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Morphological Evidence of Pulmonary Vascular Leakage Through Gaps Observed with Casting Methods and S.E.M.

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We have used selective casting methods to separate pulmonary elastin from vascular elastin in the lungs of rabbits, dogs and pigs. The lungs are digested with 0.1 N NaOH at 75℃ for 24~48 hours with frequent turning as the lungs are filled with air to about 80% of the vital capacity prior to the casting which is done at pressure of 20~50 mmHg. After vascular injections, we saw many small globular bits of casting material well separated from cast vessels and lying in the pulmonary elastin. Surface forces should make the casting material creep along the vessels even if they are not completely filled, so that the spherical shape is the one expected if the case is extruded into the parenchymal space and the air space. We conclude that this suggests that the pulmonary circulation is partially and temporarily 'open' as seen in the spleen and some other organs, rather than a completely 'closed' one as is generally accepted. At least some of these extravasations may be associated with lymphatics, although we have not proved this.

Key Words: Pulmonary circulation, selective casting method, elastin, extravasation

The lung is one of the most distensible organs in the body and can be distended from a residual volume of 1.5 L to a full capacity of 6.0 L in man. These large changes in volume must be accompanied by stretching of the component materials, particularly elastin which acts like biological rubber. We (Song and Roach, 1983) have demonstrated that aortic elastin can be otained by digesting the vessel in 0.1 N NaOH at 75℃ for 5 hours. In another series, we (Song *et al.* 1985) have also shown that combined casting and digestion studies can be used to demonstrate how small vessels such as vasa vasora travel through the elastin. In contrast to most corrosion casting, the digestion is stopped before the elastin is removed, while true corrosion casts are digested long enough that nothing but the casting material remains.

A number of studies have been done on the capillary circulation of the lung using corrosion casts, and theories range from the sheet-flow proposal of Fung and Sobin (1969) to the 'standard' capillary networks of Guntheroth *et al.* (1982). All of these theories assume that the pulmonary circulation is 'closed' and fluid does not leave normal capillaries. Contrary to these studies, it is well known that the pulmonary edema with movement of water and often blood and protein occurs with relatively small increase in pulmonary venous pressure such as those seen with mitral stenoses (Snashall and Chung, 1991). Frank hemoptysis is also quite common with mitral valve disease and suggests that the pulmonary capillaries leak, on some occasion. The alveolar-capillary membrane is very thin as one would expect with a system designed to maximize diffusion of oxygen and carbon dioxide. Thus it would not be surprising if these regions became leaky or created a partial open circulation as in the splenic sinusoids (Song, 1972). It is not well known

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on the function of the pulmonary lymphatics in clearing foreign objects such as inhaled particles or leaked red cells that reach the interstitial space. The lymphatic spread of carcinomas, particularly the breast carcinoma, shows the extent of the pulmonary lymphatics under pathological conditions.

Our present studies, initially identified which parts of the lung elastin were associated with the airwarys and with the vascular system. It also demonstrated that there may be a leakage from the pulmonary capillaries under normal conditions, suggesting that there may be a small open circulation as well as a larger closed one in the lungs of several species.

METHODS

Animals were studied immediately after the conclusion of acute experiments done with 10 mg/kg pentobarbital anesthesia, which included 2 male pigs (16 and 18 kg), 3 male dogs (14~25 kg), and 4 rabbits (2.5~3.2 kg).

A tracheotomy was done, and the chest cage was opened to the left of the sternum. The pulmonary artery was cannulated via the ventricle with the largest possible polyethylene catheter. then both lungs were inflated to approximately 80% of vital capacity and the trachea was clamped to prevent the volume changes.

