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Regulation of oxygen delivery during induced polycythemia in exercising dogs

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Lindenfeld, JoAnn, John V. Weil, Victoria L. Travis, and Lawrence D. Horwitz. Regulation of oxygen delivery during induced polycythemia in exercising dogs. *Am J Physiol Heart Circ Physiol* 289: H1821–H1825, 2005. First published July 8, 2005; doi:10.1152/ajpheart.01016.2004.—Previous studies have concluded that polycythemia decreases oxygen delivery primarily because of a large fall in cardiac output associated with a rise in systemic vascular resistance that has been attributed to increased blood viscosity. However, because other studies have shown that polycythemia may not reduce oxygen delivery, an alternative hypothesis is that cardiac output falls in response to a rising oxygen content, thereby maintaining oxygen delivery constant. To determine whether oxygen content participates in the regulation of cardiac output during polycythemia, we studied eight chronically instrumented dogs trained to exercise on a treadmill. The dogs underwent exchange transfusion with packed red blood cells containing methemoglobin, which caused an increase in hematocrit from 35 ± 1 to 50 ± 1% and in viscosity, with little change in oxygen content. The expected fall in exercise cardiac output failed to occur after exchange transfusion with red blood cells containing methemoglobin (7.5 ± 4 vs. 6.8 ± 0.5 l/min; P = not significant), and there was no rise in systemic vascular resistance. Methylene blue was then administered intravenously to facilitate conversion of methemoglobin to oxyhemoglobin, which increased oxygen content (12.8 ± 0.9 vs. 18.4 ± 0.9 vol%; $P < 0.01$) with no change in hematocrit or viscosity. Resting cardiac output did not change significantly, but there was a significant decrease in exercise output (6.8 ± 0.5 vs. 5.8 ± 0.4 l/min; $P < 0.05$). Thus we conclude that the fall in cardiac output seen in acute polycythemia results in part from the regulation of oxygen delivery and is not due solely to increased blood viscosity.

exercise; cardiac output control

ALTHOUGH POLYCYTHEMIA LEADS to an increase in the oxygen content of blood, previous studies have shown that this potential benefit on oxygen delivery is offset by a decrease in cardiac output (11, 28, 37). This fall in cardiac output has been attributed to a rise in viscosity of the polycythemic blood (11, 37). However, several considerations suggest that increased viscosity may not be the only explanation. Heart rate fails to increase with polycythemia as might be expected if the heart were confronted by an afterload burden (2, 12, 22). Whereas viscosity rises in a curvilinear fashion with increasing polycythemia, cardiac output falls in a much more linear fashion (3, 22). Similar falls in cardiac output occur both at rest and with exercise during polycythemia even though viscosity changes are minimized in exercising muscle beds (10, 22). Finally, several studies have shown that exercise capacity and maximal oxygen uptake may not fall with polycythemia as had been previously described (12, 22). These studies provide evidence that oxygen delivery is relatively constant over a wide range of hematocrit and led us to hypothesize that the fall in cardiac output that occurs with acute polycythemia may be in part a result of regulation of oxygen delivery and not solely a passive consequence of increased blood viscosity. To test this hypothesis, we altered viscosity and arterial oxygen content independently by increasing hematocrit using transfusions of red cells containing methemoglobin, which have a limited capacity for carrying oxygen. Exercise was used to stress the oxygen delivery system and amplify the effects of local regulation of vascular tone in skeletal muscle.

METHODS

Ethical policies. This study was approved by the Animal Use Committee of the University of Colorado Health Science Center.

Surgical techniques. Eight mongrel dogs weighing 30–38 kg were used in this study. After each dog was trained to run on a level treadmill, a splenectomy was performed using sterile conditions and halothane anesthesia. Splenectomy was performed because the spleen serves as an important blood reservoir in dogs and splenic constriction causes large variations in hematocrit and blood volume (32). In humans the spleen is relatively small and is not believed to have a reservoir function. The dogs were allowed to recover for a minimum of 10 days, after which each dog underwent a left thoracotomy with placement of 18-gauge polyvinyl catheters in the descending aorta and left atrium and placement of a Zepeda electromagnetic flow probe around the ascending aorta. The dogs were allowed to recover from the thoracotomy for at least 2 wk. Two days before each study, under light pentobarbital sodium anesthesia, an 18-gauge catheter was placed in a femoral vein through an external jugular vein with fluoroscopic monitoring. The opposite external jugular vein was used for placement of a 14-gauge polyvinyl catheter used for infusion of packed red blood cells. All catheters were tunneled subcutaneously and exteriorized at the back of the neck.

