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Blood sinuses in the submucosa of the large airways of the sheep†

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INTRODUCTION

We have been studying the physiology and pharmacology of the airway vasculature of the sheep by perfusing the tracheal arteries with the sheep's own blood and measuring tracheal vascular resistance (Webber, Salonen & Widdicombe, 1988*a*,*b*). At the end of some of these experiments we displayed the tracheal vasculature by injection of plastic with subsequent digestion of tracheal tissue, followed by scanning electron microscopy. This method shows a network of large blood sinuses, which we have subsequently analysed by light and transmission electron microscopy. Similar vessels occur in the bronchial wall.

Hughes (1965) described blood sinuses in the trachea of the rabbit, but they have not been reported for the sheep (see Discussion). Furthermore, even for the rabbit there are no histological studies of the sinuses and their size and distribution have not been quantified.

An abstract of some of our results has been published (Goulding, Hill, Webber & Widdicombe, 1988).

MATERIALS AND METHODS

Seven Dorset Shorthorn sheep (five ewes and two rams), approximately one year old and weighing between 25 and 40 kg, were used. The animals were anaesthetised with sodium pentobarbitone (20 mg kg^{-1}), paralysed with gallamine triethiodide (1 mg kg^{-1}) and artificially ventilated. Additional supplements of anaesthetic were given as required.

The trachea was exposed in the neck; its arterial supply and venous drainage were identified, isolated and cannulated as described elsewhere (Webber *et al.* 1988*b*). The tracheal arteries left the common carotid artery at different levels in the neck; there were usually 1–3 arteries on each side. The arteries were perfused via the common carotid artery from which they arose at a pressure as close as possible to the animal's own systemic arterial pressure, and the distribution of blood was identified by injections of Evans Blue dye into the perfusate. Usually about 8–12 tracheal rings and adjacent tissues were perfused via each artery, together with part of the adjacent oesophagus. The animals were heparinised before the start of the perfusion with 25000 units heparin i.v. (Leo Laboratories Ltd). On completion of physiological experiments for the study of tracheal circulation, the animals were killed with an overdose of pentobarbitone.

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In four sheep, segments of the trachea and bronchi, at levels ranging from the first tracheal ring to intrapulmonary bronchi less than 1·0 mm diameter, were immediately excised and fixed in 10% formalin in 0·1 M phosphate buffer for 48 hours. This period was followed by secondary fixation in 10% formalin (1 part) in saturated mercuric chloride in 0·9% saline (9 parts) for a further 48 hours. Mercury was removed with alcoholic iodine and the tissues were then embedded in paraffin wax. Sections 5μm thick were cut and stained with Harris' haematoxylin and eosin. They were examined at various magnifications using an Ultraphot research microscope (Carl Zeiss, West Germany).

Tissue samples for transmission electron microscopy (TEM) were also taken. These were fixed in 3% glutaraldehyde in 0·1 M phosphate buffer and treated with 1% osmium tetroxide in 0·1 M phosphate buffer. After embedding in resin (Araldite CY212, Emscope, UK), 90 nm sections were cut with a glass knife (LKB 8800 Ultratome III, LKB, Sweden) and examined under a Philips TEM (Philips, Holland).

In three sheep Batson's No. 17 anatomical corrosion compound (Polysciences Inc, Pennsylvania, USA) was injected via the common carotid artery into a cervical tracheal artery. In two other sheep the lungs and intrathoracic trachea were removed intact from the chest and the common bronchial (carinal) artery was catheterised. The bronchial tissue supplied by this artery was displayed by injection of 0·2–0·5 ml Evans Blue in saline. Batson's No. 17 anatomical corrosion compound was injected into the bronchial artery after the tracheal and bronchial circulations had been flushed with heparinised saline. Tracheal and bronchial segments which had been labelled with Evans Blue were removed. After the plastic had hardened, the surrounding tissues were digested with 6 M potassium hydroxide. The casts were rinsed clean with distilled water and examined under a dissecting microscope. Selected tracheal and bronchial segments were dehydrated through a graded alcohol series, mounted, dried (Critical Point Dryer, Polaron Equipment Ltd, UK), coated with gold in a sputter-coater (SEM Coating Unit E5 100, Polaron Equipment Ltd) and examined under scanning electron microscopy (SEM) (Cwikscan 100, Coates & Welter Ltd, UK) at magnifications between 100 and 2000.

In one sheep a retrograde injection of blue latex was made into a vein draining the cervical trachea. The vein, which ran alongside the tracheal arteries being perfused, was first identified by noting the appearance in it of Evans Blue following injection of the dye into the tracheal artery. The tracheal vasculature was flushed with heparinsed saline before injection of the latex. Portions of the trachea were removed, fixed and processed for light microscopy as described above.

Using sections from the four sheep fixed for light microscope studies, photomontages at a magnification of $\times 170$ were constructed for complete transverse sections of the trachea and bronchi at different levels, representative of the 1st, 5th, 18th and 23rd tracheal rings, the carina, the main, lobar segmental and subsegmental bronchi. All vessels with walls that could be characterised as 'sinus-like' (i.e. formed by a single layer of endothelium without adjacent smooth muscle) were identified by light microscopy of the original section and were marked on the photographs. Using a digitising pad (Terminal Display Systems), a microcomputer (M28, Olivetti) and appropriate software (Cambridge Electronic Design), the long and short transverse diameters, circumference and cross sectional area of each identified vessel were measured. The 'transverse diameters' given in the Results refer to the diameters at right angles to the long-axis diameters of the sinuses, since some of the vessels may have been cut obliquely. The inner circumference of each tracheal and bronchial section was also measured and used to calculate an 'effective luminal diameter' for the

airway (i.e. the diameter the airway would have if it were circular). Finally, the submucosal area of each transverse section of the airways was measured as the area bounded by the basal lamina of the epithelium and a line corresponding to the luminal boundary of the cartilages and, for the trachea, the posterior trachealis muscle.

