Effects of modest anemia on systemic and coronary circulation of septic sheep

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Bloos, Frank, Claudio M. Martin, Chris G. Ellis, and William J. Sibbald. Effects of modest anemia on systemic and coronary circulation of septic sheep. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2195–H2204, 1999.—Although a lower transfusion trigger is generally recommended, little evidence is available about the physiological mechanisms of mild anemia in diseases with an imbalance between O2 supply and O2 demand such as sepsis. This study was undertaken to describe the systemic and coronary metabolic O2 reserve in an awake sheep model of hyperdynamic sepsis comparing two different hemoglobin levels. Twenty-four hours after sheep were rendered septic by cecal ligation and perforation (CLP), blood transfusion (n = 7, hemoglobin = 120 g/l) and isovolemic hemodilution (n = 8, hemoglobin = 70 g/l), respectively, were performed. Another 24 h later, we measured hemodynamics, organ blood flows, and systemic and myocardial O2 metabolism variables at baseline and through four stages of progressive hypoxia. Maximum coronary blood flow was 766.3 ± 87.4 ml·min⁻¹·100 g⁻¹ in hemodiluted sheep group versus 422.7 ± 53.7 ml·min⁻¹·100 g⁻¹ in the transfused sheep (P < 0.01). Myocardial O2 extraction was higher in the transfusion group (P = 0.03) throughout the whole hypoxia trial. In the hemodilution group, coronary blood flow increased more per increase in myocardial O2 uptake than in transfused sheep (P < 0.01). This was accompanied by a lower left ventricular epicardial-to-endocardial flow ratio in hemodiluted sheep (1.13 ± 0.07) than in transfused sheep (1.34 ± 0.02, P < 0.05). We conclude that the lower coronary blood flow and greater myocardial O2 extraction in transfused septic sheep preserves transmyocardial O2 metabolism better in comparison to hemodiluted sheep.

Methods

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H2195
normal hemoglobin levels supported a superior ability to extract $O_2$ in the coronary circulation.

**METHODS**

Fifteen mature, male Suffolk sheep weighing 39–76 kg (average 56.7 kg) underwent instrumentation following 1 wk of acclimatization in our laboratory. On the first day of study, the study animal was anesthetized with halothane and 100% $O_2$ (5–6 l/min) via mask, after which the trachea was intubated and the sheep was ventilated with 100% oxygen. Through a left posterolateral thoracotomy and with the use of a sterile technique, a saline-filled Silastic catheter (0.125 in. OD, Dow Corning, Midland, MI) was inserted into the left atrium, secured, and exteriorized. The coronary sinus was retrogradely cannulated via the hemiazygos vein with similar grade tubing as previously described (6). Correct placement of this coronary sinus catheter was confirmed during surgery from the demonstration that its $O_2$ saturation was <30%. Using a direct cutdown technique, we then placed saline-filled Silastic catheters (0.125 in. OD) into the left femoral and carotid arteries, while the left external jugular vein was cannulated with a 8-Fr introducer (Cordis, Miami, FL).

After recovery, the sheep were placed in metabolic cages and allowed free access to food and water. Ringer lactate (4 ml·kg$^{-1}$·min$^{-1}$) was administered postoperatively to maintain adequate hydration. Analgesia was provided with meperidine, 100 mg admixed with each 1,000 ml of Ringer lactate. Catheter patency was maintained by intermittent flushes with heparinized saline (1,000 U heparin/500 ml saline).

**Experimental protocol.** Three days after the initial surgery, a 7-Fr Swan-Ganz catheter (model 93–131; American Edwards, Santa Ana, CA) was flow directed into the pulmonary artery through the jugular vein introducer (Fig. 1). A baseline, nonseptic study was then performed with the sheep in a conscious state. Systemic arterial and pulmonary artery pressures and cardiac output were measured. Blood was simultaneously obtained from the carotid artery, central vein, and coronary sinus to measure blood gases, hemoglobin, and lactate. After this nonseptic study, a partial omentectomy was performed under general halothane anesthesia. The cecum was then located, devascularized, and ligated, and the tip was incised. The abdominal wound was closed in two layers with 2–0 coated vicryl ties, and a sterile tracheostomy was performed. A 39-Fr low-pressure cuffed tracheostomy tube (Shiley) was inserted into the trachea and connected to a system providing humidified and warmed air.

