Effect of chronic sodium cyanate administration on $O_2$ transport and uptake in hypoxic and normoxic exercise

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McCanse, Web, Kyle Henderson, Tetsuya Urano, Ichiro Kuwahira, Richard L. Clancy, and Norberto C. Gonzalez. Effect of chronic sodium cyanate administration on $O_2$ transport and uptake in hypoxic and normoxic exercise. J. Appl. Physiol. 86(4): 1257–1263, 1999.—Systemic $O_2$ transport during maximal exercise at different inspired $P_{O_2}$ ($P_{O_2}$) values was studied in sodium cyanate-treated (CY) and nontreated (NT) rats. CY rats exhibited increased $O_2$ affinity of Hb (exercise $O_2$ half-saturation pressure of Hb = 27.5 vs. 42.5 Torr), elevated blood Hb concentration, pulmonary hypertension, blunted hypoxic pulmonary vasoconstriction, and normal ventilatory response to exercise. Maximal rate of convective $O_2$ transport was higher and tissue $O_2$ extraction was lower in CY than in NT rats. The relative magnitude of these opposing changes, which determined the net effect of cyanate on maximal $O_2$ uptake ($V_{O2max}$), varied at different $P_{O_2}$ ($V_{O2max}$) values was lower in normoxia (72.8 ± 1.9 vs. 81.1 ± 1.2), the same at 70 Torr $P_{O_2}$ (55.4 ± 1.4 vs. 54.1 ± 1.4), and higher at 55 Torr $P_{O_2}$ (48.0 ± 0.7 vs. 40.4 ± 1.9) in CY than in NT rats. The beneficial effect of cyanate on $V_{O2max}$ at 55 Torr $P_{O_2}$ disappeared when Hb concentration was lowered to normal. It is concluded that the effect of cyanate on $V_{O2max}$ depends on the relative changes in blood $O_2$ convection and tissue $O_2$ extraction, which vary at different $P_{O_2}$. Although uptake of $O_2$ by the blood in the lungs is enhanced by cyanate, its release at the tissues is limited, probably because of a reduction in the capillary-to-tissue $P_{O_2}$ diffusion gradient secondary to the increased $O_2$ affinity of Hb.

THE EFFECT OF CHANGES in the $O_2$ affinity of Hb on $O_2$ transport and uptake is still poorly understood. A decrease in $O_2$ affinity of Hb (rightward shift of the $O_2$ dissociation curve of Hb (ODC)) and increase in the $P_{O_2}$ necessary to obtain 50% $O_2$ saturation of Hb ($P_{50}$) occurs in acclimatization to altitude, and this is thought to be advantageous for $O_2$ transport in hypoxia, since it may facilitate $O_2$ unloading in the tissues (1, 13). On the other hand, animals indigenous to altitude (2) show high $O_2$ affinity of Hb (low $P_{50}$ and leftward ODC shift), and rats with a leftward ODC shift showed increased survival rate to extreme hypoxia (5). Studies in quiescent or contracting isolated skeletal muscles have also shown apparently contradictory results concerning $O_2$ transport, extraction, and utilization after changes in $P_{50}$ (9, 11, 15, 17).

Despite continuing interest in the subject, relatively few data are available on the effect of changes in the $O_2$ affinity of Hb on the mechanisms of systemic $O_2$ transport in intact animals, particularly during maximal exercise. Experimental data on this subject are needed, because the effects of changes in $O_2$ affinity of Hb on $O_2$ transport during exercise are not easy to predict. In general, changes in $O_2$ affinity of Hb are likely to have opposing effects on Hb oxygenation in the lungs and deoxygenation in the tissues. Furthermore, because the change in $O_2$ saturation of Hb produced by a given ODC shift is smaller at $P_{O_2}$ above ~80 Torr and below ~20 Torr than at intermediate $P_{O_2}$, the magnitude of the change in $O_2$ uptake in the lungs relative to $O_2$ release in the tissues is likely to vary at different levels of inspired $P_{O_2}$ ($P_{O_2}$). Finally, it is not clear how the changes in the different links of the $O_2$ transport system secondary to ODC shifts may interact with one another in the intact organism and whether compensatory mechanisms may exist that modify the effect of these changes.