The accuracy of the measurements may be judged from the results of 50 repeated measurements of a 1·0 × 1·0 cm square. Mean values were 98% of the true value for perimeter length and 94% of the true value for area. The coefficients of variation were 0·028 (2·8%) for perimeter length and 0·053 (5·0%) for area. Measurements of a total of 246 identified vessels in the same bronchial section by two independent observers gave identical distributions and a correlation coefficient of 0·9996 for measurements of cross sectional area. All measurements on vessels with a transverse diameter greater than 50 μm were carried out by one individual.

RESULTS

Injection studies

Arterial injection of Batson's compound filled capillaries and submucosal sinuses. Viewed from the luminal side the casts show a complex network of subepithelial mucosal capillaries (Fig. 1*a*) with sinuses lying deeper. When the superficial capillaries are partially removed (Fig. 1*b*) the sinuses are displayed with a general orientation in the long axis of the trachea. Display with SEM of the abluminal (submucosal) surface shows that the network of sinuses is complex (Fig. 2*a*, *b*). The longitudinally directed vessels communicate via circumferential connections at intervals along the airway (Fig. 2*a*). Light microscopy suggests that these circumferential vessels occur mainly in the intercartilaginous spaces (see below). Sinuses similar to those seen in the tracheal wall were found in plastic casts of the bronchial vasculature but were less frequent.

Light microscopy

Retrograde (intravenous) injection of coloured latex fills the tracheal sinuses via the tracheal veins (Fig. 3*b*). Latex does not traverse the capillary bed, the smallest vessels filled having transverse diameters of 10 μm or greater.

In transverse sections of the trachea, large vascular sinuses are seen lying mainly in the submucosa, beneath the lamina propria and in close apposition to the cartilaginous rings (Fig. 3*a*). In the region of the cartilage the vessels are usually cut transversely, indicating their longitudinal orientation across the cartilage ring. Large branches originating close beneath the basal lamina of the tracheal epithelium can sometimes be seen draining into the submucosal sinuses (Fig. 3*c*).

Sinuses of large diameter can be seen in the main bronchi and their lobar and segmental branches (Fig. 4*a*, *b*) but they become progressively fewer with each generation of branching. Their arrangement is less regular than in the trachea. In general they occur close to cartilage but they are also found between cartilage plates. In the bronchi most large sinuses lie deep to bands of smooth muscle (Fig. 4*a*, *b*).

Large sinuses are seen at the bifurcations of segmental bronchi (Fig. 4*b*) but no sinus-like vessels with diameters of 50 μm or more are seen in subsegmental bronchi.

Transmission electron microscopy (TEM)

The appearance of a small sinus under the electron microscope is illustrated in Figure 5(*a*). The absence of smooth muscle and pericytes is apparent, although sometimes very thin unidentified cells can be seen under the basal lamina (Fig. 5*b*, *c*).