Sheep were randomly allocated to either an RBC transfusion group (group T) or a hemodilution group (group H). In group H, blood was drawn from the carotid artery, and pentastarch was infused isovolemically into the external jugular vein to reach a hemoglobin level of 70 g/l. Sheep in group T received fresh packed RBCs taken from a donor sheep on the transfusion day titrated to a hemoglobin of 120 g/l. The transfusion and hemodilution interventions were completed during the first 24 h after peritoneal contamination to allow circulatory compensation to be adequately expressed before the hypoxia intervention. A pilot study demonstrated that the interval between randomization and the hypoxia study was, however, short enough to maintain the group differentiation according to different hemoglobin levels.

As previously described (5, 6, 29), CLP leads to a panperitonitis and polymicrobial bacteremia. Throughout the time between the laparotomy and the study 48 h later, pentastarch was infused to maintain left atrial pressures (LAP) at nonseptic baseline levels. Analgesia was continued as previously detailed and increased if the sheep showed discomfort.

Forty-eight hours after the CLP, sheep were connected to a semiope system to lower the inspired $O_2$ concentration ($F_{O_2}$) by mixing room air with nitrogen (Bird $O_2$ blender, Palm Springs, CA). This system was connected to a metabolic monitor (DeltaTrac II, Datex Instrumentation, Helsinki, Finland) to directly measure systemic $O_2$ consumption. We repeated all measurements described for the nonseptic study. A radioactive microsphere was then injected to allow later calculation of organ blood flows, while coronary sinus blood was obtained to calculate myocardial $O_2$ uptake and lactate extraction. Using a polarographic $O_2$ monitor (model 5570, Ventronics), we subsequently reduced the $F_{O_2}$ through up to four successive stages, adjusting to achieve a similar decrease in $C_AO_2$ between stages. The previous study by Bloos et al. (6) demonstrated the lowest $F_{O_2}$ that could be tolerated in this unanesthetized model approximated 0.1. Hemoglobin and arterial $O_2$ saturations were measured at and in between each stage to calculate the $C_AO_2$. Twenty minutes of equilibration time was allowed at each experimental level before measurements were repeated, as described in the 48-h baseline study, including the infusion of another set of radioactive microspheres. After the final stage of hypoxia, the study animal was euthanized with pentobarbital, and organs of the animal were then harvested for gamma counting to calculate organ blood flows.

The study protocol was approved by the University Council on Animal Care in accordance with the guidelines set down by the Canadian Council on Animal Care. During the experiment, all animal care was provided by a physician and qualified animal health technicians. All surgery was performed in a surgical suite certified for animal surgery.

Specific measurements and calculations. Systemic and pulmonary pressures were recorded with a two-channel monitor (model 78353A, Hewlett-Packard) and were referenced to the sheep's left atrium. Cardiac outputs were measured in triplicate by the thermodilution technique using a cardiac output computer (model 9570A, Hewlett-Packard). The cardiac output was indexed to the body surface area, and heart work was estimated as the product of cardiac index and mean aortic pressure (1). Blood gas samples were stored on ice before analysis with an ABL-3 blood gas analyzer (Radiometer, Copenhagen, Denmark).

During the experiment, the hemoglobin and the $O_2$ saturations were measured by a co-oximeter (OSM-11 Hemoximeter, Radiometer, Copenhagen, Denmark). Subsequently, hemoglobin levels were confirmed by a Coulter Cell Counter (model S, Burlington, Ontario, Canada). Lactate was measured by a Greiner G-400 Chemistry Analyzer (Switzerland).