The purpose of the present studies was to determine the effect of a leftward shift of the ODC on $O_2$ transport and uptake during maximal exercise at various $P_{O_2}$ levels. Maximal exercise was chosen, because maximal $O_2$ consumption ($V_{O2max}$) provides an accurate measurement of the capacity of the entire transport system to deliver and utilize $O_2$ under a given set of conditions; furthermore, under appropriate circumstances, it is possible to determine the conductance of one or more of the links that compose the $O_2$ transport chain. Accordingly, the effect of an experimental intervention on the transport capacity of the entire system, as well as that of the individual links, can be established, and the mechanism of action of the intervention can be determined.

Our hypothesis was that a leftward shift of the ODC would result in an increase in the maximal rate of convective $O_2$ delivery to the tissues and, at the same time, result in a decrease in the extraction of $O_2$ by the tissues. We further hypothesized that the relative magnitude of these two opposing changes would determine the effect of the ODC shift on $V_{O2max}$ and that this effect would differ at different levels of $P_{O_2}$. To test this hypothesis we used an animal, the rat, that has been...
used frequently in studies of O₂ transport under different environmental conditions (3, 7, 8, 12, 21).

**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 (CY group) and a nontreated (NT) group. Cyanate irreversibly carboxylates the amino terminal of valine and results in an increase in the O₂ affinity of Hb (4). Sodium cyanate administration was discontinued at 3 wk; 1 wk later, CY and NT animals were anesthetized using pentobarbital sodium (40 mg/kg ip); a PE-50 catheter was placed in the left carotid artery, and a PE-10 catheter was introduced 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 1 day later, after the experiment was concluded. The catheters were tunneled subcutaneously, exter-riorized at the back of the neck, cut at a length of 2 in., and flame sealed.

Exercise protocol. The exercise test took place 24 h after catheter placement. The animals were weighed, their rectal temperature was measured, and they were placed on a treadmill enclosed in an airight Lucite chamber adapted for the determination of O₂ uptake (V˙O₂) and CO₂ production (V˙CO₂) by the open-circuit method. The catheters were connected, through sampling ports located on the top of the box enclosing the treadmill, to pressure transducers. After 30 min in the treadmill, 0.5-ml arterial and mixed venous blood samples were obtained via stopcocks, the blood was replaced with homologous fresh blood from the same group (CY or NT), and the treadmill was set at a speed of 10 m/min. This speed was maintained for 2–3 min, then the treadmill was set at an angle of 10° and the speed was increased by 4 m/min every 90–120 s until V O₂max was reached. V O₂max was defined as the V O₂ after which an increase in work rate was not associated with a further increase (±5%) in V O₂.

Arterial and mixed venous blood samples were obtained during the last 45–60 s of exercise, while V O₂ and V O₂ were corrected for the rectal temperature by using tempera-
ture correction factors for rat blood (8).

Gas exchange and O₂ transport determinations. Gas enters the determination of O₂ uptake (V˙O₂) and CO₂ production (V˙CO₂) by the open-circuit method. The catheters were con-
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transfusion of plasma obtained from donor rats. The transfusion was carried out immediately before the exercise run.

Values are means ± SE. Comparisons were made between NT, CY1, and CY2 at a given $P_{IO_2}$. Statistical analysis was carried out using ANOVA. Significance was established with the $t$-test using the Bonferroni correction for multiple comparisons.

RESULTS

Although the mean body weight tended to be smaller in CY1 and CY2 than in NT rats, the difference did not reach statistical significance (Table 1). Administration of sodium cyanate resulted in a marked leftward shift of the ODC, as demonstrated in Fig. 1, which shows the arterial (SaO$_2$) and mixed venous blood O$_2$ saturation of Hb plotted as a function of the corresponding $P_{O_2}$ values observed for all groups during maximal exercise. The ODCs represented by the solid lines in Fig. 1 have $P_{50}$ values of 27.5 and 42.5 Torr for CY and NT, respectively. These values reflect the low blood pH and high temperature of maximal exercise.