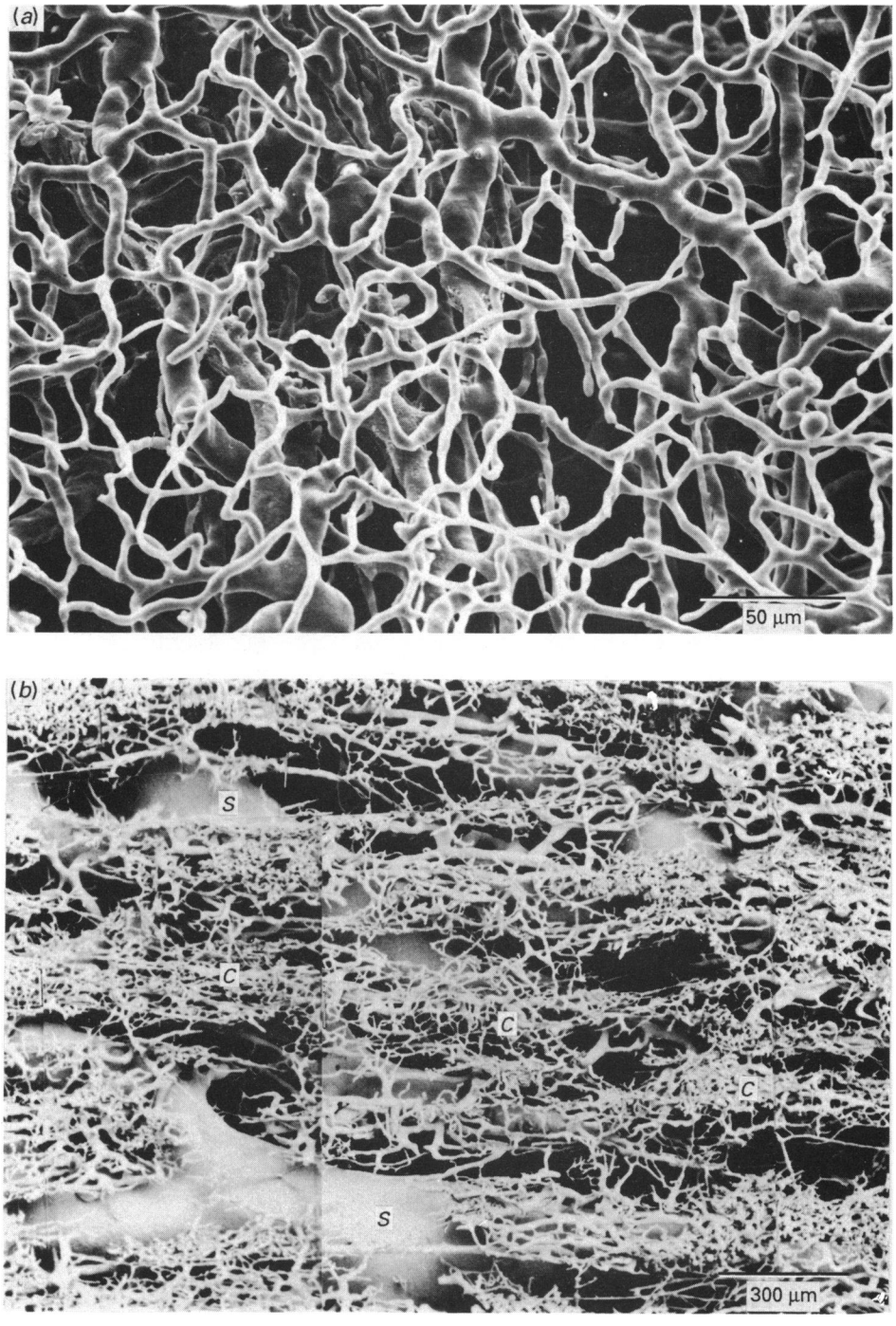


Fig. 1 (*a–b*). Scanning electron micrographs of tracheal vasculature of sheep. The vessels were filled with anatomical corrosion compound and the soft tissue was digested. (*a*) High power view from the epithelial surface showing subepithelial network of capillaries, with some larger and deeper vessels visible in the background. (*b*) Low power view from the epithelial surface, with some of the compound-filled capillary network dissected away. The remnants of the capillary network can be seen (*C*), together with large-diameter deeper sinuses (*S*). The picture is a montage.

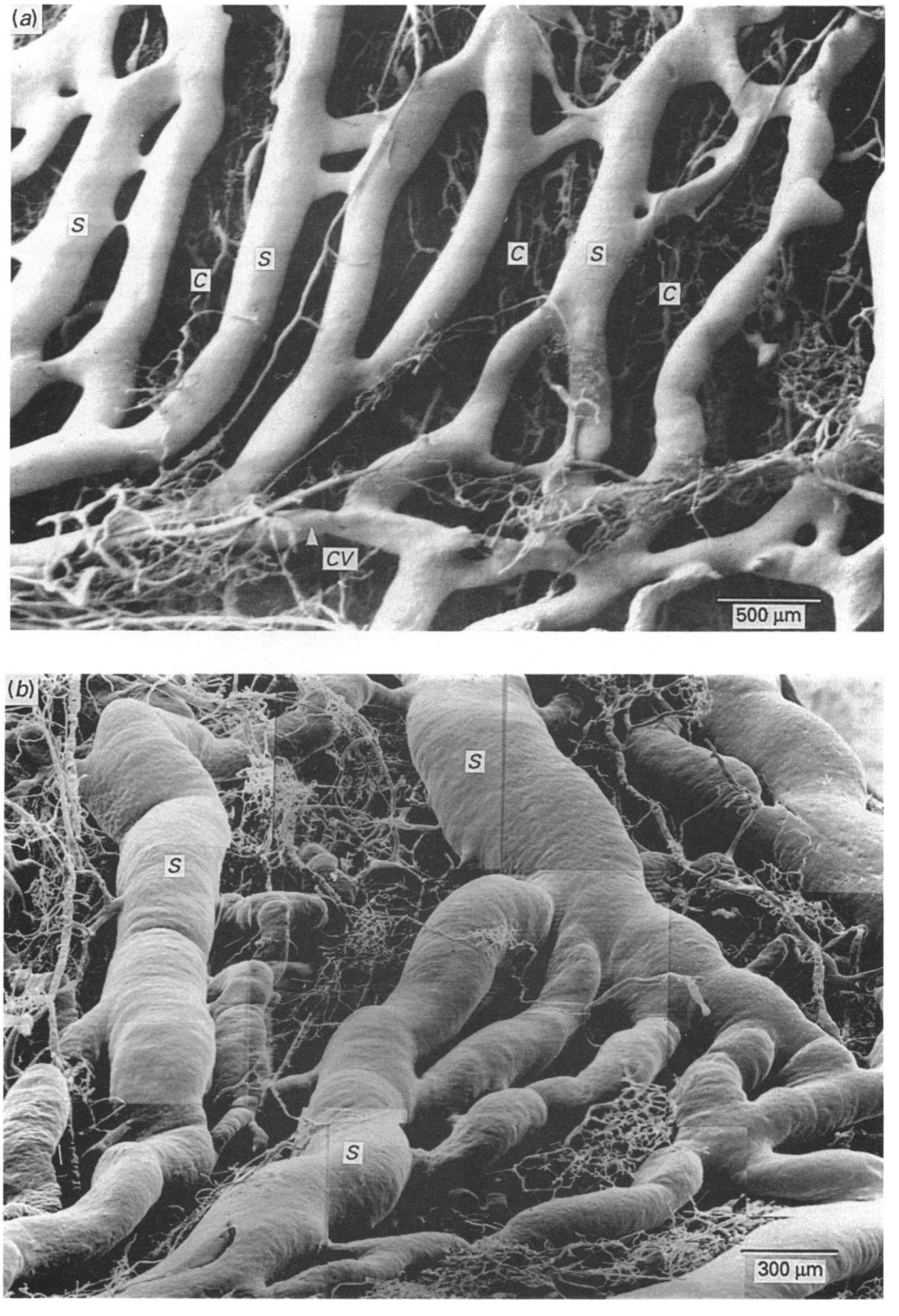
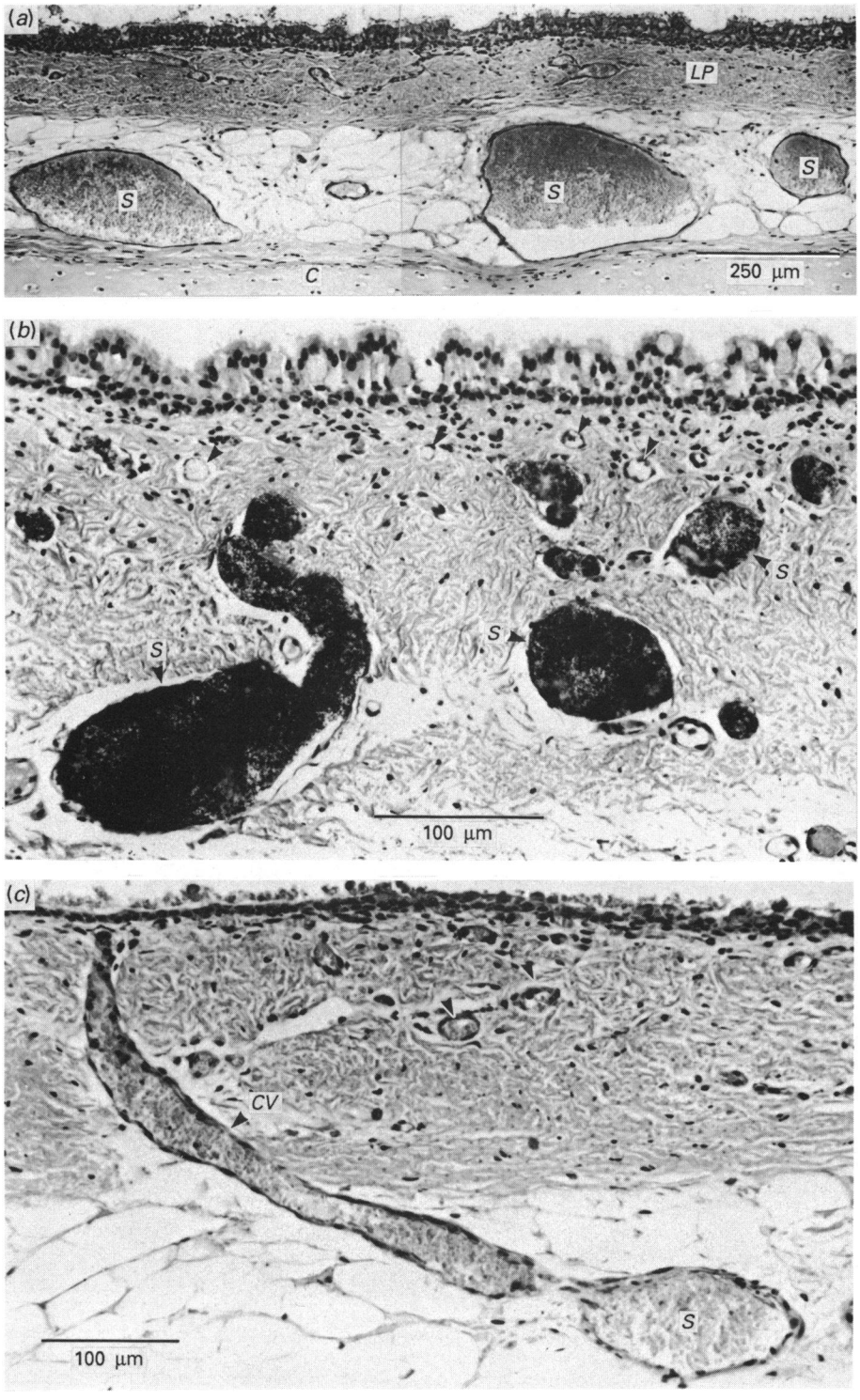


