AIRFLOW OBSTRUCTION due to airway narrowing is the hallmark of asthma (9) and is a common finding in cardiogenic (15) and noncardiogenic (30) pulmonary edema. Some of this narrowing is due to congestion of bronchial microvessels (3, 18) as well as edema and inflammatory cell infiltration of the airway wall (9, 24). The bronchial vasculature, as opposed to the pulmonary vasculature, is the likely source of the edema and the inflammatory cells because bronchial vessels are perfused at systemic pressures and the bronchial vasculature may be more permeable than the pulmonary vasculature (27). To understand mechanisms of edema formation and inflammation in airway walls, it is necessary to understand the normal distribution of bronchial blood flow and the kinetics of leukocytes in this vascular bed.

The bronchial circulation is unique in having a dual venous drainage; one fraction returns to the right atrium via bronchial and ayzygos veins and the remainder returns to the left atrium via bronchial-to-pulmonary anastomoses. In early studies, the systemic-to-pulmonary fraction was estimated to be about two-thirds of the total bronchial blood flow (20) and, in another study (1), the average systemic bronchial venous drainage was estimated to be 45% of the total bronchial arterial flow. However, these studies used surgically invasive techniques and the preparations were relatively unphysiological. The systemic-to-pulmonary fraction of total bronchial blood flow during undisturbed physiological conditions has not been measured. Therefore, the first purpose of this study was to use an indicator-dilution method to measure the systemic-to-pulmonary fraction of total bronchial blood flow in anesthetized sheep.

To accumulate in airway walls, leukocytes must first be retained by the bronchial microvessels, adhere to the endothelial barrier, and then migrate across this barrier into the interstitial space. One measure of leukocyte retention is first-pass extraction of an injected bolus of radiolabeled neutrophils. The first-pass extraction of neutrophils by the pulmonary vasculature is 70–90% (8, 10, 19, 23). There is no information available about similar measurements in the bronchial vasculature. With the use of our knowledge of the normal bronchial venous drainage pathways, the second purpose of this study was to measure the first-pass extraction of neutrophils in the bronchial vasculature.

MATERIALS AND METHODS

All studies were done by using Canadian guidelines for animal care. We studied 13 sheep (Dorset-cross, rams), weighing from 25 to 35 kg. The sheep were divided into two groups. In group 1 (n = 7), we measured the relative distribution of radiolabeled red blood cells to the right and left ventricles after injection into the bronchoesophageal artery. In group 2 (n = 6), we measured the extraction of radiolabeled neutrophils during their first pass through the vasculature of the lung after injection directly into the bronchoesophageal artery or into the right ventricle.

Surgical Preparation

Anesthesia was induced by intravenous injection of 15–20 mg/kg thiopental sodium. A tracheostomy tube was inserted; sheep were ventilated with 50% oxygen in air at a rate of ~15 breaths/min and a tidal volume of 12–15 ml/kg; and anesthesia was maintained by inhalation of 1–2% halothane. Ventilation was adjusted to keep arterial PCO2 (PACO2) between 35 and 40 Torr and arterial PO2 (PACO2) >100 Torr. A catheter was introduced into the left femoral artery for measurement of systemic arterial blood pressure and to obtain blood samples for measurement of PAO2 and PAcO2. Another catheter was inserted into the femoral vein for infusion of fluids and administration of drugs. With the use of fluoroscopy, catheters were introduced into the following vessels for later collection of blood samples: the thoracic aorta just distal to...
100 MBq of technetium-99m (99mTc) was added drop by drop. The washing procedure was repeated two more times. Then, for 10 min. The supernatant was removed and discarded, and spinning this mixture at 1,400 revolutions/min (rpm; 450 g) for 10 min. The supernatant was removed and discarded, and the washing procedure was repeated two more times. Then, 100 MBq of technetium-99m (99mTc) was added drop by drop as the concentrate was mixed gently. The radiolabeled mixture was incubated at room temperature for 10 min, and the cells were washed three times by using the procedure described above. The final concentrate was suspended in 20 ml of 0.9% saline, and 0.5 ml of this suspension was used as the injectate (~5 MBq).

