# Detailed arrangement of the bronchial arteries in the Wistar rat: A study using vascular injection and scanning electron microscopy

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SUMMARY

We have used vascular castings, light microscopy coupled with tracers, and scanning electron microscopy to define the detailed anatomy of the bronchial arteries in the Wistar rat, a rodent often used in experimental research on lung disorders; namely in those that involve vascular alterations. We found that there are two bronchial arteries in the Wistar rat and that they have a cranial origin, either from the subclavian arteries or from their primary branches. The left bronchial artery was always originated from the internal thoracic artery, ran between the thoracic aorta and the left cranial vena cava and offered branches to the thymus, trachea and esophagus. The right bronchial artery was of variable origin, and was located between the right cranial vena cava and the trachea; it gave off branches to the right cranial vena cava, phrenic nerve, trachea, esophagus and, seldomly, to the mediastinic-pericardial pleura, myocardium and caudal vena cava. In more than half of the rats, there were anastomoses between the bronchial and pulmonary arteries. The histological organization of rat bronchial arteries was different from those of humans, suggesting that there are differences in the resistance to blood flow between the two species. Scanning electron microscopy revealed that the bronchial arteries formed two plexuses surrounding the intrapulmonary airways and also supplied the *vasa vasorum* of pulmonary arteries and veins. We conclude that there are important differences in the arrangement and structure between bronchial arteries in humans and rats and that this should be taken into account whenever data from experimental studies are to be extended to humans.

**Key Words:** Bronchial arteries – Electron microscopy – Vascular casting – Lung circulation

**Abbreviations:** BA, bronchial arteries; LBA, left bronchial artery; LM, light microscopy; RBA, right bronchial artery; SEM, scanning electron microscopy.

## INTRODUCTION

The arterial vascularization of the lung is organized in two systems: a functional set from the pulmonary vessels, and nutritive vessels coming from the bronchial arteries (BA). These two types of vessels show different pathophysiological responses; namely in inflammation and tumoral disease (Grunt et al., 1986; deMello and Reid, 1991), hypoxia and hyperoxia (Hislop and Reid, 1976; Roberts et al., 1983; Matsubura et al., 1986), cor pulmonale (Sadigursky and Andrade, 1982) and emphysema (Schraufnagel and Schmid, 1988). Our own investigations have revealed that BA participate in the neoformation of vessels induced in the rat during experimental lung fibrosis (Peão et al., 1993 and 1994; Grande et al., 1997). This finding is supported by other reports using animal models of lung circu-

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lation (Hijiya, 1978; Schraufnagel, 1990; Schraufnagel et al., 1986 and 1997).

To interpret the experimental data aimed at understanding human bronchial circulation, it is helpful to have a detailed characterization of the vascular anatomy of BA in animals used as models of human disease. We and others have chosen the Wistar rat as the animal model of choice in a number of investigations regarding lung physiology and pathology (e.g. Schraufnagel and Schmid, 1987a; Schraufnagel, 1989a; Peão et al., 1995; Grande et al., 1999; Pereira et al., 1999). In the present study, we characterize the architecture of BA in the rat and compare this with the anatomy of the same arteries in humans. For this, we used vascular injection coupled with light microscopy and scanning electron microscopy. We believe that the information reported here should be taken into account whenever experimental data on BA obtained in the rat are to be used to understand human bronchial circulation.

## Materials and Methods

### *Animals*

Thirty-nine male Wistar rats were purchased from a local breeder (Gulbenkian Institute of Sciences, Oeiras, Portugal). The animals were kept in cages of two animals each and maintained under standard conditions of housing with unrestricted access to food and water, according to Portuguese law (DL 129/92 and 1005/92) and to European Union Directive no. 86/609/CEE. They were 8-weeks old and weighed 200-225 g at the time of sacrifice.

### *Casting of Bronchial Arteries (BA)*

The rats were anesthetized with 5 mg/kg of xylazine (Rompun®, Bayer Co., Amadora, Portugal) and 40 mg/kg of ketamine (Imalgene 1000®, Rhone Merieux Portuguesa, Lisboa; Portugal). The abdomen was surgically opened, the aorta cannulated and the vascular system was washed with a warm (37º C) saline solution until the efflux from the caudal vena cava was clear of blood. The thoracic cavity was exposed and the heart clamped to prevent the injected casting materials to reach the lung vasculature through the heart. The BA were filled by injection through the abdominal aorta with two casting substances: i) a mixture of barium sulphate at 20% (Polibar Rapid®) and colloidal charcoal at 2% (Pelikan China ink®) in gelatin at 30%; ii) methylmethacrylate resin (Mercox® C1-2R and C1-2B, Japan Vilene Co., Tokyo, Japan).

Before and throughout the injection of the mixture of barium sulphate, the sacrificed animals were warmed by immersion in a water bath (50º C). The casting product was also injected at 50ºC by manual injection (2.5 ml/min) until filling of the choroid vessels of the eye was observed. The rats were then placed in a cool water bath (0-4º C) to induce solidification of the gelatin. The lungs were fixed by immersion in 10% buffered formalin. In these vascular casts, the anatomy of the BA was studied under a dissecting microscope, by radiography, and by light microscopy (LM). For LM, lung samples were processed for paraffin embedding by standard methods and 5 µm sections were stained with hematoxylin-eosin and fuchsin-resorcin.