Exercise training. Each dog was trained to run on level treadmill at a speed between 5 and 9 miles/h (3.1 and 5.6 km/h). To avoid conditioning effects, no dog ran for more than 10 min three times per week.

Preparation of red blood cells containing methemoglobin. Blood was removed from donor dogs with a 13-gauge stainless steel needle placed in the apex of the left ventricle, drained into vacuum bottles containing 60 ml of acid-citrate-dextrose (ACD) for each 500 ml of whole blood, transferred to transfer packs, and spun at 1,200 *g* for 20 min to pack the red blood cells. The plasma was removed, and the red blood cells were resuspended in an equivalent amount of normal saline and mixed gently for 2 min with sodium nitrite (2.5 ml of 3 M sodium nitrite per 500 ml of red blood cells). The cells were then centrifuged at 2,100 *g* and washed three times with 300 ml of heparinized saline, washed with 6% dextran in saline, and stored in

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0.2% dextrose in saline at 4°C.(25) Aliquots of these cells were tested, and only those cells with 95–100% methemoglobin were used (5).

Hematocrit, methemoglobin, lactate, blood gases, and oxygen content. Blood for hematocrit levels was drawn from the pulmonary artery catheter, and hematocrit was measured by the microhematocrit centrifuge technique. No correction was made for trapped plasma. Methemoglobin was measured by the method of Evelyn and Malloy (5). Blood lactate was measured by a standard technique (1). Blood-gas tensions for oxygen, carbon dioxide, and measurement of pH were measured on a polaragraphic electrode (Radiometer) calibrated against gases analyzed in our laboratory by the Scholander technique and against pH using glass electrodes calibrated with standard buffers. Arterial oxygen content was measured using the Lex-O_2-Con device (Lexington Instruments), which in our hands agrees with the VanSlyke measurements within 0.1 vol%.

Blood volume. On the day before each study, 6 ml of whole blood were removed from each dog and incubated with ^{51}Cr -labeled sodium chromate in NIH-ACD for 15 min at room temperature (14). The cells were washed three times in normal saline and rediluted in plasma to the original volume. After mixing, three small aliquots were taken to determine the total number of counts injected. The remaining blood was added to a preweighed syringe and reweighed to determine the weight of the injected blood. The day of the study, 2 ml of blood were removed from the dog, and its exact volume was determined from its weight and specific gravity of blood for the observed hematocrit. Each sample was counted in a well-type gamma counter. The ^{51}Cr-labeled cells were returned to each dog through a catheter in a femoral vein on the day before the initial determination of blood volume, which was the day of the study. Initial blood volume was then determined by the formula:

$$\text{Blood volume} = \frac{\text{Total } ^{51}\text{Cr counts injected} \times \text{ml/sample}}{\text{counts per sample}}$$

Subsequent blood volumes were estimated by measuring all blood removed from the dog and counting aliquots of this blood to determine the total amount of ^{51}Cr removed. By knowing the total amount of ^{51}Cr injected initially and the amount removed, blood volume was determined at each level of the experiment without relabeling red blood cells by the formula:

$$\text{Blood volume} = \frac{\text{Total } ^{51}\text{Cr counts remaining} \times \text{ml/sample}}{\text{counts per sample}}$$

To further ensure that normovolemia was maintained, all transfusions were isovolemic exchange transfusions, a method previously shown to maintain a constant blood volume.(24)

Cardiac output and pressure. Stroke volume was calculated by the integral of the aortic flow signal using a Zepeda EDP2 electromagnetic flowmeter.(13). Cardiac output was determined by averaging stroke volume for at least six consecutive beats to reduce the effects of respiratory variation. Each electromagnetic flow probe was calibrated in vitro with normal saline before insertion in each dog. Flow calibration factors were not corrected for hematocrit. Cardiac output by electromagnetic probe was not affected at hematocrits of 33–66 in a previous study using the same equipment and techniques (22). Flow was assumed to be zero at end diastole. All signals were recorded on an eight-channel Beckman dynograph recorder. Mean arterial and left atrial pressures were measured through the implanted catheters with a Statham P23Db manometer with the zero reference at midchest level. Zero references were recorded both at rest and during exercise. Aortic flow and mean left atrial and aortic pressures were recorded continuously during exercise.

