Splenectomy impairs diffusive oxygen transport in the lung of dogs

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Abstract

The spleen acts as an erythrocyte reservoir in highly aerobic species such as the dog and horse. Sympathetic-mediated splenic contraction during exercise reversibly enhances convective $O_2$ transport by increasing hematocrit, blood volume and $O_2$ carrying capacity. Based on theoretical interactions between erythrocytes and capillary membrane (Hsia et al., J. Appl. Physiol., 86:1460-7,1999) and experimental findings in horses of a post-splenectomy reduction in peripheral $O_2$ diffusing capacity (Wagner et al., Equine Vet. J., 18 suppl: 82-89,1995), we hypothesized that splenic contraction also augments diffusive $O_2$ transport in the lung. Therefore, we have measured lung diffusing capacity ($DL_{CO}$) and its components during exercise by a rebreathing technique in 6 adult foxhounds before and after splenectomy. Splenectomy eliminated exercise-induced polycythemia, associated with a 30% reduction in maximal $O_2$ uptake. At any given pulmonary blood flow, $DL_{CO}$ was significantly lower after splenectomy due to a lower membrane diffusing capacity while pulmonary capillary blood volume changed variably; microvascular recruitment, indicated by the slope of the increase in $DL_{CO}$ with respect to pulmonary blood flow, was also reduced. We conclude that splenic contraction enhances both convective and diffusive $O_2$ transport and provides another compensatory mechanism for maintaining alveolar $O_2$ transport in the presence of restrictive lung disease or ambient hypoxia.

Key words: blood volume, red cell volume, hematocrit, lung diffusing capacity, membrane diffusing capacity, pulmonary capillary blood volume, cardiac output, maximal oxygen uptake, exercise, spleen, canine.
Introduction

The spleen is a major erythropoietic organ in invertebrate animals but has become primarily a lymphoid organ in adult reptiles, birds and mammals. In some marine diving animals or in highly aerobic land mammals (trained dogs, thoroughbred horses, etc.) that depend on a high $O_2$ delivery for survival, the spleen also functions as a blood reservoir as described by J. Barcroft (2, 3). In response to sympathetic stimulation invoked by exercise, hypoxia or hemorrhage, the spleen rapidly contracts to release extra red cells (at a hematocrit of 80-90%) into the circulation (4) and then relaxes when the stimulus recedes (28, 39). Splenic contraction increases circulating hematocrit, blood volume and $O_2$ carrying capacity during exercise 1.3 to 1.5-fold above resting levels; these indices quickly return to baseline when exercise stops (40, 42). This form of reversible autologous blood doping is an efficient way of augmenting $O_2$ delivery during periods of metabolic stress. In athletic animals, systemic hematocrit can rise from about 40% at rest to nearly 60% from rest to exercise (41, 42), and their maximal $O_2$ uptake (above 140 ml/min/kg) is almost twice that in the best endurance Olympic athletes for whom blood doping is prohibited. The rise in blood volume and hematocrit constitutes one mechanism that allows these animals to achieve supra-high exercise capacities without the complications caused by hyperviscosity since baseline hematocrit at rest remains normal. Although classically believed to enhance $O_2$ delivery, autologous red cell infusion has also been reported to enhance peripheral tissue diffusing capacity in exercising thoroughbred horses (40). The effect of red cell infusion on $O_2$ transport in the lung has not been described.
Our laboratory has investigated the compensatory mechanisms in response to gas exchange impairment caused by a) pneumonectomy and b) high altitude exposure. In both models we observed superior adaptation in the dog compared to the human subject. We speculated that auto-infusion of blood by splenic contraction contributes to a significant portion of the adaptive advantage in the dog; this mechanism could also reduce the disability imposed by existing pulmonary impairment such as the loss of a lung due to pneumonectomy. In order to understand better how dynamic autologous red cell infusion by splenic contraction regulates O$_2$ transport in athletic species, we studied trained adult foxhounds before and after splenectomy to address the following questions: 1) How much augmentation of blood volume and hematocrit occurs by splenic contraction during exercise in the foxhound? 2) How does exercise-induced splenic contraction affect pulmonary gas exchange? Our results show that splenic contraction improves not only convective O$_2$ delivery but also lung diffusing capacity and its recruitment during exercise. Splenic contraction also mitigates the reduction in lung diffusing capacity imposed by pneumonectomy.

**Methods**

**Animals.** The Institutional Animal Care and Use Committee approved all protocols and procedures. We studied 6 purpose-bred adult male foxhounds before and 7-10 months following splenectomy. Two to three years prior to the present study and as part of a separate project, two of these animals (19 and 26 kg) had undergone right pneumonectomy and four (22 to 24 kg) had undergone right thoracotomy without lung
resection (SHAM). In addition, all animals had bilateral carotid artery loops constructed under general anesthesia using an established method (32) in order to permit acute catheterization without the complications of chronic indwelling vascular catheters. The animals had been trained to run on a treadmill (see below); exercise studies were performed before and after pneumonectomy. In order to collect autologous blood, the jugular vein was catheterized under local anesthesia and one unit (250 ml) of blood was removed, separated into erythrocyte and plasma fractions, and stored at -70°C for later re-infusion. After phlebotomy, blood volume was measured by a carbon monoxide (CO) rebreathing method (below). These procedures were repeated approximately once a month for about 6 months. Iron supplementation was provided during this interval.