Vascular castings were also made by injection of the methylmethacrylate resin in order to obtain samples for scanning electron microscopy (SEM). These specimens were prepared according to Weiger et al. (1986) and Lametschwandtner et al. (1990). Briefly, manual injections were carried out using a cannula inserted into the aorta and were ended when the color of the surface of the lung changed to red. The trachea was then exposed by dissection and cannulated for intratracheal injection of the respiratory tree with methacrylate resin. Samples were then placed in a 60º C water bath for 12 hr for final resin polymerization. In order to isolate the vascular casts, lung tissue was digested by incubation in 25% sodium hydroxide at 30º C for 3-4 days. The resin casts were further cleaned in 5% formic acid for 10 min, rinsed in distilled water, and subjected to sonication. Lung lobes were isolated and their bronchovascular structures exposed with a dissecting microscope. The casts were mounted on metal stubs with conductive carbon paste. The specimens were coated with Au/Pt under vacuum and observed in a SEM (JEOL JSM-6301F, JEOL Corp., Japan).

## Results

### *Extrapulmonary Anatomy – Dissection and Radiography*

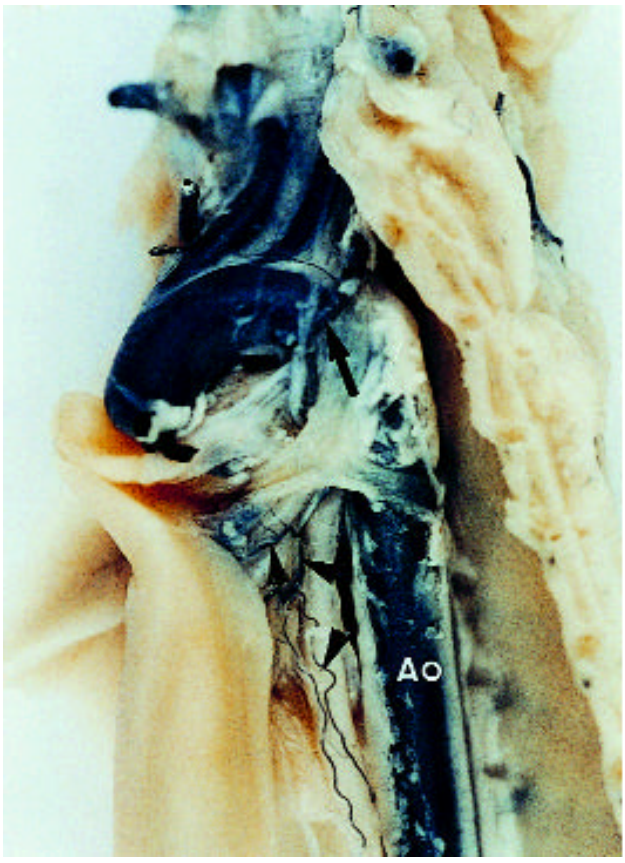
Examination under a dissecting microscope of the vascular casts of the bronchial arteries (BA) of Wistar rats revealed that there were two BA, right and left, and that these originated either from the subclavian arteries or from their primary branches. Each of the BA started near the beginning of the arterial branch of origin and followed a long caudal course through the mediastinum, supplying several thoracic structures other than the bronchi. The left bronchial artery (LBA) always originated from the internal thoracic artery. The right bronchial artery (RBA) had a variable origin: the costocervical trunk in half of the animals (in 17 of 34 rats), the right subclavian artery (in 14 of 34 rats), or the internal thoracic artery (in 3 of 34 rats).

The topography and the branches of the LBA are summarized in Fig. 1. After emerging from the internal thoracic artery, the LBA ran caudally on the ventral surface of the aortic arch, where it gave off branches to the thymus. Caudally to

the aortic arch, one branch turned medially around the aortic arch and irrigated the trachea; this branch was satellite to the left recurrent laryngeal nerve. The further course of the LBA ran between the thoracic aorta and the left cranial vena cava, where it anastomosed with a left intercostal artery, usually the ninth one. At the dorsal level of the left bronchus, the LBA originated esophageal and bronchial arteries. One or two branches of the LBA ran down along the main bronchus. On a few occasions, LBA branches to the left cranial vena cava, the mediastinic and visceral pleura were seen. The main variations in the course of the LBA are illustrated in Fig. 2.

and ran caudally in dorsal position to the bronchi in the direction of the medial, caudal and accessory lobes of the lung. The RBA gave off numerous transverse branches to the esophagus. In a few animals, we identified RBA branches to the mediastinic-pericardial pleura, to the myocardium, and to the caudal vena cava. The main variations in the course of the RBA are shown in Fig. 4.

Injection of the BA with the mixture of barium sulphate and gelatin, also resulted in the casting of the pulmonary arteries, in one or both lungs, in more than half of the animals (in 25 of 34 rats); this phenomenon was readily seen in X-ray radiographs (Fig. 5) and was the result of anasto-



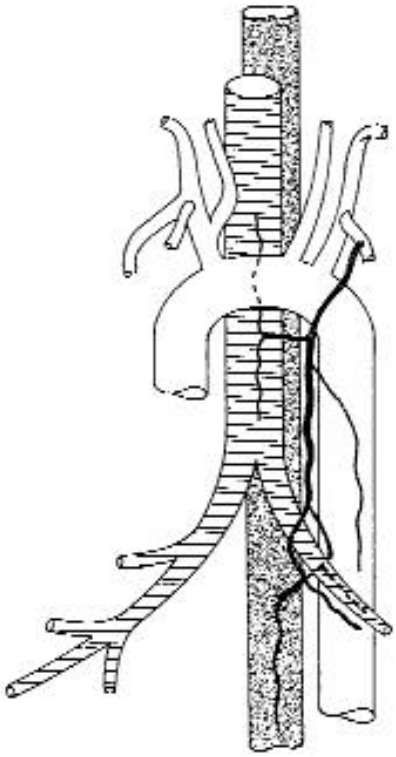



**Fig. 1.-** The most common anatomy of the left bronchial artery (LBA) in the Wistar rat is shown in a dissected specimen (A) and summarized in a diagram (B). **A:** TheLBA is seen (arrow), as well as its main branches: esophagic and bronchial arteries (arrowheads). Ao= aorta. **B:** The drawing illustrates the main anatomical features of the LBA: origin, branches and topography regarding the aorta, trachea, esophagus and bronchi.