Fig. 2(*a–b*). Low power scanning electron micrographs of tracheal vasculature of the sheep. The blood vessels have been filled with anatomical corrosion compound and the soft tissue digested away. (*a*) View from the abluminal (submucosal) surface showing network of sinuses (*S*), with subepithelial capillaries (*C*) lying deeper. The transverse direction of the trachea is across the Figure, the sinuses running mainly longitudinally. The intercartilaginous space is transverse at the bottom of the picture, showing connecting vessels (*CV*) joining the sinuses. (*b*) Montage of a scanning electron micrograph of tracheal sinuses (*S*) showing their network of connections.


(a)
LP
S
S
S
C
250 μm
(b)
S
S
S
100 μm
(c)
CV
S
100 μm

The sinus wall consists of a single layer of flattened endothelium (Fig. 5*b*). At a higher magnification (Fig. 5*c*) the nature of the tight junctions between adjacent endothelial cells can be seen. No fenestrated endothelial layers were seen.

Morphometry

A total of 5176 vessels in 18 photomontages from four sheep was examined. Of the total, 746 (14%) had transverse diameters greater than 50 μm and 55 (1%) had diameters greater than 200 μm. The frequency distributions by diameter of the tracheal and bronchial vessels, identified as having 'sinus-like' walls and compared to smaller (less than 50 μm) diameter vessels, is given in Figure 6. The trachea had more sinuses (> 50 μm diameter) compared to 'capillaries' (< 50 μm diameter) than did the bronchi (26 and 10% of totals respectively).

The circumferential and longitudinal distributions of the tracheal and bronchial sinuses are shown in Figure 7(*a*, *b*). In the trachea only six sinuses (14% of the tracheal sinuses) were related to the posterior trachealis muscle. All six occurred at the level of the first cartilaginous ring. Along the length of the trachea, sinuses lay mainly in close relationship to the cartilage. On average more were found on the left (54%) than on the right side (41%), but the difference was not statistically significant (by Student's *t*-test). In the bronchi the sinuses were scattered around the circumference of the airway but the majority (67%) were adjacent to the cartilage plates. The number of large diameter sinuses declined as the airways penetrated into the lungs (Fig. 7*b*). No sinuses with diameters greater than 50 μm were seen in airways with transverse diameters less then 1·0 mm.