Measurement of organ blood flows. As previously described in this sheep model (5, 6, 29), the microsphere technique was illustrated above.
Table 1. Effects of different hemoglobin levels on systemic hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Hemodilution Before</th>
<th>48 h After</th>
<th>Transfusion Before</th>
<th>48 h After</th>
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<tbody>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>39.0 ± 0.0</td>
<td>39.6 ± 0.2†</td>
<td>39.2 ± 0.1</td>
<td>39.8 ± 0.1†</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.31 ± 0.01</td>
<td>0.22 ± 0.01*§</td>
<td>0.30 ± 0.01</td>
<td>0.30 ± 0.00§</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>91 ± 2</td>
<td>90 ± 3</td>
<td>96 ± 4</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>Hemodynamics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>101 ± 6</td>
<td>106 ± 6</td>
<td>110 ± 6</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>3 ± 1</td>
<td>7 ± 2*</td>
<td>5 ± 1</td>
<td>7 ± 1*</td>
</tr>
<tr>
<td>LAP, mmHg</td>
<td>8 ± 1</td>
<td>10 ± 1*</td>
<td>11 ± 1</td>
<td>12 ± 1*</td>
</tr>
<tr>
<td>CI, l·min⁻¹·m⁻²</td>
<td>4.7 ± 0.2</td>
<td>7.3 ± 0.6†</td>
<td>4.9 ± 0.1</td>
<td>6.1 ± 0.2†</td>
</tr>
<tr>
<td>SVR, dyn·s⁻¹·m⁻²</td>
<td>1,723 ± 193</td>
<td>1,141 ± 148‡</td>
<td>1,731 ± 80</td>
<td>1,295 ± 50‡</td>
</tr>
<tr>
<td>Systemic O₂ metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂ delivery, ml·min⁻¹·m⁻²</td>
<td>653 ± 33</td>
<td>696 ± 59‡</td>
<td>685 ± 30</td>
<td>869 ± 37‡</td>
</tr>
<tr>
<td>O₂ uptake, ml·min⁻¹·m⁻²</td>
<td>288 ± 31</td>
<td>289 ± 19</td>
<td>293 ± 13</td>
<td>271 ± 4</td>
</tr>
<tr>
<td>O₂ extraction</td>
<td>0.44 ± 0.04</td>
<td>0.42 ± 0.02</td>
<td>0.43 ± 0.01</td>
<td>0.31 ± 0.01*§</td>
</tr>
</tbody>
</table>

Effects of hemodilution versus transfusion on central hemodynamic and O₂ metabolism variables, 48 h after cecal ligation and perforation (CLP). All data are means ± SE. PaO₂, arterial PaO₂; CVP, coronary venous pressure; LAP, left atrial pressure; CI, cardiac index; SVR, systemic vascular resistance. Significance is *P < 0.05, †P < 0.01 vs. the nonseptic baseline measurement. Significance 48 h after CLP is ‡P < 0.05, §P < 0.01 between groups.
CaO₂ was significantly greater in group T and group H than in the baseline study. By the final hypoxic stage, the overall depression in systemic O₂ delivery remained unchanged. The arterial Po₂ fell significantly in both groups compared to the baseline study. Table 2 compares the baseline study to the final hypoxic stage of selected hemodynamic and O₂ metabolism values. The final F₁₀₂ approximated 0.11 in both study groups by the end of the fourth study stage; thus the arterial Po₂ fell significantly in both groups. By the final hypoxic stage, the overall depression in CaO₂ was significantly greater in group T compared with group H (Fig. 2). Neither mean blood pressure nor mean pulmonary artery pressures were affected by the hypoxic intervention in either study group. The cardiac index was greater at baseline in group H compared with group T. Coincident with an increase in the heart rate, the cardiac index increased in both groups between the baseline and final hypoxic study stages and remained higher throughout all hypoxic stages in group H versus group T.

Table 2 also records the depression in systemic O₂ delivery that occurred between baseline and the final hypoxic study stages. During this study period, the systemic O₂ uptake remained unchanged throughout all four stages of hypoxia in group H (Fig. 3). Reducing the F₁₀₂ was accompanied by an increase in systemic O₂ extraction in both study groups (group H, P < 0.01; group T, P < 0.01). A significant group effect during hypoxia in systemic O₂ extraction (P < 0.01) was likely explained by the lower baseline value in group T because the maximal value was similar in both study groups. In both study groups, arterial lactate rose modestly but significantly by the final stage of study.

Effects of hypoxia on myocardial O₂ metabolism variables. The effect of hypoxia on myocardial O₂ metabolism is demonstrated in Figs. 4–8 and Table 3. Figure 4 shows the effect of study interventions on both myocardial O₂ uptake and heart work, the latter estimated as the mean blood pressure times cardiac index. Analysis of variance for VO₂: hypoxia effect, P < 0.01; group effect, P < 0.01. Analysis of variance for VO₂, hypoxia effect, not significant (NS); group effect, NS.
Cardiac index product. This figure demonstrates that the progressive reduction in $FIO_2$ was accompanied by significant and similar increases in myocardial work and myocardial O2 uptake in both study groups. Accordingly, coronary blood flow increased in both groups with progressive hypoxia but was significantly greater in group H throughout the entire hypoxia intervention ($P < 0.01$). The left ventricular endocardial-to-epicardial flow ratios were not affected by the hypoxic intervention in either study group, although they were significantly lower in group H than in group T during all study stages (Fig. 5). Similar to the increase in arterial lactate levels, coronary sinus lactate levels increased in both study groups during severe hypoxia (Table 3).