The topography and the branches of the RBA are shown in Fig. 3. The RBA reached the lateral surface of the trachea, where it gave off branches to the trachea, right cranial vena cava, and phrenic nerve; these latter two structures concealed the RBA, rendering its identification by dissection difficult. At the tracheal carina, the RBA offered 1-3 branches to the cranial bronchus

moses between the two arterial systems. Extrapulmonary anastomoses between the two arterial systems were found in only 3 rats (Fig. 6).

*Intrapulmonary Anatomy – Light Microscopy (LM) and Scanning Electron Microscopy (SEM)*

Positive identification by LM of BA and their branches was obtained by the identification in

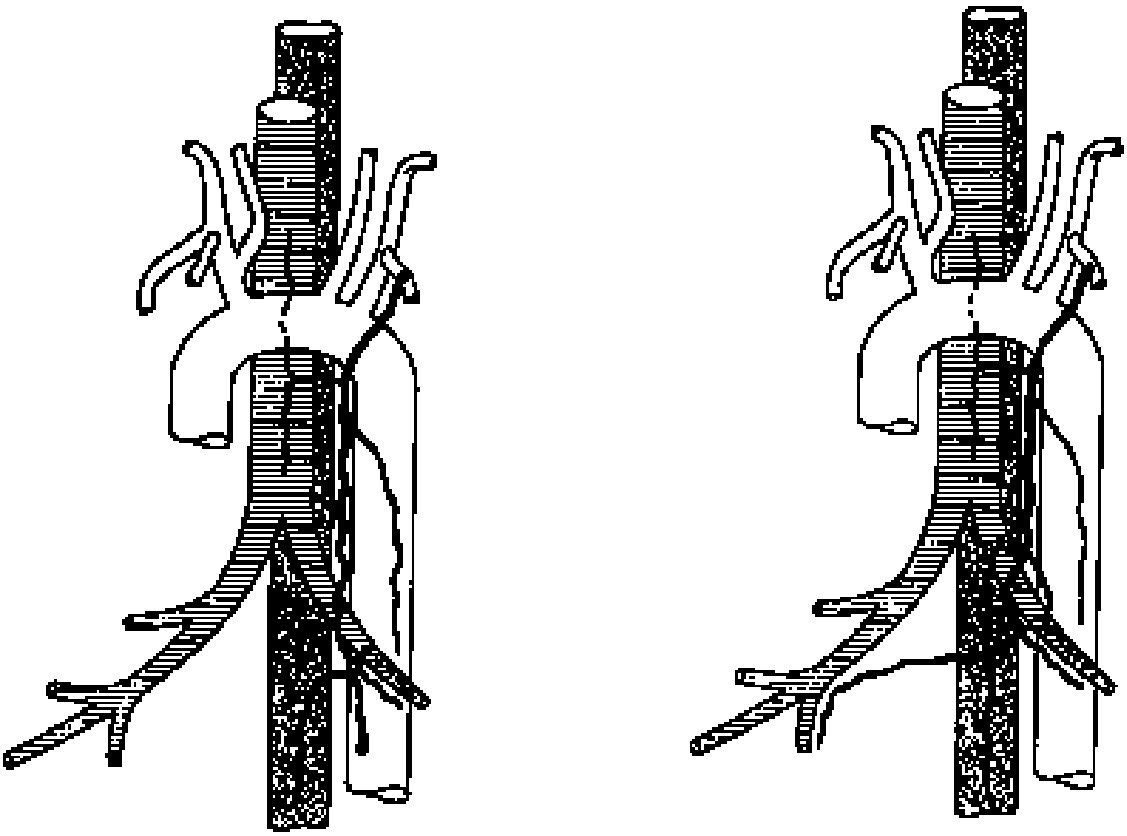


**Fig. 2.-** Diagrams illustrating two anatomical variations of the LBA. **A:** Supplementary LBA with a lower origin on the thoracic aorta (in 1 of 34 rats). **B:** Branch of the LBA to the right accessory lobar bronchus (in 2 of 34 rats).