Two different casting materials were used and produced similar results so the two groups have been pooled. The first group was infused with heparinated Ringer solution and then with Batson's methacrylate resin composed of 2.5 g Batson's monomer, 0.75 g catalyst, 0.05 g Promotor, and 1.2 g Sevriton. The second group was perfused with silicone rubber (elastomer; Dow Corning). The casting material flowed freely into the lung and the tissue gradually turned from pink to white as the small vessels filled with physiological pressures of 20~50 mmHg at the origin of the cannula. Best filling was obtained if the cannula was close to the pulmonary valve. If the cannula was more peripheral, then one lung filled better than the other.

To ensure that the casts were set firmly they were left for 24 hours, and then the lungs were removed and put in a beaker of 0.1 N NaOH kept at 75℃ (Soskel and Sandberg, 1983). The lungs were turned frequently as the air trapped in them made them float. Digestion was complete (i.e. casting material and elastin left) after 24 hours for rabbit lungs, and after 48 hours for pig or dog lungs. These specimens were then put in water where they appeared grossly to maintain their normal shapes. The elastin collapsed down onto the cast if the specimen was removed from the water. We (Roach and Song, 1988) have also used this neutral buoyancy of elastin with water to preserve arterial shape. Specimens were cut from different parts of the lungs. resuspended in water, frozen, and freeze-dried with a Virtis Freeze Drier (Model No. 10-630) at -50℃ and 0.1 Torr for 24 hours until they were completely dry. Elastin, then appeared as thin white sheets instead of the translucent ones seen in water.

The dried specimens were then mounted on aluminium SEM stubs (1.2 cm diameter; J.B. EM series Inc.), sputter coated with Au-Pd in a Polaron Instruments Inc. coater, and studied with a Philips Model 501 SEM.

RESULTS

We (Song *et al.* 1985b) have used the selective casting on the circulation or airways to allow us to identify which elastin is associated with which system. The picture in Fig. 1 shows the casting material in an arteriole or perhaps a small artery, surrounded by a fine meshwork of alveolar septal elastin. An arrow shows small blebs on the elastin surface of the arteriole or small artery. Again another

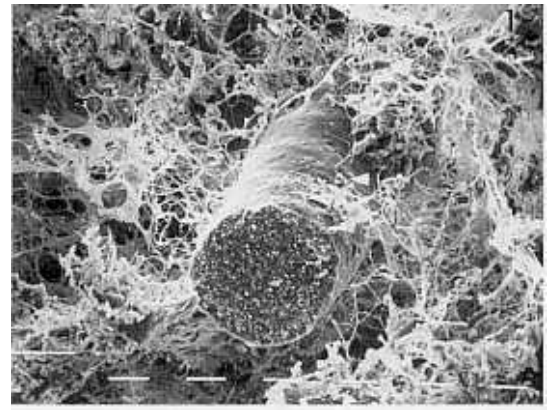

Fig. 1. *A dog's lung after 48 hours digestion in 0.1 N NaOH at 75℃ and Bastson's resin in pulmonary arteries. Fibrous elastin and elastin sheet with fenestrations are seen with small vascular elastin filled by casts. At the center of the picture a cut edge of Batson's is seen. White Bar=100 μm.*

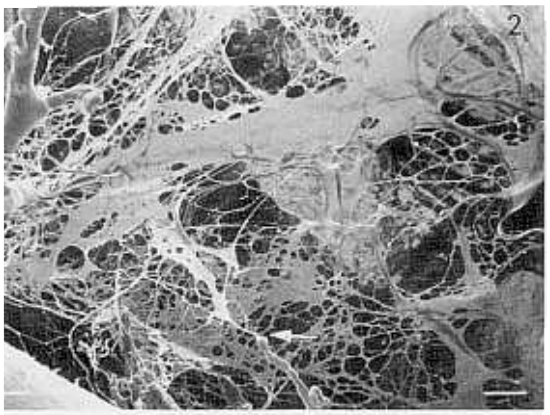

Fig. 2. A pig's lung. SEM of pulmonary elastin, the same treatment done as in dog's lung seen in fig. 1. Many small vessels in elastin sheet are filled with cast. An arrow shows a candle-tip-like vascular ending of casting material. White Bar= 100 μm.

