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

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Henderson, Kyle K., Web McCanse, Tetsuya Urano, Ichiro Kuwahira, Richard Clancy, and Norberto C. Gonzalez. 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$_O2$ (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$_{50}$; Torr) during maximal exercise was ~26 in groups 1 and 2 and ~46 in group 3. In normoxia, maximal blood O$_2$ convection (V$_{O2}$ max = cardiac output × arterial blood O$_2$ content) was similar in all groups, whereas in hypoxia V$_{O2}$ 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$_{O2}$ 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$_{O2}$ max, accordingly, their differences in V$_{O2}$ max from group 3 were attenuated or reversed. Tissue O$_2$ transfer capacity (V$_{O2}$ max/mixed venous P$_O2$) was lowest in group 1 and comparable in groups 2 and 3. We conclude that lowering Hb P$_{50}$ has opposing effects on V$_{O2}$ max and O$_2$ extraction ratio, with the relative magnitude of these changes, which varies with P$_{IO2}$, determining V$_{O2}$ max. Although the lower O$_2$ extraction ratio of groups 2 vs. 3 suggests a decrease in tissue P$_{O2}$ 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$_{O2}$) 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$_O2$ (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$_{O2}$ max) during normoxic treadmill exercise. During hypoxic exercise, however, the magnitude of the difference in V$_{O2}$ max between cyanate-treated rats and untreated controls decreased; at the lowest P$_{IO2}$ values during exercise, V$_{O2}$ max was slightly higher in the cyanate-treated rats (17). These changes in V$_{O2}$ 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$_{O2}$ max, the product of maximal cardiac output (Q$_{max}$) times the arterial blood O$_2$ concentration (C$_{A0}$)) 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 C$_{A0}$). In normoxic exercise, the leftward ODC shift increased convective O$_2$ delivery only modestly, whereas tissue O$_2$ extraction decreased substantially. Accordingly, V$_{O2}$ max was lower in the cyanate-treated rats. At lower P$_{IO2}$ 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$_{O2}$ max (17). The increase in the rate of O$_2$ delivery brought about by cyanate was exclusively due to an increase in C$_{A0}$, 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$_O2$ that results from the shift. It is not clear, however, whether other mechanisms contribute to the decrease

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in tissue \( O_2 \) 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 \( V_{O_2} \). 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 \( P_{O_2} \) secondary to the low \( Hb \) \( P_{50} \) exposure to prolonged hypoxia could result in changes that affect \( O_2 \) extraction by the tissues.

The purpose of the present experiments was to extend our observations of the effect of a decrease in \( Hb \) \( P_{50} \) on \( O_2 \) transport and maximal exercise capacity. Specifically, we investigated whether acutely elevating the \( O_2 \) affinity of \( Hb \), by exchanging the blood from untreated animals with blood with low-\( Hb \) \( P_{50} \), would have the same effect on \( O_2 \) transport as that produced by chronic ingestion of sodium cyanate. The hypothesis tested was that, if the changes in \( O_2 \) transport produced by cyanate administration in our previous experiments were due solely to the increase in \( O_2 \) 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 \( O_2 \) 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 sodium (40 mg/kg ip). A PE-50 catheter was then placed in the left carotid artery, and a PE-10 catheter was placed into the main pulmonary artery with the help of an introducer guide catheter. Adequate positioning of the pulmonary artery catheter was determined by the blood pressure tracing and verified at autopsy the day after the experiment. The catheters were tunneled subcutaneously, exteriorized at the back of the neck, cut at a length of 2 inches, and flame-sealed. During the experiments, the animals exercised maximally on a treadmill 24 h after surgery.

