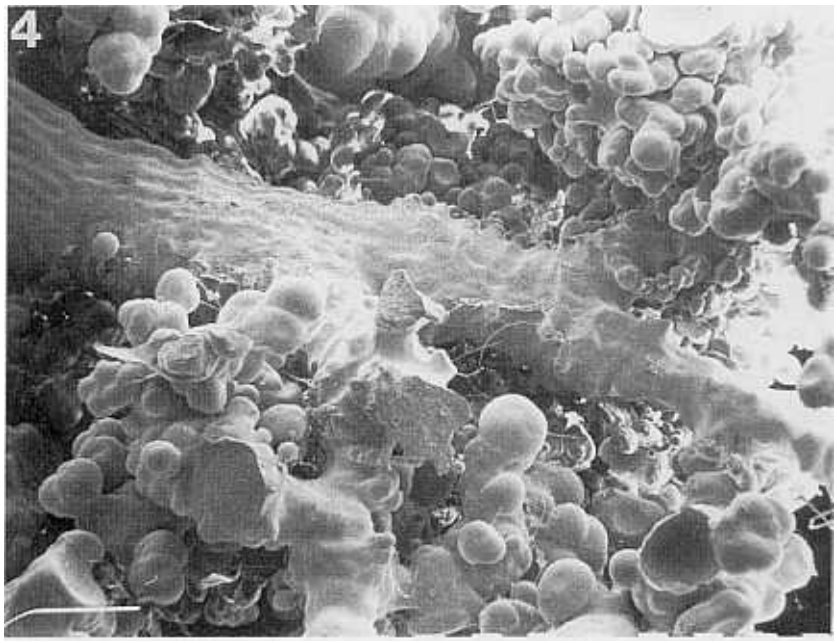

Fig. 4. *A dog's lung after maximum amount of casting material was pushed into air space and digested with hot alkaline solution for 48 hrs. Mag. ×40. White bar=100um.*

be seen infiltrated into the terminal alveoli presumably at higher pressures than anywhere else. However, the sizes of alveoli are not unique, but heterogeneous and range from 50 to 200 um in diameter. Note that the air spaces between alveoli are interconnected and formed grape cluster-like structures. In the upper center of the picture (Fig. 4) a large airway is seen. Note the longitudinal indentations on the surface of it.

Figs. 5 (a, b, c, d) show the appearance of the pulmonary elastin if no casting material is introduced into either the vascular trees or the airways. Because of the knowledge obtained with the selective casting method described above, it is now possible to see the patterns of both pulmonary and vascular elastin and to observe that they contain both sheets with holes and fibers in various sizes. Since the rabbit lungs were not inflated in Figs. 5, the air spaces seemed to be smaller. However, the air spaces surrounded by parenchymal elastin are well maintained and do not appear to have collapsed in Figs. 5 b, c, d. An area of the lower right corner of Fig. 5a is magnified in Fig. 5b and shows a ring-like structure of pulmonary elastin. The diameter is approximately 70 um and it is surrounded by fenestrated pulmonary parenchymal elastin. The structure may be a part of a terminal bronchiole or an alveolar duct. The other air spaces shown in Figs. 5 c,d are constructed by relatively solid elastin membrane, although a small portion of fibrous elastin is seen in the middle of Fig. 5c.

DISCUSSION

We have observed that collagen fibers and other connective tissues than elastin can be completely dissolved out of aortic walls when the aorta is treated in 0.1 N NaOH at 75℃ for 3 hrs (Song and Roach, 1983, 1984). However, in the case of the lung, there are more complicated problems than those in the aorta such as geometry or large differences in air