Study design. Each dog was studied on a single day. Control hemodynamic measurements were made at rest and at 3 min of steady-state exercise. Isovolemic exchange transfusion was then performed removing ~200 ml of whole blood from the dog and replacing it with an equal volume of donor packed red blood cells containing methemoglobin. Repeat exchange transfusions were performed until there was an increase in hematocrit of ~15 points. After waiting at least 45 min after the last transfusion, rest and exercise measurements were repeated at the same exercise load used during control runs. After recovery from exercise, each dog was given 1–2 mg/kg of methylene blue intravenously. After 45 min to 1 h, when oxygen content levels had increased significantly, repeat control measurements were made and exercise was repeated at the same workload used in previous runs. Exercise blood samples for arterial oxygen content, P_{O_2}, P_{CO_2}, pH, and lactate were drawn at rest and within 15 s of the end of exercise. Two normal dogs were studied on a separate day at rest and exercise for 90 min after 2 mg/kg methylene blue to control for any direct effects of methylene blue or the nitric oxide system.

Viscosity measurements. Viscosity was measured on a Brookfield cone and plate viscometer using heparinized blood and shear rates of 5–212 s^{-1} (9).

Statistics. Eight studies in eight dogs are presented from the control period, after exchange transfusion with red blood cells containing methemoglobin, and after methylene blue. These three experimental states were compared by two-way analysis of variance (3). The Student-Newman-Keuls test was used for multiple comparisons (38).

RESULTS

Methemoglobin phase. Hematocrit increased from 35 to 50% after exchange transfusion with red blood cells containing methemoglobin, but oxygen content did not change significantly (Table 1). Although the changes were not significant, there was a trend toward slight reductions in cardiac output both at rest and with exercise after exchange transfusion with red blood cells containing methemoglobin (Fig. 1). Stroke volume fell significantly both at rest and with exercise and heart rate was increased, although the increase was significant only at rest. Systemic vascular resistance did not change from control (Fig. 1*B*). Oxygen delivery fell after exchange transfusion, and this was reflected by a fall in mixed venous P_{O_2} and

Table 1. *Hemodynamic blood gases, and lactate data*

Variable	Control	MetHb	MB
Rest			
Hematocrit	35±1	50±1*	49±2*
Ca_{O_2}, vol%	14.7±0.5	13.1±1.0	18.8±0.9*†
Oxygen delivery, ml O_2/min	561±54	426±38*	570±32†
Heart rate, beats/min	133±9	156±6*	134±5†
Stroke volume, ml/beat	29±2	22±2*	24±3*
Cardiac output, l/min	3.8±0.3	3.3±0.3	3.1±0.3
Mean arterial pressure, mmHg	95±3	92±3	98±5
Pa_{O_2}, Torr	82±1	75±1*	77±2*
Pv_{O_2}, Torr	39±1	28±1*	31±1*
Mixed venous lactate, mM	1.0±0.2	1.3±0.1	1.2±0.2
Blood volume, ml	1,806±162	1,924±206	1,943±215
Exercise			
Hematocrit	35±1	50±1*	49±2*
Ca_{O_2}, vol%	14.4±0.5	12.8±0.9	18.4±0.9*†
Oxygen delivery, ml O_2/min	1,069±68	863±62*	1,042±52†
Heart rate, beats/min	212±4	224±6	195±6*†
Stroke volume, ml/beat	35±2	31±2*	30±2*
Cardiac output, l/min	7.5±0.5	6.8±0.5	5.8±0.4*†
Mean arterial pressure, mmHg	103±5	102±4	112±5*
Pa_{O_2}, Torr	79±2	78±1	79±2
Pv_{O_2}, Torr	35±2	24±1*	29±1*†
Mixed venous lactate, mM	1.8±0.6	2.5±0.5*	1.6±0.2†

Values are means ± SE. MetHb, methemoglobin; MB, methylene blue; Ca_{O_2}, arterial O_2 concentration; Pa_{O_2}, arterial P_{O_2}; Pv_{O_2}, venous P_{O_2}. *$P < 0.05$ compared with control. †$P < 0.05$ compared with MetHb.

