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Organisation and structure of the tracheal and bronchial blood vessels in the dog*

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INTRODUCTION

The tracheobronchial mucosa has an important physiological role in temperature control and humidification of inspired gas (Baile, Dahlby, Wiggs & Pare, 1985), and this control should depend on vascular adjustments. The airway vessels may also have an important function in relation to mucosal damage, nowadays closely associated with the pathogenesis of asthma (Baier, Long & Wanner, 1985; Barnes, 1986).

Although the gross anatomy of the tracheobronchial circulation has been frequently described (Marchand, Gilroy & Wilson, 1950; Cudkowicz & Armstrong, 1951; Daly & Hebb, 1966; Charan, Turk & Dhand, 1984), there are few descriptions of its detailed organisation and structure. For the canine bronchi, a capillary–venular plexus may lie under the epithelium and another may be located in the peribronchial connective tissue; in smaller bronchi these networks merge to form a single plexus (Pietra, Szidon, Leventhal & Fishman, 1971). We have recently made a three dimensional study of the airway microvasculature of the sheep (Hill, Goulding, Webber & Widdicombe, 1989), and find that this species shows striking differences from the dog, described here. An abstract of some of the results has been published (Laitinen, Laitinen, Moss & Widdicombe, 1986).

MATERIALS AND METHODS

Nineteen adult greyhounds of either sex (body weight $27{\cdot}7 \pm 0{\cdot}6$ kg) were studied. The animals were anaesthetised with intravenous sodium pentobarbitone (30 mg kg^{-1}). Fuller details of the methods are given elsewhere (Hill *et al.* 1989).

Vascular corrosion casts

In 9 dogs the caudal cervical trachea was opened and cannulated. One or both common carotid arteries were exposed at the level of the superior thyroid artery, which was isolated; arteries to tissues other than the cranial trachea were tied off. The trachea from the cricoid cartilage to tracheal rings 6–9 was usually opened in the ventral midline to visualise the mucosa. Each dog was given heparin (25000 U

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intravenously; Leo Laboratories). The tracheal arteries were then perfused with the animal's own blood. The distribution of the perfused circulation was tested by arterial injection of Evans Blue. The dog was killed with an intravenous injection of sodium pentobarbitone. One to five ml of a polymerising substance (Batson's No. 17 anatomical corrosion compound, Polysciences Inc.) was immediately injected into the tracheal artery. To inject a bronchial artery, the thorax was opened and the lungs were removed. A bronchial artery was catheterised and 0·5–2·0 ml of the polymerising substance was injected.

After the polymer had hardened the tissue was digested in 8·5 M potassium hydroxide. The casts were prepared for SEM by routine methods (Hill *et al.* 1989). They were studied using either a Jeol JSM 35C or a Coates and Welter field emission scanning electron microscope operated at 1–7 kV.

In three dogs the midline overlapping of the circulation was evaluated in the cranial trachea, which was not opened. The superior tracheal arteries on the left and right sides were injected with polymerising substance (Batson's no. 17) mixed respectively with red and blue colouring pigments. The preparation of colour casts was as described above.

Histology

Tracheobronchial specimens were taken from 16 greyhounds. The specimens were immersion-fixed for 24 hours in neutral buffered 10% formal saline, dehydrated through graded alcohols and embedded in wax. They were embedded and cut in a similar orientation to the airway. Seven μm sections were stained with haematoxylin and eosin and the numbers of microvessels in the mucosa were counted.

Transmission electron microscopy (TEM)

Specimens for TEM were dissected from the same tracheal sites as for light microscopy and from the same dogs. The specimens were immersion-fixed in phosphate-buffered 3% glutaraldehyde (Sigma) and prepared for TEM as described previously (Laitinen, 1985; Hill *et al.* 1989).

All histological and TEM specimens were coded, and examinations and vessel counts were performed blind.

RESULTS

Coloured vascular casts of the trachea

On each side of the trachea one vessel of approximately 100 μm in diameter ran circularly between adjacent cartilaginous half-rings. These vessels gave off smaller branches which formed the mucosal network. On approaching the dorsal midline, one end-branch passed cranially and the other caudally, finally contributing to a network of vessels. Final arterial branches of the two sides were occasionally interconnected.

In the ventral wall of the trachea large vessels continued from one side to the other with interconnections of vessels and with no clear midline.