**Experimental design.** On the day of the experiment, the animals treated with sodium cyanate (group 1) underwent an exchange transfusion in which 6–8 ml of blood withdrawn from the animals were exchanged for an equal volume of plasma obtained from donor rats. This was done to lower blood \( Hb \) concentration ([Hb]), which is elevated by cyanate treatment (17, 28), to a normal value of \( \sim 15 \) g/dl blood. The isovolumic exchange transfusion was carried out with the use of a technique described before (6) in which infusion through the venous catheter and withdrawal from the arterial catheter were carried out simultaneously, 1 ml at a time. The nontreated animals were randomly assigned to either of two groups: group 2 or group 3. Group 2 animals underwent an isovolumic blood-exchange transfusion using blood obtained from rats that had received 0.2% sodium cyanate in drinking water for 3 wk; blood for the transfusion was obtained 7–10 days after sodium cyanate administration to the donors was discontinued. Group 3 served as controls and consisted of untreated rats that underwent an isovolumic exchange transfusion using blood with normal \( Hb \) \( P_{50} \) obtained from untreated littersmates. In all cases, blood was obtained under 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 \( P_{O_2} \) values of 140, 70, or 55 Torr. Each rat exercised only once.

The effectiveness of the exchange transfusion with carbamylated blood in reducing \( Hb \) \( P_{50} \) of the circulating blood of the recipient was established in preliminary experiments. The mixing technique (8) was used to measure \( Hb \) \( P_{50} \) in whole blood at \( pH \) 7.40, \( P_{CO_2} \) of 40 Torr, and temperature of 37°C. In four rats treated with sodium cyanate as in group 1, \( P_{50} \) 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 \( Hb \) \( P_{50} \) of untreated rats, which is \( \sim 34 \) 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 \( V_{O_2} \) and \( CO_2 \) production (\( V_{CO_2} \)) 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 \( V_{O_2 \max} \) was reached. \( V_{O_2 \max} \) was defined as the \( V_{O_2} \) for which an increase in work rate resulted in no further increase (±5%) in \( V_{O_2} \). If a plateau in \( V_{O_2 \max} \) was not observed at the highest two workloads, the animal was discarded. Approximately 80% of all rats achieved \( V_{O_2 \max} \) as defined here. Arterial and mixed venous blood samples were obtained during the last 45–60 s of exercise, while \( V_{O_2} \) and \( V_{CO_2} \) 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 \( O_2 \) transport determinations.** The box enclosing the treadmill was airtight except for the inflow and outflow ports, which are independent of one another. \( P_{O_2} \) was adjusted to the desired level by mixing \( O_2 \) and \( N_2 \). Flow of the gas mixture entering the treadmill box was maintained constant at 20 liters ATPS/min using a Cameron Instruments precision gas flow mixer. Inflowing and outflowing \( O_2 \) concentrations and outflowing \( CO_2 \) concentration (inflowing gas was \( CO_2 \) free) were measured continuously and simultaneously with an Applied Electrochemistry \( O_2 \) analyzer and a Columbus Instruments \( CO_2 \) analyzer, respectively. The \( O_2 \) and \( CO_2 \) analyzers were calibrated with gas mixtures measured with a precision of ±0.005%. Depending on the exercise \( P_{O_2} \), the \( O_2 \) concentration of the calibrating gas mixtures was either 10 or 20%. The \( CO_2 \) concentration of the calibrating gas was 0.20%. The \( O_2 \) and \( CO_2 \) concentration differences between inflowing and outflowing gases ranged from \( \sim 0.10 \) to 0.20% during maximal exercise. The output of the \( O_2 \) 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\textsubscript{a}O\textsubscript{2} (ml/dl) and O\textsubscript{2} concentration in mixed venous blood (C\textsubscript{v}O\textsubscript{2}) were calculated from [Hb], P\textsubscript{O2}, and oxyhemoglobin saturation using an Hb-O\textsubscript{2} binding factor of 1.34 ml \textit{stp}\/g and an O\textsubscript{2} solubility coefficient of 0.003 ml \textit{stp}\/Torr\cdot dl\textsuperscript{-1}. Q (ml \cdot min\textsuperscript{-1} \cdot kg\textsuperscript{-1}) was calculated as the ratio of V\textsubscript{o2} to C\textsubscript{a}O\textsubscript{2} - C\textsubscript{v}O\textsubscript{2}. The rate of convective blood O\textsubscript{2} transport (T\textsubscript{O2}; ml \cdot min\textsuperscript{-1} \cdot kg\textsuperscript{-1}) was calculated as the product of Q times C\textsubscript{a}O\textsubscript{2}. The O\textsubscript{2} extraction ratio was calculated as C\textsubscript{a}O\textsubscript{2} - C\textsubscript{v}O\textsubscript{2} / C\textsubscript{a}O\textsubscript{2}.