**Surgery.** Following the completion of blood collections, splenectomy was performed. The animal was fasted overnight and sedated (acepromazine 0.2 mg/kg intramuscularly). Anesthesia was induced with intravenous thiopental (up to 10 mg/kg) or propofol (4 to 8 mg/kg) and maintained with isoflurane inhalation. The animal was intubated and ventilated. The abdomen was shaved and prepared with betadine and alcohol. Through a midline abdominal incision, the spleen was isolated and the splenic vessels ligated close to the hilum with silk ties. The vessels were severed between ligatures and the spleen removed. The abdomen was irrigated with sterile saline and closed with silk suture. Approximately 50-100 ml of normal saline was administrated intravenously during the operation. After surgery the animal was monitored in a recovery room until awake. Buprenorphine was administered twice daily for 2 days and as needed thereafter. Penicillin was administered daily for 7 days. The wound was dressed daily and the sutures removed in 10-14 days.
Exercise training. The physical training program has been described previously (22). Prior to undergoing splenectomy, each dog had been trained to run voluntarily on a motor-driven treadmill for 30 min a day, 3 to 5 days a week at a workload equivalent to 60-80% of predicted or measured maximal O$_2$ uptake. Dogs were considered "trained" when reproducible values of O$_2$ uptake were obtained at each speed and incline, and when a maximum speed and incline was established beyond which exercise cannot be sustained for 5 min. It took approximately 3 months to fully train a dog. Training continued throughout the course of the study and resumed two weeks after splenectomy. The present exercise studies were carried out before and 4-9 mo following splenectomy.

Breathing circuit. Each animal wore a customized, leak-free respiratory mask, which permits open mouth breathing and protrusion of the tongue to facilitate cooling and salivary drainage (1). The mask was sealed around the muzzle with a modified latex glove and duct tape. A built-in cylindrical breathing orifice connects to a set of pneumatic respiratory valves and a rebreathing bag. Inspired and expired ventilation was measured by separate heated screen pneumotachographs (model 3813, Hans Rudolph, Kansas City, MO). Expired gas concentrations were sampled continuously by a mass spectrometer distal to a mixing chamber. Minute ventilation, O$_2$ uptake, CO$_2$ production and respiratory rate were followed breath-by-breath and averaged a pre-determined number of breaths. Heart rate and rectal temperature were continuously recorded. All signals were digitized at 50 Hz.
Blood volume measurement. Before splenectomy, we measured blood volume in the conscious animal using a carbon monoxide (CO) rebreathing method, which is inexpensive and suitable for monitoring blood volumes during repeated phlebotomy to collect autologous blood. However, this method is inconvenient for use during exercise; CO also alters the oxyhemoglobin dissociation curve and precludes simultaneous blood gas measurements. After splenectomy we employed the Evans Blue dye dilution method, which is less restrictive during exercise and has less effect on the oxyhemoglobin dissociation curve but is too expensive for repeated use. Direct comparisons were made to ensure that the two techniques yielded comparable results (Table 1).

The CO rebreathing method. While wearing the respiratory mask, the animal rebreathed through a carbon dioxide absorber from a 5-liter anesthetic bag containing 100% O_2; sufficient oxygen was bled in to maintain a near-constant bag volume. Blood was drawn from a peripheral vein for the measurement of baseline fractional carboxyhemoglobin saturation, (S_Co) hemoglobin concentration ([Hb] in g/ml) and hematocrit (in fraction). Then a known volume of CO (Q_CO, about 25 ml ambient temperature and pressure, dry condition) was added to the rebreathing bag and allowed to equilibrate for 10 to 15 minutes. A repeat venous blood sample was taken to measure the change in S_CO (∆S_CO), [Hb] and hematocrit. Blood volume (Q_b, in ml) was calculated by mass balance:

\[
\frac{P_b}{760} \cdot \frac{273}{(273 + t)} \cdot Q_{CO} = [Hb] \cdot 1.39 \cdot Q_b \cdot \Delta S_{CO} \quad \text{Equation 1}
\]
Where Pb = barometric pressure (mmHg); t = room temperature (°C); 1.39 = CO binding capacity of hemoglobin (ml (STPD)/g hemoglobin).

\[
\text{Total red cell volume (in ml) = } Q_b \cdot \text{hematocrit} \tag{Equation 2}
\]

\[
\text{Plasma volume (in ml) = } Q_b \cdot (1 - \text{hematocrit}) \tag{Equation 3}
\]

*The Evans Blue dilution method.* A venous catheter was inserted into each external jugular vein under local anesthesia. Evans Blue dye (2 ml, 5 mg/ml) was injected from a calibrated syringe into one catheter; the syringe and catheters were flushed well with normal saline. Blood (3 ml) was sampled from the contralateral jugular catheter at 2, 5, 10 and 15 minutes after injection and centrifuged to separate plasma. The optical density of the dye in plasma was measured with a spectrophotometer (Beckman DU 640B, Beckman Coulter Inc., Fullerton, CA) at 620nm for first estimation of Evans Blue concentration and then repeated at 740nm in order to correct for any turbidity and free hemoglobin in the samples (15). The dye disappearance curve was used to extrapolate the changes in concentration back to the time of dye injection for calculating plasma volume. Total blood volume was calculated from plasma volume and the systemic hematocrit. Red cell volume was the difference between plasma volume and total blood volume. Measurements using this method were made post-splenectomy with the animal standing at rest and during moderate exercise (50% of maximal O₂ uptake).