For any level of $P_{IO_2}$, $P_{A_0_2}$, and $V_{A}/V_{O_2}$ were comparable in NT and CY groups (Table 1); however, the $P_{A_0_2}$-arterial $P_{O_2}$ ($P_{a_0_2}$) difference [(A-a)$P_{O_2}$] was significantly higher in CY rats (Fig. 2), which resulted in significantly lower $P_{A_0_2}$ values in CY rats than in the corresponding NT rats (Table 1). Within the CY groups, the (A-a)$P_{O_2}$ was higher in CY2 than in CY1 rats at all $P_{IO_2}$ levels. In all groups the (A-a)$P_{O_2}$ values increased with $P_{IO_2}$ (Fig. 2).

[Hb] was significantly higher in CY1 than in NT rats (Table 1). The isovolumic exchange transfusion of plasma effectively decreased [Hb] of CY2 to that of NT rats. For a given $P_{IO_2}$, O$_2$ saturation of Hb in arterial blood was higher in CY than in NT groups, despite the lower $P_{A_0_2}$ of CY rats (Table 1). No significant differences in SaO$_2$ were observed between CY1 and CY2 rats, except during normoxic exercise, when SaO$_2$ was slightly higher in CY2 rats (Table 1). $Ca_{O_2}$ was significantly higher in CY1 than in NT rats at all levels of $P_{IO_2}$ (Table 1). $Ca_{O_2}$ of CY2 rats was not significantly different from that of NT rats in normoxic exercise, and values were intermediate between NT and CY1 rats at the lower $P_{IO_2}$ levels (Table 1). Venous O$_2$ saturation of Hb and $CvO_2$ were higher in the CY groups than in the corresponding NT groups at all $P_{IO_2}$ values (Table 1). In addition, at 70 and 55 Torr $P_{IO_2}$, O$_2$ saturation in mixed venous blood and $CvO_2$ were significantly higher in CY than in CY2 rats (Table 1).

All groups showed the typical acid-base features of maximal exercise observed in this model: relatively low plasma pH, hypocapnia, and low plasma HCO$_3^-$ concentration (7, 8). During normoxic exercise, the NT group showed plasma pH 7.36 ± 0.03, $P_{AcO_2}$ 24.3 ± 0.6 Torr, and plasma HCO$_3^-$ 13.6 ± 1.3 mM. Although there was a tendency for lower pH values in CY1 and CY2 than in NT rats, this never reached statistical significance. In hypoxic exercise, $P_{AcO_2}$ of NT groups was lower (20.3 ± 0.4 and 19.9 ± 1.2 Torr at 70 and 55 Torr $P_{IO_2}$, respectively) than in normoxia, reflecting the hypoxic hyperventilation; there was no difference between these and the corresponding $P_{AcO_2}$ values of the CY groups.

