Blood flow switching among pulmonary capillaries is decreased during high hematocrit

William A. Baumgartner, Jr.,1 Amanda J. Peterson,1 Robert G. Presson, Jr.,1 Nobuhiro Tanabe,4 Eric M. Jaryszak,2 and Wiltz W. Wagner, Jr.1,2,3

Departments of 1Anesthesia, 2Cellular and Integrative Physiology, and 3Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202; and 4Department of Chest Medicine, Chiba University School of Medicine, Chiba 280, Japan

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We suspected that part of the switching process involved the continuously changing hematocrit of the blood that perfuses the pulmonary capillaries. In vivo microscopic observation of individual pulmonary capillary segments shows that capillary hematocrit varies constantly from zero, when no red blood cells are present, to high values when a train of closely spaced red blood cells passes through the segment. There are a number of elegant studies of red cell flow through the systemic microcirculation as well as through capillary tubes (5, 7, 13, 14, 19, 24–27). These studies have established that red blood cells at bifurcations tend to enter the branch with the fastest flow. Additionally, velocity differences between red blood cells decrease as the hematocrit increases. However, the pulmonary capillaries have features that differ from systemic capillaries. For example, systemic capillaries tend to be long and to branch infrequently, whereas pulmonary capillaries form a very tight mesh, in which the length of each segment hardly exceeds its width. Pulmonary capillaries, which are surrounded on two sides by alveolar airspace, are also subject to rapid opening and closing in high zone 2, where pulmonary capillary pressure just exceeds alveolar pressure. For this reason, we hypothesized that switching occurs, at least in part, because the continually changing sizes of plasma gaps between red blood cells would influence the probability of segment closure and cause individual cells to switch segments at a given junction. Thus we define switching to mean that flow suddenly stops in one branch and switches completely to another branch, a binary operation. Our hypothesis is based on the rationale that closely spaced cells would be more likely to follow the leading cell at a junction, whereas a distantly following cell would increase the chance of segment closure and switching of red blood cell flow. If this assumption is correct, then we predict that an increase in hematocrit would decrease the probability of red blood cells switching segments at each capillary junction because the average distance between red blood cells would decrease. We tested this idea by using videomicroscopy to record the perfusion patterns of alveolar capillary networks in isolated canine lung lobes that were perfused first at normal hematocrit and then in the same capillary networks at increased hematocrit to decrease the distance between the red blood cells.

METHODS

Experimental preparation. The Animal Care Committee at the Indiana School of Medicine approved these animal studies. Healthy adult male mongrel dogs (20.5 kg, SD = 2.0; n = 6) were anesthetized by

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intravenous injection of pentobarbital sodium dissolved in 0.9% saline (30 mg/kg). The animals were intubated and ventilated with room air by a constant-volume respirator (model 607D, Harvard Apparatus). After administration of heparin (1,000 U/kg), the animals were rapidly exsanguinated through a cannula (3 mm ID) placed in the left common carotid artery. During exsanguination, 120 ml of 10% dextran (40 kDa) in saline were infused (6). With the lungs inflated to a constant pressure of 5 mmHg, a 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 was placed on a microscope stand. The surface of the lobe was kept moist with saline throughout the procedure. 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. Care was taken to exclude all air bubbles from the circuit before perfusion was initiated. The time interval from complete exsanguination to reperfusion of the lobe was ~30 min. Blood was pumped (model 7522-10 pump drive and 7024-20 pump head, Masterflex) through a filter (pore size 20 μm; model 4C2423, Fenwal) to remove microaggregates, a heat exchanger (modified Avecor Cardiovascular Myotherm) to warm the blood to 37–38°C, and a windkessel to dampen pump vibrations and trap bubbles before entering the lobe (Fig. 1). Venous blood drained passively from the lobe into a reservoir. The height of the reservoir could be altered to change venous pressure. The lobe was ventilated with 6% CO₂-17% O₂-77% N₂. Expiratory pressure was set at 5 mmHg by a water overflow on the expiratory limb of the ventilator. Blood gases were sampled regularly from the pulmonary venous line and were analyzed (IRMA SL, Diametrics). Sodium bicarbonate solution (1 meq/ml) was periodically added to the venous reservoir to neutralize metabolic acid. Arterial and venous pressures were measured continuously with two transducers (model P23 XL, Statham) zeroed at the site of observation and connected to PE-200 catheters. The catheter tips were located at the distal ends of the arterial and venous cannulas.