The data are expressed as means ± SE. Comparisons were made among groups 1, 2, and 3 at a given P\textsubscript{I}\textsubscript{O2}. 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\textsubscript{2} saturation of Hb observed during maximal exercise, plotted as a function of the corresponding average P\textsubscript{I}\textsubscript{O2} 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\textsubscript{2} affinity of Hb above that of the control (group 3). Furthermore, there does not appear to be a systematic difference in Hb O\textsubscript{2} affinity between the animals of groups 1 and 2. In Fig. 1, the ODCs (represented by the solid lines) have C\textsubscript{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\textsubscript{2} affinity of Hb were equivalent in both groups.

Table 2 summarizes the O\textsubscript{2} transport variables observed during maximal exercise at P\textsubscript{I}\textsubscript{O2} values of ~140, 70, and 55 Torr. Both alveolar ventilation normalized for V\textsubscript{o2} and partial pressure of O\textsubscript{2} in alveolar air (P\textsubscript{A}\textsubscript{O2}) were similar in the three groups at each P\textsubscript{I}\textsubscript{O2} level; however, Pa\textsubscript{O2} was lower and the alveolar-to-arterial

![Figure 1. Arterial and mixed venous blood %Hb O\textsubscript{2} saturation plotted as a function of the corresponding P\textsubscript{O2}. Values are means ± SE for all 3 groups during maximal exercise. Group 1: rats treated with sodium cyanate for 3 wk and exchange-transfused with plasma so that Hb concentration was lowered to normal values. Group 2: nontreated rats exchange-transfused with carbamylated Hb blood. Group 3: nontreated rats exchange-transfused with blood obtained from nontreated donor rats. Solid lines show O\textsubscript{2} dissociation curves constructed using Hill's equation with Hb O\textsubscript{2} half-saturation pressure and an O\textsubscript{2} solubility coefficient of 0.003 ml \textit{stp} \cdot Torr\cdot dl\textsuperscript{-1}.](http://jap.physiology.org/)

**Table 1. Acid base and temperature values during exercise**

<table>
<thead>
<tr>
<th>Group</th>
<th>P\textsubscript{I}\textsubscript{O2}, Torr</th>
<th>Arterial pH</th>
<th>P\textsubscript{A}\textsubscript{CO2}, 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>
</tr>
<tr>
<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>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>140.8 ± 0.1</td>
<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>
<td>71.1 ± 0.6</td>
<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>
<td>1</td>
<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 ± 0.06</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\textsubscript{I}\textsubscript{O2} inspired P\textsubscript{O2}; arterial pH, plasma pH in arterial blood; Pa\textsubscript{CO2}, arterial blood P\textsubscript{CO2}. 

CO\textsubscript{2} meters was fed into a computer to provide determination of V\textsubscript{o2}, V\textsubscript{CO2}, and the respiratory exchange ratio every 5 s. V\textsubscript{o2} and V\textsubscript{CO2} were calculated from the inflowing and outflowing O\textsubscript{2} concentration difference, the outflowing CO\textsubscript{2} concentration, and the outflowing gas flow (expressed in ml\textit{STPD} of V\textsubscript{o2}, V\textsubscript{CO2}, and the respiratory exchange ratio every 5 s.

Arterial and mixed venous blood samples were analyzed for pH, P\textsubscript{O2}, and P\textsubscript{CO2} using appropriate electrodes at 38°C, analyzed for [Hb] and O\textsubscript{2} saturation of Hb, and corrected for the rectal temperature by using temperature correction factors for rat blood (7).
ences were observed among groups at PIO2, arterial blood hemoglobin concentration; PaO2 and PvO2, arterial and mixed venous (pulmonary arterial) PO2, respectively; CaO2 and

As exercise PIO2 decreased, the differences in VO2 max between groups became less marked: no differences were observed among groups at PIO2 of 70 Torr, whereas VO2 max of group 1 was higher than group 3 at PIO2 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 T02 max (Table 2 and Fig. 2). The extent of the leftward ODC shift on O2 extraction by the tissues was apparent at all PIO2 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 PIO2 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 PIO2 decreased, the difference between the experimental groups and group 3 decreased, with no significant difference observed between the three groups at PIO2 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 cyanate administration (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 at all PIO2 values (Table 1), 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

Fig. 2. Maximal rate of O2 consumption (VO2 max) plotted as a function of the maximal rate of convective blood O2 delivery (T02 max), calculated as maximal cardiac output (Qmax) × arterial blood O2 content (Cao2). The slope ΔVO2 max/ΔT02 max is equal to the average O2 extraction ratio (Cao2 - CvO2/Cao2). Values are means ± SE of maximal exercise values. Regression lines were calculated from individual values. See Fig. 1 legend for description of groups. PIO2.

