Splenic contraction during exercise reversibly enhances convective O₂ transport by increasing hematocrit, blood volume, and O₂-carrying capacity. Based on theoretical interactions between erythrocytes and capillary membrane (Hsia CCW, Johnson RL Jr, and Shah D. J Appl Physiol 86: 1460–1467, 1999) and experimental findings in horses of a postsplenectomy reduction in peripheral O₂-diffusing capacity (Wagner PD, Erickson BK, Kubo K, Hiraga A, Kai M, Yamaya Y, Richardson R, and Seaman J. Equine Vet J 18, Suppl: 82–89, 1995), we hypothesized that splenic contraction also augments diffusive O₂ transport in the lung. Therefore, we have measured lung diffusing capacity (DLCO) and its components during exercise by a rebreathing technique in six adult foxhounds before and after and before splenectomy. Splenectomy eliminated exercise-induced polycythemia, associated with a 30% reduction in maximal O₂ uptake. At any given pulmonary blood flow, DLCO was significantly lower after splenectomy owing to a lower membrane diffusing capacity, whereas pulmonary capillary blood volume changed variably; microvascular recruitment, indicated by the slope of the increase in DLCO with respect to pulmonary blood flow, was also reduced. We conclude that splenic contraction enhances both convective and diffusive O₂ transport and provides another compensatory mechanism for maintaining alveolar O₂ transport in the presence of restrictive lung disease or ambient hypoxia.

Address for reprint requests and other correspondence: R. L. Johnson, Jr., Dept. of Internal Medicine, Univ. of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9034.
permit acute catheterization without the complications of chronic indwelling vascular catheters. The animals had been trained to run on a treadmill (see Exercise Training below); exercise studies were performed before and after pneumonectomy. 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 reinfusion. After phlebotomy, blood volume was measured by a CO rebreathing method (see Blood Volume Measurement below). These procedures were repeated approximately once a month for ~6 mo. Iron supplementation was provided during this interval.

**Surgery**

After the completion of blood collections, splenectomy was performed. The animal was fasted overnight and sedated (acepromazine 0.2 mg/kg im). 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 were ligated close to the hilum with silk ties. The vessels were severed between ligatures, and the spleen was removed. The abdomen was irrigated with sterile saline and closed with silk suture. Approximately 50–100 ml of normal saline were administered intravenously during the operation. After surgery the animal was monitored in a recovery room until awake. Buprenorphine was administered daily for 7 days. The wound was dressed daily, and the sutures were removed in 10–14 days.

**Exercise Training**

The physical training program has been described previously (22). Before undergoing splenectomy, each dog had been trained to run voluntarily on a motor-driven treadmill for 30 min a day, 3–5 days a week at a workload equivalent to 60–80% of predicted or measured maximal O2 uptake. Dogs were considered “trained” when reproducible values of O2 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 ~3 mo to fully train a dog. Training continued throughout the course of the study and resumed 2 wk after splenectomy. The present exercise studies were carried out before and 4–9 mo after 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, O2 uptake, CO2 production, and respiratory rate were followed breath by breath and averaged over a predetermined 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 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).

**CO rebreathing method.** While wearing the respiratory mask, the animal rebreathed through a carbon dioxide absorber from a 5-liter anesthetic bag containing 100% O2; sufficient O2 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 (SCO), hemoglobin concentration (Hb), in g/dl, and hematocrit (in fraction). Then a known volume of CO (QCO, ~25 ml ATPD) was added to the rebreathing bag and allowed to equilibrate for 10–15 min. A repeat venous blood sample was taken to measure the change in SCO (ΔSCO), [Hb], and hematocrit. Blood volume (Qb, in ml) was calculated by mass balance:

$$\frac{Pb}{760} \cdot \frac{273}{273 + t} \cdot Q_{CO} = [Hb] \cdot 1.39 \cdot Q_{b} \cdot \Delta S_{CO}$$

where Pb is barometric pressure (mmHg), t is room temperature (°C), and 1.39 is CO binding capacity of hemoglobin [ml (STPD)/g hemoglobin].

