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MICROVASCULARIZATION OF THE PLEURA IN RATS AND GUINEA PIGS**

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Abstract

The microvascularization of the visceral and parietal pleura was studied in rats and guinea pigs using vascular corrosion casts and scanning electron microscopy.

The visceral pleura was shown to be devoid of a vascular bed of its own. The capillary meshwork observed on the surface of the lung belongs to the pulmonary parenchyma. The parietal pleura, by contrast, possesses its own capillary network with an appropriate arterial supply and a venous drainage. The parietal pleural capillaries cover the costal regions completely, whereas the intercostal spaces are only provided by interspersed small patches of capillaries. That the feeding arteries of the parietal pleura are connected to the systemic circulatory system, supports the well-known fact that the parietal pleura is the main site for production of pleural fluid.

Key words: Visceral pleura, Parietal pleura, Microvascularization, Scanning electron microscopy, Vascular corrosion casts, Rat, Guinea pig, Mercox

**This work is dedicated to Professor Dr. Wilhelm FIRBAS on the occasion of his 50th birthday.

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Introduction

Experimental findings under physiological conditions indicate that the parietal pleura is responsible both for the production and re-absorption of pleural fluid, while the visceral pleura only insignificantly contributes to pleural fluid exchange (Miserocchi, 1991). Conversely, Agostoni and Piiper (1962) concluded that the pleural liquid is formed by parietal pleura and re-absorbed by visceral pleura. Earlier investigations (von Hayek, 1970) suggested that the human visceral pleura receives its blood supply from the pulmonary circulation. Other studies, however, distinguished between thick and thin visceral pleura and depending on the thickness of the organ they postulated both the systemic and pulmonary circulation as the sources of pleural blood supply. The visceral pleura in sheep and human for example is thick and is suggested to receive its blood supply from the bronchial arteries (Miller, 1937; McLaughlin *et al.*, 1961; Nagaishi, 1972; Albertine *et al.*, 1982). Animals with a thin visceral pleura, on the other hand, lack a systemic source of blood to their visceral pleura (McLaughlin *et al.*, 1961). This short review of the literature makes it clear that the function and blood supply of the two pleural membranes are still controversial. These problems were a challenging matter to use microvascular corrosion casting in order to shed light on the topic.

Material and Methods

Twenty adult Sprague Dawley rats (200-250 g body weight) and 17 guinea pigs (Cavia porcellus, 200-250 g body weight) of both sexes were used for corrosion casting. The animals were anaesthetized with ether, and thorax and abdomen were opened by a median cut. To obtain parietal pleural casts, in one half of the animals a plastic catheter (Argyle 0.8x19 mm, Sherwood Medical, St. Louis, MO, USA) connected to a two-way connector (LS-2B, Braun-Melsungen, Germany) was introduced into the aortic arch. To obtain visceral pleural casts, in the other half of animals, the cannula was inserted into the inferior vena cava at the level of the renal

veins. In the first group of animals, the common carotid arteries and the subclavian arteries were clamped just after arising from the aortic arch, the abdominal aorta at the level of the kidneys and the inferior vena cava was incised. In the second group the abdominal aorta was opened at the level of the kidneys. The systemic and the pulmonary circulatory system were rinsed (using manual pressure) in both groups with at least 80 ml 42° C warm heparinized Tyrode solution (5,000 IU /l) until the efflux of the cannulated inferior vena cava (first group) and of the abdominal aorta (second group) was clear.

Mercox CL-2B (Dainippon Ink and Chemicals, Tokyo, Japan) diluted with monomeric methylmethacrylate (v/v:4/1, final volume 20 ml; Hodde, 1981) was immediately injected through the cannulas. Thereafter the animals were left at room temperature for 2 hours and then placed into a 60° C water bath for final polymerization of the resin overnight. After solidification of the resin, chest walls and the lungs with adhering mediastinal vessels were dissected out and macerated in a 15% potassium hydroxide solution at 40° C for 2 days or longer. The cast specimens were cleaned in 5% formic acid for 30 minutes, rinsed in distilled water, and frozen in a small volume of water. Some of the frozen specimens were cut into 1-2 mm thick slices, using a specially adapted circular saw at -20° C. All specimens were freeze-dried, mounted onto copper foils and fixed by specimen stubs with conductive silver paste (Lametschwandtner *et al.*, 1980). The specimens were evaporated with carbon and gold for 3 seconds, then sputtered with gold for 600 seconds (Aharinejad *et al.*, 1989, 1990a) and examined with a Cambridge Stereoscan 90B SEM, using accelerating voltages of 10 to 15 kV.