Although myocardial O2 delivery increased as the $FIO_2$ was reduced in hemodiluted and transfused sheep ($P < 0.01$), there were no group differences during any of the hypoxic study periods (Fig. 6). At baseline, myocardial O2 extraction was similar in group H (0.78 ± 0.04) and group T (0.79 ± 0.02) study groups but was greater in group T throughout the entire hypoxia intervention ($P = 0.03$). ANOVA did not find an overall statistical change over the time course, suggesting a different behavior of myocardial O2 extraction over time. However, the interaction lacked statistical significance. Figure 7 demonstrates that coronary sinus PO2 was greater in group H compared with group T at any level of arterial PO2 ($P < 0.01$). The maximum increase in coronary blood flow during the hypoxic intervention was significantly greater in group H (766.3 ± 87.4 ml·min⁻¹·100 g⁻¹) compared with group T (422.7 ± 53.7 ml·min⁻¹·100 g⁻¹; $P < 0.01$). Figure 8 compares changes in coronary blood flow and myocardial O2 uptake as the $FIO_2$ was reduced and demonstrates that the level of this relationship was greater in group H than that in group T studies ($P < 0.01$). The slopes of the two regression lines also differed ($P < 0.01$), thus demonstrating that coronary blood flow increased more in group H than in group T sheep when myocardial O2 needs rose.

Effects of hypoxia and hemoglobin level on regional O2 delivery relationships. Figure 9 demonstrates that the hypoxic intervention was accompanied by an increase in the regional O2 delivery ($QO_2$) to the heart in both groups. $QO_2$ to the brain was not affected by the hypoxic trial. Both groups reduced $QO_2$ to the gallbladder, kidneys, and spleen. There was a statistically significant reduction in regional $QO_2$ to the liver, pancreas, rumen, and small and large gut in the transfusion group only. The hemodilution group only showed a tendency to reduce $QO_2$ to these organs without being statistically significant.

Table 3. Effects of hypoxia on coronary circulation

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Last Stage</th>
</tr>
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<tbody>
<tr>
<td>Coronary blood flow, ml·min⁻¹·100 g⁻¹</td>
<td>198 ± 24†</td>
<td>715 ± 84†‡</td>
</tr>
<tr>
<td>Hemo</td>
<td>137 ± 14§</td>
<td>451 ± 108‡</td>
</tr>
<tr>
<td>Trans</td>
<td>20.6 ± 3.5</td>
<td>15.0 ± 3.2*</td>
</tr>
<tr>
<td>CS lactate, mmol/l</td>
<td>0.2 ± 0.0</td>
<td>1.2 ± 0.4†</td>
</tr>
<tr>
<td>Hemo</td>
<td>27.3 ± 3.1</td>
<td>16.7 ± 5.3*‡</td>
</tr>
<tr>
<td>Trans</td>
<td>0.3 ± 0.1</td>
<td>0.7 ± 0.2†</td>
</tr>
</tbody>
</table>

Effects of hypoxia on coronary O2 metabolism variables comparing baseline study versus the last stage of hypoxia by the groups Hemo versus Trans. ScSO2, coronary sinus O2 saturation; CcSO2, coronary sinus O2 content; CS lactate, coronary sinus lactate. Analysis of variance: *P < 0.05, †P < 0.01 (hypoxia effect); ‡P < 0.05 (group effect); §there was no significant interaction.

Fig. 5. Left ventricular endocardial-to-epicardial blood flow ratios. Because there is no interaction or time effect, bars represent flow ratios of all stages for each group. Flow ratios differ with $P = 0.026$.