Total red cell volume (in ml) = Qb · hematocrit

Plasma volume (in ml) = Qb · (1 – hematocrit)

**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 min 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, Fullerton, CA) at 620 nm for first estimation of Evans blue concentration and then repeated at 740 nm 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

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

<table>
<thead>
<tr>
<th>Method</th>
<th>CO 1 Pre-Rest</th>
<th>CO 1 Post-Rest</th>
<th>CO 2 7 mo Pre-Rest</th>
<th>CO 2 7 mo Post-Rest</th>
<th>CO 3 7 mo Pre-Rest</th>
<th>CO 3 7 mo Post-Rest</th>
<th>Evans Blue 1 8 mo Pre-Rest</th>
<th>Evans Blue 1 8 mo Post-Rest</th>
<th>Evans Blue 2 8 mo Pre-Rest</th>
<th>Evans Blue 2 8 mo Post-Rest</th>
</tr>
</thead>
<tbody>
<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>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood volume, ml/kg</td>
<td>107±13</td>
<td>106±15</td>
<td>103±20</td>
<td>109±18</td>
<td>102±20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell volume, ml/kg</td>
<td>46±6</td>
<td>44±8</td>
<td>42±8</td>
<td>44±9</td>
<td>42±9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma volume, ml/kg</td>
<td>61±7</td>
<td>62±8</td>
<td>62±13</td>
<td>65±10</td>
<td>61±12‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measurements were obtained in the conscious animal standing at rest or during exercise (50% of maximal workload). Values are means ± SD; combined data from all animals (n = 6) were used in paired t-test. CO, carbon monoxide. See text for explanation of groups. *P < 0.05 and †P < 0.01 vs. presplenectomy. ‡P < 0.05 Evans blue exercise vs. Evans blue rest postsplenectomy.
plasma volume and the systemic hematocrit. Erythrocyte 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 O₂ 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 predetermined constant level. The treadmill grade was incremented by 5% every 2 min until maximal workload was reached, signaled by volitional termination, heart rate exceeding 300 beats/min, 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 were drawn from the carotid artery for measuring lactate (YSI, 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 prefilled 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% C¹³O, 8–9% He, 30% O₂, in a balance of N₂ or in a balance of 100% O₂. At end expiration, a pneumatic valve was switched so the animal inspired from the bag and then rebreathed for 6–8 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 (V̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̇̍
purposes we assumed that plasma volume remained constant and used the measured changes in hematocrit to index the change in circulating erythrocyte volume at peak exercise. In all animals before splenectomy, systemic hematocrit increased as O₂ uptake increased; after splenectomy hematocrit no longer increased and actually decreased from rest to peak exercise (Fig. 1). Before splenectomy, there was a direct relationship between changes in erythrocyte volume and O₂ uptake in both groups of animals (Fig. 2). From pre- to postsplenectomy, blood volume estimated at peak exercise was 19 and 32% lower and erythrocyte volume 40 and 33% lower in pneumonectomized and Sham-operated animals, respectively (Table 2).

**Maximal O₂ Uptake After Splenectomy**

Minute ventilation, O₂ uptake, and CO₂ output at peak exercise were significantly lower in pneumonectomized animals compared with Sham-operated animals (Table 2). Postsplenectomy maximal O₂ uptake declined by a similar extent in Sham-operated and pneumonectomized animals (30 and 25%, respectively) compared with presplenectomy. There was a direct relationship between maximal O₂ uptake and circulating erythrocyte volume estimated at exercise; the slope of this relationship was similar between Sham-operated and pneumonectomized animals as well as between foxhounds and published reports of Thoroughbred horses studied before and after splenectomy (40) (Fig. 3). In comparison, circulating erythrocyte 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 After Splenectomy**

The absolute measurement of DmCO 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 postsplenectomy, DmCO, DmCO, Vc, as well as the DLo2 estimated from DmCO and Vc declined by a similar extent in both groups of animals compared with their respective presplectomy control values (Fig. 4). Pulmonary capillary blood volume measured at a given pulmonary blood flow changed variably, and the difference from pre- to postsplenectomy did not reach statistical significance. In addition, the slope of the relationships of DmCO and DmCO with respect to pulmonary blood flow was significantly lower postsplenectomy, 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 physiological interaction between blood volume and pulmonary O₂ uptake by diffusion. After splenectomy, circulating hematocrit and blood volume were normal at rest, but exercise-induced polycythemia was eliminated. Hematocrit actually declined slightly postsplenectomy. These changes correspond to a 25–30% reduction in maximal O₂ uptake in both Sham-operated and pneumonectomized animals compared with Sham-operated animals (Table 2).

