Perfusion heterogeneity in the pulmonary acinus


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THE ACINUS, the functional unit of pulmonary ventilation, contains ∼10,000 alveoli supplied by a single respiratory bronchiolone (17). Although acinar ventilation has been studied in detail (5, 6, 9, 10), there is less information about acinar perfusion. Several lines of evidence strongly suggest that acinar perfusion is likely to be heterogeneous. First, a dye bolus flowing from the main pulmonary artery to subpleural arteriodes is dispersed in a way that suggests the presence of stream tubes within the arterial tree (2), a characteristic that could cause heterogeneity of blood flow into the acinus. A second potentially important contributor to acinar perfusion heterogeneity is the fact that local regions may have inputs from multiple sources. These may come from supernumerary arteries, the vessels of the pulmonary arterial tree that branch more frequently than the away tree (3). Third, stratification of perfusion has been found in secondary pulmonary lobules, with the lowest flow going to the distal part of the lobules (12, 19). Fourth, there is clear evidence of a distribution of transit times through the capillary network (4, 7, 8, 11). Thus the potential variability of blood flow into the acinus, the likely stratification of perfusion within the acinus, and the distribution of capillary-transit times all suggest that individual pulmonary acini are likely to be perfused heterogeneously. If that is true, heterogeneous perfusion not only would have implications in terms of normal gas exchange but would also have potential clinical importance when acinar volume increases, for example, in panacinar emphysema. In that case, successful matching of ventilation to perfusion, which is normally thought to occur on an interacinar level, would have to occur on an intra-acinar level.

Much of the problem in studying acinar perfusion comes from the difficulty in measuring pulmonary blood flow within so small a volume of tissue. In vivo microscopy alone has the required resolving power. With that technique, it is possible to observe the flow patterns in subpleural venules that directly drain capillary blood after it has traversed the acinus and reached the surface of the lung. Using this approach, we videotaped the passage of fluorescent dye boluses through the tributary branches of Y-shaped subpleural venules. From these observations, some deductions could be made about the uniformity of acinar perfusion. For example, simultaneous passage of the dye through each of the tributary branches would suggest either that acinar flow was homogeneous or, alternatively, that intra-acinar variations in blood flow cancelled each other. Dissimilar dye transits would favor perfusion heterogeneity. Either result would test the hypothesis that pulmonary acini are heterogeneously perfused.

METHODS

Animal preparation. Healthy adult male mongrel dogs (18–27 kg; n = 17) were anesthetized by using pentobarbital sodium (30–40 mg/kg iv), intubated, and mechanically ventilated with room air via a constant-volume respirator (Harvard Apparatus model 607D). After heparinization (1,000 U/kg), the animals were rapidly exsanguinated through a cannula (3 mm ID) placed in the left common carotid artery. The lungs were inflated to a constant airway pressure of 5 mmHg, a left thoracotomy was performed, and the left upper lobe was excised to provide access to the left lower lobe. The left lower lobar artery was cannulated with a Teflon fluorinated ethylene polypropylene cannula (6 mm ID), and the left lower lobe bronchus was clamped to maintain constant inflation. The left lower lobe was then excised, along with a cuff of left atrium, and it was placed on a microscope stand. The left atrial cuff was secured around another Teflon fluorinated ethylene polypropylene cannula (10 mm ID), and the lobe was perfused with autologous, heparinized whole blood (hematocrit = 30–41%). The time interval from complete exsanguination to reperfusion of the lobe was <30 min. Blood was pumped (Masterflex 7522–10 pump drive and 7024–20 pump head) through a windkessel to dampen pump vibrations and trap bubbles, a filter (20-µm pore size; Fenwal 4C7700) to remove macroaggregates, a heat exchanger (Bentley HE-30) to warm the blood to 37–38°C, and finally a dye injection loop before entering the lobe (Fig. 1). Venous blood drained passively from the lobe into a reservoir. The height of
the tubing between the vein and the reservoir could be altered to change venous pressure. The lobe was ventilated with 6% CO₂-17% O₂-77% N₂ at a tidal volume of 100 ml. End-expiratory pressure was set to 5 mmHg by using a water overflow on the expiratory limb of the ventilator. Arterial and venous pressures were measured continuously with two transducers (model P23 XL; Statham) zeroed at the site of microcirculatory observation and connected to polyethylene tubing (PE-200), the tips of which were located at the ends of the arterial and venous cannulas. Airway pressure was measured intermittently (P23 XL transducer; Statham). All measurements were made under zone 2 conditions: pulmonary arterial pressure, 10–15 mmHg; airway pressure, 5 mmHg; and pulmonary venous pressure, 1 mmHg. Pump flow rate was 400 ml/min.