Results

Before presenting our data it should be emphasized that the findings on parietal and visceral pleura were identical in guinea pigs and rats. For categorization of vessels as arteries, veins and capillaries, the following criteria were used: branching order of vessels (Anderhuber *et al.*, 1989; Aharinejad *et al.*, 1990c) and endothelial cell nuclei imprints (Miodonski *et al.*, 1976; Hodde *et al.*, 1977). In addition, both routes used in this study to perfuse the target organs led to filling of the lung vasculature. However, the parietal pleural microvascular bed together with that of intercostal spaces could be filled via the aortic arch only as the other tributaries of the aortic arch were clamped.

Visceral surface

On the whole surface of the lung a dense capillary network is present, which shows an indistinct lobular arrangement with veins localized in the center of each of these lobules (Fig. 1). A proper arterial feeding of the capillary systems is missing, the venous drainage, by contrast, is evident. The veins in their course are joined by capillaries, as they, increasing in size, descend towards the deeper parenchymal parts (Fig. 1).

Micrographs of the sectioned lung corrosion casts reveal areas around the larger airways and also at the bordering regions to the adventitia of larger vessels, which clearly resemble the capillary pattern described above (Fig. 2).

Costal surface

The microangioarchitecture of the parietal pleura differs from that of the lung visceral surface. The supplying and draining main vessels have a segmental arrangement (Fig. 3) which reflects the anatomy of the ribs and intercostal spaces. Each segment is provided with a main supplying artery and an accompanying draining vein, the posterior intercostal vessels, running along the inferior margin of the corresponding rib. The latter, at regular distances (in average 1.5 mm), give rise to first order arteries and veins at right angles (Fig. 3). First order arteries have an average diameter of 75 μm, they arise either in front or behind intercostal veins. One first order artery, in general, is accompanied by two first order veins, supernumerary veins, however, may be seen. From the first order arteries and veins, second order vessels arise which capillarize to form the parietal pleural capillary bed (Fig. 4). This capillary meshwork is composed of uniform, over longer distances running capillaries, although several interconnections and loops are present. The capillary casts are relatively delicate, their surface is smoothly outlined and the diameter remains constant along their course.

The pleural capillary network covers the regions above the ribs homogeneously, sometimes also overlapping the posterior intercostal arteries (Figs. 3, 4). The regions of the intercostal spaces, by contrast, can be clearly distinguished by characteristic corkscrew-shaped muscle capillaries. Only circumscribed patches of pleural capillaries are present in the intercostal areas, subjacent to which intercostal muscle capillaries lie (Fig. 3).

Discussion

In general, criteria for a proper vascular bed are: a supplying artery, a draining vein and an interposed capillary network between these vessels. Considering these criteria, a proper vascular bed of the visceral pleura has to be disregarded. Our findings clearly show that supplying arteries failed to be present on the lung surface, where visceral pleural vessels are usually expected to occur. On the other hand, although veins were seen, they do not exclusively drain the most superficial capillaries