Cell Labeling

Group 1. Arterial blood (20 ml) was collected in a heparinized tube for labeling of red blood cells. Sodium glucoheptanate solution (0.3 ml; DuPont-Merck, Billerica, MA) was mixed with the whole blood, and this mixture was allowed to incubate at room temperature for 10 min. Then, the cells were washed by adding 20 ml of 0.9% saline, mixing gently, and spinning this mixture at 1,400 revolutions/min (rpm; 450 g) for 10 min. The supernatant was removed and discarded, and the washing procedure was repeated two more times. Then, 100 MBq of technetium-99m (99mTc) was added drop by drop as the concentrate was mixed gently. The radiolabeled mixture was incubated at room temperature for 10 min, and the cells were washed three times by using the procedure described above. The final concentrate was suspended in 20 ml of 0.9% saline, and 0.5 ml of this suspension was used as the injectate (~5 MBq).

Group 2. Red blood cells were radiolabeled as described above for group 1. To isolate neutrophils, 50 ml whole blood were drawn into a syringe containing 8 ml of ACD solution (citric acid 8 g/l, sodium citrate anhydrous 22 g/l, and dextrose 24.5 g/l, all diluted in sterile, distilled H2O). This mixture was spun at 2,500 rpm (1,300 g) for 5 min, and the supernatant was removed (~5 cm above the buffy coat) and discarded. Then, the buffy-coat layer was removed by pipetting carefully, and it was saved for separation of cells by using an elutriator as previously described (7). The proportion of neutrophils in the final sample was 97–99%.

Radiolabeling Neutrophils

The neutrophils were diluted to give a concentration of 5 × 10^6/ml. Next, 5 MBq of 111In-labeled oxine were added drop by drop and mixed in gently. The mixture was incubated at room temperature for 30 min and mixed gently 3–4 times during the incubation. After the incubation, 5 ml of neutrophil buffer were added and mixed in gently, and this mixture was spun at 1,400 rpm (450 g) for 10 min. The supernatant was removed, the pellet was resuspended in 5 ml polymorphonuclear neutrophil buffer, and 0.5 ml of this suspension was used for each injetate.

Experimental Protocol

After surgery, when the sheep were physiologically stable, we recorded vascular pressures (systemic arterial pressure, pulmonary arterial pressure, right atrial pressure), cardiac output in triplicate, and PA,CO2 and PA,02.

Group 1. To measure the relative distribution of bronchial blood flow to the right and left ventricles, we used the following protocol: 99mTc-labeled red blood cells (0.5 ml, ~5 MBq) were loaded into the lumen of the Cobra catheter. At time 0, the red blood cells were infused into the bronchial artery by using an infusion pump set at a rate of 2 ml/min. Saline (0.9%) was used as the infusate. Four seconds before injection of the red blood cells, a fraction collector was started, and samples of blood were collected simultaneously via catheters situated in the aorta, left ventricle, right ventricle, and inferior vena cava (Fig. 1). The sampling time was 2 s/sample, and 20 samples of blood were obtained from each collection site. The average volume of a sample was ~1 ml. After collection of the blood samples, cardiac output and vascular pressures were measured again. After ~30 min, this protocol was repeated.

Group 2. Hemodynamics and Pa,CO2, and Pa,O2 were measured as described for group 1. To measure the extraction of neutrophils, we used the following protocol: 111In-labeled neutrophils (0.5 ml, ~0.5 MBq) were added to and mixed with 99mTc red blood cells (0.5 ml, ~5 MBq), and these were loaded into either the Cobra catheter or the right ventricular catheter. A fraction collector was started 4 s before infusion of the radiolabeled cells; in the case of bronchial arterial injection, blood samples were collected from the aorta, the left ventricle, the inferior vena cava, and the right ventricle. In the case of right ventricular injection, blood samples were collected from the left ventricular catheter. Four seconds after the fraction collector was started, the labeled cells were infused into the bronchial artery or the right ventricle at a rate of 2 ml/min by using 0.9% saline as the infusate. Cardiac output and vascular pressures were measured again after the fraction collector was stopped. The first bronchial arterial injection of cells was followed by a right ventricular injection and then a final bronchial arterial injection of cells. There was a 15-min pause after the first and second injections to allow baseline counts of 99mTc and 111In to equilibrate.

Groups 1 and 2. After the final measurements, the sheep were deeply anesthetized and killed by injection of saturated potassium chloride into the right ventricle. The radioactivity in the blood samples was measured by using a Beckman gamma counter (model 800), and appropriate corrections were made to account for background, radioactive decay, and overlap.