Data collection. The lobe was suspended by two small spring-backed paper clips attached to opposite edges of the lobe (18) and raised until the uppermost pleural surface (the diaphragmatic surface in this orientation) came into contact with a transparent window. A 1.3 cm² area on the surface of the lobe was observed through the window, which was surrounded by a vacuum ring to prevent lateral movement (13, 15). The subpleural microcirculation under the window was observed with a modified Olympus BH2 reflectance microscope coupled to a Leitz Ultropak illuminator and a ×11 objective. Bright-field illumination through the Ultropak illuminator was provided by a 200-W mercury arc lamp mounted on an optical bench. This light source was heavily filtered with a combination of dichroic infrared-reflecting filters, broad band-pass ultraviolet-absorbing filters to prevent tissue damage, and a narrow band-pass interference filter to illuminate the field only with the mercury green line (546 nm). This wavelength was absorbed by hemoglobin, thereby increasing the contrast between the erythrocytes and surrounding tissue (14). Illumination for fluorescence microscopy was provided by a 100-W mercury arc mounted on a sidearm of the Olympus microscope. This light was also filtered by dichroic infrared-reflecting filters and ultraviolet-absorbing filters. The light from this arc passed through a blue band-pass exciter filter (410–480 nm) and a high-pass dichroic mirror (cutoff wavelength = 480 nm) that reflected the exciting light down through the objective onto the subpleural microcirculation beneath the window. Emitted light from the lung passed back through the objective, the dichroic mirror, and a yellow high-pass barrier filter (cutoff wavelength = 510 nm). Video recordings of the subpleural microcirculation were made with a Panasonic AU650 MI1 videorecorder and a Cohu (model 5510) intensified charge-coupled device camera that was attached to the microscope with a Nikon zoom adapter (model CCTV 79444).

In 11 lobes, microscopic fields were selected in which there was a venule that had two tributary branches forming a Y shape (2–14 Y-shaped venules per lobe; 82 total). While these fields were observed with fluorescence microscopy, test injections of dye [fluorescein isothiocyanate conjugated to 70-kDa dextran (Sigma Chemical), 10 mg/ml of 0.9% saline] were made by using a loop just proximal to the lobar artery. Each limb of the loop contained a volume of 25 ml and was controlled by a solenoid pinch valve (Cole Parmer NO/C-1367–92/93) on its downstream end. On one limb, the valve was open when deenergized, whereas on the other limb, the valve was closed. The normally closed limb was loaded with a 1-ml bolus of dye, and, when the solenoids were energized, blood flow was diverted through the dye-containing limb, thereby washing the dye into the lobar artery. In this way, the bolus of dye was rapidly introduced into the arterial circulation without the pressure increase or movement of the microscopic field that occurred when high-pressure injections were made directly into the lobar pulmonary artery.

The passage of dye through the field was videotaped, and elapsed time in milliseconds was recorded on the videotape images by a Panasonic WJ-810 time-date generator that was activated by the same switch that energized the solenoids of the injection loop. The black level of the camera and the gain...
of the camera and intensifier were adjusted according to these test injections of dye to maximize the contrast between the baseline brightness of the microscopic field before dye entered the circulation and the peak brightness during passage of dye through the microcirculation.

After the test injections, we recorded the passage of two separate dye injections per venule during end expiration. Dye-dilution curves were obtained by replaying the recordings and sampling image brightness at 30 Hz from rectangular areas over the venular lumens (referred to as vessel windows) and from areas over the adjacent alveoli (background windows) with a frame-grabber board (Data Translation DT-2851) interfaced with a microcomputer (Dell 486, 50 MHz). The windows were moveable and of adjustable size. To obtain a dye-dilution curve for each of the two tributaries of the Y-shaped venules, the recordings of each injection were replayed twice, sampling over a different branch each time.