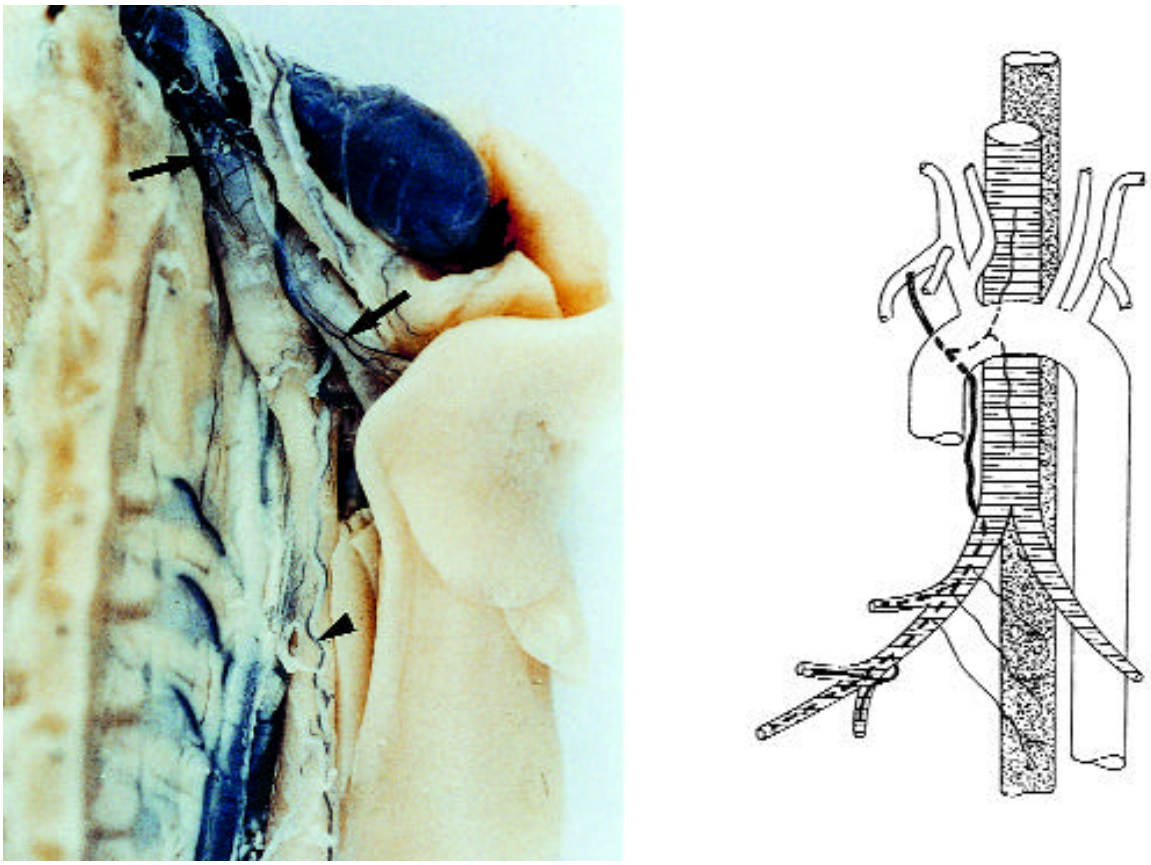


**Fig. 3.-** The most common anatomy of the right bronchial artery (RBA) in the Wistar rat is shown in a dissected specimen (A) and summarized in a diagram (B). **A:** The tracheal (upper arrow), cranial bronchial (lower arrow) and esophagic (arrowhead) branches are seen. **B:** The main branch of RBA originates from the costocervical trunk and the topography of the bronchial, tracheal and esophagic branches is illustrated.

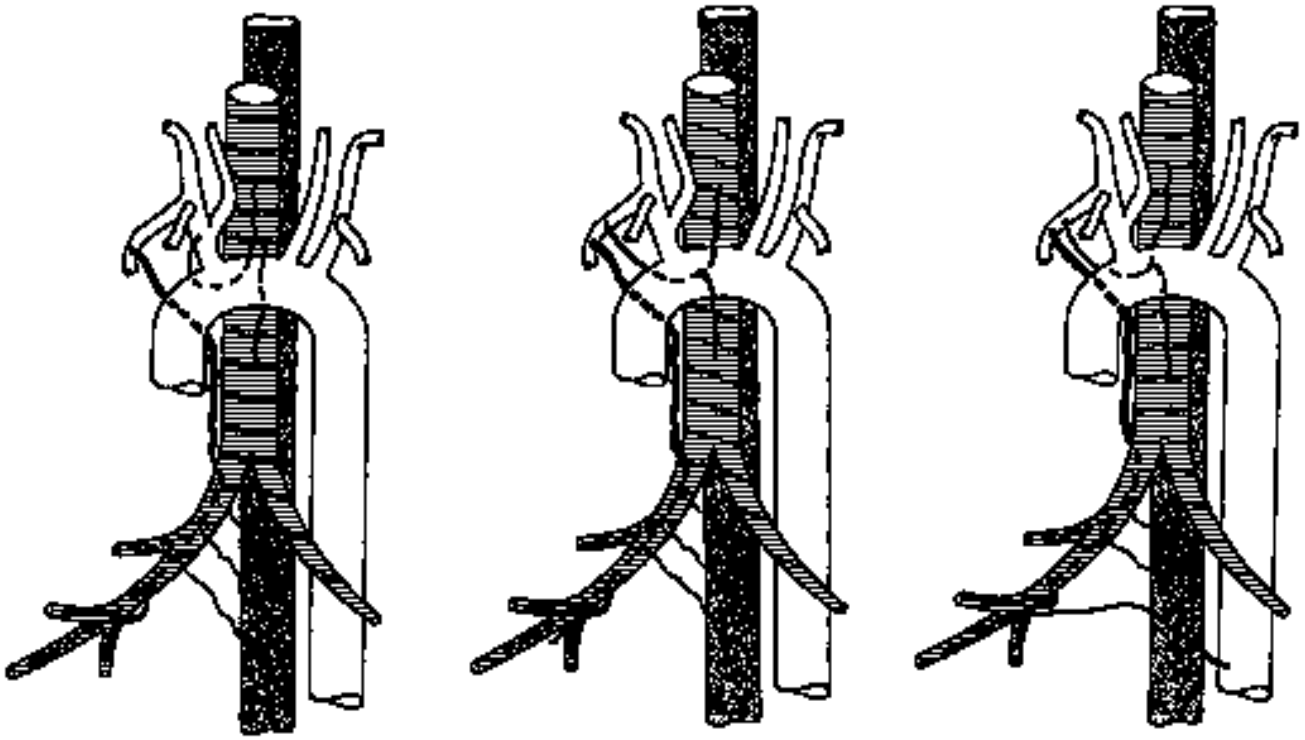


**Fig. 4.-** Diagrams illustrating three anatomical variations of the RBA: **A**: the tracheal branch arising from the right subclavian artery (in 2 of 34 rats). **B**: the tracheal branch originating directly from the costocervical trunk (in 2 of 34 rats) and **C:** supplementary RBA coming from the thoracic aorta (in 3 of 34 rats).

their lumen of black charcoal particles that had been added to the barium sulphate marker (Fig. 7). The intrapulmonary branches of the BA showed a muscular wall with two circular layers, lacking elastic lamina. In the animals with evidence of anastomoses between the pulmonary and bronchial arteries, we found that the cast product was present in the lumen of both types of arteries. The vascular wall of the pulmonary artery branches was different from that of the BA vessels because they depicted a thicker muscular wall and presented an internal elastic lamina (Fig. 8).