The effect of cyanate administration on VO$_2$max varied depending on the $P_{IO_2}$ as well as the [Hb]: in normoxic exercise VO$_2$max was highest in NT, intermediate in CY1, and lowest in CY2 rats (Table 2); at 70 Torr $P_{IO_2}$ there was no difference in VO$_2$max between NT and CY1 rats, whereas CY2 rats showed the lowest VO$_2$max (Table 2). At 55 Torr $P_{IO_2}$, VO$_2$max was highest in CY1 rats, with no significant difference between NT and CY2 rats. The
Table 2. Systemic O2 transport and hemodynamic parameters during maximal exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>VO2max, m(^3)(\cdot)min(^{-1})(\cdot)kg(^{-1})</th>
<th>TO2max, m(^3)(\cdot)min(^{-1})(\cdot)kg(^{-1})</th>
<th>O2ER</th>
<th>Qmax, m(^3)(\cdot)min(^{-1})(\cdot)kg(^{-1})</th>
<th>HRmax, beats/min</th>
<th>MABP, mmHg</th>
<th>PAP, mmHg</th>
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</thead>
<tbody>
<tr>
<td>NT</td>
<td>81.1 ± 1.2</td>
<td>104.7 ± 2.3</td>
<td>0.777 ± 0.012</td>
<td>539 ± 12</td>
<td>587 ± 3</td>
<td>138 ± 6</td>
<td>22.9 ± 0.8</td>
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<tr>
<td>CY1</td>
<td>72.8 ± 1.9*</td>
<td>122.6 ± 5.1*</td>
<td>0.598 ± 0.015*</td>
<td>565 ± 15</td>
<td>589 ± 2</td>
<td>138 ± 8</td>
<td>27.0 ± 1.0*</td>
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<tr>
<td>CY2</td>
<td>61.2 ± 1.2*</td>
<td>99.0 ± 1.6*</td>
<td>0.618 ± 0.007*</td>
<td>538 ± 24</td>
<td>568 ± 17</td>
<td>137 ± 4</td>
<td>28.0 ± 1.6*</td>
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<tr>
<td>Exercise PIO2 = 140 Torr</td>
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<tr>
<td>NT</td>
<td>54.1 ± 1.4</td>
<td>64.3 ± 1.9</td>
<td>0.844 ± 0.014</td>
<td>572 ± 23</td>
<td>581 ± 5</td>
<td>128 ± 3</td>
<td>28.0 ± 1.4</td>
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<tr>
<td>CY1</td>
<td>55.4 ± 1.4</td>
<td>95.0 ± 4.8*</td>
<td>0.594 ± 0.020*</td>
<td>569 ± 22</td>
<td>578 ± 6</td>
<td>130 ± 3</td>
<td>27.7 ± 0.7</td>
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<tr>
<td>CY2</td>
<td>48.6 ± 1.0*</td>
<td>74.0 ± 2.0*</td>
<td>0.656 ± 0.007*</td>
<td>540 ± 19</td>
<td>568 ± 13</td>
<td>130 ± 5</td>
<td>30.0 ± 1.8</td>
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<td>Exercise PIO2 = 70 Torr</td>
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<tr>
<td>NT</td>
<td>40.4 ± 1.9</td>
<td>45.7 ± 2.2</td>
<td>0.883 ± 0.007</td>
<td>546 ± 28</td>
<td>592 ± 7</td>
<td>122 ± 1</td>
<td>27.5 ± 0.9</td>
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<tr>
<td>CY1</td>
<td>48.0 ± 0.7*</td>
<td>83.8 ± 2.4*</td>
<td>0.575 ± 0.015*</td>
<td>556 ± 17</td>
<td>583 ± 8</td>
<td>125 ± 4</td>
<td>28.8 ± 0.6</td>
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<tr>
<td>CY2</td>
<td>36.9 ± 1.3*</td>
<td>59.9 ± 2.9*</td>
<td>0.619 ± 0.018*</td>
<td>529 ± 32</td>
<td>570 ± 12</td>
<td>122 ± 3</td>
<td>27.0 ± 1.2</td>
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<td>Exercise PIO2 = 55 Torr</td>
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Values are means ± SE. VO2max, maximal rate of O2 uptake; TO2max, maximal rate of convective O2 delivery, i.e., product of cardiac output and arterial blood O2 content; O2 ER, O2 extraction ratio, i.e., VO2max/TO2max; Qmax, maximal cardiac output; HRmax, maximal heart rate; MABP, mean systemic arterial blood pressure; PAP, mean pulmonary arterial pressure. *Significant difference between CY and NT at same PIO2; †significant difference between CY1 and CY2 at same PIO2.

The major observation of the present studies was that the leftward ODC shift resulted in an increase in the rate of maximal convective O2 delivery to the tissues; this effect was potentiated in the CY1 groups by the elevated [Hb] but was still evident in hypoxic exercise in CY2 rats, where [Hb] was normal. The effect of the elevated TO2max on the other hand, was offset by a decrease in the O2 extraction ratio, such that the resulting VO2max depended on the relative magnitude of these two opposing changes, which, in turn, varied with the prevailing PIO2.

Limitations of the experimental model. Sodium cyanate administration affected the O2 transport system at several levels: the pulmonary gas exchange, the rate of convective O2 transport by blood, and the O2 extraction by the tissues. These effects can largely be explained on the basis of the leftward shift of the ODC.