Table 2. O2 transport variables during maximal exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>PIO2 Torr</th>
<th>PaO2 Torr</th>
<th>A-aPO2</th>
<th>VO2 /VO2</th>
<th>VO2max</th>
<th>T02max</th>
<th>[Hb] g/dl</th>
<th>PIO2</th>
<th>PaO2</th>
<th>PV02</th>
<th>Cao2</th>
<th>Cvo2</th>
<th>O2 ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>140</td>
<td>116.4 ± 3.2</td>
<td>24.0 ± 2.9</td>
<td>27.5 ± 1.5</td>
<td>59.4 ± 1.7</td>
<td>94.0 ± 2.2</td>
<td>13.8 ± 0.8</td>
<td>92.4 ± 2.5</td>
<td>39.6 ± 2.2</td>
<td>27.5 ± 2.6</td>
<td>15.0 ± 0.3</td>
<td>95.0 ± 2.2</td>
<td>22.7 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>111.8 ± 1.9</td>
<td>21.4 ± 2.2</td>
<td>28.0 ± 1.8</td>
<td>72.4 ± 2.6</td>
<td>102.5 ± 4.7</td>
<td>14.9 ± 0.4</td>
<td>96.7 ± 1.4</td>
<td>26.0 ± 1.1</td>
<td>18.4 ± 0.4</td>
<td>2.9 ± 0.4</td>
<td>0.854 ± 0.015</td>
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</tr>
<tr>
<td>3</td>
<td>110.1 ± 0.2</td>
<td>13.4 ± 0.3</td>
<td>24.7 ± 1.8</td>
<td>87.6 ± 2.2</td>
<td>103.9 ± 3.2</td>
<td>16.0 ± 0.5</td>
<td>35.6 ± 1.1</td>
<td>16.4 ± 0.8</td>
<td>14.7 ± 0.4</td>
<td>4.7 ± 0.2</td>
<td>0.650 ± 0.009</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are means ± SE; n = no. of animals. A-aPO2, “ideal” alveolar PO2; A-aPO2, alveolar-to-arterial blood PO2 difference; VO2/VO2, arterial blood ventilation (ml STPD·min⁻¹·kg⁻¹) normalized for O2 consumption (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 and Cvo2, arterial 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).
transfusion resulted in equivalent changes in Hb O2 affinity. This is supported by the in vitro measurements (see METHODS), which showed no significant difference in whole blood standard P50 between cyanate-treated rats and rats transfused with carbamyalted blood. The agent utilized to lower Hb P50 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 O2 transport variables between groups 2 and 3 can be explained solely on the basis of the changes in O2 affinity of Hb, whereas differences between groups 1 and 2 reflect additional effects of chronic cyanate treatment and/or chronic elevation of O2 affinity of Hb.

**Systemic O2 transport and VO2 max.** The central observation of the present studies is that producing an equivalent decrease in Hb P50 by either transfusion of blood with carbamyalted Hb or by chronic administration of sodium cyanate had similar directional effects on the O2 transport system (Table 2): in both cases, the changes in T02 max were offset by those in tissue O2 extraction, with the resulting VO2 max depending on the relative magnitude of these opposing influences (Fig. 2 and Table 2). During normoxic exercise, the negative effect of lowering Hb P50 on VO2 max was relatively large because the reduction in O2 extraction was not opposed by an increase in T02 max (Table 2, Fig. 2). At lower PiO2 values, the effect on T02 max increased relative to the decrease in O2 extraction, and the decrease in VO2 max compared with control was attenuated or even reversed (Table 2, Fig. 2). The observation of a dissociation between VO2 max and the rate at which O2 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 O2 transport system (29), which indicate that the rate of convective O2 delivery to the tissues is not the only determinant of VO2 max.