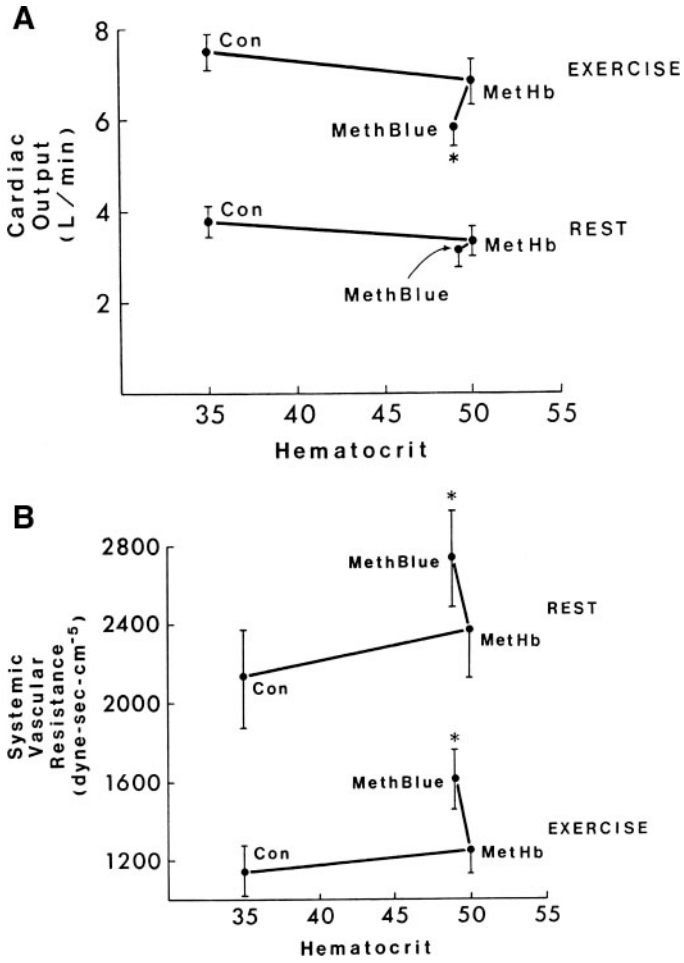

Fig. 1. *A*: cardiac output vs. hematocrit (%) at rest and with exercise. *B*: systemic vascular resistance at rest and with exercise. Con, control; MetHb, methemoglobin; MethBlue, methylene blue. *$P < 0.01$.

an increase in mixed venous lactate, the latter being significant only with exercise. Arterial P_{O_2} dropped slightly at rest but did not change with exercise.

Methylene blue phase. Intravenous methylene blue was administered to convert the methemoglobin to hemoglobin. After methylene blue administration, 98% of the hemoglobin was oxyhemoglobin. Hematocrit after methylene blue was not significantly different from the methemoglobin phase of polycythemia but remained significantly higher than the normocythemic control. Oxygen content increased markedly after methylene blue administration vs. both the methemoglobin polycythemic phase and the control. Cardiac output fell during exercise compared with both the control and methemoglobin phase. A trend toward a fall in resting cardiac output after methylene blue was not significant (Fig. 1). Stroke volume remained less than control but was unchanged from the methemoglobin phase of the study. Heart rate decreased to control values at rest and during exercise was even lower than during control studies. Systemic vascular resistance increased compared with both the control and methemoglobin phases (Fig. 1*B*). Oxygen delivery returned to control values after methylene blue as did mixed venous lactate. Mixed venous P_{O_2} increased compared with methemoglobin values but did not return to control levels. Arterial P_{O_2} was not changed during exercise but was slightly lower than control at rest.

Table 2. *Viscosity of red blood cells containing primarily methemoglobin before and after methylene blue*

		Shear Rate, s^{-1}					
Viscosity	Hct	5	11	21	42	106	212
Dog 1							
Before MB	56	24.7	15.9	11.3	8.3	6.3	5.4
After MB	55	18.5	13.1	8.8	7.4	5.5	4.9
Dog 2							
Before MB	51	18.6	12.2	9.8	7.8	6.1	5.3
After MB	51	16.4	12.2	10.3	8.1	6.3	5.6

Values are given in cPo.

Viscosity was measured in two dogs after transfusion with red blood cells containing methemoglobin both before and after methylene blue and was unchanged (Table 2). Blood volumes were unchanged from control (1,806 ± 162 ml) to the methemoglobin phase (1,925 ± 206 ml) to after methylene blue (1,943 ± 215 ml).

Two dogs instrumented in the same fashion as the other dogs in this study were exercised twice before and 90 min after methylene blue (2 mg/kg) without altering hematocrit. Methylene blue did not change any hemodynamic measurements (Table 3).