![Fig. 1. Blood-sampling sites](http://jap.physiology.org/ for explanation). Pa, pulmonary artery; Pv, pulmonary vein; Br-P, bronchial-to-pulmonary; RA, right atrium; LA, left atrium.)
Calculations

**Group 1. Systemic-to-Pulmonary Fraction.** The fraction of the radiolabeled red blood cells injected into the bronchial artery that was recovered from the left ventricle was calculated by dividing the total radioactive counts of the red blood cells (RBC counts) reaching the left ventricle by the total radioactivity of the injected red blood cells. To eliminate the recirculated portion on the left ventricular time-radioactivity curve, the initial part of the descending limb was fitted to a gamma variate function and extrapolated to zero counts. The sum of the counts under this fitted curve was used to calculate total left ventricular recovery of RBC counts.

\[ \text{Total LV recovery (RBC counts)} = \text{sum of LV counts (cardiac output/sampling flow rate)} \]

where LV is left ventricular, and sampling flow rate is 30 ml/min.

Total injected RBC counts were calculated by multiplying counts in replicate samples (5 µl) of the initial injectate and extrapolating to obtain the total counts of the initial injectate volume. The fraction of bronchial blood flow returning to the left ventricle (systemic-to-pulmonary fraction) is then calculated as

\[ \text{Systemic-to-pulmonary fraction} = \frac{\text{total LV recovery of RBC counts}}{\text{total injected RBC counts}} \]

**Group 2. Neutrophil Extraction.** To calculate the extraction of neutrophils, relative to red blood cells, in their first pass through the bronchial circulation, the radioactivity of each blood sample was plotted against time and the left and right ventricular time-radioactivity curves were fitted to gamma variate functions. Each curve was then standardized by dividing the counts at each time point by the total injected radioactivity. Areas under each curve were calculated and used to calculate neutrophil extraction as follows:

\[ \text{Neutrophil extraction} = 1 - \frac{\text{area under standardized neutrophil curve}}{\text{area under standardized red blood cell curve}} \]

**RESULTS**

**Hemodynamics, PaCO2, and PaO2**

Vascular pressures, cardiac output, PaCO2, and PaO2 were within the normal physiological range and did not change throughout the experimental period.

**Group 1**

Recovery of labeled red blood cells from the left ventricle, the aorta, the inferior vena cava, and the right ventricle in one sheep is shown in Fig. 2. Labeled red blood cells are first detected in the left ventricle; the aortic curve, however, is almost superimposed on the left ventricular curve, which is an indication of the small intravascular volume between these two sampling sites. In contrast, the appearance of labeled red blood cells in the inferior vena cava and right ventricle is considerably delayed. An example of a gamma variate function fitted to a left ventricular time-radioactivity curve is shown in Fig. 3. The sum of the counts under this fitted curve was used to calculate the total RBC counts returning to the left ventricle. The percentage of the injected RBC counts that return to the left ventricle is the systemic-to-pulmonary fraction. The mean values (±SD) for the systemic-to-pulmonary fraction for the first and second injections were 90 ± 8 and 86 ± 10%, respectively, and they were not different from each other. No distinct early appearance of RBC counts, which might represent bronchial-to-systemic venous drainage, was detected. If there was a bronchial...
arterial-systemic venous fraction, it was either too small to detect as a distinct peak or it was delayed and obscured by the recirculating counts.

Group 2

The recovery of labeled neutrophils from the left ventricle, aorta, inferior vena cava, and right ventricle in one sheep is shown in Fig. 4. In shape and timing, these curves are very similar to the curves for the red blood cells (Fig. 2). However, when the left ventricular curves for red blood cells and neutrophils are standardized by dividing by the total counts injected (Fig. 5), there is a considerable difference in recovery between red blood cells and neutrophils. The mean values for extraction of neutrophils for the two injections into the bronchial artery were 54 ± 8 and 62 ± 14%, respectively, and they were not different from each other. An example of the standardized left ventricular recovery of labeled red blood cells and neutrophils across the pulmonary vasculature, after right ventricular injection of the cells, is shown in Fig. 6. The mean value for extraction of neutrophils across the pulmonary vasculature was 82 ± 5%. This was considerably greater than the first-pass extraction through the bronchial vasculature (P < 0.05).

DISCUSSION

The first finding of this study is that at least 85% of the systemic blood flow to the lung and intraparenchymal airways returns via bronchial-to-pulmonary anastomoses to the left ventricle. Our results show that a greater proportion of the systemic blood flow to the lung drains to the left ventricle than has been previously reported (20). In contrast to earlier studies, in which pulmonary and systemic bronchial venous return were compared (12), this study was done under undisturbed physiological conditions and with an intact pulmonary circulation. Thus these results are probably a more accurate representation of the in vivo distribution of bronchial venous return.

