Acute vs. chronic effects of elevated hemoglobin O$_2$ affinity on O$_2$ transport in maximal exercise

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Acute vs. chronic effects of elevated hemoglobin O$_2$ affinity on O$_2$ transport in maximal exercise. J Appl Physiol 89: 265–272, 2000.—These studies were conducted to compare the effects on systemic O$_2$ transport of chronically vs. acutely increased Hb O$_2$ affinity. O$_2$ transport during maximal normoxic and hypoxic (inspired P$_{O_2}$ (P$_{Io2}$) = 70 and 55 Torr, respectively) exercise was studied in rats with Hb O$_2$ affinity that was increased chronically by sodium cyanate (group 1) or acutely by transfusion with blood obtained from cyanate-treated rats (group 2). Group 3 consisted of normal rats. Hb O$_2$ half-saturation pressure (P$_{50}$) during maximal exercise was ~26 in groups 1 and 2 and ~46 in group 3. In normoxia, maximal blood O$_2$ convection (V$_{O_2_{max}}$ = cardiac output × arterial blood O$_2$ content) was similar in all groups, whereas in hypoxia V$_{O_2_{max}}$ was significantly higher in groups 1 and 2 than in group 3. Tissue O$_2$ extraction (arteriovenous O$_2$ content/arterial O$_2$ content) was lowest in group 1, intermediate in group 2, and highest in group 3 (P < 0.05) at all exercise P$_{Io2}$ values. In normoxia, maximal O$_2$ utilization (V$_{O_2_{max}}$) paralleled O$_2$ extraction ratio and was lowest in group 1, intermediate in group 2, and highest in group 3 (P < 0.05). In hypoxia, the lower O$_2$ extraction ratio values of groups 1 and 2 were offset by their higher V$_{O_2_{max}}$, accordingly, their differences in V$_{O_2_{max}}$ from group 3 were attenuated or reversed. Tissue O$_2$ transfer capacity (V$_{O_2_{max}}$/mixed venous P$_{O_2}$) was lowest in group 1 and comparable in groups 2 and 3. We conclude that lowering Hb P$_{50}$ has opposing effects on V$_{O_2_{max}}$ and O$_2$ extraction ratio, with the relative magnitude of these changes, which varies with P$_{Io2}$ determining V$_{O_2_{max}}$. Although the lower O$_2$ extraction ratio of groups 2 vs. 3 suggests a decrease in tissue O$_2$ diffusion gradient secondary to the low P$_{50}$, the lower O$_2$ extraction ratio of groups 1 vs. 2 suggests additional negative effects of sodium cyanate and/or chronically low Hb P$_{50}$ on tissue O$_2$ transfer.

hemoglobin oxygen dissociation curve; leftward oxygen dissociation curve shift; chronic sodium cyanate administration; convective blood oxygen delivery; tissue oxygen extraction; tissue oxygen transfer capacity

CHANGES IN THE OXYGEN AFFINITY of hemoglobin (Hb) tend to influence the uptake of O$_2$ (V$_{O_2}$) by the blood in the lungs in one direction and its release in the tissues in the opposite direction. Because the magnitude of these effects varies at different levels of inspired P$_{O_2}$ (P$_{Io2}$), the net effect of a change in the O$_2$ affinity of Hb on systemic O$_2$ transport is difficult to predict and is still the subject of controversy (2, 11, 14, 20, 22, 24, 31). We recently showed (17) that increasing the O$_2$ affinity of Hb in rats [leftward shift of the O$_2$ dissociation curve (ODC); decrease in Hb O$_2$ half-saturation pressure (P$_{50}$)] by chronic administration of sodium cyanate resulted in a decrease in maximal O$_2$ utilization (V$_{O_2_{max}}$) during normoxic treadmill exercise. During hypoxic exercise, however, the magnitude of the difference in V$_{O_2_{max}}$ between cyanate-treated rats and untreated controls decreased; at the lowest P$_{O_2}$ values during exercise, V$_{O_2_{max}}$ was slightly higher in the cyanate-treated rats (17). These changes in V$_{O_2_{max}}$ were the net result of two opposing factors: an increase in the rate of the maximal convective delivery of O$_2$ to the tissues [V$_{O_2_{max}}$], the product of maximal cardiac output (Q$_{max}$) times the arterial blood O$_2$ concentration (Ca$_{O_2}$)] on one hand, and, on the other, a decrease in the O$_2$ extraction by the tissues, as represented by the O$_2$ extraction ratio (the ratio of the arteriovenous O$_2$ content difference to the Ca$_{O_2}$). In normoxic exercise, the leftward ODC shift increased convective O$_2$ delivery only modestly, whereas tissue O$_2$ extraction decreased substantially. Accordingly, V$_{O_2_{max}}$ was lower in the cyanate-treated rats. At lower P$_{O_2}$ values during exercise, the effect of the ODC shift on convective O$_2$ delivery increased, whereas that on O$_2$ extraction remained relatively constant, thus minimizing or even reversing the overall negative effect on V$_{O_2_{max}}$ (17).