Fig. 4. Changes of heart work (BPM, beats/min; Cl, cardiac index; A) and myocardial VO2 (B) over change of CaO2 during hypoxia trial. Analysis of variance for heart work estimated by product of mean blood pressure and cardiac index: hypoxia effect, $P < 0.01$; group effect, NS. Analysis of variance for myocardial VO2: hypoxia effect, $P < 0.05$; group effect, NS. C, Hemodilution; ○, transfusion.
DISCUSSION

Critical appraisal has shown considerable variation in both practice and opinion regarding the appropriate RBC transfusion trigger in sepsis (1, 42). Because this may be related to a paucity of basic research, we designed an experiment to measure and compare the effect of two clinically relevant RBC transfusion strategies on the metabolic O2 reserve of circulation in septic sheep. We found that isovolemic hemodilution to maintain hemoglobin levels between 75 and 80 g/l during sepsis imposed changes on the adaptation of circulation to acute hypoxia, which have not been previously reported in healthy models. Novel information from this experiment is the demonstration that maintaining normal hemoglobin levels by RBC transfusion was accompanied by a greater coronary flow reserve, a better intramyocardial blood flow distribution, and a greater capacity to extract O2 in the septic coronary circulation.

Background. Sepsis is characterized by a quantifiable tissue injury, which may lead to the multiple organ dysfunction syndrome. Sepsis is also a hypermetabolic state, where tissue O2 needs are markedly increased. Where research has linked outcomes from sepsis with an inability to match O2 delivery to these elevated tissue O2 needs (43), treatment strategies have emphasized optimizing increasing systemic O2 delivery by 1) increasing the cardiac output with intravascular volume expansion and/or inotropic therapy, and/or 2) increasing CaO2 with RBC transfusion and measures that improve arterial oxygenation (i.e., supplemental O2 and positive end-expiratory pressure). Because it is not uncommon to find modest anemia in septic patients, the appropriateness of transfusing RBCs to increase tissue O2 availability has been frequently discussed (2, 9, 43) yet not explicitly examined.
A finely regulated control system distributes O₂ delivery to match the metabolic O₂ need of the tissue. Because some organs have a limited ability to increase O₂ extraction, isovolemic hemodilution in health is accompanied by an increase in convective O₂ delivery to the heart and brain (22, 30, 38, 43). Such compensation is facilitated by a redistribution of O₂ delivery away from organs with a greater O₂ extraction reserve, such as the splanchnic circulation (22, 28). The effectiveness of this compensation is evident in recent guidelines which propose that anemia in previously healthy patients can be tolerated to hemoglobin levels as low as 60–70 g/l (2).

In contrast to health, an argument may be made that the RBC transfusion trigger should be higher in sepsis. For example, sepsis is a hypermetabolic process, and increasing the metabolic rate in healthy animal models elevates the optimal hematocrit of the gut (22). Second, sepsis is characterized by circulatory abnormalities that impact on tissue O₂ delivery, including a limited cardiac output reserve (4), an impaired capacity of O₂ delivery from the splanchnic organs to the heart and brain (5), and microcirculatory dysfunction which limits O₂ extraction capacity (24). We have assessed adequacy of the circulatory reserve when anemia complicates sepsis in different approaches. We found that 1) the hemoglobin concentration exerted independent and negative effects on regional O₂ delivery in septic sheep (17); 2) sepsis in sheep depresses the capacity to increase both coronary blood flow and myocardial O₂ extraction during acute hypoxia, compared with control sheep (6); and 3) the ability to appropriately increase myocardial O₂ delivery during acute hypoxia in septic rats occurred only in animals transfused to maintain hematocrit levels >45% (30). In contrast and confirming previous work (10, 20), nonseptic rats maintained myocardial O₂ delivery reserve to hypoxia with hematocrits <30%. 4) The hemoglobin dissociation curve was shifted left in septic sheep compared with that in nonseptic study conditions (7). Taken together, these data are indirect evidence that sepsis may elevate the “optimal” hemoglobin concentration, which would be clinically acknowledged as a need to transfuse RBCs earlier in sepsis than in health.

The current experiment was therefore designed to test the hypothesis that isovolemic hemodilution to create modest anemia in mature sheep rendered septic by CLP would depress the ability of the heart to support hemodynamic circulation in this syndrome. We used a large animal model of sepsis complicating peritoneal contamination as previously described by our laboratory (5, 6, 29). This model reproduces the circulatory lesion, which has been reported at both the regional (5) and microregional (29) levels. To obviate potential confounding effects of anesthetic agents on both regional and microregional circulations, this study was carried out with the experimental animal awake (10). After baseline studies, we then exposed the animals to acute hypoxia to determine whether the usual metabolic O₂ reserve of the circulation was altered by hemoglobin status (3, 6, 40). Because the hypoxic intervention was accompanied by a modest increase in the arterial lactate concentration, it is probably reasonable to conclude that acute compensation to maintain O₂ availability in this animal model was exceeded during the final hypoxic study stage (31).