The three-dimensional structure of the lung caused detectors placed over the venules to measure light emitted not only from dye within the venular lumens but also from dye in the surrounding alveolar capillaries. To obtain curves that accurately reflected the concentration of dye in the venular lumens at each instant in time, it was necessary to subtract light emitted by dye in the capillaries (background window signal) from the venous window signal, using the method of Presson et al. (8). In each animal, the background-corrected dye-dilution curves from the duplicate injections were aligned at the injection time and then averaged to produce a single curve for each branch. The baseline segment of each of these average curves, before dye entered the vessel, was set to zero, and the tail of each curve (--5% of the area under the curve) was extrapolated to baseline as a monoexponential function. Finally, the area under each curve was set to unity.

The mean appearance time (MT) from the time of injection was calculated as

$$MT = \frac{1}{n-1} \sum_{i=1}^{n} \frac{t_i + t_{i+1}}{2} \left( (t_{i+1} - t_i) + \frac{1}{2} (t_i \right)$$

where $t_1$ was the time when the intensity of the dye-dilution curve (I) became $>$0, and $t_0$ was the time when the curve returned to baseline. The percentage difference in MT between the fast branch and the slow branch ($\Delta MT$) of a venular pair was calculated as

$$\Delta MT(\%) = \frac{MT_{slow} - MT_{fast}}{MT_{slow} + MT_{fast}} \times 100$$

where $MT_{slow}$ was the MT of the slow tributary branch, and $MT_{fast}$ was the MT of the fast tributary branch. We divided by the average of $MT_{slow}$ and $MT_{fast}$, as if flow were homogeneous. From Eq. 2, when $MT_{slow} = MT_{fast}$ (homogeneous perfusion), $\Delta MT = 0$%.

Arteriolar heterogeneity. To determine how heterogeneous the dye bolus was when it arrived in the subpleural microcirculation, we recorded the passage of dye through all arterioles (diameter = 50 µm) under the window (4–12 arterioles in each of 9 lobes; total, 53 arterioles). That information permitted the estimation of the separate effect that arteriolar input had on acinar perfusion homogeneity. Because the daughter branches of an arteriole were fed by a single parent, they had the same MT distribution as the parent. For this reason, we calculated the difference in MT between pairs of different arterioles to get an idea of the general variation of input to the subpleural microcirculation being studied. We computed in each lobe the difference for all possible pairs of arterioles under the window (total, 157 pairs for all lobes). The data collection and methods of analysis were the same as those used for the venules.

Statistics. We used the paired t-test to compare the physiological variables at the beginning of the venular measurements with those at the end of the venular measurements and the variables at the beginning of the arteriolar measurements with those at the end of the arteriolar measurements. We then compared the average physiological variables during the venular measurements to the average variables during the arteriolar measurements by using the t-test for independent samples. The $\chi^2$ test was used to compare the distribution of arteriolar appearance-time differences to the distribution of venular appearance-time differences. The mean and variance of the venular appearance-time differences were compared with the corresponding mean and variance of the arteriolar appearance-time differences with a t-test for independent samples. We accepted $P < 0.05$ as significant.