The increase in the rate of O$_2$ delivery brought about by cyanate was exclusively due to an increase in Ca$_{O_2}$, because Q was not modified by sodium cyanate administration. The reason for the decrease in O$_2$ extraction, however, is less clear. It is likely that the leftward ODC shift tends to limit the diffusion of O$_2$ from the tissue capillary to the cell due to the reduced capillary P$_{O_2}$ that results from the shift. It is not clear, however, whether other mechanisms contribute to the decrease
in tissue O₂ extraction in the animals chronically treated with sodium cyanate. The observed decrease in the respiratory capacity of mitochondria isolated from livers of mice treated with sodium cyanate (21) suggests that, at least in some tissues, cyanate may interfere with cellular Vo₂. Alternatively, chronic cyanate administration results in “hypoxia-like” effects (28), such as polycythemia and pulmonary hypertension. These effects are likely due to the reduced tissue Po₂ secondary to the low Hb P₅₀; exposure to prolonged hypoxia could result in changes that affect O₂ extraction by the tissues.

The purpose of the present experiments was to extend our observations of the effect of a decrease in Hb P₅₀ on O₂ transport and maximal exercise capacity. Specifically, we investigated whether acutely elevating the O₂ affinity of Hb, by exchanging the blood from untreated animals with blood with low-Hb P₅₀, would have the same effect on O₂ transport as that produced by chronic ingestion of sodium cyanate. The hypothesis tested was that, if the changes in O₂ transport produced by cyanate administration in our previous experiments were due solely to the increase in O₂ affinity of Hb and not to other effects, the results of acute and chronic leftward shifts of the ODC should be the same.

METHODS

Animal model. Male Sprague-Dawley rats weighing 225–250 g were randomly assigned to two groups: a group that received 0.2% sodium cyanate in the drinking water for 3 wk and a nontreated group. Cyanate irreversibly carbamylates the amino terminus of valine and results in an increase in the O₂ affinity of Hb (1). Sodium cyanate administration was discontinued at 3 wk; 7–10 days later, sodium cyanate-treated and nontreated animals were anesthetized with pentobarbital anesthesia; −12–14 ml of blood were withdrawn from each donor, after which the animal was euthanized with an anesthetic overdose. Exchange transfusion was carried out within 30 min of blood withdrawal from the donor. Approximately 20 ml of blood were exchange-transfused in each animal from groups 2 and 3.

One hour after the transfusion was finished, the animals exercised according to the protocol described below. Each experimental group was subdivided into three subgroups of six to seven animals each, which exercised at PVT values of 140, 70, or 55 Torr. Each rat exercised only once.

The effectiveness of the exchange transfusion with carbamylated blood in reducing Hb P₅₀ of the circulating blood of the recipient was established in preliminary experiments. The mixing technique (8) was used to measure Hb P₅₀ in whole blood at pH 7.40, Pco₂ of 40 Torr, and temperature of 37°C. In four rats treated with sodium cyanate as in group 1, P₅₀ was 21.7 ± 0.9 Torr; this value was not significantly different from that of 22.4 ± 0.7 Torr obtained in the blood of four rats that had undergone exchange transfusion with 22 ± 1.2 ml of blood obtained from cyanate-treated rats (as in group 2). This represents a substantial decrease from the normal value of standard Hb P₅₀ of untreated rats, which is ~34 Torr (8).

Exercise protocol. After measurement of rectal temperature, the animals were placed on a treadmill enclosed in an airtight Lucite chamber adapted for the determination of Vo₂ and CO₂ production (Vco₂) using the open-circuit method (8). The catheters were connected, through sampling ports located on the top of the box enclosing the treadmill, to pressure transducers. After 30 min on the treadmill, arterial and mixed venous blood samples were obtained via stopcocks. The blood was replaced with homologous fresh blood from the same group, and the treadmill was set at a speed of 10 m/min. This speed was maintained for 2–3 min, after which the treadmill was set at an angle of 10° and the speed increased by 4 m/min every 90–120 s, until VVo₂ max was reached. VVo₂ max was defined as the Vo₂ for which an increase in work rate resulted in no further increase (±5%) in VVo₂. If a plateau in VVo₂ max was not observed at the highest two workloads, the animal was discarded. Approximately 80% of all rats achieved VVo₂ max as defined here. Arterial and mixed venous blood samples were obtained during the last 45–60 s of exercise, while VVo₂ and VVco₂ values remained steady. The box enclosing the treadmill was opened, and the rectal temperature was determined within 30 s of termination of exercise.