**Maximal oxygen uptake.** Under local anesthesia a 5-Fr catheter was inserted into a jugular vein, flushed with heparinized saline, sutured to the skin and connected to a manifold for blood sampling. After a 5-min warm-up period with the animal running at 6
mph, 0% grade, the treadmill speed was elevated to a pre-determined constant level. The treadmill grade was incremented by 5% every 2 minutes until maximal workload is reached, signaled by volitional termination, heart rate exceeding 300 bpm or rectal temperature exceeding 41°C. Before exercise, during the last 30 s of each workload and every 2 min for 6 min after cessation of exercise, 3 ml of blood was drawn from the carotid artery for measuring lactate (YSI, Inc., Yellow Springs, OH), hemoglobin concentration (OSM-3, Radiometer, Copenhagen, Denmark) and hematocrit (by microcapillary centrifuge).

**Rebreathing measurement during exercise.** These methods have been described in detail elsewhere (23, 37). A rebreathing bag was pre-filled with a volume of gas equal to (average tidal volume at a given exercise intensity + 200 ml ATPD). The rebreathing gas mixture contained 0.6% acetylene, 0.3% C\(^{18}\)O, 8-9% He, 30% O\(_2\), in a balance of N\(_2\) or in a balance of 100% O\(_2\). At end-expiration, a pneumatic valve was switched so the animal inspired from the bag and then re-breathed for about 8 s while gas concentrations were monitored at the mouth. At a respiratory rate of 100-120 breaths/min during heavy exercise we could reliably obtain 8-10 breaths in 5 s; 90% mixing was accomplished within 2-3 breaths. Lung volume (BTPS) was measured from helium dilution, pulmonary blood flow from the slope of the exponential acetylene disappearance, tissue volume from the extrapolated intercept of the acetylene relationship to zero time, and DL\(_{CO}\) from the slope of exponential C\(^{18}\)O disappearance (5, 6). From DL\(_{CO}\) measured at the two different O\(_2\) tensions we calculated DM\(_{CO}\) and Vc by the Roughton-Forster technique, using the values of Θ\(_{CO}\) in dog blood from Holland (18, 19) for a rectal temperature of 39°C:
\[ \frac{1}{\Theta_{CO}} = \frac{\text{hematocrit}}{45} (0.929 + 0.0042 \cdot P_{A02}) \]  
Equation 4

From the estimates of DL\(_{CO}\), DM\(_{CO}\) and Vc at a given cardiac output, a standardized DL\(_{CO}\) (DL\(_{CO\text{-std}}\)) was calculated at a constant hematocrit of 45% and alveolar P\(_{O2}\) of 120 mmHg. In addition, the equivalent membrane and lung diffusing capacities for oxygen (DM\(_{O2}\) and DL\(_{O2}\), respectively) were calculated from DM\(_{CO}\) and Vc using the published value of \(\theta_{O2} = 3.9 \theta_{CO}\) (43) and the ratio of diffusivity between O\(_2\) and CO based on molecular weights and solubility, i.e., DM\(_{O2}\) = 1.23 DM\(_{CO}\). One can reasonably assume this \(\theta_{O2}\) value when alveolar oxyhemoglobin saturation falls below ~85%, a level reached in dogs at heavy exercise even breathing room air at sea level (27). We have previously shown in dogs an empirical correlation between DL\(_{O2}\) estimated from DM\(_{CO}\) and Vc by the Roughton-Forster method and that estimated invasively by the multiple inert gas elimination technique during hypoxic exercise (27).

Data analysis. Results were normalized by body weight and expressed as mean±SD. Between-group comparisons were performed by 2-tailed unpaired t-test and analysis of variance; pre- to post-splenectomy comparisons were performed by paired t-test or repeated measures ANOVA (StatView v.5.0, SAS Institute, Cary, NC). DL\(_{CO}\), DM\(_{CO}\) and Vc were plotted with respect to pulmonary blood flow; the slopes and intercepts of individual regression lines were compared as described by Zar (45). Because there was no significant difference between pneumonectomized and SHAM animals in the responses to splenectomy, data from all animals were combined for the analysis of post-splenectomy changes relative to pre-splenectomy using each animal as its own control. Where the absolute measurement in pneumonectomized animals differs
significantly from that in SHAM-operated animals, the results were summarized separately for each group to ensure an accurate report of their respective means and variance. A p value ≤0.05 was considered significant.

Results

Hematology measured at rest and moderate exercise. These results did not differ between pneumonectomized and SHAM-operated animals; therefore they were pooled for pre- to post-splenectomy comparisons (Table 1). The resting hematocrit was significantly reduced after splenectomy compared to before. Blood volumes measured at rest by the CO rebreathing method before (pre-splenectomy, CO #1) and on two occasions 7 mo post-splenectomy (CO #2 and CO #3) are shown; duplicate post-splenectomy results were highly reproducible (CO #2 vs. CO #3). Blood volumes were measured at rest by the Evans Blue dilution method at approximately 8 mo post-splenectomy at rest (Evans Blue #1) and during moderate exercise (50% maximal O₂ uptake, Evans Blue #2). Blood volumes measured at rest by the two independent techniques were similar (CO #2 and #3 vs. Evans Blue #1). Splenectomy did not significantly alter circulating blood, plasma or red cell volumes at rest (CO #1 vs. CO #2, CO #3 or Evans Blue #1). Following splenectomy, circulating blood and red cell volumes did not increase from rest to exercise while plasma volume declined by ~6%.