RESULTS

Of the 82 Y-shaped venules, the 164 tributaries were binned into either the fast or slow tributary, which were not different in diameter [50.0 ± 1.6 (SE) and 50.2 ± 1.8 µm, respectively (P > 0.94)]. Their recipient vessel had an average diameter of 72.6 ± 24.8 µm. The MT difference between tributary venular branches (Eq. 2) was divided into five groups: 0–5, 5–10, 10–15, 15–20, and >20% (Fig. 2). This distribution was right skewed, with a mean difference of 6.5 ± 0.6% (SE) and a median difference of 5.8%. In 42.7% of the sample, there was a minimal MT difference (<5%) between the tributary branches. When the electronically measured MT difference was small, it was not possible to observe with the unaided eye a difference between tributary branches during videotape replay (Fig. 3, left). In 41.5% of the cases, the MT difference was between 5 and 10%. When the difference between branches was >10% (only 15.8% of the cases; Fig. 2), it was easy to detect the difference. In the more extreme cases, in which the MT difference was >15% (7.3% of cases), the dye had nearly drained from the faster branch before the dye arrived in the slower branch (Fig. 3, right).
The distribution of venular appearance-time differences was different from the arteriolar appearance-time difference distribution under the same conditions \((P < 0.01; \text{Fig. 4})\). The average appearance-time difference for the venules \([0.67 \pm 0.06 \text{ (SE) s}]\) was significantly greater than the average appearance-time difference for the 53 arterioles studied \((0.38 \pm 0.02 \text{ s}; P < 0.01)\), indicating dispersion of the dye by the capillary bed; i.e., the capillary bed added to appearance-time differences already present among the arteriolar inputs to the capillary bed. The variance of the venular appearance-time differences \((0.27 \text{ s}^2)\) was also greater.
The MT differences between venular tributary branches were small in almost all cases. In 42.7% of the observed venular pairs, the difference (Eq. 2) was <5% (Fig. 2). We classified perfusion of venular pairs with differences <5% as homogeneous because it was not possible to visually detect a difference between flow through tributary branches during videotape replay, and the dye curves were essentially superimposable (Fig. 3, left). In another 41.5% of cases (Fig. 2), there was a small but visually unimpressive difference in dye passage between tributary branches, reflected by a 5–10% difference in MT. Only when the difference between branches was >10% was it easy to detect the difference visually. This group was classified as clearly heterogeneous, but it was a minority of cases (<15.8%). Only 1.2% of venular tributaries had MT differences of >20%.

The finding of small appearance-time differences between venular tributary branches indicating homogeneous acinar perfusion was unexpected because several lines of evidence have suggested that acinar perfusion would be heterogeneous. Dawson et al. (2) showed that blood arriving in the acinus via different arterioles could have different appearance times. In addition to this variation in input to the acinus, stratification of perfusion has been shown to occur in the secondary pulmonary lobule (12, 19). Additionally, there are anatomical grounds to expect a distribution of transit times through the capillary network, because there are a range of capillary path lengths (4, 11), an expectation confirmed by videomicroscopy (1, 7, 8, 16). On the basis of these findings, we hypothesized that there would be significant heterogeneity of perfusion within a single acinus.

Our interpretation of the results assumes that, in each case, the tributary branches of a Y-shaped venule drained the same acinus. To test this assumption, we compared the MT differences of the largest venules with those of the smallest venules, reasoning that the largest venules would be most likely to drain more than one acinus. The MT difference of the largest branches (65–90 µm, 5.6 ± 1.0% [SE], n = 15) was not different (P = 0.36, unpaired t-test) from that of the smallest branches (20–35 µm, 7.2 ± 1.5%, n = 14). This result strongly implies that all the branches studied drained single acini.

We also assumed that the results obtained from the isolated lobe are similar to results in the intact dog. In pilot studies of intact dogs, we found it more difficult to maintain a stable catheter position at the site of injection (main pulmonary artery) and a constant cardiac output between injections. However, there was a similar distribution of venular appearance-time differences between tributary branches. The MT difference was 0–5% in ~40% of venular branch pairs and was 5–10% in ~35% of pairs (n = 20 pairs), results similar to data reported here for the isolated lobes. Therefore, we think our results are reasonably representative of the intact animal. Our results are also consistent with previous values obtained from the isolated canine lobe preparation. In the present study, the MT in arterioles was 6.8 s, and the MT from injection to venules was 10.6 s, a difference of 3.8 s, which is the mean capillary-transit time. This value is close to the 3.5 s reported earlier at the same flow rate in the isolated dog lobe (8).

In conclusion, despite several lines of evidence suggesting that acinar perfusion would be heterogeneous, our findings based on venular drainage patterns demon-

![Graph](http://jap.physiology.org/)
strate that acinar perfusion is homogeneous. Acinar perfusion is homogeneous in the majority of cases and therefore matches acinar ventilation, which is also thought to be homogeneous (5, 6, 9, 10).

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