Gas exchange and O₂ transport determinations. The box enclosing the treadmill was airtight except for the inflow and outflow ports, which are independent of one another. PVT was adjusted to the desired level by mixing O₂ and N₂. Flow of the gas mixture entering the treadmill box was maintained constant at 20 liters ATA/min using a Cameron Instruments precision gas flow mixer. Inflowing and outflowing O₂ concentrations and outflowing CO₂ concentration (inflowing gas was CO₂ free) were measured continuously and simultaneously with an Applied Electrochemistry O₂ analyzer and a Columbus Instruments CO₂ analyzer, respectively. The O₂ and CO₂ analyzers were calibrated with gas mixtures measured with a precision of ±0.005%. Depending on the exercise PVT, the O₂ concentration of the calibrating gas mixtures was either 10 or 20%. The CO₂ concentration of the calibrating gas was 0.20%. The O₂ and CO₂ concentration differences between inflowing and outflowing gases ranged from ~0.10 to 0.20% during maximal exercise. The output of the O₂ and
Systemic arterial pressure and pulmonary arterial pressure (Pap) were recorded continuously, with mean pressures obtained by electronic integration. Heart rate was obtained directly from the systemic arterial blood pressure tracing.

\( C_{\text{O}_2} \) (ml/dl) and \( O_2 \) concentration in mixed venous blood (\( C_{\text{V}_2} \)) were calculated from \( [Hb] \), \( P_{\text{O}_2} \), and oxyhemoglobin saturation using an \( Hb-O_2 \) binding factor of 1.34 ml \( \text{STPD}/g \) and an \( O_2 \) solubility coefficient of 0.003 ml \( \cdot \) Torr \( \cdot \) dl \( ^{-1} \). \( Q \) (ml \( \cdot \) min \( ^{-1} \) \( \cdot \) kg \( ^{-1} \)) was calculated as the ratio of \( V_{\text{O}_2} \) to \( C_{\text{A}_2}-C_{\text{V}_2} \). The rate of convective blood \( O_2 \) transport (\( T_{\text{O}_2} \); ml \( \cdot \) min \( ^{-1} \) \( \cdot \) kg \( ^{-1} \)) was calculated as the product of \( Q \) times \( C_{\text{A}_2} \). The \( O_2 \) extraction ratio was calculated as \( C_{\text{A}_2}-C_{\text{V}_2} / C_{\text{A}_2} \).

The data are expressed as means ± SE. Comparisons were made among groups 1, 2, and 3 at a given \( P_{\text{O}_2} \). Statistical analysis was carried out using a one-way ANOVA. Significance was established with the \( t \)-test, using the Bonferroni correction for multiple comparisons. A \( P \) value of <0.05 indicated a significant difference.

**RESULTS**

Figure 1 shows the average arterial and mixed venous blood values of \( O_2 \) saturation of \( Hb \) observed during maximal exercise, plotted as a function of the corresponding average \( P_{\text{O}_2} \) values. It is clear that chronic administration of sodium cyanate (group 1) and transfusion of carbamylated blood into normal rats (group 2) were effective in substantially increasing the \( O_2 \) affinity of \( Hb \) above that of the control (group 3). Furthermore, there does not appear to be a systematic difference in \( Hb \) \( O_2 \) affinity between the animals of groups 1 and 2. In Fig. 1, the ODCs (represented by the solid lines) have \( P_{50} \) values of 26.2 Torr for groups 1 and 2 and 45.7 Torr for group 3. These values reflect the low blood \( pH \) and high temperature of maximal exercise. Table 1 shows that exercise induced similar changes in acid-base balance and temperature in groups 1 and 2, indicating that the effects of these factors on the \( O_2 \) affinity of \( Hb \) were equivalent in both groups.

Table 2 summarizes the \( O_2 \) transport variables observed during maximal exercise at \( P_{\text{O}_2} \) values of ~140, 70, and 55 Torr. Both alveolar ventilation normalized for \( V_{\text{O}_2} \) and partial pressure of \( O_2 \) in alveolar air (\( P_{\text{A}_2} \)) were similar in the three groups at each \( P_{\text{O}_2} \) level; however, \( P_{\text{A}_2} \) was lower and the alveolar-to-arterial