Blood and red cell volumes estimated at peak exercise. Since we could not directly measure plasma volume at peak exercise and the measured change in plasma volume during moderate exercise was small (Table 1), for practical purposes we assumed that
plasma volume remained constant and used the measured changes in hematocrit to index the change in circulating red cell volume at peak exercise. In all animals before splenectomy, systemic hematocrit increased as O₂ uptake increased; following splenectomy hematocrit no longer increased and actually decreased from rest to peak exercise (Fig. 1). Before splenectomy, there was a direct relationship between the changes in red cell volume and oxygen uptake in both groups of animals (Fig. 2). From pre- to post-splenectomy, blood volume estimated at peak exercise was 19% and 32% lower and red cell volume 40% and 33% lower in PNX and SHAM-operated animals, respectively (Table 2).

Maximal oxygen uptake following splenectomy. Minute ventilation, O₂ uptake and CO₂ output at peak exercise were significantly lower in pneumonectomized animals compared to SHAM-operated animals (Table 2). Post-splenectomy maximal O₂ uptake declined by a similar extent in SHAM-operated and PNX animals (30% and 25%, respectively) compared to pre-splenectomy. There was a direct relationship between maximal O₂ uptake and circulating red cell volume estimated at exercise; the slope of this relationship was similar between SHAM-operated and PNX animals as well as between foxhounds and published reports of thoroughbred horses studied before and after splenectomy (40) (Fig. 3). In comparison, circulating red cell volume changes relatively little from rest to exercise in human athletes and maximal O₂ uptake is only moderately enhanced when blood volume is elevated by autologous blood transfusion (8, 9) (Fig. 3).

Pulmonary diffusing capacity following splenectomy. The absolute measurement of DL_{CO} and its components at a given pulmonary blood flow was significantly lower in
pneumonectomized animals than in SHAM-operated animals (Table 3). At a given pulmonary blood flow post-splenectomy, DL\textsubscript{CO}, DM\textsubscript{CO}, Vc as well as the DL\textsubscript{O2} estimated from DM\textsubscript{CO} and Vc declined by a similar extent in both groups of animals compared to their respective pre-splenectomy control values (Figure 4). Pulmonary capillary blood volume measured at a given pulmonary blood flow changed variably and the difference from pre- to post-splenectomy did not reach statistical significance. In addition, the slope of the relationships of DL\textsubscript{O2} and DM\textsubscript{CO} with respect to pulmonary blood flow was significantly lower post-splenectomy indicating impaired alveolar microvascular recruitment (Fig. 4 and Table 4).

Discussion

Summary of results. This study directly demonstrates the effect of splenectomy on exercise performance of foxhounds and provides the first experimental evidence of physiologic interaction between blood volume and pulmonary oxygen uptake by diffusion. Following splenectomy, circulating hematocrit and blood volume were normal at rest but exercise-induced polycythemia was eliminated. Hematocrit actually declined slightly upon exercise post-splenectomy. These changes correspond to a 25-30% reduction in maximal oxygen uptake in both SHAM-operated and pneumonectomized animals. At any given cardiac output, lung and membrane diffusing capacities were significantly reduced following splenectomy, while pulmonary capillary blood volume changed variably. The slope of recruitment in lung and membrane diffusing capacity with respect to pulmonary blood flow was also reduced following splenectomy,
indicative of impaired alveolar microvascular recruitment. The SHAM and pneumonectomy groups exhibited similar responses to splenectomy. Thus, the auto-infusion of red cells by splenic contraction significantly augmented convective as well as diffusive oxygen transport.

Critique of methods. The present report is one component of a chronic series that required 5 years to complete. The two survival operations (thoracotomy with or without pneumonectomy and splenectomy) were separated by 2.5 to 3 years. In pneumonectomized animals, splenectomy did not impair maximal O\textsubscript{2} uptake more than in SHAM-operated animals and post-splenectomy maximal oxygen uptake remained relatively high (~70 ml/min/kg). Because of the chronicity and the consistent response pattern to splenectomy in 6 animals, we did not feel it necessary to study more pneumonectomized animals. All animals continued regular exercise training throughout the study and remained healthy, fit and well socialized. With special permission from our institution, two animals were adopted by staff members at the end of the study.

Our estimates of hematocrit (43.4\%) and red cell volume (46.3 ml/kg) in unsedated resting animals pre-splenectomy were significantly higher than that reported in sedated or anesthetized adult dogs (average hematocrit 37.2–39.8\% and red cell volume 29.3–34.2 ml/kg) (13, 26), consistent with the basal sympathetic tone that keeps the spleen partially contracted in the awake resting state. We estimated the red cell volume at peak exercise from the measured hematocrit assuming that resting plasma volume remained unchanged during exercise. From rest to moderate exercise we found only a modest change (-6\%) in plasma volume post-splenectomy in these foxhounds, while Sarelius (33) had reported no change or a modest increase in plasma volume (0 to
+11.8%) in splenectomized greyhounds. These modest changes in plasma volume do not alter our conclusions in the present study.