The cross sectional areas of the sinuses were also measured, summed and expressed as percentages of the submucosal areas of each transverse section of an airway. The means $\pm$standard deviations of these percentages were $5{\cdot}4 \pm 2{\cdot}2$% for the trachea and $1{\cdot}4 \pm 1{\cdot}9$% for the bronchi. In sites where there were concentrations of sinuses the percentages were as high as 25%. Expressed as sinuses per mm of mucosal length, the mean value for trachea was 0·79 sinuses mm^{-1} and for bronchi 0·41 sinuses mm^{-1}. Expressed as sinuses per mm^2 of mucosal area the mean values were 1·98 sinuses mm^{-2} for trachea and 0·97 sinuses mm^{-2} for bronchi.

DISCUSSION

A sinus may be defined as "a channel for the passage of blood which has not the coats of an ordinary blood vessel..." (Stedman, 1976). The term is appropriate to the vessels we have described here. We have avoided the term 'sinusoid' in deference to pleas to restrict the use of this term to large diameter capillaries with incomplete walls and with reticuloendothelial cells in close proximity (Henderson & Daniel, 1984). In analysing our results we have taken an arbitrary diameter of 50 μm for quantitation. Vessels larger than this were certainly sinuses as defined above; smaller vessels would include capillaries, post-capillary venules and sinuses, so we have probably underestimated the total numbers of sinuses.

Fig 3(*a–c*). Light micrographs of tracheal mucosa and vasculature of the sheep, stained with haematoxylin and eosin. (*a*) Transverse section showing large sinuses (*S*) lying between the lamina propria (*LP*) and the cartilage (*C*). Smaller vessels can be seen in the lamina propria. The sinuses are full of blood cells. (*b*) Micrograph showing sinuses (*S*) filled with blue latex by retrograde injection into the tracheal vein. Subepithelial capillaries (arrowheads) can be seen empty of latex. (*c*) Micrograph of a sinus (*S*) with a connecting vessel (*CV*) reaching almost to the epithelium. Subepithelial capillaries (arrowheads) can be seen.

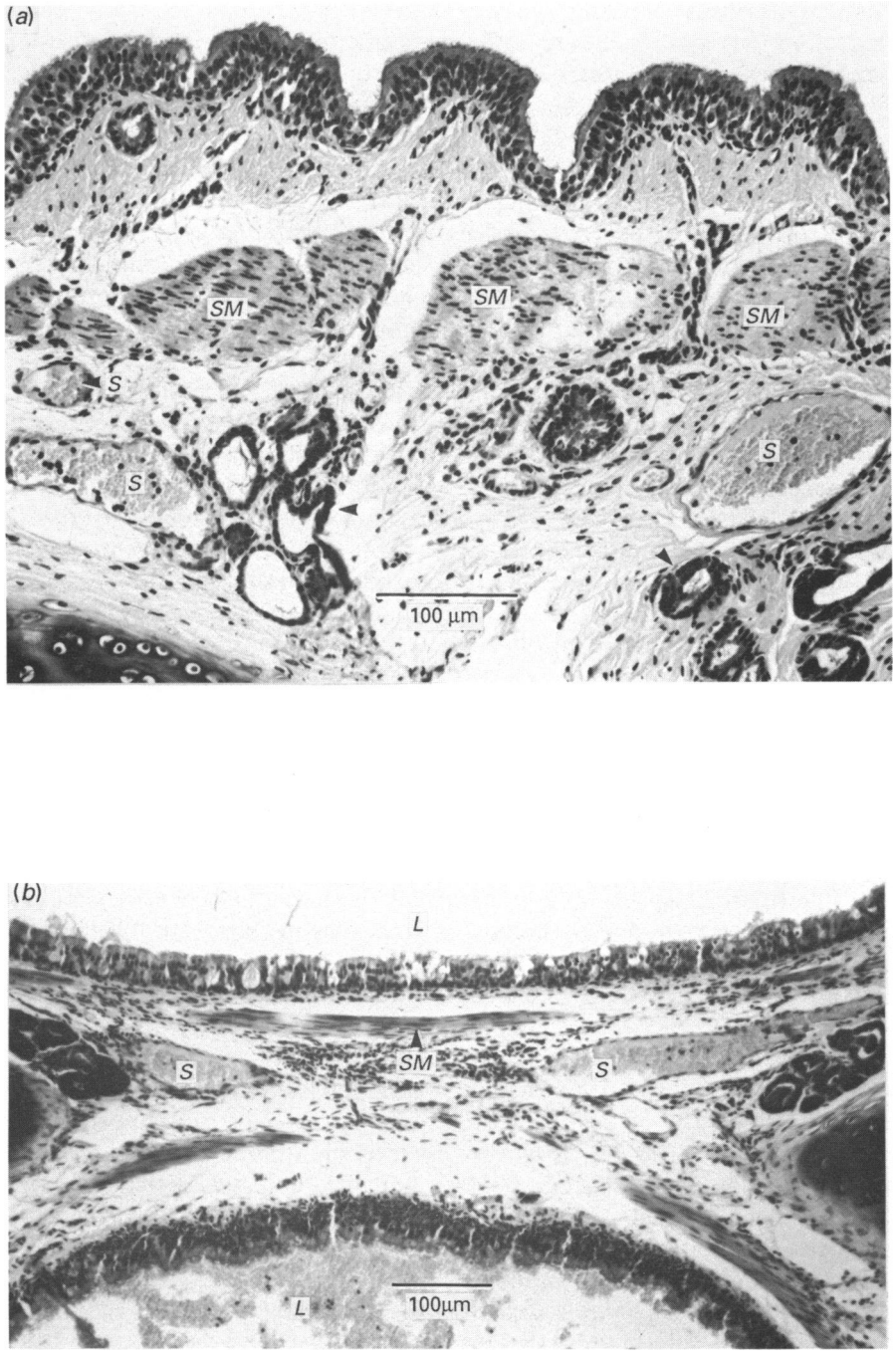


Fig 4(*a–b*). Light micrographs of bronchial mucosa of the sheep, stained with haematoxylin and eosin. (*a*) Sinuses (*S*) lie under smooth muscle bands (*SM*) close to glands (arrowheads) and cartilage (bottom left). (*b*) Sinuses (*S*) in the spur of tissue between two bronchial lumens (*L*). Smooth muscle bands (*SM*) can be seen under the epithelium. Edges of cartilage can be seen on the far right and far left.