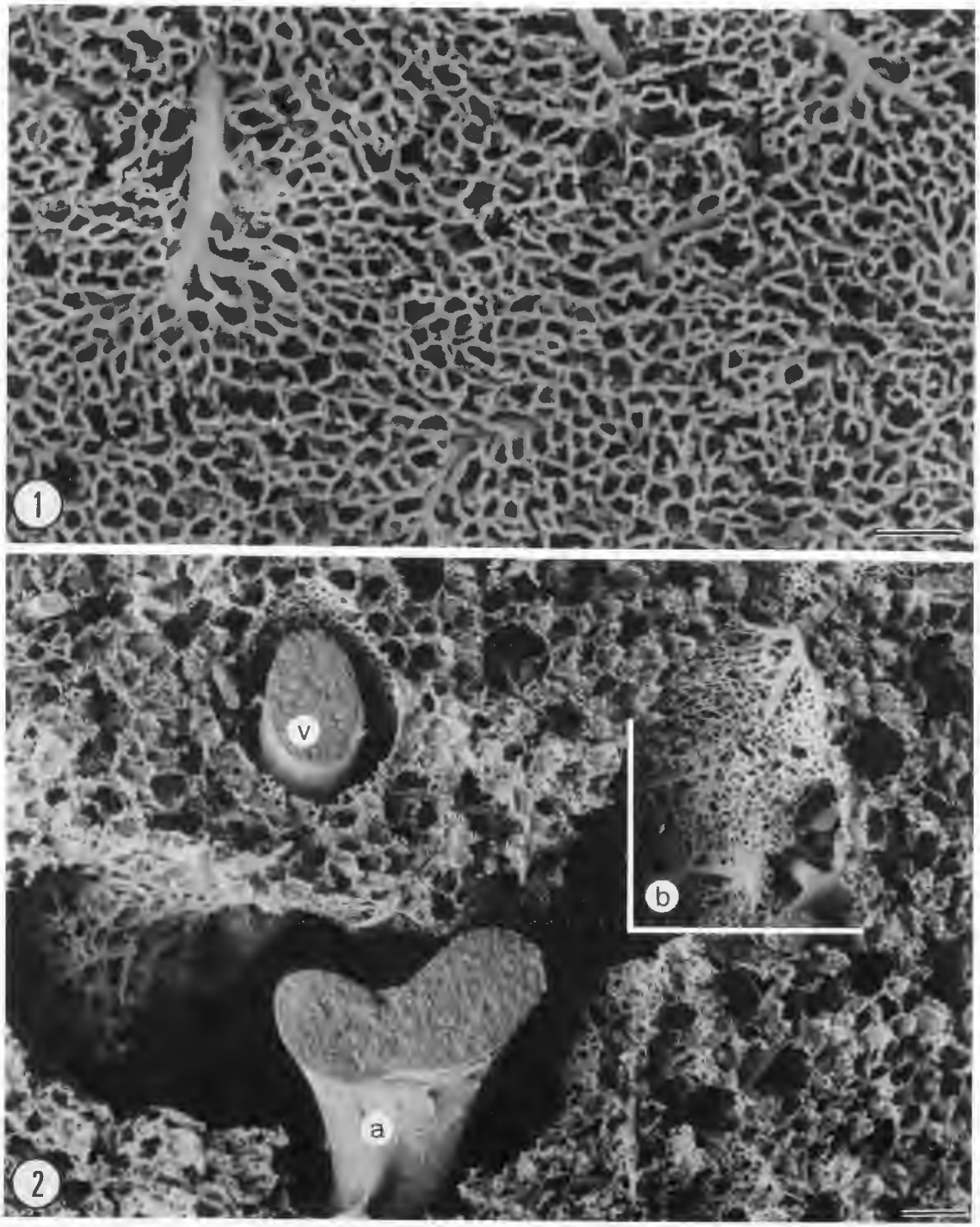

Figure 1. Subpleural capillary network of the rats lung showing a lobular organization. Supplying arterioles are missing. The capillaries drain into small venules which run towards the depth of the lung parenchyma. These veins lead to interlobular veins, their localization is assumed to indicate the connection of interlobular septa to the pleural connective tissue layer. Bar = 100 μm.