The possible role of cyanate effects not due to the leftward ODC shift is likely to be minor. In the present experiments the CY rats gained less weight than their littermates during the 3 wk of cyanate administration; however, during the following week their weight gain exceeded that of the NT group, such that by the time the experiments were performed there were no significant weight differences between NT and CY rats (Table 1). The rapid elimination of cyanate from the basis of the leftward shift of the ODC. The possible role of cyanate effects not due to the leftward ODC shift is likely to be minor. In the present experiments the CY rats gained less weight than their littermates during the 3 wk of cyanate administration; however, during the following week their weight gain exceeded that of the NT group, such that by the time the experiments were performed there were no significant weight differences between NT and CY rats (Table 1). The rapid elimination of cyanate from the body (4) suggests that cyanate levels would be negligible 1 wk after cessation of cyanate treatment. Essentially no effects other than those secondary to Hb carbamylation have been detected in several species (4). On the other hand, a decrease in respiratory capacity of isolated liver mitochondria was demonstrated in mice treated with a higher sodium cyanate concentration than used here (16). Whether this effect is localized to the liver or extends to other tissues such as skeletal muscle is not clear; in the same study, no effects of cyanate on resting whole body VO2 were observed.

Mitochondria and O2 transportation to VO2max. There was no difference in VO2max between NT and CY rats (Table 1). The rapid elimination of cyanate from the body (4) suggests that cyanate levels would be negligible 1 wk after cessation of cyanate treatment. Essentially no effects other than those secondary to Hb carbamylation have been detected in several species (4). On the other hand, a decrease in respiratory capacity of isolated liver mitochondria was demonstrated in mice treated with a higher sodium cyanate concentration than used here (16). Whether this effect is localized to the liver or extends to other tissues such as skeletal muscle is not clear; in the same study, no effects of cyanate on resting whole body VO2 were observed.

Ventilation and pulmonary gas exchange. There was no difference in VoA/Vo2max between NT and the corresponding CY groups (Table 1), indicating that the ventilatory response to exercise is not influenced by cyanate administration at any of the PIO2 values studied. Previous studies have shown that cyanate does not modify the ventilatory response to hypoxia at rest (3). The present data extend these findings to the exercise condition and indicate that neither the ventilatory response to exer-
cise nor the ventilatory response to hypoxia in conditions of maximal exercise is affected by cyanate.

Despite comparable ventilatory responses, PaO₂ was significantly lower and the (A-a)PO₂ was higher in CY than in NT rats; this effect was larger in the CY2 groups, where [Hb] was lower than in CY1 rats (Fig. 2). A lower resting PaO₂ in rats chronically treated with cyanate has been reported (3, 12, 19). The mechanisms responsible for this are not clear. The increase in (A-a)PO₂ as PlO₂ increased (Fig. 2) suggests a contribution of ventilation-perfusion (VA/Qc) mismatch. The presence of pulmonary hypertension in the CY animals would support a VA/Qc mismatch as responsible for the elevated (A-a)PO₂, since the vascular remodeling that accompanies prolonged pulmonary hypertension could modify pulmonary blood flow distribution and lead to increased VA/Qc heterogeneity. On the other hand, previous studies from our laboratory showed that rats with pulmonary hypertension due to chronic environmental hypoxia do not present a larger (A-a)PO₂ than control animals exercising in hypoxic or normoxic conditions (7, 8). It is possible that the low P₅₀ as well as the presence of low VA/Qc units may contribute to the low PaO₂ of the CY rats: computer models of gas exchange indicate that a leftward shift of the ODC will exaggerate the effect on (A-a)PO₂ produced by a low VA/Qc distribution (20). Additionally, the larger (A-a)PO₂ of CY2 than of CY1 rats may result in part from diffusion limitation, since pulmonary diffusing capacity is influenced by [Hb], which was lower in CY2 rats. Regardless of its mechanism, the decrease in PaO₂ tends to offset the beneficial effect of the leftward shift of the ODC on the oxygenation of Hb in the lungs.