<table>
<thead>
<tr>
<th>Group</th>
<th>( P_{\text{O}_2} ), Torr</th>
<th>Arterial pH</th>
<th>( P_{\text{CO}_2} ), Torr</th>
<th>Rectal Temperature, °C</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139.6 ± 0.4</td>
<td>7.30 ± 0.05</td>
<td>30.1 ± 0.9</td>
<td>39.8 ± 0.2</td>
<td>6</td>
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<td>2</td>
<td>139.7 ± 0.7</td>
<td>7.32 ± 0.07</td>
<td>27.3 ± 1.5</td>
<td>39.2 ± 0.4</td>
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<tr>
<td>3</td>
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<td>7.34 ± 0.06</td>
<td>28.4 ± 1.1</td>
<td>39.2 ± 0.8</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
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<td>7.30 ± 0.07</td>
<td>19.8 ± 1.1</td>
<td>38.7 ± 0.5</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>68.5 ± 0.3</td>
<td>7.29 ± 0.08</td>
<td>19.7 ± 1.1</td>
<td>38.2 ± 0.4</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>68.6 ± 0.4</td>
<td>7.29 ± 0.06</td>
<td>19.7 ± 1.0</td>
<td>38.2 ± 0.7</td>
<td>7</td>
</tr>
<tr>
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<td>54.3 ± 0.2</td>
<td>7.32 ± 0.09</td>
<td>18.0 ± 0.6</td>
<td>37.9 ± 0.5</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>55.3 ± 0.3</td>
<td>7.29 ± 0.04</td>
<td>17.4 ± 0.8</td>
<td>38.5 ± 0.6</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>54.1 ± 0.6</td>
<td>7.29 ± 0.04</td>
<td>18.7 ± 0.6</td>
<td>37.8 ± 0.6</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \) = no. of animals. Group 1 were rats treated with sodium cyanate for 3 wk; exchange-transfused with plasma so that \( Hb \) concentration \([Hb]\) was lowered to normal values. Group 2 nontreated rats were exchange-transfused with carbamylated \( Hb \) blood. Group 3 nontreated rats were exchange-transfused with blood obtained from nontreated donor rats. \( P_{\text{O}_2} \) inspired \( P_{\text{O}_2} \); arterial \( pH \), plasma \( pH \) in arterial blood; \( P_{\text{CO}_2} \) arterial blood \( P_{\text{CO}_2} \).
HEMOGLOBIN P50 AND MAXIMAL EXERCISE CAPACITY

Table 2. O2 transport variables during maximal exercise

| Group | P50 | Torr | PaO2 | Torr | A-aPO2 | Torr | Va/Vo2 | Vo2max | T2max | [Hb], g/dl | P50 | Torr | PaO2 | Torr | CaO2 | m/dl | CvO2 | m/dl | O2 ER |
|-------|-----|------|------|------|-------|------|--------|--------|-------|--------|-----|------|------|------|-------|------|------|------|
| 1     | 140 | 116.4±3.2 | 24.0±2.0* | 27.7±1.5 | 59.4±1.7* | 94.0±2.2 | 13.8±0.8 | 92.4±2.5 | 25.3±0.6 | 18.6±1.0 | 6.7±0.6* | 0.640±0.010* |
| 2     | 111.8±1.9 | 21.4±2.2* | 28.0±1.8 | 72.4±2.6* | 102.5±4.7 | 15.0±0.3 | 95.0±2.2* | 22.0±0.9* | 19.4±0.7 | 5.8±0.4* | 0.698±0.013* |
| 3     | 110.1±2.1 | 13.4±0.3 | 24.7±1.8 | 87.6±1.4 | 103.9±3.2 | 14.9±0.9 | 96.7±1.4 | 26.0±1.1 | 18.4±0.4 | 2.9±0.4 | 0.845±0.015 |

Values are means ± SE; n = no. of animals. PaO2, arterial blood PO2; A-aPO2, arterial-to-alveolar PO2 difference; Va/Vo2, alveolar ventilation (ml BTPS·min⁻¹·kg⁻¹); VO2, maximal VO2 (ml STPD·min⁻¹·kg⁻¹); VO2max, maximal VO2 (ml STPD·min⁻¹·kg⁻¹); [Hb], arterial blood hemoglobin concentration; PaO2, arterial and mixed venous (pulmonary arterial) PO2, respectively; CaO2, arterialized and mixed venous blood O2 content, respectively; CvO2, arterialized and mixed venous blood O2 content, respectively; O2 ER, O2 extraction ratio. *Significant difference between group 1 or 2 and the corresponding control group 3 (P < 0.05); †significant difference between group 1 and the corresponding group 2 (P < 0.05).

Po2 gradient (A-aPo2) was higher in both groups 1 and 2 compared with that in control group 3. In all cases, the A-aPo2 tended to decrease as P50 decreased.