As expected, injection of Mercox® resin into the aorta resulted in the permeation and casting of the whole blood vasculature of lungs. This finding is in accordance with previous studies on the visualization of lung vessels by injection of this resin (Schraufnagel, 1989b; Peão et al., 1993).

Intrapulmonary branches of BA were identified around bronchovascular structures, forming two vascular plexuses that were best seen in SEM preparations. They surrounded the airways, showing submucosal and peribronchial topographies (Fig. 9). The peribronchial plexus was made up of anastomosing arterioles giving off branches that passed through the muscularis layer to form a second layer under the epithelium. The inner plexus was dense and consisted of a loose network of vessels 9-22 µm in diameter. In some rats, the BA gave off branches to

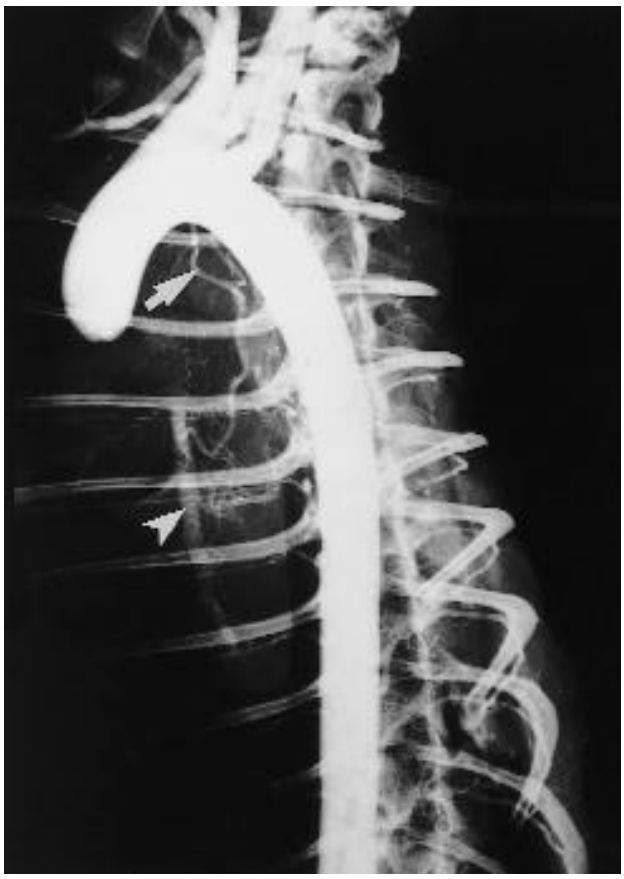


**Fig. 5.-** X-ray radiography of the RBA casted with barium sulphate (arrow). There is also filling of the pulmonary artery (arrowhead), indicating anastomoses between the bronchial and pulmonary arteries in the rat.

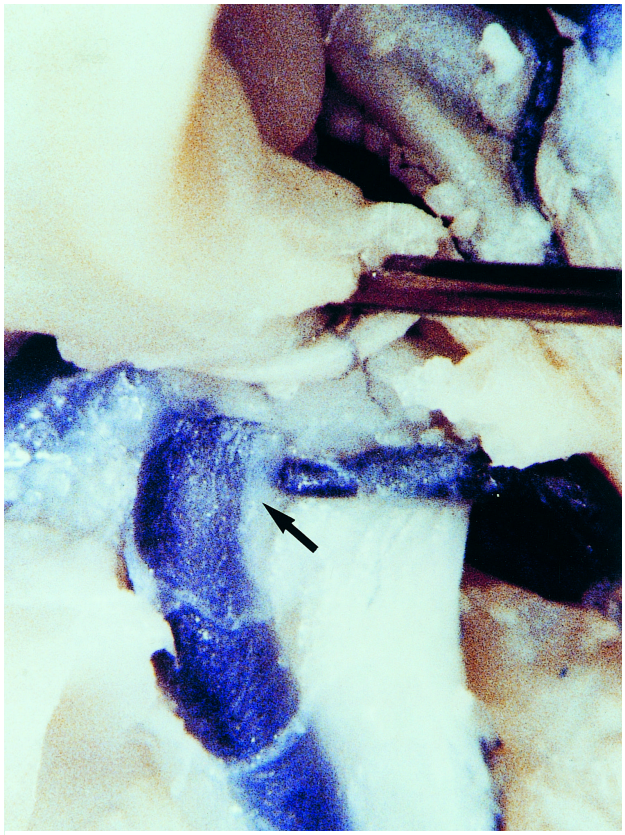


**Fig. 6.-** Dissection showing an extrapulmonary anastomosis (arrow) between the bronchial artery and the pulmonary artery in the rat (x8).

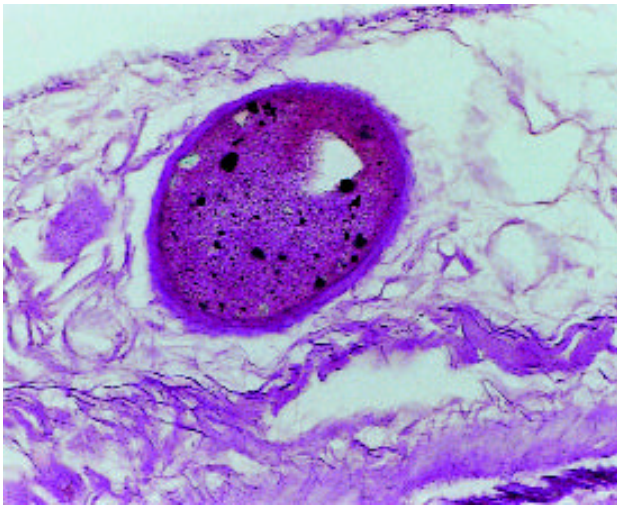


**Fig. 7.-** Light micrograph of an intrapulmonary branch of a bronchial artery in the rat. The casting product (charcoal particles) is seen filling the lumen of the artery (Fuchsin-resorcin stain, x 400).

the walls of the pulmonary artery and vein, in the form of *vasa vasorum*, that was organised in a single layer in the artery and in three layers in the vein (Fig. 10). BA also supplied branches to the visceral pleura near the pulmonary hilus.