In the early 1960s, ingenious techniques were devised to make the first measurements of the fraction of total bronchial blood flow returning to the left and right ventricles. Using anesthetized dogs, Martinez de Latorna et al. (20) adapted the aortic pouch technique of Horisberger and Rodbard (12), designed to measure total bronchial blood flow. They collected pulmonary venous blood from the left lung after ligation of the left main pulmonary artery to allow calculation of the systemic-to-pulmonary fraction of bronchial blood flow. To calculate total systemic-to-pulmonary flow, they extrapolated the value obtained from the left lung to both lungs and compared it with the total arterial flow from the aortic pouch measured with a flowmeter. They calculated an average systemic-to-pulmonary blood flow fraction of 67%, which is ~20% less than we measured in sheep. Aramendia et al. (1) also adapted the aortic pouch technique to measure a fraction of blood flow returning to the right ventricle in anesthetized dogs. They cannulated and ligated the azygos vein just above the diaphragm and measured the bronchial venous return from this vessel by using another flowmeter. The average bronchial venous return was 45% of the bronchial arterial flow, which is approximately three times greater than we estimated. The problem with the aortic pouch technique and its variations is that it is rela-
Martin et al. (19) showed that sand times less deformable than red blood cells (29). Microvascular segments and are approximately a thousand times less deformable than red blood cells (29). Martin et al. (19) showed that ~80% of neutrophils are delayed relative to red blood cells during a single passage of the pulmonary circulation in dogs. Doerschuk et al. (8) have shown that mechanical factors play an important role in inflammatory cell sequestration within the pulmonary microvasculature. Neutrophils are significantly bigger than a considerable fraction of pulmonary vascular segments and are approximately a thousand times less deformable than red blood cells (29).

In summary, we found that 88% of the blood flow in the bronchial arterial system returns to the left heart via bronchial-to-pulmonary anastomoses. In addition, extraction of neutrophils in the bronchial microvasculature is ~20–30% less than in the pulmonary microvasculature. This difference may be explained by anatomic differences between the bronchial and pulmonary microvascular beds, differences in the hydrodynamic dispersal forces between these two microvascular beds, or may simply be a reflection of sequestration of neutrophils within the pulmonary microvasculature while they are traversing systemic-to-pulmonary anastomotic pathways.

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Finally, expression of adhesion molecules in the bronchial vasculature could be different from their expression in the pulmonary vasculature. It is known that cytokine-inducible adhesion molecules are present in the microvasculature of humans (25). The extraction of neutrophils in the bronchial microvasculature may have significance for the pathophysiology of airway diseases. Neutrophils and eosinophils are known to play a role in the pathogenesis of asthma, chronic obstructive pulmonary disease, and bronchiectasis (14, 17, 28). Further insight into the mechanisms involved in neutrophil sequestration within the bronchial microvasculature will help in the understanding of the pathogenesis of these diseases.

Very little is known about the transit time and sites of sequestration of neutrophils in the bronchial microvasculature, and there are no published data regarding neutrophil traffic through other systemic organs. Interpretation of our results, which show a significant delay of neutrophils after injection into the bronchial microcirculation, is difficult, given the anatomic arrangement of the bronchial and pulmonary microvasculature beds. Anatomic and physiological studies show that bronchial capillaries anastomose with the pulmonary circulation at the precapillary, capillary, and postcapillary levels (4). However, there is considerable variation among species. Bronchopulmonary anastomoses in sheep are similar to those of humans and are more numerous on the pulmonary arterial side than on the venous side (6, 26). Bronchial vessels could anastomose with the precapillary pulmonary vessels, in which case neutrophils, in their transit through the pulmonary microvasculature, would behave the same way as cells injected directly into the right ventricle. Similarly, cells traversing capillary-to-capillary anastomoses after traversing the bronchial microvascular bed would enter the pulmonary microvasculature and be retained in a fashion similar to those injected directly into the pulmonary circulation. Alternatively, the 50–60% retention of neutrophils after an injection into the bronchial artery could represent a delay of neutrophils within the bronchial microvasculature itself. The bronchial vasculature is perfused at a higher pressure than the pulmonary vasculature, and, therefore, the hydrodynamic dispersal forces (mechanical influence of pressure, flow, and vascular caliber) (13) in the bronchial microvasculature may be greater than those in the pulmonary microvasculature. The anatomic arrangement of bronchial capillary segments is quite different from that of pulmonary capillaries (21), and it may be that, on average, these vessels are wider or more distensible than those of the pulmonary vasculature.