**Table 2. Data at peak exercise**

<table>
<thead>
<tr>
<th>Animal Group Pre-or Postsplenectomy</th>
<th>Sham</th>
<th>Post-splenectomy</th>
<th>Combined Pre vs. Post</th>
</tr>
</thead>
<tbody>
<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, breaths/min</td>
<td>113±53</td>
<td>105±29</td>
<td>88±30</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>279±12</td>
<td>287±29</td>
<td>245±41</td>
</tr>
<tr>
<td>Lactate, mM/l</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</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§</td>
<td>135±15</td>
<td>109±10</td>
<td>115±12</td>
</tr>
<tr>
<td>Red cell volume, ml/kg§</td>
<td>72±11</td>
<td>43±5</td>
<td>60±4</td>
</tr>
</tbody>
</table>

Values are means ± 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.
animals. At any given cardiac output, lung and membrane diffusing capacities were significantly reduced after splenectomy, whereas pulmonary capillary blood volume changed variably. The slope of recruitment in lung and membrane diffusing capacities were significantly reduced after splenectomy, indicative of impaired alveolar microvascular recruitment. The Sham and pneumonectomy groups exhibited similar responses to splenectomy. Thus the autoinfusion of erythrocytes by splenic contraction significantly augmented convective as well as diffusive O2 transport.

Critique of Methods

The present report is one component of a chronic series that required 5 yr to complete. The two survival operations (thoracotomy with or without pneumonectomy and splenectomy) were separated by 2.5 to 3 yr. In pneumonectomized animals, splenectomy did not impair maximal O2 uptake more than in Sham-operated animals and postsplenectomy maximal O2 uptake remained relatively high (~70 ml·min⁻¹·kg⁻¹). 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 erythrocyte volume (46.3 ml/kg) in unsedated resting animals presplenectomy were significantly higher than that reported in sedated or anesthetized adult dogs (average hematocrit 37.2–39.8% and erythrocyte 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 erythrocyte 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 postsplenectomy in these foxhounds, whereas 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 DmCO (20); the significance of this effect in vivo is unknown. In the Roughton-Forster method, errors in DmCO may be introduced if cardiac output is reduced during the component breathing a high O2 concentration; an overestimate of DmCO and an underestimate of Vc may result owing to the fact that DlCO 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 DlCO.

Diffusing capacity for O2 can also be estimated by invasive methods during hypoxic exercise, e.g., by MIGET. We have previously shown an empirical agreement between DlO2, estimated by the Roughton-Forster method and that estimated by the MIGET in dogs during hypoxic exercise (27). This agree-

![Graph showing the relationship between circulating RBC volume and maximal O2 uptake.](Image)