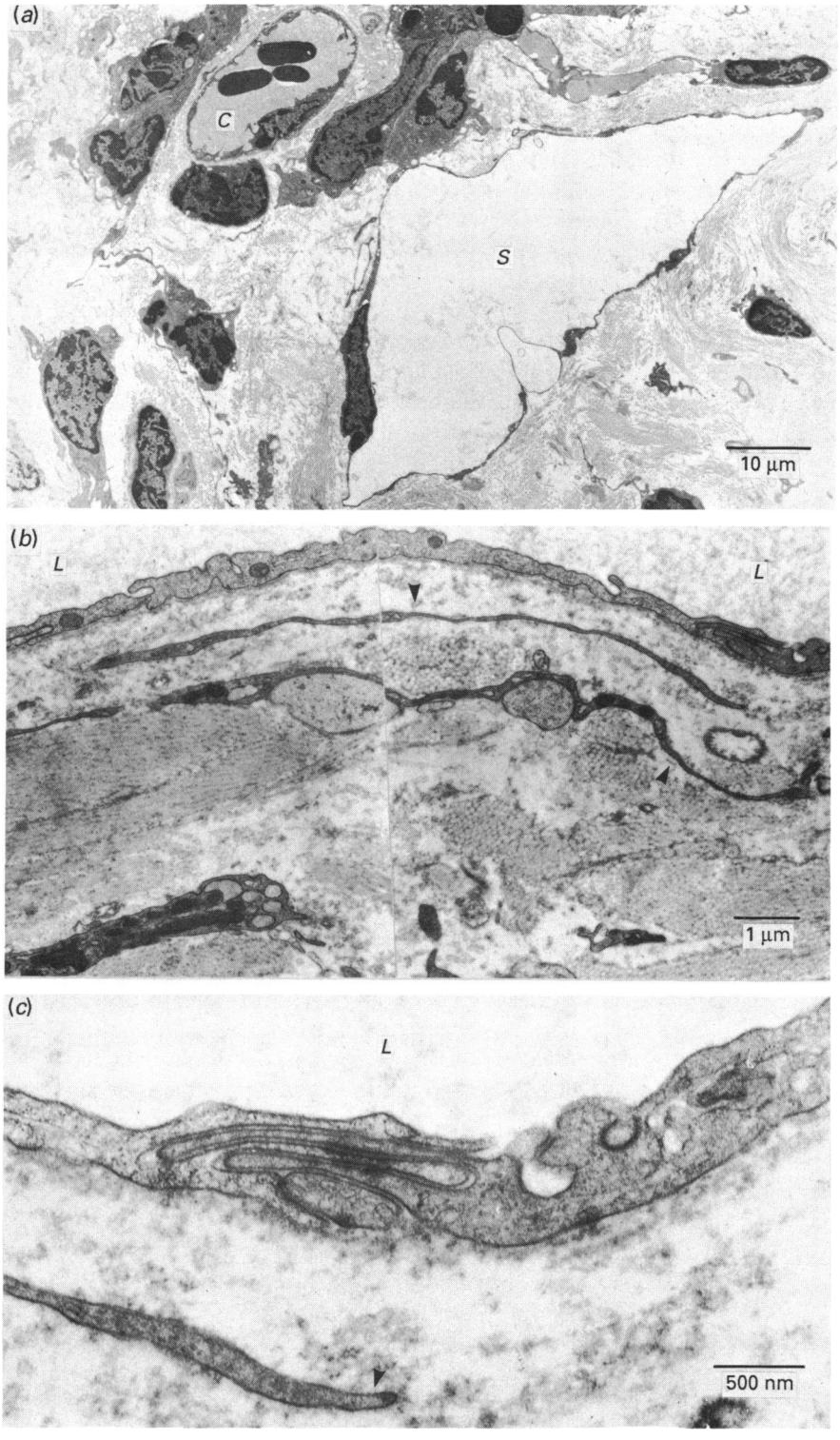
There have been a number of thorough studies of the gross and subgross anatomy of the pulmonary and bronchial (but not the tracheal) circulations in sheep (Turk, Charan & Czartolemny, 1985; Charan, Turk & Dhand, 1984; McLaughlin, 1983; Magno & Fishman, 1982; Albertine, Wiener-Kronick, Roos & Staub, 1982; May, 1970; McLaughlin, Tyler & Canada, 1961). None of these authors mentions the plexus of blood sinuses that we have described.

Hughes (1965) described a similar network of blood sinuses in the trachea of the rabbit. He described the sinuses as being "fed by venules draining radially outward from the superficial capillary network of the mucosa". He also stated that, in the rabbit, "the sinuses...drain into collecting veins which run circumferentially with the arteries, in the intercartilaginous spaces to pierce the tracheal wall on its lateral aspect". Our findings suggest a similar arrangement in the sheep. Hughes (1965) also briefly reported the results of comparative studies of the tracheal microcirculation in man, rhesus monkey, dog, cat, guinea-pig and rat as well as the rabbit. He studied neither the histology nor the ultrastructure of the vessels, nor the vasculature of the sheep.