The lobe was suspended by two small spring-backed clips attached to opposite edges of the lobe and was 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 of the observed area (29, 33). Suspending the lobe against the window in this manner prevented compression of the subpleural alveoli by the window and allowed free downward expansion of the lobe during ventilation. The remainder of the lobar surface was covered with a thin plastic sheet to prevent drying and to slow the transpleural diffusion of gas.

Data acquisition. The subpleural alveoli under the window were illuminated by a 200-W mercury arc lamp, heavily filtered to prevent tissue damage with a combination of dichroic infrared reflecting filters, broad band-pass ultraviolet-absorbing filters, and a narrow band-pass interference filter to illuminate the field with only the mercury green line (546 nm). This wavelength is absorbed by hemoglobin, thereby increasing the contrast between the red blood cells and the surrounding tissue (30). Video recordings of the subpleural microcirculatory perfusion patterns were made with a surface-illuminating microscope (Leitz Ultropak) connected to a charge-coupled device television camera (model TM-840, Videoscope) and a video recorder (model SVO-5800, Sony). On average, the final magnification of the video microscope system was X560.

In each preparation, a microscopic field containing three to five neighboring subpleural alveoli was selected for observation in which the capillary segments were clearly visible. The pump flow rate was set to perfuse about one-half of the capillaries, a control hematocrit value was measured, ventilation was adjusted to 3–4 breaths/min, and the field of alveoli was video recorded for 8 min. The hematocrit was then raised by using a hemoconcentrator (Hemocor HPH 400, Minntech). To maintain a constant number of perfused and unperfused segments, pump flow was again adjusted to perfuse about one-half of the capillaries, and the field was recorded for another 8 min. The time elapsed between video recordings was ~40 min. Blood-gas and perfusion pressure measurements were made every 5 min. After completion of the recordings, perfusion pressure was increased until all of the capillaries appeared to be perfused, and the field was video recorded for 1 min. Finally, a video recording of a calibration slide was made.

![Fig. 1. Schematic of perfusion circuit. Pa, alveolar pressure; Ppa, pulmonary arterial pressure; Ppv, pulmonary venous pressure; CCD, charge-coupled device.](image-url)
Data analysis. The video recordings were replayed, and all capillary segments were traced onto a sheet of clear transparency film placed over a video monitor. A capillary segment was defined as any vessel that passed red blood cells single file between junctions with other vessels or between a junction and the alveolar boundary. Each capillary segment was then numbered. The 8-min video recordings were broken into 120 4-s observation periods. For every 4-s period, the state of perfusion (on or off) was determined for each capillary segment. A capillary segment was considered to be perfused (on) if one or more red blood cells passed through the segment during that period. A switch was recorded whenever the state of perfusion of a segment changed from one 4-s observation period to the next. The total number of switches for each alveolus was counted for each condition (normal and high hematocrit).

We also computed the capillary perfusion index (31–33) for each 4-s observation period as follows. We measured the total length of the perfused capillaries from the transparency film with a digitizing pad (Truegrid 1017, Houston Instruments), planimetry software (SigmaScan, Jandel Scientific), and an IBM-compatible computer. The areas of the observed alveolar walls were also measured by using the same system. Because the subpleural alveolar facets in the upper lung can be approximated by flat disks with an average diameter of 110 μm and an area of 10,000 μm² (32, 33), the alveolar wall area was divided by 10,000 μm² to obtain the number of average walls in the observed alveolar facet. This normalization permitted us to compare results between individual alveoli and between animals. Defined mathematically, the capillary perfusion index (1) is

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\text{Capillary perfusion index (μm) = } \frac{\sum \text{perfused capillary lengths (μm)}}{\text{alveolar wall area (μm²)/10}^{8} \text{ (μm²)}}
\]

The level of capillary recruitment can be easily estimated from the capillary perfusion index. For example, a capillary perfusion index of 32 μm can be visualized as a capillary pathlength that would cross about one-third of the 110-μm diameter of an average alveolar facet (Fig. 2, left), whereas a capillary perfusion index of 307 μm would mean that an average alveolar wall could be crossed almost three times at its diameter (Fig. 2, right). For each 8-min recording, we calculated the average capillary perfusion index as the mean of the 120 4-s observation periods.