Table 3. Rebreathing data at heavy exercise

<table>
<thead>
<tr>
<th></th>
<th>Pre- or Postsplenectomy</th>
<th>Sham</th>
<th>Post</th>
<th>Combined</th>
<th>Pre vs. Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>23.0±0.9</td>
<td>25.5±3.5</td>
<td>22.7±2.5</td>
<td>28.4±2.7</td>
<td>*</td>
</tr>
<tr>
<td>O2 uptake, ml·min⁻¹·kg⁻¹</td>
<td>108.43</td>
<td>74.18</td>
<td>81.15</td>
<td>56.6±18</td>
<td></td>
</tr>
<tr>
<td>CO2 output, ml·min⁻¹·kg⁻¹</td>
<td>98.42</td>
<td>60.14</td>
<td>71.15</td>
<td>41.7</td>
<td></td>
</tr>
<tr>
<td>Ventilation, l·min⁻¹·kg⁻¹</td>
<td>6.9±3.3</td>
<td>5.4±1.2</td>
<td>5.6±1.3</td>
<td>4.0±0.8</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>191±15</td>
<td>254±13</td>
<td>275±5</td>
<td>239±17</td>
<td></td>
</tr>
<tr>
<td>Pulmonary blood flow, ml·min⁻¹·kg⁻¹</td>
<td>735±92</td>
<td>708±175</td>
<td>558±132</td>
<td>568±227</td>
<td></td>
</tr>
<tr>
<td>End-expiratory lung volume, ml/kg</td>
<td>117±23</td>
<td>101±17</td>
<td>92±17</td>
<td>71±26</td>
<td></td>
</tr>
<tr>
<td>End-inspiratory lung volume, ml/kg</td>
<td>166±19</td>
<td>149±21</td>
<td>137±12</td>
<td>106±42</td>
<td></td>
</tr>
<tr>
<td>DlCO2, ml·min⁻¹·mmHg⁻¹·kg⁻¹</td>
<td>1.92±0.17</td>
<td>1.57±0.36</td>
<td>1.16±0.47</td>
<td>0.89±0.27</td>
<td>†</td>
</tr>
<tr>
<td>DmCO2, ml·min⁻¹·mmHg⁻¹·kg⁻¹</td>
<td>2.84±0.35</td>
<td>2.11±0.43</td>
<td>1.57±0.58</td>
<td>1.14±0.28</td>
<td>†</td>
</tr>
<tr>
<td>Vc, ml/kg</td>
<td>6.40±0.73</td>
<td>8.85±4.69</td>
<td>4.07±0.87</td>
<td>7.98±5.26</td>
<td>‡</td>
</tr>
<tr>
<td>DlO2, ml·min⁻¹·mmHg⁻¹·kg⁻¹</td>
<td>3.12±0.34</td>
<td>2.37±0.52</td>
<td>1.75±0.63</td>
<td>1.34±0.36</td>
<td>‡</td>
</tr>
<tr>
<td>Septal volume, ml/kg</td>
<td>28.9±1.14</td>
<td>25.2±4.2</td>
<td>13.2±1.5</td>
<td>12.9±4.7</td>
<td></td>
</tr>
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Values are means ± SD: Sham (n = 4), pneumonectomized (n = 2). DlCO2, lung CO-diffusing capacity; DmCO2, diffusing capacity of the alveolar-capillary membrane; Vc, pulmonary capillary blood volume; DlO2, lung O2-diffusing capacity. Data from all animals were combined for paired t-test (n = 6): *P < 0.05, †P < 0.01, ‡P < 0.001 presplenectomy vs. postsplenectomy.

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Fig. 4. Relationships of lung CO-diffusing capacity (DL_{CO}; A), membrane CO-diffusing capacity (Dm_{CO}; B), capillary volume (Vc; C), and lung O₂-diffusing capacity (DL_{O₂}) derived from Dm_{CO} and Vc (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. postsplenectomy.
Table 4. Average slope of recruitment with respect to pulmonary blood flow

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<thead>
<tr>
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<th>Pre-</th>
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<td>DMCO</td>
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<td>2.37±0.38</td>
<td>1.72±0.42</td>
<td>1.18±0.87</td>
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<td>Vc</td>
<td>4.88±3.42</td>
<td>9.08±4.89</td>
<td>6.69±0.60</td>
<td>13.68±3.98</td>
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<tr>
<td>DL&lt;sub&gt;O2&lt;/sub&gt;</td>
<td>3.71±0.85</td>
<td>2.62±0.38</td>
<td>2.06±0.36</td>
<td>1.52±0.80</td>
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Values are means ± SD; Sham (n = 4), pneumonectomized (n = 2). The units are as shown in Figure 4. Data from all animals were combined (n = 6) for statistical comparison. *P < 0.05 prespleenectomy vs. postspleenectomy.

Splenectomy also holds in the present animals (unpublished observations).

**Spleenic Contribution to Exercise Capacity**

In aerobic animals. Joseph Barcroft noted in the 1920s that the spleen of dogs and cats contracts in response to physiological stress (2, 3) and thought the contraction might mobilize erythrocytes to enhance O<sub>2</sub> transport. In seals, horses, and dogs (4, 14, 38), the spleen sequesters nearly 50% of total erythrocyte volume or 13% of blood volume at rest at a hematocrit of 85–90%. α-Adrenergic-mediated splenic contraction during exercise, hypoxia, or blood loss releases the sequestered erythrocytes into the circulation. Wainet et al. (39) studied unanesthetized instrumented dogs during exercise before and after splenectomy and reported that mobilization of erythrocytes raised systemic hematocrit from ~40% at rest to ~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<sub>2</sub> 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<sub>2</sub> uptake in mongrel dogs before and after splenectomy and again before and after administering α-adrenergic receptor blockade to prevent splenic contraction. They found a 13% reduction in maximal O<sub>2</sub> uptake after splenectomy, significantly less than our finding of 25–30% reduction in Sham and pneumonectomized foxhounds. The difference between our finding and that of Wainet et al. and Longhurst et al. most likely reflects differences in aerobic conditioning; these investigators studied mongrel dogs with a presuplenectomy maximal O<sub>2</sub> uptake of 95 ml·min<sup>−1</sup>·kg<sup>−1</sup> 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<sub>2</sub> uptake of 148 ml·min<sup>−1</sup>·kg<sup>−1</sup>, similar to that in our Sham-operated foxhounds (144 ml·min<sup>−1</sup>·kg<sup>−1</sup>). In these horses, the average reduction in maximal O<sub>2</sub> uptake by splenectomy (31%) is also identical to that in Sham-operated foxhounds.