Vo2 max during normoxic exercise was lowest in group 1, intermediate in group 2, and highest in group 3. The differences among groups were significant in all cases. As exercise P50 decreased, the differences in Vo2 max between groups became less marked: no differences were observed among groups at P50 of 70 Torr, whereas Vo2 max of group 1 was higher than group 3 at P50 of 55 Torr (Table 2 and Fig. 2). No differences in CaO2 among groups were observed in normoxia, whereas CaO2 was significantly higher in groups 1 and 2 than in group 3 at both levels of hypoxia (Table 2). Because maximal Q was not influenced by any of the experimental interventions (Table 3), the changes in CaO2 were translated into proportionate changes in T2max (Table 2 and Fig. 2). The effect of the leftward ODC shift on O2 extraction by the tissues was apparent at all P50 values: O2 extraction ratio was highest in group 3, intermediate in group 2, and lowest in group 1 (Table 2). The differences between groups were significant at all P50 levels.

Table 3 shows hemodynamic variables for the three groups. The major effect is shown in Pap: groups 1 and 2 both show pulmonary hypertension during normoxic exercise; as P50 decreased, the difference between the experimental groups and group 3 decreased, with no significant difference observed between the three groups at P50 of 55 Torr.

DISCUSSION

Experimental design. The design of the present study allowed us to separate the effects of an acute leftward shift in the ODC from possible additional effects of chronic cyanate administration. Similar exercise Hb P50 values (Fig. 1) were obtained by either chronic administration of sodium cyanate (group 1) or by transfusion of blood containing carbamylated Hb to normal recipients (group 2). Because exercise was accompanied by similar changes in acid-base balance and temperature in both groups 1 and 2, it can be concluded that the extent to which these factors modified Hb P50 was equivalent in both groups and that both cyanate administration and blood

Table 3. Hemodynamic variables during maximal exercise

<table>
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<tr>
<th>P50</th>
<th>Group</th>
<th>Qmax</th>
<th>ml·min⁻¹·kg⁻¹</th>
<th>SAP, mmHg</th>
<th>Pap, mmHg</th>
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<td>1</td>
<td>512±27</td>
<td>131±6</td>
<td>29±0.4*</td>
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</tr>
<tr>
<td>2</td>
<td>525±26</td>
<td>139±5</td>
<td>30±1.3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>554±28</td>
<td>127±3</td>
<td>24±0.4</td>
<td></td>
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</tr>
<tr>
<td>70</td>
<td>1</td>
<td>556±16</td>
<td>130±4</td>
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<td>540±18</td>
<td>125±4</td>
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<td>526±39</td>
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<td>32±1.4</td>
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<tr>
<td>3</td>
<td>553±27</td>
<td>117±7</td>
<td>28±0.9</td>
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</tbody>
</table>

Values are means ± SE; Qmax, maximal cardiac output; SAP, mean systemic arterial blood pressure; Pap, mean pulmonary arterial blood pressure. *Significant difference between group 1 or 2 and the corresponding control group 3 (P < 0.05); †significant difference between group 1 and the corresponding group 2 (P < 0.05).
transfusion resulted in equivalent changes in Hb O$_2$ affinity. This is supported by the in vitro measurements (see METHODS), which showed no significant difference in whole blood standard P$_{50}$ between cyanate-treated rats and rats transfused with carbamylated blood. The agent utilized to lower Hb P$_{50}$ was the same in both groups 1 and 2, thus ensuring that the same conformational changes occurred in the Hb molecule in both cases. Treatment with sodium cyanate was discontinued 7–10 days before the experiments in the animals of group 1 as well as in the blood donors of group 2 to ensure elimination of sodium cyanate (1). The influence of possible nonspecific effects of the exchange transfusion was ruled out because all groups underwent transfusion. In addition, group 1 underwent plasma, rather than whole blood, exchange transfusion, which helped eliminate the confounding effect of elevated [Hb] that results from chronic sodium cyanate treatment (15, 17, 28). Finally, care was taken to avoid changes in blood volume during the exchange transfusions. Given these design features, differences in O$_2$ transport variables between groups 2 and 3 can be explained solely on the basis of the changes in O$_2$ affinity of Hb, whereas differences between groups 1 and 2 reflect additional effects of chronic cyanate treatment and/or chronic elevation of O$_2$ affinity of Hb.