Animals allocated to group T had a mean study hemoglobin that approximated normal hemoglobin levels in sheep, whereas the mean study hemoglobin in group H animals was similar to values proposed as target values above which no transfusion is necessary in recent clinical guidelines (2). Because we have recently found that sepsis lengthens the time required to ensure steady-state conditions in the central and regional circulations (18), we completed interventions to distinguish anemic versus nonanemic sheep 24 h before the hypoxic intervention. We used RBCs stored in CPDA-1 (citrate, phosphate, dextrose, adenine), taken from a donor sheep on the same day as RBC transfusion, because Marik and Sibbald (26) noted that reduced RBC deformability complicating storage may limit tissue O₂ availability in sepsis.

Hemoglobin levels and systemic circulation’s metabolic O₂ reserve. Before the hypoxic intervention, both study groups demonstrated a circulatory profile that is typical of sepsis. Demonstrating that anemia imposed an added stress on the central circulation, group H sheep had a higher cardiac index than group T sheep, and this difference was maintained across all subsequent study stages. As O₂ delivery was sequentially reduced during acute hypoxia, an expected increase in systemic O₂ extraction was similar in both study groups. In a previous study by Bloos et al. (6), it was confirmed that sepsis in this animal model depressed the capacity to extract O₂. Data from the current experiment therefore demonstrate that the hemoglobin status of the animal does not influence the progression of this lesion. Because measurement of systemic O₂ extraction reflects the algebraic sum of changes occurring within individual organ circulations, it is possible that changes according to hemoglobin status might have occurred in individual organ circulations. Whereas hemodilution is known not to reduce regional O₂ delivery by increase of organ blood flows (38), severe hypoxia reduced regional O₂ transport to all noncardiac organs observed during this study regardless of the hemoglobin level. In a previous hypoxia study (6), Bloos et al. found that sham animals redistributed QO₂ from the gut to the heart, whereas septic sheep did not. Bloos et al. suggested that septic sheep were unable to increase O₂ extraction sufficiently to give up blood flow. In this study, we found a significant reduction in regional QO₂ to the gut in the transfusion group only. This might reflect a normalization of the O₂ extraction reserve of the gut in the transfusion group. However, this remains speculation because we did not measure the regional O₂ extraction of the gut.

Hemoglobin levels and coronary circulation’s metabolic O₂ reserve. Reducing O₂ content was accompanied by an increase in the cardiac index times blood pressure product, a surrogate end point for heart work (1). A parallel increase in myocardial O₂ uptake was the
consequence of an increase in flow work and confirms that the metabolic coupling of O₂ availability to changing O₂ needs remains intact in hyperdynamic sepsis. However, the mechanism by which myocardial O₂ uptake was supported differed according to the study subject's hemoglobin status. Thus 1) coronary blood flow was greater at all levels of myocardial O₂ uptake in group H compared with group T animals, and 2) the maximum myocardial O₂ extraction achieved was greater in group T compared with group H animals.

In health, hemodilution is accompanied by an increase in coronary blood flow, which may be greater than necessary to satisfy myocardial O₂ needs (3). Such relative overperfusion has been attributed to the drop of blood viscosity with hemodilution (39) and is accompanied by a maldistribution of coronary blood flows from subendocardial to subepicardial layers after severe hemodilution (3, 14). In our study, this subepicardial redistribution of coronary blood flows occurred already after mild hemodilution. Therefore, the greater dependence on using coronary blood flow reserve to support myocardial oxygenation in hemodiluted animals is a pattern consistent with the effects of hemodilution alone, albeit occurring at an earlier stage.

This enhanced dependence on coronary flow reserve in hemodiluted animals could also be explained by an effect of anemia to impair the O₂ extraction reserve of the heart. Hemodilution is normally accompanied by a modest increase in myocardial O₂ extraction (14), whereas Bloos et al. (6) previously found that sepsis blunts this compensation. The current study is consistent with our previous experiment, namely, an acute reduction in myocardial O₂ availability was not accompanied by a significant increase in the O₂ extraction of this organ. However, we did find that the capacity to augment myocardial O₂ extraction was greater in sheep transfused to normal hemoglobin levels, when normalized to CaO₂ (Fig. 4).