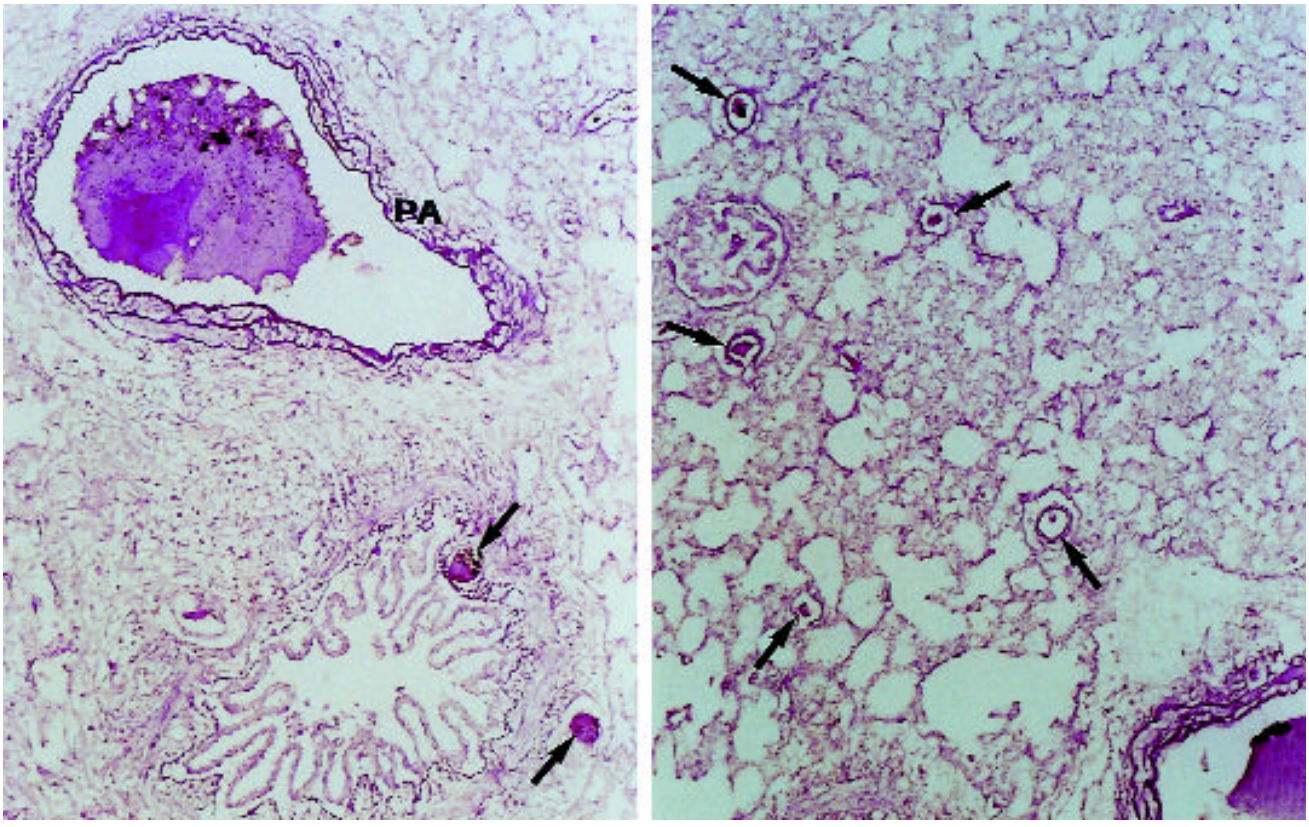



**Fig. 8.-** **A:** Light micrograph of the pulmonary artery (PA) and of the branches of BA (arrows). **B:** The elastic internal lamina is clearly seen in the wall of branches of the pulmonary artery (arrows) (Fuchsin-resorcin stain, x 100).

## Discussion

The purpose of this study was to define the detailed anatomical arrangement of bronchial arteries (BA) in the Wistar rat, an animal that has been adopted as a model in lung vascular research. The data reported here will be of help whenever rats are employed in experimental work aimed at answering questions related to the pathophysiology of human lung circulation.

Our investigations confirmed a previous macroscopic report stating that the rat has two BA (Hebel and Stromberg, 1963), a feature in conflict with the three BA usually found in humans (Warwick and Williams, 1973; Netter, 1980). The origin of rat BA is more cranial than that observed in BA of other mammals, including humans. The statistical study of Liebow (1965) reported that 43% of RBA of man were of intercostal origin, usually arising from the first intercostal artery, whereas the LBA was always originated from the aorta. Only infrequently does the human BA originate from the initial portion of the aorta, i.e., above the first intercostal artery or from the subclavian artery. The internal mammary and coronary arteries are also rare origins of BA in humans. In the Wistar rat, we found that a BA coming from the aorta was also a rare event and corresponded to a supernumerary artery. Also, we never detected rat BA having intercostal arteries as origin; nevertheless, on the left side, the intercostal arteries could contribute to the bronchial irrigation, since anastomoses between the LBA and a left intercostal artery were present. It can therefore be concluded that the origin of BA is clearly different in the Wistar rat and in humans.