**Systemic O$_2$ transport and V$\dot{O}_{2_{\text{max}}}$**. The central observation of the present studies is that producing an equivalent decrease in Hb P$_{50}$ by either transfusion of blood with carbamylated Hb or by chronic administration of sodium cyanate had similar directional effects on the O$_2$ transport system (Table 2): in both cases, the changes in T$\dot{O}_{2_{\text{max}}}$ were offset by those in tissue O$_2$ extraction, with the resulting V$\dot{O}_{2_{\text{max}}}$ depending on the relative magnitude of these opposing influences (Fig. 2 and Table 2). During normoxic exercise, the negative effect of lowering Hb P$_{50}$ on V$\dot{O}_{2_{\text{max}}}$ was relatively large because the reduction in O$_2$ extraction was not opposed by an increase in T$\dot{O}_{2_{\text{max}}}$ (Table 2, Fig. 2). At lower P$_{O_2}$ values, the effect on T$\dot{O}_{2_{\text{max}}}$ increased relative to the decrease in O$_2$ extraction, and the decrease in V$\dot{O}_{2_{\text{max}}}$ compared with control was attenuated or even reversed (Table 2, Fig. 2). The observation of a dissociation between V$\dot{O}_{2_{\text{max}}}$ and the rate at which O$_2$ is delivered to the tissues supports previous observations in isolated skeletal muscles (11, 20) and intact animals (17), as well as theoretical analysis of the O$_2$ transport system (29), which indicate that the rate of convective O$_2$ delivery to the tissues is not the only determinant of V$\dot{O}_{2_{\text{max}}}$.

A recent theoretical analysis (30) highlighted the relative insensitivity of V$\dot{O}_{2_{\text{max}}}$ to changes in Hb P$_{50}$ in humans. Although it would appear that this contradicts our results, closer examination of both the present data and the theoretical predictions shows otherwise. For instance, Fig. 1 of Ref. 30 shows that, during normoxic exercise, V$\dot{O}_{2_{\text{max}}}$ remains essentially unchanged as standard P$_{50}$ is increased from the normal value of 26.8 Torr to ~40 Torr. On the other hand, a decrease in P$_{50}$ from the normal value to ~15 Torr results in a decrease in V$\dot{O}_{2_{\text{max}}}$ of ~20%. Thus, although the effect is relatively small, i.e., almost halving P$_{50}$ results in a decrease in V$\dot{O}_{2_{\text{max}}}$ of only 20%, it is not negligible in these conditions. In the present experiments, a similar relative percent reduction in actual P$_{50}$ (from ~46 to ~26 Torr) also resulted in an ~20% decrease in V$\dot{O}_{2_{\text{max}}}$ during normoxic exercise, from 87.6 to 72.4 ml·min$^{-1}$·kg$^{-1}$ from group 3 to group 2, respectively. Accordingly, after the species differences in standard Hb P$_{50}$ values [26 Torr in humans and 34 Torr in rats (8)] are accounted for and because the theoretical results (30) are presented in terms of standard P$_{50}$ (i.e., P$_{50}$ at pH 7.4, P$_{CO_2}$ = 40 Torr, and temperature $= 37^\circ$C) vs. actual P$_{50}$ values in this study, it is apparent that the effects on V$\dot{O}_{2_{\text{max}}}$ of a decrease in P$_{50}$ observed in this study during normoxic conditions are not substantially different from those predicted. In addition, the theoretical model predicts that the effect on V$\dot{O}_{2_{\text{max}}}$ of changing P$_{50}$ decreases as exercise P$_{O_2}$ decreases, which is also consistent with our data in the present study (Table 2 and Fig. 2) and a previous study (17). Finally, the model predicts that the net V$\dot{O}_{2_{\text{max}}}$ effect of a change in P$_{50}$ is the result of the relative magnitude of the opposing changes in arterial blood Hb O$_2$ saturation on one hand and of tissue O$_2$ extraction on the other (30), which is in agreement with the present results and the previous findings (17).

A second important observation of the present experiments is that, although the direction of the changes was the same, there were quantitative differences between the effects of chronic cyanate administration (group 1) and those of transfusion of blood with carbamylated Hb (group 2): although arterial blood oxygenation levels and the rate of T$\dot{O}_{2_{\text{max}}}$ were similar in both groups, the O$_2$ extraction ratio was slightly but significantly lower in group 1 than in group 2 at all P$_{O_2}$ values (Table 2 and Fig. 2). Insight into the mechanisms responsible for the decrease in O$_2$ extraction may be obtained by examining the relationship between V$\dot{O}_{2_{\text{max}}}$ and mixed venous P$_{O_2}$ (Fig. 3). Because most of the O$_2$ utilization during maximal exercise takes place in the exercising muscles (16), the P$_{O_2}$ of mixed venous blood largely reflects the P$_{O_2}$ of the blood draining the exercising muscles. Under these conditions, skeletal muscle cell P$_{O_2}$ is near zero (3, 19), and changes in mixed venous P$_{O_2}$ during maximal exercise reflect changes in the blood-to-cell P$_{O_2}$ diffusion gradient. Within the framework of these assumptions, the relationship between V$\dot{O}_{2_{\text{max}}}$ and venous P$_{O_2}$ can be considered an indication of the O$_2$ transfer capacity of the tissues, a composite parameter determined by all the processes involved in the flow of O$_2$ from the tissue capillaries to the mitochondria (29). It is apparent that the relationship between V$\dot{O}_{2_{\text{max}}}$ and venous P$_{O_2}$ in group 2 is similar to that of the control group 3, suggesting that lowering Hb P$_{50}$ per se does not alter tissue O$_2$ transfer capacity. This being the case, the lower V$\dot{O}_{2_{\text{max}}}$ observed in group 2 with respect to control group 3 in normoxia can be interpreted as being the result of the decreased capillary-to-cell P$_{O_2}$ gradient: as the leftward shift of the ODC lowers the capil-
lary PO₂, the diffusion gradient is reduced and cannot support the O₂ flux from capillary to cell; as a consequence, VO₂\text{max} decreases. At the lower PIₐ values, the decreased O₂ extraction ratio is compensated by the higher VO₂\text{max} (Table 2 and Fig. 2), and VO₂\text{max} does not change as much. The slope of the line relating VO₂\text{max} and venous PO₂ for the animals of group 1, on the other hand, is lower than that corresponding to the other two groups, suggesting a reduction in the tissue O₂ transfer capacity: for a given PO₂ gradient, the rate at which O₂ is transferred to the cells is lower.