Simulation studies have suggested that O₂-dependent distribution of CO flux across the erythrocyte membrane could alter DM CO (20); the significance of this effect in vivo is unknown. In the Roughton-Forster method, errors in DM CO may be introduced if cardiac output is reduced during the component breathing a high O₂ concentration; an overestimate of DM CO and an underestimate of Vc may result owing to the fact that DL CO measured at different O₂ tensions will not be matched to the same cardiac output. This source of error is eliminated in our rebreathing technique by the simultaneous measurement of pulmonary blood flow (using acetylene) with DL CO.

Diffusing capacity for O₂ can also be estimated by invasive methods during hypoxic exercise, e.g., by the multiple inert gas elimination technique (MIGET). We have previously shown an empirical agreement between DL O₂ estimated by the Roughton-Forster method and that estimated by the MIGET in dogs during hypoxic exercise (27). This agreement also holds in the present animals (unpublished observations).

Splenic contribution to exercise capacity

In aerobic animals: Joseph Barcroft noted in the 1920’s that the spleen of dogs and cats contracts in response to physiologic stress (2, 3) and thought the contraction might mobilize red cells to enhance O₂ transport. In seals, horses and dogs (4, 14, 38), the spleen sequesters nearly 50% of total erythrocyte volume or 13% of blood volume at a hematocrit of 85-90%. Alpha-adrenergic mediated splenic contraction during exercise, hypoxia or blood loss releases the sequestered erythrocytes into the
circulation. Vatner et al. (39) studied untethered instrumented dogs during exercise before and after splenectomy and reported that mobilization of erythrocytes raised systemic hematocrit from about 40% at rest to about 49% at peak exercise; after splenectomy, hematocrit no longer increased but the capability for severe exercise in the dog appeared identical to that before splenectomy, leading to the conclusion that the spleen contributed to the exercise response by raising hematocrit and O$_2$ transport sufficiently to obviate the necessity for reducing visceral blood flow but did not actually augment exercise capacity (39). Later, Longhurst et al (31) measured exercise capacity and maximal O$_2$ uptake in mongrel dogs before and after splenectomy and again before and after administering alpha-adrenergic receptor blockade to prevent splenic contraction. They found a 13% reduction in maximal O$_2$ uptake after splenectomy, significantly less than our finding of 25-30% reduction in SHAM and PNX foxhounds. The difference between our finding and that of Vatner et al. (39) and Longhurst et al. (31) most likely reflects differences in aerobic conditioning; these investigators studied mongrel dogs with a pre-splenectomy maximal O$_2$ uptake of 95 ml/min/kg of body weight. On the other hand, the thoroughbred horses studied by Wagner et al. (40) and shown in Fig. 3 reach a maximal O$_2$ uptake of 148 ml/min/kg, almost identical to that in our SHAM-operated foxhounds (144 ml/min/kg). In these horses, the average reduction in maximal O$_2$ uptake by splenectomy (31%) is also identical to that in SHAM-operated foxhounds.

In humans: Compared to aerobic species, the small human spleen has a volume around 100 ml at rest (35) and serves mainly as an immunologic organ and a site for erythrocyte breakdown and iron storage. Even so, maximal exercise in normal subjects
induces a 58% to 66% reduction in spleen volume (29, 36); total body erythrocyte and blood volume does not change from pre- to post-exercise but plasma volume decreases by 18.9% associated with a 4 to 5% absolute increase in hematocrit (36). Maximal O₂ uptake correlates with hemoglobin mass and blood volume, which are 35-40% higher in endurance athletes (average 15.3 g/kg and 107.1 ml/kg, respectively) than in untrained persons (average 11.0 g/kg and 78.3 ml/kg, respectively) (17). These average values in athletes are similar to our measurements in foxhounds. The change in maximal O₂ uptake due to blood volume manipulation by venesection or autologous transfusion in human subjects is modest and variable, about 10–13% (8, 9).

**Splenic contribution to lung diffusing capacity.** Splenic contraction is generally thought to augment convective O₂ transport by increasing cardiac output as well as the O₂ carry capacity of blood. Splenectomy in dogs abolishes the increase in cardiac output induced by acute hypoxemia (30). The increase in DL_CO with blood volume or hematocrit is usually attributed to a higher Vc and pulmonary blood flow that recruits previously closed capillaries and enlarges the hemoglobin sink for CO uptake. However, a comparative study from our laboratory found a much larger increase of DL_CO and DM_CO in exercising foxhounds than in average human subjects (42), leading us to suggest that polycythemia from splenic contraction could potentially explain the superior recruitment of diffusing capacity in dogs, a consequence of the physical properties of erythrocytes as discrete particles non-uniformly distributed within and among capillaries. Geiser et al. (11) found a 36% higher steady-state DL_O₂ in excised rabbit lungs perfused with hemoglobin solutions than in lungs perfused with red cell suspensions at the same concentration; these investigators (11) attributed the higher
DL\textsubscript{O2} to the elimination of diffusion resistance offered by unstirred layers around the flowing red cells although it is equally possible that packaging hemoglobin into discrete erythrocytes imposed additional membrane resistance to O\textsubscript{2} uptake. About the same time, Federspiel (10) demonstrated in a two-dimensional capillary model a lower computed DL\textsubscript{O2} when the spacing between red cells is increased due to lower fluxes across the tissue-erythrocyte barrier, also supporting an effect of hematocrit on membrane resistance.