DISCUSSION

While both viscosity and oxygen content normally increase with polycythemia, we sought to change these variables independently. We found that changes in cardiac output and systemic vascular resistance correlate strongly with the oxygen content of blood and are not solely regulated by changes in blood viscosity. When polycythemia was created by isovolemic exchange transfusion with cells containing methemoglobin, viscosity increased without an increase in arterial oxygen content. Although viscosity was not measured at baseline, viscosity is known to consistently increase with increasing hemoglobin or hematocrit (26, 31, 33). Despite this increased viscosity, cardiac output and systemic vascular resistance (systemic vascular resistance) were unchanged from values at normal hematocrits. This was particularly clear during exercise. However, when oxygen content was increased after methylene blue without altering viscosity, exercise cardiac output decreased and systemic vascular resistance increased substantially. Thus, in this hematocrit range, systemic vascular resistance and cardiac output are actively regulated according to tissue oxygen demand and are not governed by blood viscosity

Table 3. *Exercise hemodynamics before and after methylene blue in two dogs*

	HR, beats/min	CO, l/min	MAP, mmHg	LAP, mmHg	SVR, dyn·s·cm^{-5}
Control 1	190	4.6	113	6.0	1,878
Control 2	178	4.8	103	5.0	1,633
Control Post-MB 1 (90 mins)	190	5.4	120	6.5	1,703
Control Post-MB 2 (90 mins)	190	5.2	120	5.5	1,769

HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; LAP, mean left atrial pressure; SVR, systemic vascular resistance.

levels. However, if oxygen content were the sole factor involved in regulating cardiac output, a rise in the cardiac output would have been expected when oxygen delivery decreased after exchange transfusion with red blood cells containing methemoglobin. Therefore, it is possible that viscosity has some role, albeit a minor role, in the regulation of cardiac output. A less likely possibility is that the multiple exchange transfusions led to some myocardial depression, preventing a rise in cardiac output.

Our data demonstrating that systemic vascular resistance and cardiac output are regulated in great measure by oxygen delivery support recent studies clarifying the regulation of the arterial microcirculation (7, 16, 35). Nitric oxide reacts with hemoglobin under physiological conditions to form *S*-nitrosohemoglobin. At high oxygen concentrations nitric oxide remains bound to hemoglobin, whereas at low oxygen concentrations nitric oxide is preferentially released, causing vasodilation and a decrease in systemic vascular resistance and an increase in cardiac output.

We have concluded that the increased systemic vascular resistance and decreased cardiac output that followed the conversion of methemoglobin to oxyhemoglobin with methylene blue were due to an increase in the oxygen content of the red blood cells. However, the possibility that the effect of methylene blue altered resistance primarily through inhibition of nitric oxide needs to be addressed. Whereas intravenous methylene blue is highly effective in converting methemoglobin to hemoglobin and is used for this purpose clinically, methylene blue inhibits guanylate cyclase in in vitro vascular strip preparations and in in vivo models (22). Inhibition of guanylate cyclase prevents nitric oxide signaling (21, 27). At issue is whether in our experiments administration of methylene blue to dogs with methemoglobinemia could have increased systemic vascular resistance through decreases in nitric oxide-mediated vasodilation rather than through a physiological response to the concomitant increase in oxygen-carrying capacity. We believe that a direct effect on nitric oxide sufficient to increase systemic vascular resistance substantively in our experiments is an unlikely mechanism for several reasons. First, we specifically addressed this issue by treating two dogs that did not have methemoglobinemia with methylene blue. There was no consistent increase in systemic vascular resistance in these dogs given the same dose of methylene blue with the same time course as the dogs with methemoglobinemia. Therefore, it would appear that the rise in systemic vascular resistance was related to reversal of methemoglobin to hemoglobin and not to a direct effect on endothelial nitric oxide through inhibition of guanylate cyclase. Second, methylene blue is rapidly metabolized and has a short half life in blood. In one report, the plasma half-time of a 2 mg/kg iv dose of methylene blue was <8 min (4). In clinical settings when methylene blue is administered for vascular effects in shock, it is given as a prolonged intravenous infusion of at least 30 min, not as a bolus. By giving this dose and waiting 30–45 min, we very likely avoided any substantive vascular effect of a dose given as a bolus over 1–2 min. Finally, even with prolonged intravenous infusions the effects of methylene blue on systemic vascular resistance have been inconsistent. For example Loeb and Longnecker (23) reported that with intravenous dosing in intact, anesthetized rats, methylene blue administration decreased systemic vascular resistance, whereas, in contrast, *N*-monomethyl-L-arginine administration increased systemic vascular resistance. Evgenov et al. (6) observed no effects of infusions of methylene blue alone on hemodynamics in lambs, although infusions of methylene blue did blunt endotoxin-induced vasodilation. Zhang et al. (39) studied anesthetized mongrel dogs during endotoxic shock. In the control animals, low doses of methylene blue at 2.5 mg/kg resulted in trivial increases in systemic vascular resistance and decreases in cardiac output of $<5\%$, whereas changes in our study were 30–40%. Thus we believe that the hemodynamic changes we noted are not likely to be due to the effects of methylene blue on the nitric oxide system.