Vascular casts of trachea and bronchi

Scanning EM revealed that the casts of vessels running between the tracheal cartilaginous half-rings were regular in diameter (80–100 μm) and had small fusiform depressions (presumably nuclear) on their surface, thus exhibiting typical arteriolar structure (Rhodin, 1974). These long straight arterioles gave rise to narrower arterioles which branched and supplied a profuse subepithelial capillary plexus in the mucosa (Fig. 1). The capillaries were drained by small venules converging into larger venules, identified by the smooth surface of their casts and irregular size (Rhodin, 1974).

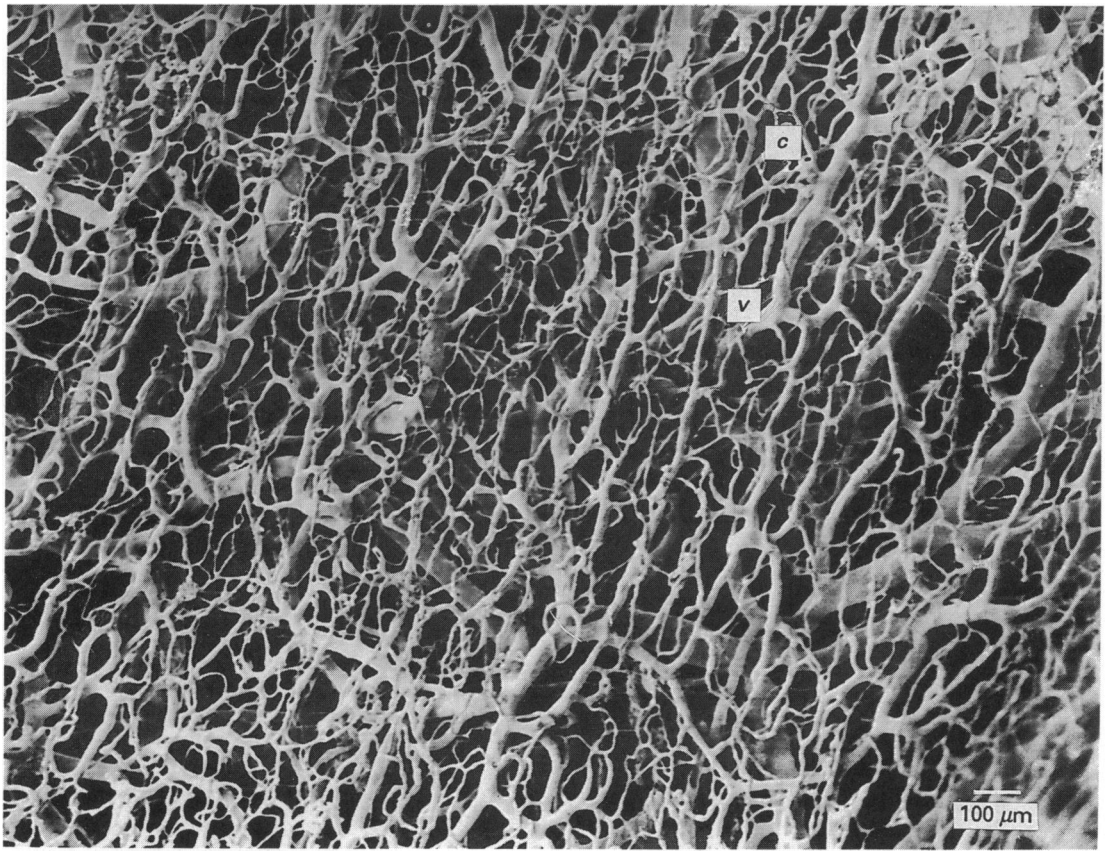

Fig. 1. Scanning electron microscopy picture taken from the luminal side of a vascular cast from the lateral wall of the trachea over the fifth cartilage half-ring. A rich vascular network is shown in the tracheal mucosa. Small superficial capillaries (*c*) are draining into deeper veins (*v*). ×75; bar = 100 μm.

Thus two networks were present in the trachea. Closest to the airway lumen was a superficial and very extensive network formed by capillaries and their loops. A less copious plexus was formed in the deeper half of the mucosa from which branches originated to supply and drain the superficial mucosal network. Larger arteries and veins ran outside the cartilage in the tracheal wall but no network was formed. No arteriovenous anastomoses were seen.

At least two arteries (0·5–1·0 mm diameter) accompanied each bronchus (0·5–5·0 mm diameter), running in the peribronchial connective tissue. They gave branches of equal size to the dividing bronchi and regularly gave off small branches which penetrated the bronchial wall, supplying the mucosal layer. As in the trachea, a dense network of vessels was situated in the mucosa close to the airway lumen (Fig. 2). Many long capillaries, with clearer longitudinal orientation than was seen in the trachea, followed the length of the bronchus and were connected by transverse capillary loops. The network was obvious even in the smallest (0·5 mm diameter) bronchi examined. The capillaries merged to form venules which extended into the deeper mucosal plexus. Arteriolar and venular structures ran close to each other but the main bronchial arteries were not accompanied by veins. No arteriovenous anastomoses were observed.