It is conceivable that the increase in blood viscosity that would have accompanied transfusion to normal hemoglobin levels in the sheep (21) explains the ability of this group to extract O₂ in the circulation of the heart at greater levels than in the anemic group. Sepsis is characterized by elevated blood flows (4), impaired arteriolar reactivity (5), and a loss of RBC-perfused capillaries (24). The usual microvascular response to normal isovolemic anemia includes changes in both microcirculatory hematocrit and in RBC flow distribution, because of a decline in both vascular hindrance and blood viscosity (35). Routing an elevated blood flow through circulatory networks with fewer perfused capillaries would boost RBC flow rates in remaining perfused capillaries and, by shortening transit times, potentially impede O₂ extraction. An increased blood viscosity in group T could therefore have led to greater RBC transit times, compared with group H, and thereby provided more time for capillary O₂ exchange to occur. The left-shifted O₂ dissociation curve in septic sheep would further be of disadvantage during low transit times (7). We did not measure myocardial transit times or directly assess the microcirculation of the heart, but the higher coronary sinus PO₂ at any level of arterial PO₂ in the hemodilution group might confirm such microcirculatory differences between the two study groups. Crystal (13) also proposed that excessively elevated RBC flow rates could explain an inability of the right ventricle to maximally extract O₂ during hemodilution. It is also possible that greater nitric oxide release in the peripheral microcirculations in group T versus group H could have provided further cytoprotective function in this model (13). Experiments from our laboratory support the possibility that increasing RBC levels minimizes progression of the septic microcirculatory injury. In a study demonstrating that significant increases in the number of stopped-flow capillaries was time dependent in septic rats, post hoc analysis demonstrated a negative relationship between the number of stopped-flow capillaries and the systemic hemoglobin concentration (34).

Methodological considerations. This study did not use a nonseptic control group to prove that CLP induced sepsis in the animals of this experiment. Sepsis is defined as the invasion of microorganisms and/or their toxins into the bloodstream together with the host response (8). CLP is known to produce panperitonitis and polymicrobial bacteremia. The presence of peritonitis was confirmed in each animal during the postmortem examination. Thus the animals had a focus of infection producing bacteremia. Our data support that there was also a systemic response to CLP: both groups required fluid resuscitation to maintain LAP. This was accompanied by an increase in cardiac index and a drop of systemic vascular resistance, both changes typical of sepsis. All animals demonstrated a statistically significant increase in body temperature. This model is well validated to produce sepsis in different models (41). In our laboratory, this model has proven to produce hyperdynamic sepsis in sheep.

We demonstrated that alterations of the coronary circulation in sepsis is aggravated during hemodilution. However, we did not prove that this alteration produces myocardial tissue ischemia. Coronary lactate levels increased similarly in both study groups. The problems of applying transmyocardial lactate metabolism to identify myocardial ischemia in this model has been discussed in detail in a previous paper by Bloos et al. (6). Briefly, the myocardium uses lactate as a nutrient. If arterial lactate delivery increases as it happens during severe hypoxia, the myocardium starts to increase myocardial lactate extraction. Thus we may observe an unchanged myocardial net lactate extraction despite cardiac ischemia. Proving tissue ischemia in a disease like sepsis is still not possible (37) and remains inferential.

The electrocardiogram is commonly used to identify an inadequate coronary perfusion. Sepsis causes a maldistribution of coronary blood flow within the heart (19), producing myocardial areas with very high as well as very low regional blood flow. The data of this study suggest that hemodilution aggravates maldistribution of coronary blood flow. It is not established whether S-T segment analysis can identify such an injury.
In summary, this is the first study to examine the effects of two clinically relevant hemoglobin levels on systemic and regional O2 delivery in an animal model of normotensive hyperdynamic sepsis. Transfusing to a normal hemoglobin level does not change the systemic O2 extraction reserve that is depressed by sepsis. The metabolic coupling between myocardial O2 need and coronary blood flow remained intact in both groups. However, mild hemodilution inflicted changes on the regional and microregional blood flow of the heart, which are considered unfavorable and are usually only seen after severe hemodilution. In hemodiluted septic animals, these changes include a lower coronary flow reserve, a redistribution of coronary blood flow to the subepicardial layer, and a lower myocardial O2 extraction.

This study was supported by a grant from the Heart and Stroke Foundation of Canada (Grant B2433). Current address for F. Bloos: Klinik f. Anästhesiologie und Intensivtherapie, Klinikum der Friedrich-Schiller-Universität, Jena 07740, Germany.

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Received 15 July 1998; accepted in final form 2 June 1999.

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