Although the increases in hematocrit in this study were modest, they were adequate to provoke changes in cardiac output and systemic vascular resistance. Viscosity increases to a much greater degree above a hematocrit of 60, and it is possible that at higher levels of hematocrit viscosity plays a more dominant role in the regulation of cardiac output.

Only red blood cells in which hemoglobin was completely converted to methemoglobin were used in this study because partial oxidation may shift the oxyhemoglobin dissociation curve to the left, limiting oxygen delivery to an even greater degree than predicted by the oxygen content (15, 25). Blood viscosity was not changed when methemoglobin was converted to oxyhemoglobin in two animals over a wide range of shear rates. This was not surprising because methemoglobin does not effect the functional integrity of human red blood cells (15).

We conclude that the cardiac output response to polycythemia is, at least in major part, a function of oxygen delivery. Our data demonstrate that sensing of arterial oxygen content effects those hemodynamic changes. Other data suggest that changes in arterial oxygen saturation also produce systemic hemodynamic changes. Overall, there is considerable evidence to support the notion that cardiac output and systemic vascular resistance are linked to oxygen delivery, even though responses to different forms of hypoxemia may differ. Cardiac output increases in response to hypoxic hypoxemia when P_{O_2} falls without a change in viscosity (18, 19, 29). However, the effects of decreased arterial P_{O_2} on the chemoreceptors may lead to different results than when oxygen content is decreased by other means (36). Anemic hypoxemia has been less well studied. Although arterial P_{O_2} does not change in this circumstance, hematocrit, viscosity, and oxygen content fall and cardiac output increases. In this circumstance, the cardiac output increase has been attributed to the decrease in blood viscosity so that the effects of decreased oxygen delivery alone, without a change in P_{O_2} or viscosity, have been unclear (20). Of three previous studies of methemoglobinemia, in two there were rises in cardiac output with stable hematocrit (8) and in one there was no change (25). This latter study was performed in anesthetized, artificially ventilated animals where the response to hypoxemia may be inhibited (18). Results of studies with carbon monoxide have been variable perhaps because it also shifts the oxygen-hemoglobin dissociation curve to the left, creating more severe tissue hypoxia (36, 38). However, it has been reported that cerebral blood flow increases more with carbon monoxide hypoxemia than with hypoxic or anemic hypoxemia when oxygen contents are equivalent (17). In addition. others have observed increases in cardiac output and skeletal muscle flow in normal human subjects during reduction in oxyhemoglobin by carbon mon-

oxide (7). Perhaps these observations illustrate regulation of blood flow to preserve tissue P_{O_2} in the face of the combined threats of decreased oxygen content and a left-shifted oxygen-hemoglobin dissociation curve. Finally, in studies of generalized hypoxemia, autoregulation occurred in response to decreased arterial oxygen content despite complete denervation (34). Our study separates the effects of viscosity and oxygen content and systemic hemodynamic changes and demonstrates that sensing of oxygen content is a significant factor in the hemodynamic response to polycythemia. Thus, although there is some variation, studies of various forms of hypoxemia have supported the concept that systemic vascular resistance and cardiac output are regulated in accordance with tissue oxygen delivery and not solely influenced by blood viscosity.

The sensitivity of the electromagnetic flow probe is reported to be affected by hematocrit. However, these changes usually are unimportant in the hematocrit range under study here, especially in electromagnetic flow probes with high input impedance such as we used in this study (30). Indeed, we have found no consistent changes in probe sensitivity even at higher hematocrits (22).

This study demonstrates that systemic vascular resistance and cardiac output are regulated by oxygen content during modest levels of polycythemia.

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