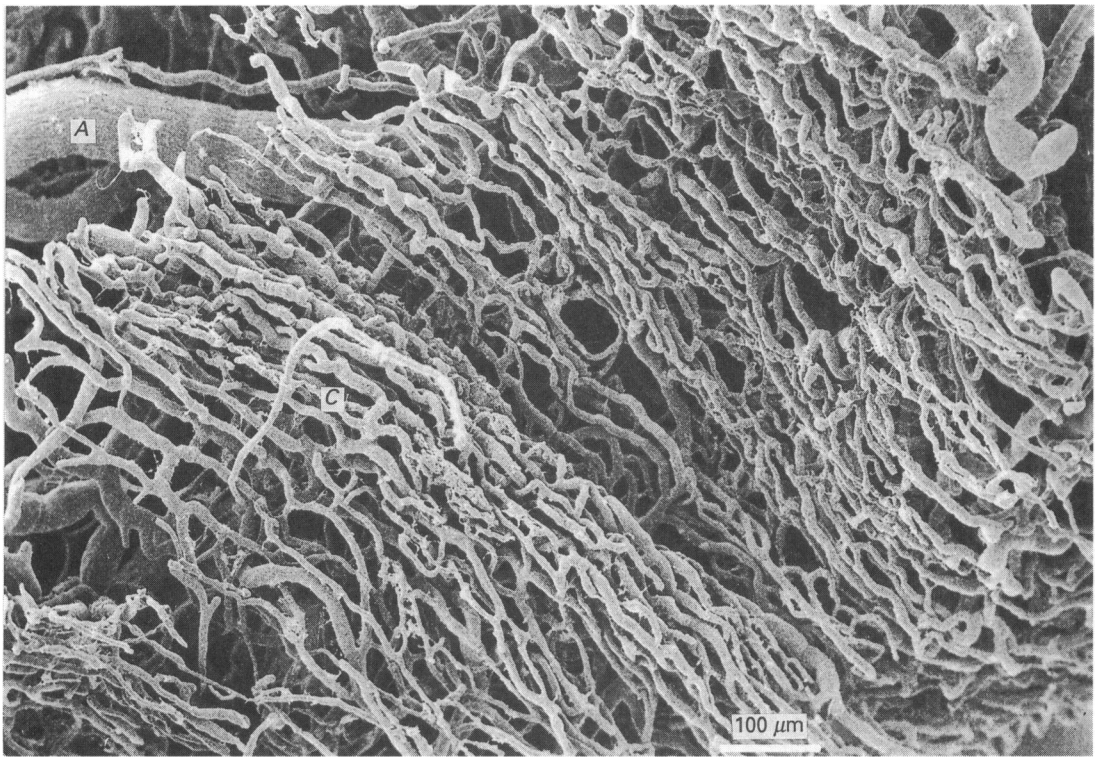

Fig. 2. Scanning electron micrograph of the microvascular network in the mucosa of a 3 mm diameter bronchus which has been opened to expose the mucosa. The bronchus runs from upper left to lower right, and has been cut at both ends. The capillaries (*C*) are longitudinally arranged along the course of the bronchus. *A*, arteriole. × 190; bar = 100 μm.

Histology

Light microscopy revealed a pattern of vessels which confirmed the findings with SEM. The numbers of capillary–venular structures in the trachea and bronchi were greater in the superficial half of the mucosa compared to the deep half (62 ± 8 compared to $22 \pm 7{\cdot}5$ vessels/mm^2; means ± sems, n = 14, $P < 0{\cdot}01$). There were only a few arterioles in the mucosa with more in the deep mucosa ($1{\cdot}0 \pm 0{\cdot}40$ vessels/mm^2) than in the superficial mucosa ($0{\cdot}3 \pm 0{\cdot}27$ vessels/mm^2). These general patterns of distribution were similar in all parts of the trachea and bronchi. Some lymphatic vessels were seen, usually in the deeper half of the mucosa. Most blood vessels, especially those close to the epithelial basement membrane, were 10 μm in diameter or less. Larger venules (30–50 μm diameter) and a few arterioles (15–40 μm diameter) were usually situated in the deeper half of the mucosa. Arterioles of 40–100 μm diameter were regularly seen in the tracheal tissue between the cartilaginous half-rings.