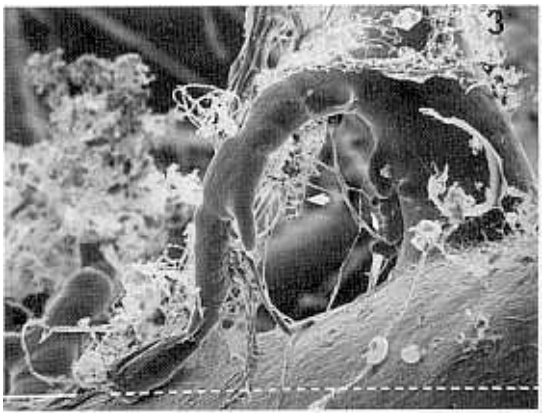

Fig. 3. Another pig's lung. A corrugated shape of cast filled an artherial branch showing incomplete filling and uneven hardening. White Bar= 10 μm.

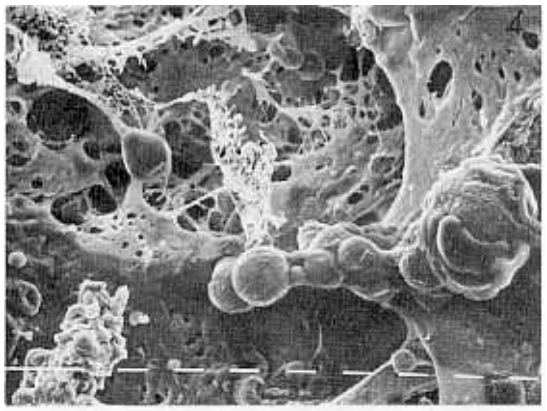

Fig. 4. A rabbit's lung. SEM of pulmonary elastin. Many globular protrusions are seen and a cluster of capillary network is seen at the left lower corner of the pictures. White Bar= 10 μm.

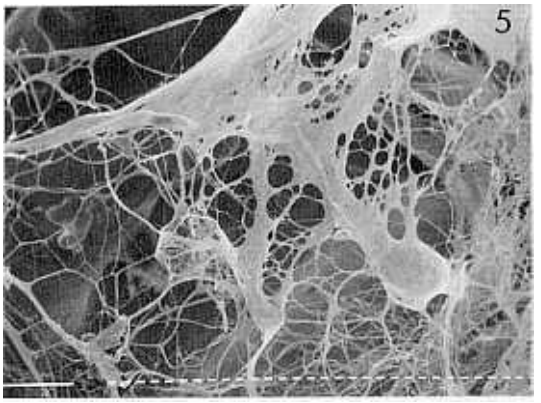

Fig. 5. A pig's lung showing divisions of elastin sheet and a ball-like bulge of cast at the end. White Bar= 10 μm.

picture in Fig. 2 shows the casting material in smaller vessels which are only partially filled along with the netlike structure of interstitial elastin. It is noted that the terminals, where the vessels are incompletely filled, are rounded as would be expected because of high surface forces.

By contrast, pictures shown in Fig. 3 and 4 show small bulbous arising from the sides of vessels and often appearing to be coated with the interstitial elastin often at the bronchioles and small alveolar ducts. In Fig. 5, there appears to be a ball of casting material encased in airway elastin at some distance from any vessels filled with casting material.

In the center of Fig. 6 we can observe two small drops of casting material (white arrow) which are located far from any vascular cast, but appear to be related to the airway elastin. Two other small droplets of casting material are also seen on a thread in the lower right corner of Fig. 6, and again at a considerable distance from any cast vessel. It is rare to