The mechanism underlying the effect of chronic sodium cyanate administration on tissue O₂ transfer capacity is not clear. Because Hb P₅₀ values were essentially the same in both groups, the differences between groups 1 and 2, although small, could be the result of direct effects of cyanate on sites different from the Hb molecule. The demonstration of a decreased oxidative capacity of mitochondria isolated from liver of cyanate-treated mice (21) could support a direct effect of cyanate on tissue VO₂, independent of its effects on the Hb molecule. A decreased muscle oxidative capacity would be evidenced by a lower O₂ extraction ratio and lower VO₂\text{max}-to-venous PO₂ ratio. Alternatively, the lower O₂ extraction ratio could be the result of the chronically elevated Hb O₂ affinity. Chronic cyanate administration produces hypoxia-like effects such as polycythemia and elevated blood [Hb] and pulmonary hypertension (Tables 2 and 3 and Refs 15, 17, and 28); the polycythemia is due to increased erythropoietin release (15). These features suggest that the chronic leftward shift of the ODC results in tissue hypoxia, even in a normoxic environment. Previous data from our laboratory (17) suggest that chronic cyanate administration has a negative effect on tissue O₂ transfer capacity that is offset by the increase in [Hb]: lowering [Hb] of cyanate-treated rats to normal values resulted in a further decrease in the O₂ extraction ratio during hypoxic exercise (17). Furthermore, for a comparable venous PO₂, VO₂\text{max} was lower in the cyanate-treated rats with normal blood [Hb] than in both control rats and cyanate-treated rats with high blood [Hb], whereas there were no differences between the latter two groups (17). A positive effect of [Hb] on tissue O₂ transfer capacity is supported by the decrease in skeletal muscle O₂ diffusing capacity observed in exercising humans after the lowering of blood [Hb] (23) and the opposite effect seen in rats when blood [Hb] is increased (6). In this respect, we have observed (5) that chronic exposure to hypobaric hypoxia does not influence O₂ extraction ratio or tissue O₂ transfer capacity despite blood [Hb] levels that were substantially higher than those observed here, further suggesting that exposure to chronic hypoxia results in a decrease in tissue O₂ extraction that is offset by the increase in blood [Hb]. The net effect of chronic hypoxia on tissue O₂ extraction may be the result of the interaction of several opposing mechanisms. On the one hand, the increased [Hb] should tend to improve O₂ transfer from capillary to cell. In addition, chronic hypoxia results in a decrease in muscle fiber size mainly due to loss of myofibrillar proteins, without a concomitant decrease in the size of the capillary network (12), which results in a relative increase in capillary density. Interestingly, rats chronically treated with sodium cyanate show similar morphological features in skeletal muscle (13): a decrease in fiber cross-sectional area, an increase in capillary density, and no change in the capillary-to-fiber ratio. It is generally thought that these features should facilitate muscle O₂ transfer by decreasing the distance between capillary and fiber. However, recent studies (9) showed that limb immobilization in dogs, which also results in a lower skeletal muscle fiber cross-sectional area and unchanged capillary-to-fiber ratio, fails to influence muscle O₂ diffusing capacity and actually results in a flatter VO₂\text{max}/venous PO₂ relationship compared with control animals. These latter findings suggest that the decreased capillary-to-cell distance that occurs in prolonged hypoxia is not necessarily translated into an improved muscle O₂ transport.