It is arguable that the preliminary experiments conducted in the sheep may have made the sinuses more prominent through vasodilation or increased venous pressures. However, such experiments could not result in the formation of the vessels *de novo*. Furthermore, sinuses with the same size and concentration, and of the same structural appearance, were found in those parts of the trachea which were not experimentally perfused. The vessels we call sinuses cannot be lymphatics, since they contain red cells (unless first perfused with saline), the cellular structure of their walls is quite different from that of lymphatics of similar diameter, and the injection studies show that they communicate on the one hand with the tracheal arterial supply and on the other with the tracheal venous drainage (retrograde injection). We have not defined their relationship with capillary networks; this will require three dimensional histological analysis. With the plastic casts we did not see capillary networks converging on the tracheal sinuses, and the general appearance of their junctions with other vessels at the intercartilaginous areas is consistent with their being supplied by arterioles and draining into venules. However, this conclusion is tentative.

The sinuses are most conspicuous in the tracheal wall, where their most notable characteristics are their large size, the complexity and extent of the network they form, and the constancy of their anatomical relationships to cartilage. The function of the sinuses has yet to be determined. We suggest three hypotheses that might repay investigation.

First, they might have a role in the conditioning of inspired air, helping to warm and humidify it. Although the sheep is an obligatory nose-breather except in severe panting (Hales & Webster, 1967), by the time inspired air reaches the trachea it need not be completely warmed and moistened (Hanna & Scherer, 1986). Hughes (1965) suggested that, when inspired air was very humid, heating the tracheal air would be more dependent on convection and radiation and offered this as an explanation for the large blood sinuses and sparsity of submucosal glands in the rabbit trachea. However the nasal vasculature would be more important in this respect. Furthermore the most efficient vascular system for conditioning inspired air would probably be formed by arteriovenous anastomoses, as seen in the nose. The structure of the sinuses does not appear ideal for a system controlling temperature and humidity of respired gas, although their volume might act as an adjustable heat reservoir. The dog, not an obligatory nose-breather, might be expected to have a tracheal vasculature more


(a)
C
S
10 μm
(b)
L
L
1 μm
(c)
L
500 nm

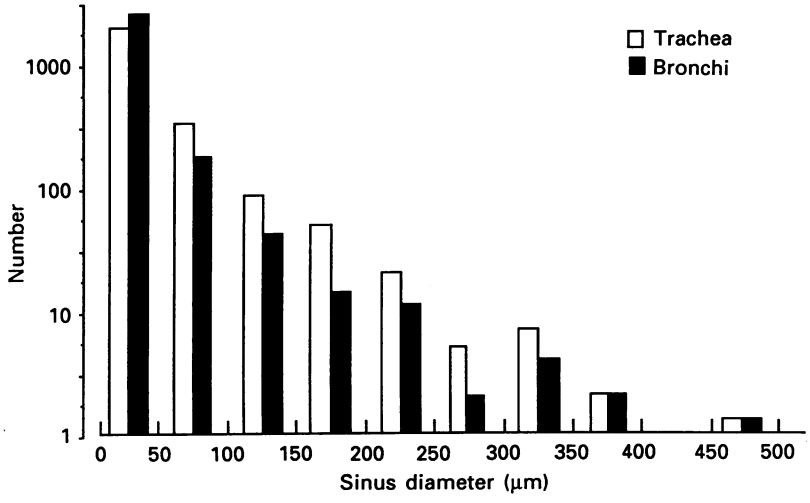


Fig 6. Histogram showing the numbers of vessels, including sinuses (defined as having diameters greater than 50 μm) and smaller vessels (diameters less then 50 μm), compared with vessel diameters for the trachea and bronchi. The ordinate scale is logarithmic, to permit presentation of all values. For vessels of diameter less than 50 μm, more were counted for the bronchi than for the trachea. For vessels of diameter greater than 50 μm, i.e. blood sinuses, for nearly all diameters more were counted for the trachea than for the bronchi.

important for the conditioning of gas, yet it has far fewer or no blood sinuses (Laitinen, Laitinen, Moss & Widdicombe, 1987).

Alternatively, the sinuses may play a role as a blood reservoir in prolonging the time blood is in the mucosa. This would allow more time for inactivation of mucosal metabolites released into the blood before they could reach potential sites of action such as tracheal smooth muscle or even, via the right heart, the pulmonary vasculature. The relationship of the vessels to the relatively 'insensitive' cartilage might be significant in this regard.

Thirdly, the sinuses may have a mechanical role. Their relationship to cartilage means that any dilation of these vessels is likely to be directed inwards, encroaching on the airway lumen. During an act such as coughing, intrathoracic pressure rises, impeding venous return and potentially engorging the blood sinuses. Any resultant reduction in airway diameter could result in an increased velocity of the airflow, making the cough more effective and improving the clearance of mucus or particulate matter.

At present these hypotheses are purely speculative. What is not in doubt is that in all the sheep we have examined there is an extensive plexus of large submucosal sinuses in the trachea and its major branches. Preliminary studies show that sinuses are also

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Fig 5(*a–c*). (*a*) Transmission electron micrograph to show a small sinus (*S*) in the tracheal submucosa of a sheep. The sinus has been washed out with saline, which has not removed blood from a smaller vessel (*C*) at the top left. The sinus has a single-cell endothelial wall, with no smooth muscle or pericytes. An endothelial bleb is seen on the lower right border. (*b*) High power transmission electron micrograph to show endothelium of a blood sinus in the tracheal mucosa of the sheep. The lumen (*L*) is at the top. Thin unidentified cells (arrowheads) are visible below the endothelium, but no smooth muscle is present. (*c*) Higher powered picture of the junction between two endothelial cells, corresponding to the upper right part of Fig. 5(*b*). Tight junctions can be seen along the tortuous intercellular connection. A thin unidentified cell (arrowhead) is also visible. Lumen (*L*) at top.