To further explore physical interactions between alveolar tissue and erythrocyte membranes, we used a finite element method to model alveolar capillary CO uptake. Our results showed that as the number of capillary erythrocytes increase (i.e., increasing hematocrit), endothelial and erythrocyte membranes become more uniformly matched and CO flux increases up to a hematocrit of 40-45% (20). As more alveolar capillaries open to erythrocyte traffic (i.e., increasing blood volume and flow), effective endothelial and erythrocyte surfaces progressively increase and contribute to further elevations in CO flux (24). Above a hematocrit of ~45%, DL\textsubscript{CO} or DM\textsubscript{CO} per erythrocyte progressively declines as the erythrocytes become crowded and the anatomical match between tissue and erythrocyte membranes becomes less optimal, eventually imposing an upper limit to CO flux (20). Flow-related distortion of erythrocytes can reduce hemodynamic resistances while at the same time reducing DM\textsubscript{CO} by making portions of the erythrocyte surface less accessible to diffusion (21). At a given average capillary hematocrit, non-uniform distribution of erythrocytes within a capillary or among separate capillaries could potentially impair overall CO uptake by more than 30% (24). These simulation data suggest that alveolar-capillary recruitment involves more than opening.
capillaries or increasing hematocrit but also optimizing erythrocyte distribution to more effectively match erythrocyte and tissue membranes for gas exchange. Simulation also predicts that $DM_{CO}$ would change with hematocrit independent of changes in $Vc$. The present study provides direct in vivo evidence to corroborate these predictions by showing that post-splenectomy reduction of lung diffusing capacity was predominantly due to a reduction of the membrane component while $Vc$ either increased or did not change. Our results complement data from rats (12) showing that increasing hematocrit by isovolemic red cell exchange transfusion enhances peripheral tissue diffusing capacity as well as data from horses (40) that splenectomy impairs peripheral tissue diffusing capacity during exercise.

**Risk-free polycythemia.** Although an elevated circulating blood volume increases cardiac preload and raises cardiac output by the Starling mechanism, the associated increase in vascular resistance and cardiac afterload tend to curtail cardiac output. A high hematocrit could cause non-uniform distribution of blood flow and reduce gas exchange efficiency in the lung and muscle (7, 25). In human subjects chronic polycythemia caused by primary hematological overproduction or secondary to mountain sickness or severe lung disease is associated with hyperviscosity, which compromises cerebral blood flow and increases the risks of ischemia and thrombosis (16, 44). Athletes who blood dope via autologous transfusion or exogenous erythropoietin administration risk systemic hypertension and sudden death (34). Clinically, a level of hematocrit similar to that observed at maximal exercise in foxhounds or horses (~60%) is considered sufficiently dangerous to require phlebotomy. In contrast, periodic exercise-induced polycythemia in dogs or horses does not incur
these risks. The aerobic animal develops polycythemia only for the duration of heightened O\(_2\) demand when systemic vascular resistance is normally minimized. When O\(_2\) demand subsides, circulating blood volume and hematocrit quickly normalize as the extra erythrocytes return to the relaxing spleen. In contrast, the blood-doping athlete and the polycythemic patient must tolerate an elevated blood volume and hematocrit at all times regardless of O\(_2\) requirements or vascular tone. Thus, the evolutionary significance of the splenic reservoir in aerobic species lies not only in the enhancement of O\(_2\) transport on demand but also in its protective role against the development of polycythemia-associated complications.

A potential source of compensation. In addition to augmenting oxygen transport during acute episodes of increased metabolic demand, the splenic red cell reservoir constitutes another compensatory mechanism that facilitates chronic adaptation to diffusion impairment imposed by ambient hypoxia or prior pneumonectomy. For example, we have noted that dogs acclimate to high altitude much more readily than average human lowlanders. While it takes weeks to months for hypoxia-induced polycythemia and hypervolemia to fully develop in the average lowlander acclimatizing to high altitude and a similar time to return to normal when the hypoxic stress recedes, these changes could occur in dogs within minutes, allowing these animals to chase their prey up and down a mountain with minimal changes in hypoxic acclimatization during the exertion.

We conclude that reversible autologous red cell infusion via splenic contraction during exercise is an important physiological mechanism for enhancing convective as well as diffusive oxygen transport in the aerobic animal at sea level. The dynamic increase in hematocrit and red cell volume enhances both pulmonary as well as peripheral (muscle)
diffusing capacities by enlarging the hemoglobin pool for oxygen uptake and release, and by more effectively matching the membrane surfaces between tissue capillaries and red cells for gas exchange. Further investigation should address whether exercise training or chronic hypoxia enlarges the splenic reservoir, whether a large splenic reservoir improves acclimatization to hypoxia, and whether splenectomy shifts the balance of compensatory mechanisms during chronic hypoxia towards non-hematological sources, such as a greater ventilatory response and/or accelerated growth and remodeling of the alveolar gas exchange units and muscle capillaries.
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Figure legends

Figure 1. Circulating hematocrit increased from rest to exercise pre-splenectomy but not post-splenectomy. Regression lines are shown for each animal. Pre-splenectomy: open circles, solid lines; post-splenectomy: solid circles, dashed lines.