In the tracheobronchial adventitia outside the cartilages, few arterioles and venules were observed and even fewer capillaries.

Transmission electron microscopy

No vessels were seen in the epithelium. Within 100 μm of the epithelial basement membrane there was a high concentration of capillaries ($286 \pm 29{\cdot}3$ vessels/mm^2; n = 9) with a smaller number of venules and few arterioles. The capillaries came very

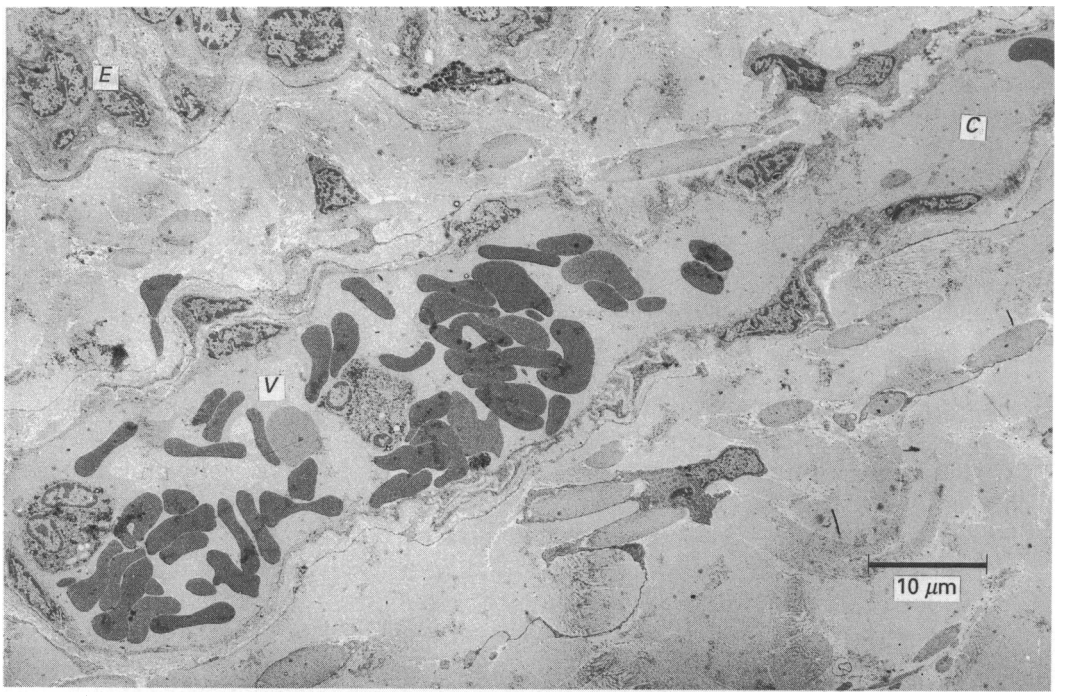

Fig. 3. Transmission electron micrograph of a longitudinally cut capillary (*C*) and post-capillary venule (*V*) in the dog bronchial mucosa. The vessel runs close to the epithelial (*E*) basement membrane. ×2500; bar = 10 μm.

close (5 μm) to the epithelial basement membrane. They had no fenestrae or discontinuities between endothelial cells; their luminal diameters were 4–10 μm. Occasional pericytes were present.

The postcapillary venules, ranging in diameter from 10–30 μm, were located with the capillaries in the luminal half of the mucosa (Fig. 3), but they were mainly deeper than the capillaries. Their walls consisted of endothelial cells, but the edges of adjacent cells often overlapped and junctional specialisations were often absent. The endothelium was surrounded by a basal lamina and usually an incomplete layer of pericytes. Larger collecting venules with diameters up to 50 μm and a complete layer of pericytes were located mainly in the deeper part of the mucosa.

Arterioles with a luminal diameter of 10–70 μm were found in the mucosa at 100 μm or more from the epithelial basement membrane. The endothelium was surrounded by a basal lamina and one layer of smooth muscle. They were associated with venules and sometimes nerve bundles. Some lymphatic capillaries and vessels were seen deep in the mucosa. The lymphatics had a very thin endothelium with an incomplete basal lamina.