A recent theoretical analysis (30) highlighted the relative insensitivity of VO2 max to changes in Hb P50 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, VO2 max remains essentially unchanged as standard P50 is increased from the normal value of 26.8 Torr to ~40 Torr. On the other hand, a decrease in P50 from the normal value to ~15 Torr results in a decrease in VO2 max of ~20%. Thus, although the effect is relatively small, i.e., almost halving P50 results in a decrease in VO2 max of only 20%, it is not negligible in these conditions. In the present experiments, a similar relative percent reduction in actual P50 (from ~46 to ~26 Torr) also resulted in an ~20% decrease in VO2 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 P50 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 P50 (i.e., P50 at pH 7.4, PCO2 = 40 Torr, and temperature = 37°C) vs. actual P50 values in this study, it is apparent that the effects on VO2 max of a decrease in P50 observed in this study during normoxic conditions are not substantially different from those predicted. In addition, the theoretical model predicts that the effect on VO2 max of changing P50 decreases as exercise PiO2 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 VO2 max effect of a change in P50 is the result of the relative magnitude of the opposing changes in arterial blood Hb O2 saturation on one hand and of tissue O2 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 carbamyalted Hb (group 2): although arterial blood oxygenation levels and the rate of T02 max were similar in both groups, the O2 extraction ratio was slightly but significantly lower in group 1 than in group 2 at all PiO2 values (Table 2 and Fig. 2). Insight into the mechanisms responsible for the decrease in O2 extraction may be obtained by examining the relationship between VO2 max and mixed venous PO2 (Fig. 3). Because most of the O2 utilization during maximal exercise takes place in the exercising muscles (16), the PO2 of mixed venous blood largely reflects the PO2 of the blood draining the exercising muscles. Under these conditions, skeletal muscle cell PO2 is near zero (3, 19), and changes in mixed venous PO2 during maximal exercise reflect changes in the blood-to-cell PO2 diffusion gradient. Within the framework of these assumptions, the relationship between VO2 max and venous PO2 can be considered an indication of the O2 transfer capacity of the tissues, a composite parameter determined by all the processes involved in the flow of O2 from the tissue capillaries to the mitochondria (29). It is apparent that the relationship between VO2 max and venous PO2 in group 2 is similar to that of the control group 3, suggesting that lowering Hb P50 per se does not alter tissue O2 transfer capacity. This being the case, the lower VO2 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 PO2 gradient: as the leftward shift of the ODC lowers the capi-
Chronic cyanate administration has a negative effect on 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 oxygen extraction that is offset by the increase in blood [Hb].

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 tissue oxidative capacity would be evidenced by a lower O2 extraction ratio and lower VO2max-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, VO2max 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 oxygen 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 VO2max/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 VO2max/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.

**Fig. 3.** VO2max plotted as a function of mixed venous (pulmonary arterial) PO2 (PV O2). 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-aP₀₂, 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-aP₀₂ indicates a decrease in the efficacy of pulmonary gas exchange. The fact that there was no difference in A-aP₀₂ 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-aP₀₂ could be the result of ventilation-perfusion distribution heterogeneity, incomplete alveolo-capillary P₀₂ equilibration, or a combination of both. Alveolo-capillary P₀₂ equilibration could be impaired by a leftward ODC shift as a result of a combination of the lower venous P₀₂ and the steeper slope of the ODC. Theoretical analysis by Piiper and Scheid (18) shows that the likelihood of complete alveolo-capillary P₀₂ equilibration is inversely related to the slope of the blood O₂ absorption curve. A steeper ODC would result in smaller P₀₂ increments as blood O₂ saturation increases along the pulmonary capillary. On the other hand, the positive correlation observed between A-aP₀₂ and P₀₂ 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 P₀₂ offsets the increase in O₂ saturation of Hb, particularly in hypoxia, thus limiting the beneficial effects of the leftward ODC shift on V₀₂ 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 P₀₂ level at which exercise takes place. Our results indicate that a decrease in P₅₀ results in decreases in V₀₂ max in normoxia; this effect decreases in magnitude as P₁O₂ 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 P₀₂, 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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