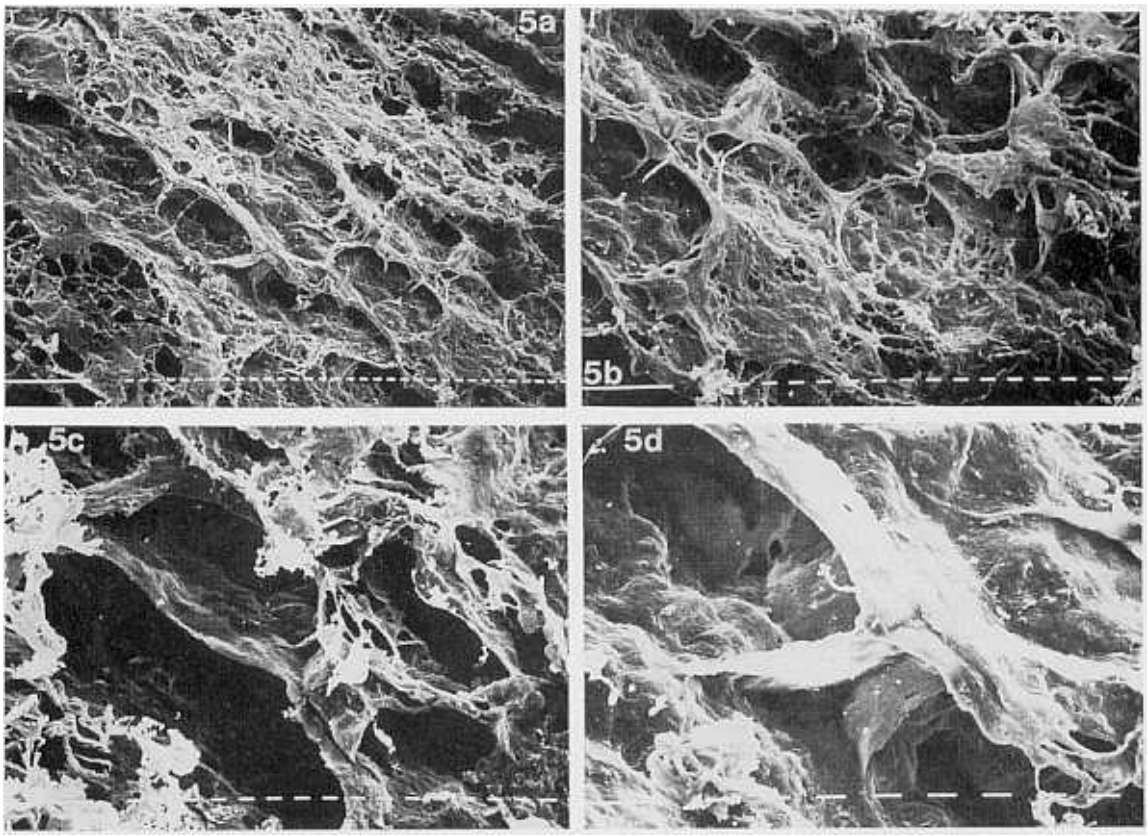

Fig. 5. *Structures of rabbit lung elastin after hot alkaline treatment for 24 hrs.*
(a) Lung elastin including pleural and vascular elastin is shown as membranes with holes and fibers. Mag. ×160. White bar=10um.
(b) A magnified view of the right lower corner of Fig. 5a is showing a round ring form of elastin which suggests a part of airway duct. Mag. ×320. White bar=10um.
(c) Another area of airway space shows pockets made of elastin sheets and junctions where vascular and airway elastin could join together. Mag. ×320. White bar=10um.
(d) Further magnification of the elastin junction cannot separate vascular elastin from airway or alveolar elastin. Mag. ×640. White bar=10um.

volumes between inflation and deflation. Furthermore there are at least 3 different sources of tissues for elastin, e.g. pleura, interstitial or parenchymal and vascular. In addition, because of air spaces in the organ it is difficult to immerse the organ completely in the alkaline solution and therefore we tried longer hours of digestion between 10 and 72 hrs.

In earlier studies done by Wang and Ying (1977), lungs were treated in the alkaline solution for weeks at room temperature, while Pierce and Ebert (1965) treated the lung for brief periods of only 30 min at 100℃. We have found, in this study that to obtain clear and purified specimens of pulmonary elastin, it took 48 hrs in 0.1 N NaOH at 70~75℃ for the whole lung of most species we studied. In a recent study, Soskel and Sandburg (1983) compared six different methods of extracting elastin from lung tissues and recommended the hot alkaline treatment as the best to recover the intact elastin even quantitatively.

It is obvious that SEM pictures taken from various lungs in this study represent the insoluble components mainly including elastin as of connective tissues and casting materials exclusively.

With selective casting followed by hot alkaline digestion, we have now clearly demonstrated that it is possible to separate the vascular from the pulmonary elastin. Both appear to have some sheet-like structures and some fibers, and there appear to be many connections between the two. It may be possible that elastin is so adherent to each other to form linkage aftrer digestion. At present study there is no confirmed evidence to deny the presence of connections. Another fact is that, at least at this level of resolution, there do not seem to be gross differences between the three species we have studied in pictures of elastin, but there are considerable differences between the cast (Figs. 1~4) and uncast (Fig. 5) lungs as the casting material can preserve the geometry to some extent. In general the vascular casts are the best as the casting does not have to displace air in the lung as the vessels are continuous while the alveoli have dead ends at the sac.

The pleural elastin shown in Figs. 1 (a, b, c, d) is more sheet-like than the parenchymal elastin (Figs. 2 b, c, d) which appears to have many large fenestrations (Fig. 2d), presumably for alveolar ducts or pores. Note that this method does not allow us to tell if the holes exist in vivo as they could well be filled with collagen, cement substance, smooth muscle cells, or small vessels in airways which are not sheathed with elastin.

The pictures shown on Figs. 2 (a, b, c, d) illustrate clearly how elastin formed the airways above the terminal bronchioles. Matsuda *et al.* (1987) described the presence of elastin in alveolar ducts and mouths of alveolar sacs. In fact at the level of trachea, elastin can be seen only surrounding the cartilage bones in this study, and after 24 hrs digestion, the elastin of the trachea disappeared. It is not clear whether the elastin in trachea is immature or less cross-linked like tropoelastin which are easily soluble. The web-like fibrous elastin shown in Figs. 2 (b, c) was described by Pierce and Ebert (1965) with light microscopy.

The air spaces shown in Fig 5 appeared to be shrunken because the lungs were partially collapsed when compared with those shown on Figs. 3 and 4. However, since the elastin is defined as one of the soft skeletal tissues, or scleroproteins (Andreotti *et al.* 1980), the alveolar and airway structures should maintain the basic framework as seen in Figs 3 and 4. The alveoli seen in Fig. 3c from a rabbit lung measured around 80 to 110 um in diameter, though the normal surface structures seen from undigested lungs (Greenwood and Holland, 1972; Gronioski *et al.* 1972; Nowell and Tyler, 1971) may not be the same as those seen from digested lungs.

Length-tension relationships such as those done by Sugihara *et al.* (1971) show that the lung is very distensible and so it is not surprising that large amounts of elastin are present. However, in view of heterogeneous combinations of collagens and elastin such as in pulmonary emphysema (Cardoso *et al.* 1993) it can be well postulated that gas inspired may unevenly distributed throughout different alveoli. Also in future studies it is planned to determine how much of this distensibility is tied to contents of the vascular elastin and how much to the lung parenchymal elastin.

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