Statistics. Pulmonary arterial pressure, pump flow rate, the number of switches, the capillary perfusion index, and arterial blood-gas measurements were tested for differences among the two hematocrit levels with the two-tailed paired t-test. The two levels of hematocrit were compared by using the one-tailed, paired t-test. P < 0.05 was considered significant. All observations are reported as means ± SE.

RESULTS

After baseline measurements, the hematocrit was increased from 35.8 ± 1.6 to 54.8 ± 1.7% (P < 0.01; Table 1). The average number of switches per individual alveolus over the course of the 8-min observation period decreased from 245 ± 30 during normal hematocrit to 160 ± 24 during high hematocrit (P < 0.01; Fig. 3), a 37.9 ± 6% decrease. As expected, pulmonary arterial pressure increased when hematocrit was raised due to increased blood viscosity (Table 1). Because capillary recruitment is a function of pressure and because our goal was to match the level of recruitment under the two conditions, pump flow rate was lowered during high hematocrit (Table 1). In this way, we successfully maintained a constant capillary perfusion index: 177 ± 24 μm during normal hematocrit and 172 ± 12 μm during high hematocrit (Fig. 4; P = 0.86) Blood gases measured before and after the two 8-min observation periods remained in the normal range (Table 1).

DISCUSSION

We compared the frequency with which red blood cells switched among segments as they crossed alveolar-capillary networks when hematocrit was normal and when it was elevated. The frequency of switching significantly decreased with an increase in hematocrit. This observation supports our hypothesis that switching occurs, at least in part, because the continually changing sizes of plasma gaps between red blood cells influences the probability of segment closure, which would cause individual cells to switch segments at a given junction.

Fig. 2. Capillary perfusion index (CPI). Left: 6 subpleural alveoli perfused at low pressure are minimally recruited. Right: same 6 alveoli perfused at high pressure are now highly recruited.

Fig. 3. Comparison of switch count between normal and increased hematocrit. Each line is 1 alveolus. In 22 of 26 alveoli, switching decreased when perfused with increased hematocrit. ▪. Average number of switches for all alveoli ± SE.

<table>
<thead>
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<th>Variable</th>
<th>Normal Hematocrit</th>
<th>Increased Hematocrit</th>
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<td>Hematocrit, %</td>
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<td>54.8±1.7*</td>
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<td>Pump flow rate, ml·min⁻¹·kg⁻¹</td>
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<td>Ppa, mmHg</td>
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<tr>
<td>pH</td>
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<td>7.39±0.01*</td>
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Values are means ± SE for 6 dogs. Ppa, pulmonary arterial pressure; Ppv, pulmonary venous pressure; PaO₂, arterial P O₂; PaCO₂, arterial P CO₂. *Significantly different from normal hematocrit, P < 0.05 (by paired, 2-tailed t-test).
In testing this hypothesis, we made several assumptions. First, we assumed that the alveolar networks were perfused equally during normal and increased hematocrit. As we were measuring the frequency of red blood cell switching among capillary segments, we concentrated on having the same level of capillary recruitment for both conditions rather than maintaining equivalent lobar perfusion pressures or flow rates. We matched capillary recruitment levels visually during the experiment by altering the pump flow rate (Table 1). Within each dog, the capillary perfusion index varied over time, but on average, the difference was small and not significant (Fig. 4). These data show that the transmural pressures within the capillary network in each alveolus were similar, because the level of capillary recruitment is controlled by the capillary transmural pressure (9).

One design consideration in these experiments was selecting the observation period needed to accurately assess the switching frequency. Previously, our laboratory had observed alveolar-capillary networks for a period of 1 min (2, 4, 9, 10, 17, 18, 21–23, 32). If a single red blood cell moved through a capillary segment during that period, we considered it to be perfused. Although practical, this time period underestimated the switching frequency, because numerous switches do occur in <1 min (33). As we reduced the time of observation to very short periods, however, we found that another error occurred in the opposite direction. To illustrate this error, consider three capillary segments connected in series. If a red blood cell traveling through the feeding segment was separated by a large enough plasma gap from a second red blood cell traveling through the draining segment, the middle segment would be considered unperfused because no red blood cell was perfusing it. When the first red blood cell entered the middle segment, that segment would be considered perfused. If both red blood cells separated by the plasma gap could be observed to be moving along a single path at the same velocity, the observer would readily deduce that the middle segment was perfused, albeit with plasma alone. Unfortunately, it was not always possible to make this kind of deduction accurately. Through trial and error in an earlier study (33), our laboratory determined that, if a 4-s observation period was used, the switching could be reasonably assessed while avoiding overestimation of switching by counting plasma gaps as unperfused segments.