Circulatory convective O₂ transport. Cyanate administration resulted in an increase in CaO₂ through an increase in [Hb] and in O₂ saturation of Hb. The relative contribution of these two factors depended on the PlO₂: during normoxia, CaO₂ increased largely as a result of the elevated [Hb]; as PlO₂ decreased, the contribution of the increased SaO₂ to the higher blood O₂ content of the CY rats became more important. Elevated [Hb] in normoxic environments is a characteristic of cyanate treatment (19) and is associated with elevated serum erythropoietin levels (12). This feature, as well as the pulmonary hypertension and the blunted hypoxic pulmonary hypertensive response observed by us during maximal exercise (Table 2) and by others in resting conditions (19), is characteristic of chronic cyanate administration. These "hypoxia-like" effects (19) are probably the result of the leftward shift of the ODC, which results in lower Po2 values needed to unload O₂ in the tissues.

Maximal Q (Qmax) of the CY groups did not differ significantly from that of their NT counterparts (Table 2). This suggests that the decrease in O₂ extraction associated with the leftward shift of the ODC did not influence myocardial oxygenation to an extent that could result in a deterioration of myocardial performance that would be evidenced, in turn, by a decrease in Qmax. Previous studies in resting conditions showed that cyanate treatment did not modify Q, coronary blood flow, or flow to various organs of rats exposed to PlO₂ values comparable to those of the present study (21). Because Qmax was not affected, the changes in CaO₂ produced by cyanate administration were translated into proportionate changes in the maximal rate of convective O₂ delivery to the tissues.

VO₂max. The effect of cyanate on VO₂max depended on the PlO₂ and, for any PlO₂ level, on the [Hb]. Cyanate treatment decreased VO₂max in normoxia; the magnitude of this decrease was reduced as PlO₂ was lowered. Eventually the effect of cyanate on VO₂max was reversed, with VO₂max of CY1 rats being higher than that in NT rats at the lowest PlO₂. When the confounding effect of the elevated [Hb] was eliminated, VO₂max was lower at all PlO₂ values in the CY rats, with the difference between NT and CY2 rats decreasing as PlO₂ was reduced (Table 2).

The effect of cyanate on VO₂max at various PlO₂ and [Hb] values can be explained if the relationship between VO₂max and To₂max is considered (Fig. 3). ΔVO₂max/ΔTo₂max is the average O₂ extraction ratio, which is represented by the slope of the solid lines fitting the NT and CY groups. In the CY and NT groups, changes in To₂max were accompanied by proportionate changes in VO₂max; however, as a result of the reduced O₂ extraction of the CY groups (Table 2), VO₂max was lower in CY than in NT rats at comparable levels of To₂max. Accordingly, VO₂max of the CY groups was ultimately determined by the balance between the opposing changes in To₂max and in the O₂ extraction by the tissues. The relative magnitude of these changes varied according to the prevailing PlO₂ and, for a given PlO₂, on the [Hb], with the negative effect of cyanate on VO₂max being moderated as PlO₂ decreased and [Hb] increased. Because the negative effect of the leftward ODC shift on VO₂max is reduced as PlO₂ decreases, it is conceivable that the increase in

![Graph showing VO₂max vs. To₂max](http://example.com/vo2max_graph.png)
T$_{O2}^{max}$ may eventually outweigh the decrease in O$_2$ extraction when extremely low P$_{O2}$ values are reached and that a beneficial effect of a leftward ODC shift may occur in these conditions. This could explain the higher survival rate of CY rats exposed to a barometric pressure of 223 Torr, the equivalent of an altitude of 9,000 m (5). Nevertheless, our results clearly show that if [Hb] is maintained constant, a leftward shift of the ODC has a negative impact on V$^\prime$O$_2^{max}$ over a wide range of PIO$_2$ values.

A recent theoretical analysis (23) showed that optimal P$_{50}$, defined as the P$_{50}$ that results in the highest V$_{O2}^{max}$, decreases as P$_{IO2}$ decreases. This is in general agreement with the directional changes observed in the present experiments; however, the predicted sensitivity of V$_{O2}^{max}$ to changes in P$_{50}$ was very low, with V$_{O2}^{max}$ at the various P$_{IO2}$ values changing $\pm$5% over a fairly wide range of P$_{50}$ values. Part of the discrepancy between the present data and the theoretical predictions appears to be due to the difference in the changes in O$_2$ extraction as a function of P$_{IO2}$; in the present experiments the O$_2$ extraction ratio remained relatively constant, for a given P$_{50}$ value, at the three different P$_{IO2}$ values; in the model predictions, however, O$_2$ extraction decreased as P$_{IO2}$ decreased. This behavior would tend to limit the effect on V$_{O2}^{max}$ of a change in P$_{50}$.