Figure 2. Changes in the estimated red cell volume are shown with respect to O$_2$ uptake during exercise before splenectomy. Assuming plasma volume was unchanged from rest to exercise, the increase in hematocrit directly reflects a similar proportional increase in red cell volume in SHAM and pneumonectomized (PNX) animals. Regression lines are through pooled data; SHAM, solid line; right PNX, dashed line.

Figure 3. Comparison of the relationship of maximal O$_2$ uptake to circulating red cell volume in individual foxhounds before and after splenectomy (solid lines; SHAM - open circles, post-PNX - open squares), in horses before and after splenectomy (dashed line, solid circles, mean±SD) from Wagner et al. (40), and in human athletes before and after autologous blood transfusion from Ekblom et al. (8) (solid triangles).

Figure 4. The relationships of DL$_{CO}$ (panel A), DM$_{CO}$ (panel B), Vc (panel C) and DL$_{O2}$ derived from DM$_{CO}$ and Vc (panel D) to pulmonary blood flow pre- and post-splenectomy are shown for SHAM-operated (left) and pneumonectomized (right) animals. Means±SD. *p<0.05 pre- vs. post-splenectomy.
Figure 1.

[Graph showing circulating hematocrit (%) vs. oxygen uptake (ml/min/kg) with data points and trend lines for Pre-SPX and Post-SPX conditions.]
Figure 2.

Change in Red Cell Volume (ml/kg) vs. Oxygen Uptake (ml/min/kg)

- SHAM
- R PNX
Figure 3.
Figure 4.  

**A**  

**SHAM**  

- **Pulmonary Blood Flow (mL.min⁻¹.kg⁻¹)**  

**B**  

- **DLCO (mL.min⁻¹.mmHg⁻¹.kg⁻¹)**  

**C**  

- **Vc (mL.kg⁻¹)**  

**D**  

- **DLCO (mL.min⁻¹.mmHg⁻¹.kg⁻¹)**  

**Pneumonectomy**  

- **Pulmonary Blood Flow (mL.min⁻¹.kg⁻¹)**  

- **NS**
Table 1. Hematocrit and blood volumes measured at rest and moderate exercise

<table>
<thead>
<tr>
<th>Method</th>
<th>CO #1</th>
<th>CO #2</th>
<th>CO #3</th>
<th>Evans Blue #1</th>
<th>Evans Blue #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomy</td>
<td>Pre-</td>
<td>7 mo Post-</td>
<td>7 mo Post-</td>
<td>8 mo Post-</td>
<td>8 mo Post-</td>
</tr>
<tr>
<td>Workload</td>
<td>Rest</td>
<td>Rest</td>
<td>Rest</td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.4 ± 1.5</td>
<td>41.5 ± 2.3</td>
<td>40.3 ± 2.1 *</td>
<td>40.2 ± 2.4 *</td>
<td>40.6 ± 1.3 †</td>
</tr>
<tr>
<td>Blood vol. (ml/kg)</td>
<td>107 ± 13</td>
<td>106 ± 15</td>
<td>103 ± 20</td>
<td>109 ± 18</td>
<td>102 ± 20</td>
</tr>
<tr>
<td>Red cell vol. (ml/kg)</td>
<td>46 ± 6</td>
<td>44 ± 8</td>
<td>42 ± 8</td>
<td>44 ± 9</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>Plasma vol. (ml/kg)</td>
<td>61 ± 7</td>
<td>62 ± 8</td>
<td>62 ± 13</td>
<td>65 ± 10</td>
<td>61 ± 12 ¶¶</td>
</tr>
</tbody>
</table>

Measurements were obtained in the conscious animal standing at rest or during exercise (50% of maximal workload).

Mean±SD, combined data from all animals (n=6) were used in paired t-test: * p<0.05 and † p<0.01 vs. pre-splenectomy.

¶¶ p<0.05 Evans Blue exercise vs. Evans Blue rest post-splenectomy.
Table 2. Data at Peak Exercise

<table>
<thead>
<tr>
<th>Animal group</th>
<th>SHAM</th>
<th>POST-PNEUMONECTOMY</th>
<th>Combined Pre- vs. Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- or Post-splenectomy</td>
<td>Pre-</td>
<td>Post-</td>
<td>Pre-</td>
</tr>
<tr>
<td>Number of animals</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ventilation (L.[min.kg]⁻¹)</td>
<td>6.7 ± 3.8</td>
<td>5.3 ± 2.2</td>
<td>4.2 ± 1.5</td>
</tr>
<tr>
<td>O₂ uptake (ml.[min.kg]⁻¹)</td>
<td>144 ± 49</td>
<td>101 ± 42</td>
<td>95 ± 36</td>
</tr>
<tr>
<td>CO₂ output (ml.[min.kg]⁻¹)</td>
<td>139 ± 55</td>
<td>91 ± 46</td>
<td>82 ± 36</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>113 ± 53</td>
<td>105 ± 29</td>
<td>88 ± 30</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>279 ± 12</td>
<td>287 ± 29</td>
<td>245 ± 41</td>
</tr>
<tr>
<td>Lactate (mM.L⁻¹)</td>
<td>5.3 ± 3.9</td>
<td>3.0 ± 2.0</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Hemoglobin (g.dL⁻¹)</td>
<td>17.8 ± 1.4</td>
<td>13.8 ± 0.5</td>
<td>17.2 ± 0.4</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>52.6 ± 3.1</td>
<td>39.3 ± 1.1</td>
<td>50.5 ± 2.1</td>
</tr>
<tr>
<td>Blood volume (ml.kg⁻¹) #</td>
<td>135 ± 15</td>
<td>109 ± 10</td>
<td>115 ± 12</td>
</tr>
<tr>
<td>Red cell volume (ml.kg⁻¹) #</td>
<td>72 ± 11</td>
<td>43 ± 5</td>
<td>60 ± 4</td>
</tr>
</tbody>
</table>

Mean±SD. Data from all animals were combined for paired t-test (n=6): * p<0.05, † p<0.01, § p<0.001 pre-splenectomy vs. post-splenectomy.