DISCUSSION

Our results show two networks of vessels of different types and sizes in the mucosa of both the trachea and bronchi. A rich network of capillaries runs close to the epithelial basement membrane. The capillaries converge to form venules which extend to a deeper mucosal plexus formed by larger venules and arterioles. Previous studies (McLaughlin, Tyler & Canada, 1961; Pietra & Magno, 1978) describe the microcirculation of the larger bronchi of the dog as an arteriolar and venular plexus

outside the muscle layer in the peribronchial connective tissue and a capillary and venular plexus in the submucosa between muscle and epithelium. They do not describe a subepithelial capillary–venular plexus, the pictures often showing vascular cul-de-sacs indicative of unfilled vessels. The capillary vascular casts described by us represent true vessels, since the dimensions of the casts correspond to those generally accepted (Rhodin, 1974) and to those seen in light and electron microscopy, and cul-de-sacs indicating unfilled vessels were rare or absent. The casts showed no evidence of vascular disruption. The trachea and bronchi have similar vascular patterns, except that in the bronchi the capillaries have a more conspicuous longitudinal orientation.

Both light and electron microscopy confirm the subepithelial location of many capillaries and some venular structures, corresponding to the luminal network seen with casts and SEM. The counts of vessels per unit area performed with electron microscopy exceeded those with light microscopy, but this is not surprising because the former was restricted to a layer 60–240 μm thick under the epithelium where the vessels are concentrated. In addition the higher resolution of electron microscopy would reveal vessels not apparent with light microscopy. In agreement with Pietra & Magno (1978) for the bronchi, no fenestrated capillaries were found. In man fenestrated capillaries have been described close to the epithelium in areas where epithelial neuroendocrine cells were located (Laitinen, 1988); such cells were not seen in the dog (Laitinen, Laitinen & Widdicombe, unpublished observations).

Although arteriovenous anastomoses occur in the nose (Malm, Sundler & Uddman, 1980; Lung, Phipps, Wang & Widdicombe, 1984), no such vessels were found in the present study either in the tracheal or in the bronchial mucosal circulations. However, for the tracheal vascular bed, cross-over between opposite sides was shown by injection of coloured plastics. This clearly cannot apply to the bronchi. In physiological experiments drugs injected into the cranial tracheal artery on either side have a contralateral effect on vascular resistance (Laitinen, Laitinen & Widdicombe, 1987).

Hill *et al.* (1989) have described the structure of the tracheobronchial vasculature in the submucosa of the sheep. This species has, in addition to a subepithelial capillary plexus, a copious network of large blood sinuses, up to 500 μm in diameter, deep in the mucosa close to the airway cartilages. These were not seen, nor have they been described by others, in the dog. We have seen them in man, but have not quantitated them (Hill, Goulding, Webber & Widdicombe, unpublished results). They have been described for the rabbit (Hughes, 1965). Why man, sheep and rabbit should have them, but not the dog, is not clear; their function is unknown.

The physiological role of the bronchial circulation has classically been related to the nutrition of the bronchi (Daly & Hebb, 1966). Recently the function of the bronchial circulation has been widened to include the regulation of heating and the humidification of inspired air (Baile *et al.* 1985; Baier *et al.* 1985), which would apply mainly to the nose and the central airways. The bronchi have a similar vascular network to that of the trachea, and the microcirculation may serve as a transport system redistributing mediators from one part of the airway to another; it may also play a role in controlling the distribution and clearance of drugs given by aerosol. The development of oedema of the airway wall will depend on its vasculature (Pietra *et al.* 1971; Baier *et al.* 1985; Persson & Erjefalt, 1988) and the effects of inflammatory and other mediators on tracheal vascular resistance and on tracheal mucosal thickness have been measured in dogs (Laitinen *et al.* 1986, 1987).

The present study establishes that in the canine mucosa of both the trachea and the

bronchi there is a copious network of microvessels, close to the epithelium. This finding may help to interpret pathophysiological findings attributed to the mucosa.

SUMMARY

We have studied the tracheal and bronchial microcirculation of dogs. Plastic casts of the vessels were studied by scanning electron microscopy. A dense network of small vessels ran in the trachea and bronchi close to the tracheobronchial lumen around and along the airway wall. Under light and transmission electron microscopy, capillaries lay just deep to the epithelial basement membrane. Small postcapillary venules were found deeper in the mucosa. Arterioles occurred regularly between the cartilaginous plates in the trachea but there were only a few in the tracheobronchial mucosa itself and none was closer than 100 μm to the epithelial basement membrane. The diameter of most vessels in the airway mucosa was less than 30 μm. No arteriovenous anastomoses were seen. Apart from a possible physiological role in conditioning inspired air, the profuse subepithelial microvasculature may act as a means of uptake and distribution of mediators and also as a barrier between the lumen and the airway smooth muscle.

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