The distribution of the extrapulmonary branches of BA in the rat recalls what is observed in other mammals: they cross the mediastinal space and supply several thoracic structures other than the bronchi. In the present work, the LBA supplied the thymus, esophagus and trachea, and the RBA gave off numerous branches aimed at the esophagus, trachea, right cranial vena cava and phrenic nerve and, infrequently, the mediastinic-pericardial pleura, myocardium and caudal vena cava. In humans, BA also send branches to mediastinal structures such as the esophagus, hilar lymph nodes and vagus nerve (Harris et al., 1962a). The irrigation of these extrapulmonary structures reflects the

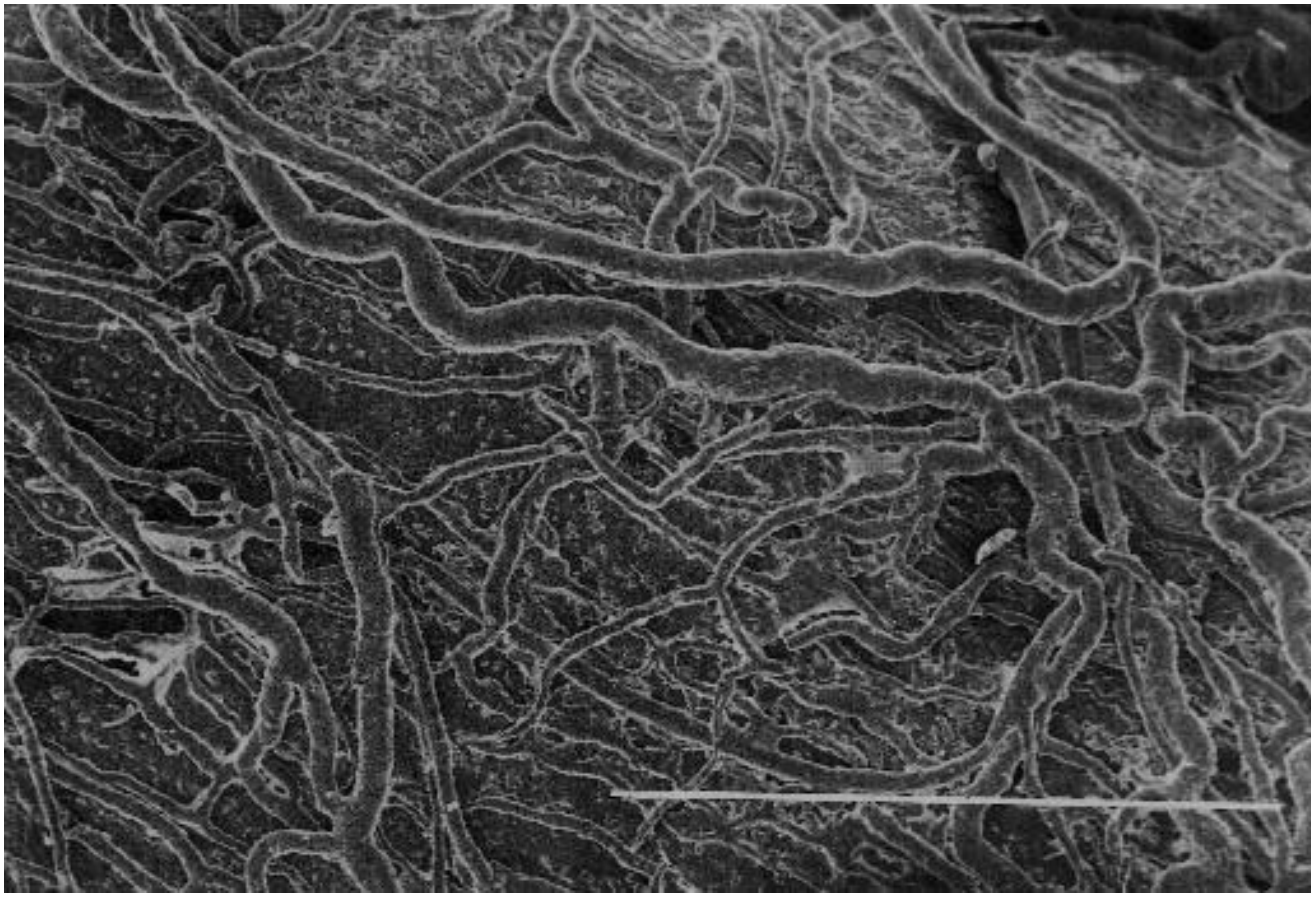


**Fig. 9.-** Scanning electron micrograph of intrapulmonary bronchial arteries (BA) located in the bronchial wall. The distance between the bronchial lumen (casted surface) and the vessels indicates that BA form two vascular plexuses with submucosal and peribronchial topographies. Bar = 1 mm.