A major conclusion of the present study is that the rate at which O$_2$ is consumed by an intact animal during maximal exercise can be dissociated from the rate of convective O$_2$ delivery to the tissues (Fig. 3). Although a dissociation of V$_{O2}^{max}$ from T$_{O2}^{max}$ has been demonstrated in isolated skeletal muscle (9, 10, 15), we have no knowledge of such an observation in intact animals. These results show that, as it was shown in isolated skeletal muscle, T$_{O2}^{max}$ is not the only determinant of V$_{O2}^{max}$ in intact animals.

Tissue O$_2$ extraction. The low O$_2$ extraction ratio of the CY groups agrees with previous observations of a reduction in tissue O$_2$ extraction and an increase in the rate of convective O$_2$ transport needed to maintain “critical” V$_{O2}$ during progressive hypoxia in anesthetized, cyanate-treated dogs (24). Studies on cyanate-treated dogs exercising submaximally also showed elevated convective delivery of O$_2$ in hypoxia and a reduced O$_2$ extraction (18). However, the effect of cyanate on V$_{O2}^{max}$ was not determined in those studies.

A question that remains is, Why did the O$_2$ extraction ratio fall with sodium cyanate administration? In other words, what prevented the venous blood flowing through the contracting muscles from reducing its O$_2$ saturation of Hb? If this were to occur, the arteriovenous O$_2$ content difference and the O$_2$ extraction would increase (Fig. 4), and, other things being equal, V$_{O2}^{max}$ would increase.

Barring a direct depressing effect of cyanate on muscle oxidative capacity, the low O$_2$ extraction ratio could be the result of the leftward shift of the ODC, which would determine that, to lower O$_2$ saturation of Hb in the tissue capillaries, P$_{O2}$ must be lowered to levels that would compromise the PO$_2$ gradient between the capillary and the cell, thus limiting O$_2$ diffusion between those two sites (Fig. 4). This explanation implies that tissue O$_2$ diffusion is a critical determinant of...
VO_{2max}, a concept that has been advanced by Wagner and colleagues (9, 10, 15, 22). If venous PO_{2} is considered a reflection of the average tissue capillary PO_{2} and if the skeletal muscle cell PO_{2} during maximal exercise is assumed to be very low (6, 14), then venous PO_{2} values should reflect the PO_{2} diffusion gradient from capillary to cell (9, 10, 15, 22). In the present case, mixed venous PO_{2} (PV_v) is taken as representative of muscle effluent PO_{2}. Although this is a simplification, the contribution of nonmuscle tissues to VO_{2} during maximal exercise is minimal, and PV_v should still largely reflect the PO_{2} of working locomotory muscles. If VO_{2max} were in part limited by tissue O_{2} diffusion, a positive correlation between VO_{2max} and PV_v should be observed. That this is the case is shown in Fig. 5, where VO_{2max} is plotted as a function of PV_v for all the experimental groups. Within the constraints of the assumptions mentioned above, the positive relationship between VO_{2max} and PV_v is consistent with the notion that tissue diffusion limitation is one of the determinants of VO_{2max} in intact animals: a larger PO_{2} gradient between capillary and cell is needed to effect a larger O_{2} transfer between those sites.

In summary, chronic administration of sodium cyanate to rats resulted in a leftward shift of the ODC and marked changes in the O_{2} transport system. The net effect of these changes on VO_{2max} was the result of a balance between an increase in the rate of convective O_{2} delivery and a decrease in the O_{2} extraction ratio, the relative magnitude of which depended on the P_{O2} and the [Hb]. The decrease in O_{2} extraction is consistent with a limitation of tissue O_{2} diffusion secondary to the leftward shift of the ODC, which would compromise the PO_{2} diffusion gradient between capillaries and muscle cells. The data support the notion that VO_{2max} of intact animals is determined by the interaction between the rate of O_{2} delivered to the tissue capillaries and the rate of O_{2} diffusion from capillaries to cells.

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REFERENCES