In addition to structural changes, prolonged hypoxia could influence the VO₂\text{max}/venous PO₂ relationship by changes in muscle oxidative capacity. Data on this subject are controversial, and it is possible that the discrepancy may reflect differences in species and in severity and duration of exposure to hypoxia. A loss of muscle mitochondria (12) and a decrease in muscle oxidative capacity (25) have been observed in both humans and rats exposed to prolonged hypoxia. These changes should have a negative effect on O₂ transfer. On the other hand, studies in animals indigenous to high altitude (10, 26, 27) and in sea-level animals acclimatized to simulated altitude (4) demonstrated increased muscle oxidative capacity and increased myoglobin concentration. It is clear that further studies are necessary to determine the possible effect of prolonged hypoxia on muscle O₂ transfer in exercise.

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**Fig. 3.** VO₂\text{max} plotted as a function of mixed venous (pulmonary arterial) PO₂ (PvO₂). Values are means ± SE of maximal exercise values. Regression lines were calculated from individual values. Data from groups 1 and 2 were pooled to calculate regression line. See Fig. 1 legend for description of groups.
Pulmonary circulation and gas exchange. Pulmonary hypertension was observed in both groups 1 and 2 during normoxic exercise. In group 1, no further increases in Pap were observed during hypoxia, suggesting a blunting of the hypoxic pulmonary vasoconstriction (Table 3). This agrees with previous observations in cyanate-treated rats (17, 28). The high Pap in group 2 in normoxia (Table 3) suggests that the ODC shift results in pulmonary vascular smooth muscle hypoxia severe enough to elicit pulmonary vasoconstriction. This was accompanied by a further increase in Pap during hypoxic exercise, suggesting that hypoxic pulmonary vasoconstriction was not blunted in this case. These differences may result from the differences between the effects of acute and chronic hypoxia in the pulmonary circulation, where hypoxic vasoconstriction and vascular remodeling play different roles in the pulmonary hypertension.

An interesting result of the present experiments is the effect of the increased O₂ affinity of Hb on A-aPO₂ , which was higher in both groups 1 and 2 than in group 3. In the presence of unchanged alveolar ventilation and PaO₂, an increase in A-aPO₂ indicates a decrease in the efficacy of pulmonary gas exchange. The fact that there was no difference in A-aPO₂ values between groups 1 and 2 shows that the gas exchange defect is due to the leftward shift of the ODC and not to additional effects of chronic sodium cyanate administration. The higher A-aPO₂ could be the result of ventilation-perfusion distribution heterogeneity, incomplete alveolarcapillary PO₂ equilibration, or a combination of both. Alveolarcapillary PO₂ equilibration could be impaired by a leftward ODC shift as a result of a combination of the lower venous PO₂ and the steeper slope of the ODC. Theoretical analysis by Piiper and Scheid (18) shows that the likelihood of complete alveolarcapillary PO₂ equilibration is inversely related to the slope of the blood O₂ absorption curve. A steeper ODC would result in smaller PO₂ increments as blood O₂ saturation increases along the pulmonary capillary. On the other hand, the positive correlation observed between A-aPO₂ and P₅₀ values (Table 1) is consistent with increased ventilation-perfusion distribution heterogeneity; however, the reason that both acute and chronic ODC shifts result in comparable decreases in pulmonary gas exchange efficacy via ventilation-perfusion mismatch is not readily apparent. Independent of the mechanism responsible for the impaired pulmonary gas exchange, it is interesting to note that the negative effect on PaO₂ offsets the increase in O₂ saturation of Hb, particularly in hypoxia, thus limiting the beneficial results of the leftward ODC shift on VO₂ by the blood in the lungs.

In summary, the main relevance of the present results is that an acute increase in the O₂ affinity of Hb, isolated from any other factors, has a definite effect on systemic O₂ transport during exercise. This effect, which is relatively large in normoxic and decreases substantially in hypoxic conditions, can be reasonably predicted from the extent of the change in Hb P₅₀ and the PO₂ level at which exercise takes place. Our results indicate that a decrease in P₅₀ results in decreases in VO₂max in normoxia; this effect decreases in magnitude as P I O₂ decreases and could even be reversed in severe hypoxia. The fact that the net effect of the ODC shift is the result of a balance between opposing mechanisms, the magnitude of which depends on the prevalent PO₂, may help reconcile the apparently contradictory results obtained in the past. In addition, the present data show that the decrease in tissue O₂ extraction is largest after chronic elevation of Hb O₂ affinity, suggesting a direct effect either of cyanate or of prolonged hypoxia on tissue O₂ transfer. The results of this study show that there is no unambiguous answer to the question of whether an increase or decrease in P₅₀ is most beneficial for systemic O₂ transport and utilization during exercise. Perhaps the most effective conditions to maximize O₂ transport are obtained not when large overall changes in P₅₀ are produced, as it was done in this and other studies, but in conditions when O₂ affinity of Hb increases as blood flows through the lungs and decreases as it flows through the tissues. These conditions, of course, normally occur as a result of the acid-base and temperature changes that take place at those sites during exercise.

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