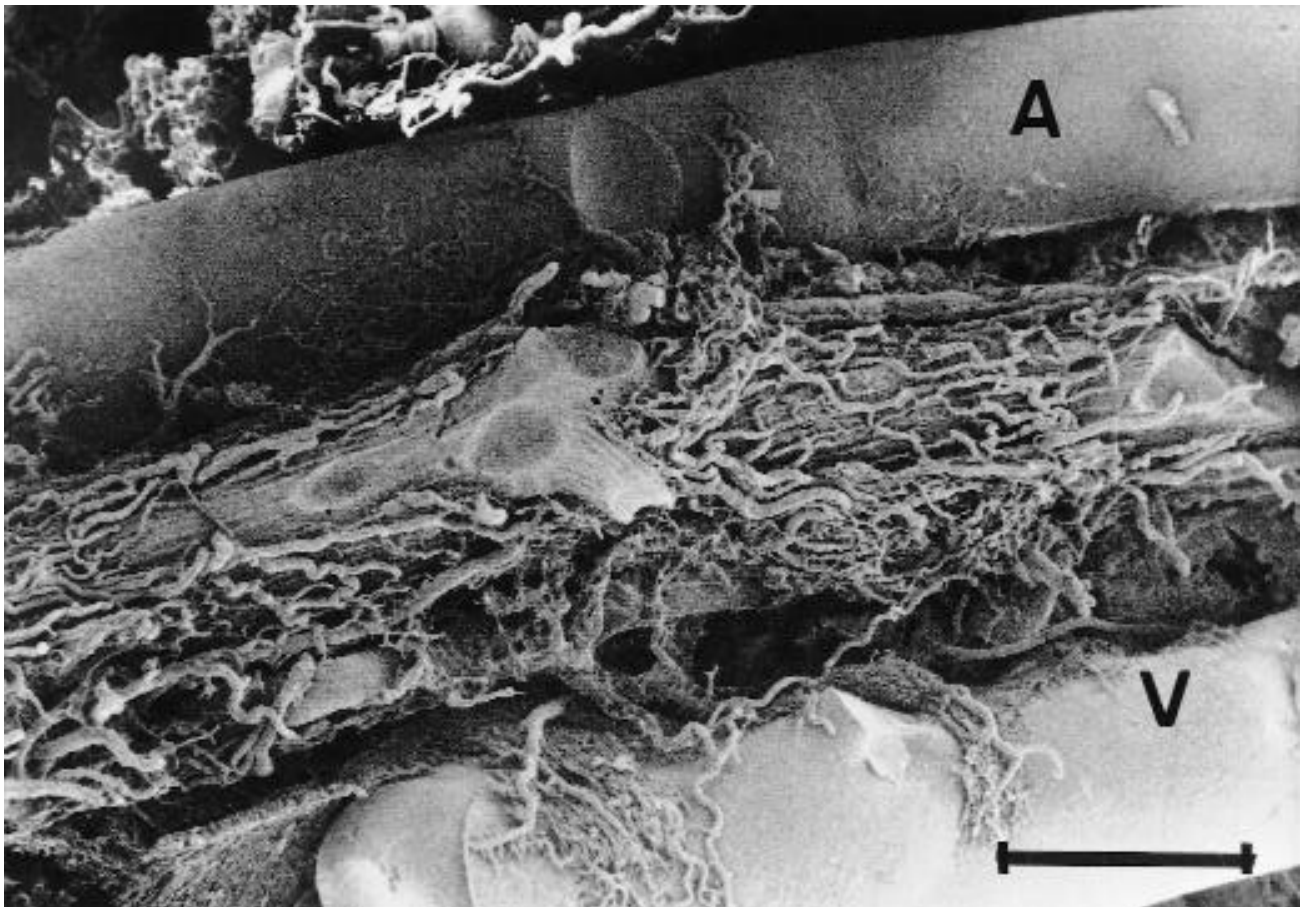



**Fig. 10.-** Scanning electron micrograph of casts of pulmonary vasculature and airways. Note the dense bronchial vascular plexus surrounding the bronchus and the casted pulmonary artery (A) and vein (V) showing *vasa vasorum* on their surface. Bar = 1 mm.

embryologic origin of BA: during the fourth week of human embryonic development, the lungs, trachea and esophagus are supplied by branches from paired dorsal aortas which form capillary plexuses around all of these structures (Tobin, 1952). However, the different origins of the blood supply of these organs suggest that there are links between BA and a variety of other systemic arteries. In fact, when obstruction of a major BA occurs, loss of blood supply may be overcome by inflow from vascular anastomoses with other vessels (Deffebach et al., 1987). Of all tissues supplied by extrapulmonary branches of BA, it is important to underline the mediastinic-pericardial pleura and myocardium, because this suggests that there are anastomoses between BA and the coronary circulation in the rat. There are reports of anastomoses between bronchial and coronary arteries; this vascular arrangement may work as a collateral source of blood that reaches the myocardium (Arvidsson and Moberg, 1966; Bjork, 1966; Moberg, 1968). Thus, the Wistar rat may offer a model of coronary-bronchial arterial anastomoses that may be of interest to study the role of these anastomoses in heart and lung disorders in humans.

Our observations on the general architecture of the intrapulmonary branches of BA were in general agreement with data from other authors (Miller, 1945; Deffebach et al., 1991; Peão et al., 1994). In addition, SEM revealed a BA origin for the *vasa vasorum* of the pulmonary veins, thus strengthening the previous report by Othani (1980).

In the Wistar rat, the histological organization of the vascular wall of BA is characteristic of systemic arteries of rodents and different from that of human BA, that have a thick media layer, as well as a developed internal elastic lamina (Harris et al., 1962a; Wagenvoort et al., 1964). Interestingly, we also observed that the vascular wall of the pulmonary arteries of the rat showed a different histology from that of pulmonary arteries in humans: in the latter, the pulmonary arteries have a thin media layer, a wide lumen and the media is bound by two distinct elastic lamina (Alexander and Jensen, 1963; Wagenvoort et al., 1964). Taken together, the different structural arrangements of both bronchial and pulmonary arteries in humans and rat suggest a different resistance to blood flow in the two species.

We found that the majority (73%) of the Wistar rats had pulmonary arteries filled with the barium marker that, due to the diameter of its particles, is not able to cross capillaries (Schraufnagel, 1987). Thus, filling of the pulmonary

arteries after barium injection of systemic arteries shows that there are precapillary anastomoses between systemic and pulmonary vessels in the rat, hence resembling what occurs in humans (Lindskog and Liebow, 1953; Grande, 1958; von Hayek, 1960; Harris et al., 1962b).

In conclusion, this study establishes the main anatomical similarities and dissimilarities between the bronchial circulation of rat and humans and suggests that the bronchial vascular data obtained in rodents should be carefully considered before being extended to the angiology of the bronchi and lung of humans.

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