In humans. Compared with aerobic species, the small human spleen has a volume around 100 ml at rest (35) and serves mainly as an immunological organ and a site for erythrocyte breakdown and iron storage. Even so, maximal exercise in normal subjects induces a 38–66% reduction in spleen volume (29, 36); total body erythrocyte and blood volume do not change from pre- to postexercise, but plasma volume decreases by 18.9% associated with a 4–5% absolute increase in hematocrit (36). Maximal O<sub>2</sub> 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 O<sub>2</sub> uptake due to blood volume manipulation by venesection or autologous transfusion in humans is modest and variable, ~10–13% (8, 9).

**Spleenic Contribution to Lung Diffusing Capacity**

Spleenic contraction is generally thought to augment convective O<sub>2</sub> transport by increasing cardiac output as well as the O<sub>2</sub>–carrying capacity of blood. Splenectomy in dogs abolishes the increase in cardiac output induced by acute hypoxemia (30). The increase in DL<sub>CO</sub> 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<sub>CO</sub> and Dm<sub>CO</sub> 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 nonuniformly distributed within and among capillaries. Geiser and Betthiger (11) found a 36% higher steady-state DL<sub>O2</sub> in excised rabbit lungs perfused with hemoglobin solutions than in lungs perfused with erythrocyte suspensions at the same concentration. These investigators (11) attributed the higher DL<sub>O2</sub> to the elimination of diffusion resistance offered by unstirred layers around the flowing erythrocytes, although it is equally possible that packaging hemoglobin into discrete erythrocytes imposed additional membrane resistance to O<sub>2</sub> uptake. About the same time, Federspiel (10) demonstrated in a two-dimensional capillary model a lower computed DL<sub>O2</sub> when the spacing between erythrocytes is increased because of 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 increases (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<sub>CO</sub> or Dm<sub>CO</sub> 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<sub>CO</sub> by making portions of the erythrocyte surface less accessible to diffusion (21). At a given average capillary hematocrit, nonuniform 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 $D_m CO_2$ would change with hematocrit independent of changes in $V_c$. The present study provides direct in vivo evidence to corroborate these predictions by showing that postsplenectomy reduction of lung diffusing capacity was predominantly due to a reduction of the membrane component, whereas $V_c$ either increased or did not change. Our results complement data from rats (12) showing that increasing hematocrit by isovolemic erythrocyte 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 nonuniform 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 administration risk systemic hypertension and sudden death (34). Athletes who blood dope to enhance their performance may be at risk of these complications. However, these risks are typically associated with hemoglobin levels above 60% which are rare in healthy individuals. Therefore, it is important to consider the potential benefits and risks of polycythemia as a means of compensation.

A Potential Source of Compensation

In addition to augmenting $O_2$ transport during acute episodes of increased metabolic demand, the splenic erythrocyte 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 acclimatize to high altitude more readily than average human lowlanders. Although 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 erythrocyte infusion via splenic contraction during exercise is an important physiological mechanism for enhancing convective as well as diffusive $O_2$ transport in the aerobic animal at sea level. The dynamic increase in hematocrit and erythrocyte volume enhances both pulmonary as well as peripheral (muscle) diffusing capacities by enlarging the hemoglobin pool for $O_2$ uptake and release, and by more effectively matching the membrane surfaces between tissue capillaries and erythrocytes 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 toward nonhematological 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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DISCLAIMER

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REFERENCES

13. Grover RF, Johnson RL Jr, McCullough RG, McCullough RE, Hofmeister SE, Campbell WB, and Reynolds RC. Pulmonary hyper-