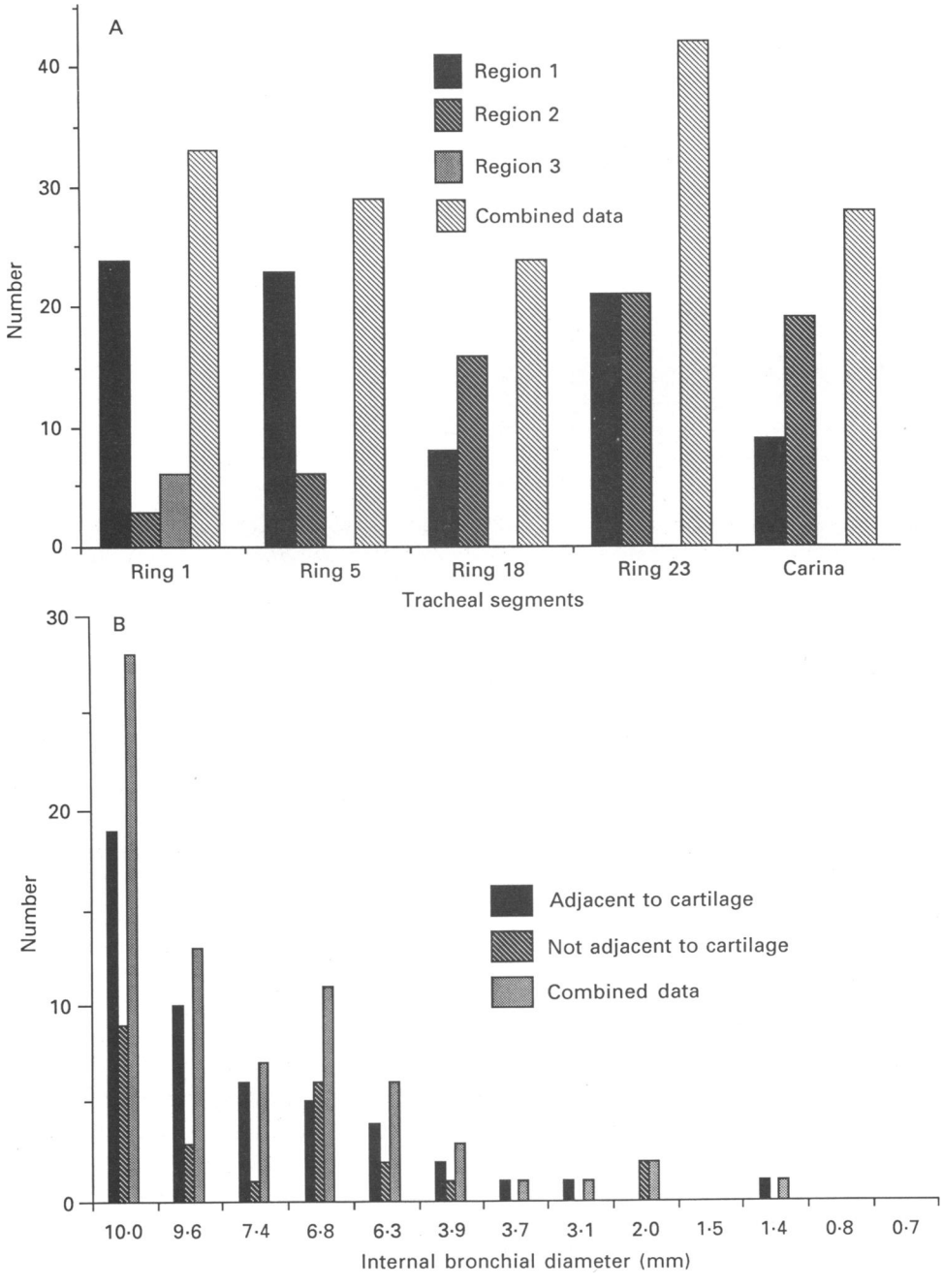

Fig 7(A–B). Quantification of sinuses in the submucosa of sheep airways. (A) Tracheal sinuses showing number of vessels greater than 50 μm in diameter, at different levels of the trachea, and at the carina. Ring 1 is immediately below the cricoid cartilage. Region 1, left wall of trachea overlying cartilage; Region 2, right wall; Region 3, mucosa overlying posterior trachealis muscle. The last region had only six sinuses, all of them at the level of Ring 1. Although clear differences between the two sides can be seen at most levels, the total number of sinuses (combined data) does not vary significantly with tracheal position. (B) Corresponding results for bronchial sinuses, showing numbers of sinuses greater than 50 μm in diameter. The abscissa shows the measured internal bronchial diameters in mm. Sinuses are divided into those adjacent to cartilage, those not adjacent to cartilage, and the combined data. About two thirds of the sinuses were adjacent to cartilage, and the total number of sinuses varied with bronchial diameter, no sinuses being seen in bronchi smaller than 1·4 mm diameter.

present in the walls of the human trachea and bronchi (P. Hill, D. Goulding, S. E. Webber & J. G. Widdicombe, unpublished results).

SUMMARY

We have studied the airway vasculature in sheep using light and transmission electron microscopy, as well as arterial and venous (retrograde) injections of anatomical corrosion compound and latex. Vascular casts were viewed by scanning electron microscopy. There is a complex network of blood sinuses of large diameter (up to 500 μm) in the submucosa of the large airways. The vessels have thin walls formed by a single layer of flattened endothelium with tight junctions and without pericytes or smooth muscle cells. Characteristically the sinuses lie between the cartilage and lamina propria of the trachea or between cartilage and smooth muscle in the bronchi. Sinuses of greater than 50 μm transverse diameter are not found in airways less than 1·0 mm across.

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