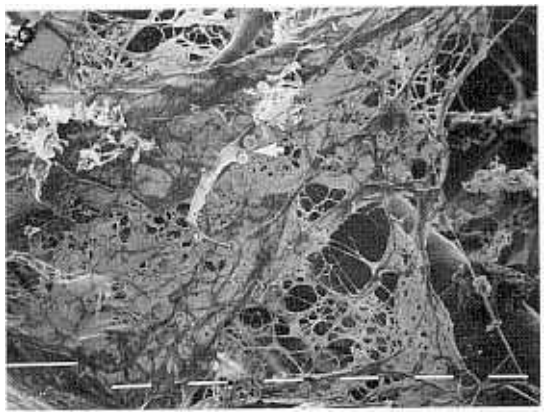

Fig. 6. A pig's lung. SEM of pulmonary elastin. Small vessels running through parenchymal elastin are filled with casting material. An arrow at the center of the picture shows two globular forms of cast protrusions. White Bar = 10 μm.

Fig. 7. A dog's lung. A fenestrated elastin sheet is lying under a cast-filled vessel and a few globular masses appear to be aggregated around the vasculature. White Bar = 10 μm.

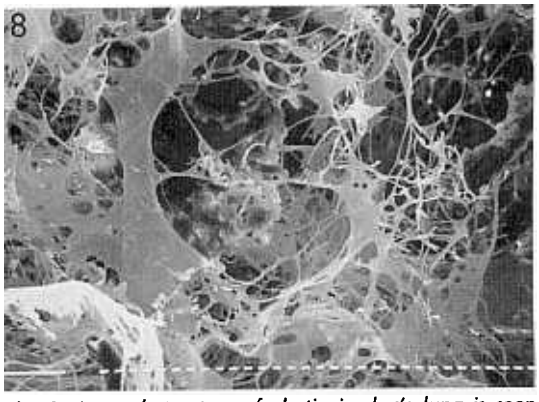

Fig. 8. An oval structure of elastin in dog's lung is seen and behind this, there are many small blebs from casted vessel. White Bar = 10 μm.

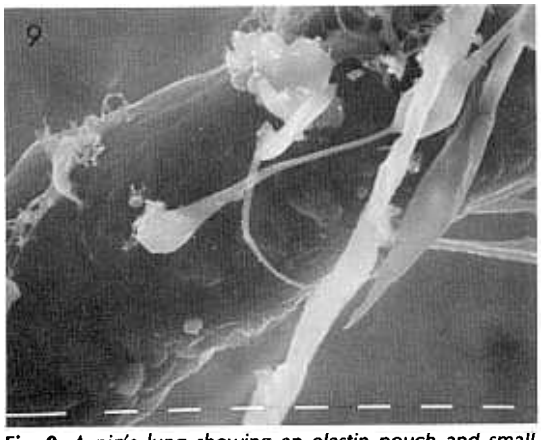

Fig. 9. A pig's lung showing an elastin pouch and small blebs are shown at the entrance of the pouch. White Bar = 10 μm.

observe arterioles or capillaries in the vessel shown with casting material in it as the branches usually arise in a tree-like pattern. On other occasions, as shown in Fig. 7, the small round blebs of casting material are found adjacent to a capillary filled with casting material. These blebs which are about 5 μm in diameter could be mistaken for alveoli, except that they are too small. In addition, no casting material was injected into the airways. The picture in Fig. 8 shows another set of these blebs of casting material arising from a capillary with casting material in it. The airway elastin is seen near where as fenestrated sheets with many holes and adjacent fibers.

On a few occasions, as shown in Figs. 9 and 10 we have seen these small globules of casting material linked by what appears to be long slender stalks which run parallel to the arterial branch. These stalks are much too small to be veins, but could be lymphatics although this is not easy to prove at the present time. In this case the stalks are less than 3 μm in diameter. Surface forces cannot explain these