Another assumption we made is that increased hematocrit led to a decrease in the distance between red blood cells in the capillaries. Elegant measurements of capillary hematocrit have been made in systemic capillaries and small tubes by using transillumination (5, 7, 13, 14, 19, 24–27). Because the lung is too thick for transillumination, we were forced to use epi-illumination. Transillumination benefits from the differences in absorbance of light by different cells, particularly the hemoglobin in red blood cells. In contrast, the reflectivity of the red blood cells with epi-illumination is very similar to the surrounding tissue, which means that the red blood cells do not stand out with much contrast (although we used the mercury green line at 546 nm to enhance the contrast as much as possible). Under epi-illumination, red blood cell presence is detected more by movement than by any other feature. With epi-illumination, we could not resolve vessel walls accurately, observe platelets (which might tell us whether a capillary that was not perfused by red blood cells was perfused by plasma), use dual-slit photometry to determine velocities, or apply other techniques that require the high resolution and contrast of transillumination. Our surface-illuminating microscope system did not provide sufficient resolution to measure the hematocrit within the capillaries directly because the exposure time of each television frame was too long to arrest the motion of the moving red blood cells. However, we were able to count the number of large plasma gaps (length >3 red blood cells) between cells passing through individual capillary segments (n = 13) over a period of 5 min. The number of large plasma gaps when perfused by normal hematocrit was significantly greater than when the hematocrit was increased (5.6 vs. 1.8 gaps/min, respectively; P = 0.02), providing an estimate of the expected decrease in distance between red blood cells when hematocrit was elevated. This measurement provides reasonable assurance that the average distance between red blood cells was decreased when hematocrit was raised.

The final assumption is that the flow characteristics on the surface of the lung approximate the characteristics in the interior of the lung. Short et al. (28) demonstrated that despite the anatomic differences in diameter and density between subpleural and interior capillaries, subpleural capillary recruitment accurately reflected the recruitment of the interior capillaries. Furthermore, Capen et al. (3) demonstrated that capillary transit times on the surface of the lung were similar to whole lung capillary transit times. These observations suggest that subpleural and interior capillary networks behave similarly.

Switching of perfusion among pulmonary capillaries has been observed by a number of investigators (1, 17, 32–34), but the mechanism remains unknown. Our laboratory’s strategy over a series of studies has been either to correlate switching with hemodynamic variables (17) or to hold an increasing number of variables constant (33). When flow rate, perfusion pressure, and ventilation were held constant in isolated, pump-perfused lobes, switching continued unabated (33). This led us to suspect that plugging of capillary segments by leukocytes could be a significant contributor (8, 11, 12, 15, 16, 20).
However, in the pump-perfused lobes, the white cell count dropped dramatically due to leukocyte margination on the perfusion tubing (normal vs. pump perfused), but switching continued. We wondered whether indeed the switching was a random event, but recent findings showed that it was not random. Furthermore, the switching was independent among capillary networks in neighboring alveolar walls, even when perfusion pressure, flow, and ventilation were held constant, and the alveoli were fed by the same arteriole and drained by the same venule (33). These data suggest that the mechanism of switching lies within each alveolar capillary network. The specific mechanism remains unknown.

In summary, these data are consistent with the idea that increased hematocrit decreases the probability that red blood cells will switch between segments in the pulmonary capillary bed. This observation combined with previous work (33) show that switching is a characteristic of the capillary networks within alveolar walls. This does not solve the issue of whether the switching is caused entirely by an active mechanism or is a passive, inherent characteristic of particulate fluid traversing a complex network. However, the present study supports the idea that a passive mechanism plays a role. Nevertheless, it is an incomplete test of the hypothesis, for a hematocrit of 60% does not entirely eliminate the space between red blood cells. Nor does this study completely rule against the possibility of an active component. The results do demonstrate that highly flexible red blood cells, encountering dozens of junctions as they traverse from arterioles to venules, are less likely to switch between capillary segments if the distance between cells is decreased. Such a mechanism is a passive, inherent characteristic of the system requiring no active control.

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GRANTS

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