Figure 2. View on a section through lung parenchyma of the rat. The micrograph shows casts of pulmonary artery (a), pulmonary vein (v) and large cavities corresponding to the bronchial tree. Where lung parenchyma borders against airway regions, a capillary network is seen which closely resembles the capillaries on the pleural surface (b). A similar situation is found towards the adventitial layer of a small pulmonary vein (v). Bar = 200 μm.


ics
cr
f o v
f o a
plv
pla
3
pc
f o a
4a
4
fov
4b

Figure 3. Pleural aspect of the guinea pig's chest wall shows characteristic vascular pattern of costal region (cr) and intercostal space (ics). Note the close association of posterior intercostal artery (pia) and vein (piv), as well as regular distances between outbranching first order arterioles (foa) and venules (fov). In the region of the intercostal space the characteristic parallel arrangement of muscle capillaries is seen in the depth. In circumscribed areas the muscle capillaries are covered by a superficial capillary network, the pattern of which resembles that seen in the costal region nearby. Boxed area is shown at higher magnification in Fig. 4. Bar = 500 μm.

Figure 4. Higher magnification of the area outlined in Fig. 3. Proximal branches of first order vessels (foa, fov) supply and drain the pleural capillary bed (pc). Note the anastomoses (white arrowheads) between consecutive first order venules (fov) and between the first order venules lying bilaterally to a first order arteriole (arrows). Bar = 200 μm. **Inset Figure 4a**. Parietal pleural capillary bed. Note the terminal branching of a deep ascending arteriole (arrow). Bar = 100 μm. **Inset Figure 4b**. First order venule (fov) showing a deep incision (arrowhead) which may be caused by a valve, and sphincter-like constriction (arrow). Bar = 50 μm.

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of the lung but are also joined by lung parenchymal capillaries. In this manner, these veins are a common drainage route both for the most superficial and deeper parenchymal capillaries as the veins descend towards the hilum as initial interlobular veins. Hence, the visceral pleura is concluded to possess no proper vascular bed. The absence of a capillary network in the visceral pleura of the studied species might not be surprising. McLaughlin *et al.* (1961) studied the vascularization of the cow, sheep, pig, monkey, cat, dog, and horse pleura. They distinguished between "thin" and "thick" pleural membranes. Thin pleura (monkey, cat, dog), they concluded, does not have a proper vascular bed. Our observations confirm their findings but it should be mentioned that in the study of McLaughlin and coworkers Latex was used as an injecting mass. Considering the viscosity of Latex and its inefficiency to fill the capillary pathways, it was a challenge to prove whether the absence of a capillary system in species with a thin pleura was due to the methodological problems.

The most superficial capillaries are parenchymal capillaries of the lung and appear as visceral pleural capillaries when viewing the lungs cast surface (Böck *et al.*, 1991). The same considerations are valid where lung parenchyma borders against adventitial connective tissue of larger vessels, airways or interlobular septa, which is observed in sectioned lung casts (Fig. 2). These regions are assumed as "internal surfaces of lung parenchyma", the microvascular bed of which is identical with that seen at the surface of the lungs corrosion casts. These observations confirm the findings of Guntheroth *et al.*, (1982) in rats, who described a capillary pattern around branches of pulmonary arteries and between the latter and the alveolar capillary network similar to that of the pleura. The spatial arrangement of alveolar septa and the flat connective tissue layers cause the different appearance of capillary networks around alveoli (basket form) and that adjacent to the pleura or bordering areas of lung parenchyma towards adventitial connective tissue of pulmonary vessels and airways (flat).

The above statement that the visceral pleura is devoid of a proper microvascular bed is evident for guinea pigs and rats as studied in this contribution. In larger animals, nevertheless, branches of the bronchial arteries are shown to take their course through the visceral pleura, in regions close to the hilus of the lung and from there to the costal side (in sheep; Albertine *et al.*, 1982). It should be stressed that in the experimental protocol of Albertine and co-workers Microfil was used as the injection mass. These authors only visualized vessels with diameters of at least 200 μm or larger, observed under the dissection microscope, a capillary network failed to be shown. This particular situation needs further investigations.

In the parietal pleura, by contrast, all criteria of a proper vascular bed are fulfilled for the entire rib region and for circumscribed spots atop the intercostal muscles. A precise affiliation of the observed capillary meshwork in both costal and intercostal regions, however, is not clear. It should be the object of further investigations to discern whether or not the capillaries also belong to the periosteal layer in the costal region, or to adipose tissue in the intercostal region. In human, parietal pleural arterioles were observed, as the posterior intercostal arteries were injected with Latex (Aharinejad *et al.*, 1990b). This may suggest the comparability between human and other species, at least when it concerns the vascularization of the parietal pleura.