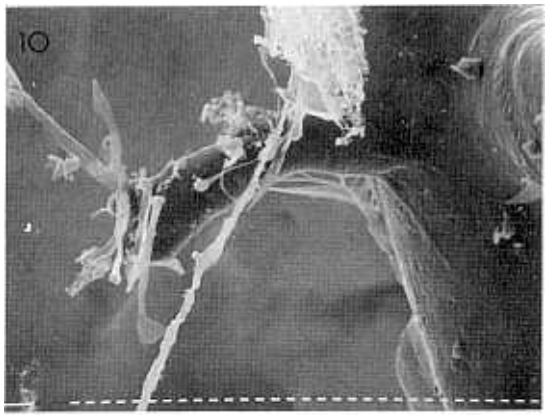

Fig. 10. A pig's lung showing an arterial branch with cast in and then a bleb attached to a slender neck paralled to the arterial branch. White Bar=10 μm.

shapes. These apparent leaks were rarely seen in the rabbit but appeared to be quite common in the lungs of the pigs and dogs.

DISCUSSION

Many attempts have been made to cast the complete vasculature of lungs. Frasca *et al.* (1978) obtained beautiful casts with their own ideal mixture of vinyl chloride co-polymers. Kratky and Roach (1984) have tested the shrinkage of a variety of casting materials used in large arteries and have varied the viscosity to avoid shearing damage to the surface if the cast was hardened too soon and was pushed through small vessls. The tapered blunt ends are typical of inadequate filling (Fig. 2) and the grooves on the edges of the cast vessel could be due to local muscle contraction or anisotropic shrinkage in Fig. 3. The blebs in the other pictures are unlikely to be artifacts. To our present knowledge, there are no blind sacs arising from pulmonary capillaries. We have seen these shapes on airway casts presumably from alveolar filling, but these are much larger ($80 \sim 180$ μm compared to $2 \sim 5$ μm) (Wang and King, 1977).

Although extravasation in healthy adult animals may seem inconceivable, it is not impossible to have seepage of larger molecules by an outward filtration process in the pulmonary microvascular system according to many other physiologists (DeFouw *et al.* 1983; DeFouw and Chinard, 1983; Parker *et al.* 1986; Risberg *et al.* 1982). However, this permeation should occur only at the capillary level as Wayland (1984) pointed out in his review. Castenholz *et al.* (1982) demonstrated the leakage of casting materials into the extravascular spaces around brain vessels and defined them as "plastic strips". Our findings in this study show that globules rather than strips appear to penetrate through small holes in the elastin sheet (Song and Roach, 1983, 1984, 1985a) and to protrude if all the vessels are "closed".

It is apparent that since the surface energy of the casting material is higher than that of the vessel surfaces, the casting material spreads efficiently over the wall. This explains why the outlines of endothelial cells are seen so clearly on casts (Kratky and Roack, 1987). However, the surface energy of the casting material is high compared to that of air, and so if the casting material is in air, it will form a sphere to minimize the surface energy of the whole system. This makes it seems very likely that the round drops are in the air spaces rather than in the vessels, while the drops sometimes appear to be covered (at least on one side) by elastin as shown in Fig. 5. If the drops were completely surrounded by elastin which appears to be in sheets or fibers, it would be more apt to take on their shape rather than to be spherical. The long narrow stems which appear to be attached to some of the blebs (e.g. Figs. 9 and 10) are very small, and in some cases, the long narrow stems seem to have casting material in the blebs. We speculate that they may be lymphatics, but have not proved their existence. However, the picture is compatible with the descriptions of pulmonary lymphatic channels (Risberg *et al.* 1982; Roberge *et al.* 1985; Rodrigues Grande *et al.* 1983).

The data presented suggest that there may be physiological leakages from the pulmonary vessels into the air spaces in normal lungs from a few species. Some of these leakages appear to come from fairly large vessels; some from capillaries and possibly from lymphatics. We are unable to conclude from this study if the leakage occurs through physiological 'pores' or through tiny damaged areas. However, due to their frequent appearances and consistent shapes we believe that they are not artifacts. Therefore, it is evident that the pulmonary circulation is not always 'closed' but 'open' as seen in the spleen (Song, 1972; Kashimura, 1985), brain, liver, kidney, and in the coronary systems (Majno, 1965; Simionescu *et al.* 1983).

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