# Blood and red cell volumes were calculated from the average plasma volume of each animal measured awake at rest and the hematocrit measured at peak exercise, assuming a constant plasma volume.
Table 3. Rebreathing data at heavy exercise

<table>
<thead>
<tr>
<th>Pre- or Post-splenectomy</th>
<th>SHAM</th>
<th>POST-PNEUMONECTOMY</th>
<th>Combined Pre- vs. Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-</td>
<td>Post-</td>
<td>Pre-</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>23.0 ± 0.9</td>
<td>25.2 ± 3.5</td>
<td>22.7 ± 2.5</td>
</tr>
<tr>
<td>O₂ Uptake (ml.[min.kg]⁻¹)</td>
<td>108 ± 43</td>
<td>74 ± 18</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>CO₂ output (ml.[min.kg]⁻¹)</td>
<td>98 ± 42</td>
<td>60 ± 14</td>
<td>71 ± 15</td>
</tr>
<tr>
<td>Ventilation (L.[min.kg]⁻¹)</td>
<td>6.9 ± 3.3</td>
<td>5.4 ± 1.2</td>
<td>5.6 ± 1.3</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>193 ± 15</td>
<td>254 ± 13</td>
<td>275 ± 5</td>
</tr>
<tr>
<td>Pulmonary blood flow (ml.[min.kg]⁻¹)</td>
<td>735 ± 92</td>
<td>708 ± 175</td>
<td>558 ± 132</td>
</tr>
<tr>
<td>End-expiratory lung volume (ml.kg⁻¹)</td>
<td>117 ± 23</td>
<td>101 ± 17</td>
<td>92 ± 17</td>
</tr>
<tr>
<td>End-inspiratory lung volume (ml.kg⁻¹)</td>
<td>166 ± 19</td>
<td>149 ± 21</td>
<td>137 ± 12</td>
</tr>
<tr>
<td>DLCO (ml.[min.mmHg.kg]⁻¹)</td>
<td>1.92 ± 0.17</td>
<td>1.57 ± 0.36</td>
<td>1.16 ± 0.47</td>
</tr>
<tr>
<td>DMCO (ml.[min.mmHg.kg]⁻¹)</td>
<td>2.84 ± 0.35</td>
<td>2.11 ± 0.43</td>
<td>1.57 ± 0.58</td>
</tr>
<tr>
<td>Vc (ml.kg⁻¹)</td>
<td>6.40 ± 0.73</td>
<td>8.85 ± 4.69</td>
<td>4.07 ± 0.87</td>
</tr>
<tr>
<td>DLO₂ (ml.[min.mmHg.kg]⁻¹)</td>
<td>3.12 ± 0.34</td>
<td>2.37 ± 0.52</td>
<td>1.75 ± 0.63</td>
</tr>
<tr>
<td>Septal volume (ml.kg⁻¹)</td>
<td>28.9 ± 11.4</td>
<td>25.2 ± 4.2</td>
<td>13.2 ± 1.5</td>
</tr>
</tbody>
</table>

Mean±SD: SHAM (n=4), PNX (n=2).
Data from all animals were combined for paired t-test (n=6): *p<0.05, †p<0.01, §p<0.001 pre-splenectomy vs. post-splenectomy.
Table 4. Average slope of recruitment with respect to pulmonary blood flow

<table>
<thead>
<tr>
<th>Pre-or Post-splenectomy</th>
<th>SHAM</th>
<th>POST-PNEUMONECTOMY</th>
<th>Combined Pre- vs. Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-</td>
<td>Post-</td>
<td>Pre-</td>
</tr>
<tr>
<td>DL(_{CO})</td>
<td>2.18 ± 0.60</td>
<td>1.63 ± 0.38</td>
<td>1.44 ± 0.17</td>
</tr>
<tr>
<td>DM(_{CO})</td>
<td>3.45 ± 0.71</td>
<td>2.37 ± 0.38</td>
<td>1.72 ± 0.42</td>
</tr>
<tr>
<td>Vc</td>
<td>4.88 ± 3.42</td>
<td>9.08 ± 4.89</td>
<td>6.69 ± 0.60</td>
</tr>
<tr>
<td>DL(_{O2})</td>
<td>3.71 ± 0.85</td>
<td>2.62 ± 0.38</td>
<td>2.06 ± 0.36</td>
</tr>
</tbody>
</table>

Mean±SD: SHAM (n=4), PNX (n=2). The units are as shown in Figure 4. Data from all animals were combined (n=6). * p<0.05 pre-splenectomy vs. post-splenectomy.