Physiological experiments under normal conditions have shown that production of pleural fluid is mainly provided by the parietal pleura, and the same is true for its re-absorption (Miserocchi, 1991). The preferential production of pleural fluid by the parietal pleura becomes plausible when the systemic blood pressure in its vascular bed is considered. The visceral pleura, by contrast, is equipped with capillaries connected to a low pressure system. Fluid re-absorption is mainly carried out by the costal pleural lymphatics which can increase their flow rates up to 40 times to compensate for an equal increase in filtration rates. Such a dramatic increase in pleural fluid reabsorption rates is suggested to be brought about by particular openings of lymphatics

towards the pleural cavity, the so-called stomata (Albertine *et al.*, 1984; Wang, 1988).

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Discussion with Reviewers

D.E. Schraufnagel: For years, investigators (including myself) have struggled with the question of whether the bronchial or pulmonary arteries supply the pleura. This paper answers the question simply: neither. It does not have its own blood supply. The pleura in these animals is a thin structure that closely approximates the alveolar capillaries and can obtain its nutriment from them. Why have we not thought of this sooner?

Authors: The blood supply of visceral pleura via bronchial arteries has been demonstrated in species with a thick pleura, using macrovascular injection methods. In species with a thin pleura, no capillaries could be visualized in this organ (McLaughlin *et al.*, 1961). However, the dye used does not pass through capillaries. Therefore it cannot be decided whether or not the absence of filled capillaries is due to the size and viscosity of the injected mass. The use of Mercox now assure us to obtain complete replica of the microvascular bed. In other words, our study now provides the proof for the absence of capillaries in the thin pleura of small laboratory animals.

O. Ohtani: How can you identify the pleural capillaries as such?

Authors: The most superficial capillary network was

assumed as being related to the parietal pleura.

O. Ohtani: You have described that the intercostal spaces are only provided by interspersed small patches of capillaries. However, in the intercostal spaces shown in Fig. 3, there are many blind-ended capillaries which are apparently the results of incomplete filling. Did you also obtain similar results by light microscopy of dye (e. g., India ink)-injected specimens?
Authors: Incomplete filling of the most superficial capillaries in the intercostal spaces cannot be excluded. In the case of an incompletely filled cast, one has to consider even more extended capillarized areas in the parietal pleural region. This would further support our findings on the vascularization of the parietal pleura. Indian ink injection method was applied to human parietal pleura but not to laboratory animals.

N.S. Wang: I do not see the rib in Fig. 3. If the rib was completely macerated away, is there any possibility that some of these vessels in the parietal pleura represent intra-costal vasculatures? One normally does not see too many capillaries in a densely fibrosed tissue, such as perichondrium.
Authors: We agree. In the costal region, indicated "cr" in Fig. 3, the parietal pleura and the periosteal or perichondrial layer are in close spatial proximity to each other. Therefore, the capillary network shown in the costal region may well belong to both these structures.

K.H. Albertine: Although Microfil has a somewhat higher viscosity than Mercox, both compounds have provided excellent microvessel filling results. A problem with Mercox, which the authors did not mention, is that casts are so fragile once the maceration step is completed that the casts of the smallest microvessels break, thereby limiting the number of useful preparations.
Authors: We agree that Microfil generally may fill the capillaries. In our experiments, however, we often observed incompletely filled casts when Microfil was the injection medium. In those cases, we repeated the experiments with Mercox and got an excellent filling. This phenomenon may be due to the higher viscosity of Microfil as compared with Mercox. We also agree that the casts prepared by injection of Mercox are somewhat fragile. The handling procedure of macerated specimens certainly alters the number of capillaries but this problem is also present during the preparation of Microfil-injected specimens. To limit this source of artifacts, we use special paper for removing the specimens from the maceration medium. In addition, those casts which are damaged during the preparation procedure were excluded from the analysis in the present approach.