Myoglobin $O_2$ tension determined from measurements of carboxymyoglobin in skeletal muscle

R. F. COBURN AND L. B. MAYERS

Department of Graduate Physiology and Cardiovascular-Pulmonary Division, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

COBURN, R. F., AND L. B. MAYERS. Myoglobin $O_2$ tension determined from measurements of carboxymyoglobin in skeletal muscle. Am. J. Physiol. 220(1): 66-74. 1971.—We have developed a method of estimating muscle intracellular oxygen tension in the anesthetized cat. In the present study we have used this method to determine the steady-state distribution of CO between blood and muscle as a function of arterial $P_0_2$. These data are required in order to compute mean myoglobin $P_0_2$. At the beginning of each experiment 50-75 ml of 99.9% CO was slowly administered via the rebreathing system; this resulted, after uptake via the lungs and mixing in the body stores, in a blood carboxyhemoglobin percent saturation (HbCO) of 12.1-23.5. This raised the MbCO and made possible a more accurate analysis of CO in muscle biopsy specimens. Chromium 51-labeled erythrocytes were also injected intravenously at the beginning of each experiment to allow quantitation of hemoglobin in the muscle biopsy specimens (27). We skinned the hindlegs and partially dissected the hamstring muscle, taking care not to disturb vascular or nervous tissue. Thirty minutes before the muscle was to be biopsied, we adjusted the $P_0_2$ tension of the gas in the rebreathing system so that the arterial $P_0_2$ ranged from 50 to 172 mm Hg in 10 experiments and from 28 to 38 mm Hg in five “hypoxemia” experiments. After arterial and mixed venous blood samples were drawn, the hamstring muscle was suddenly clamped, isolating the middle third of the muscle from its blood supply, and 10-20 ml of muscle were rapidly dissected and placed in a nitrogen-filled container. The biopsy specimen was weighed, homogenized under nitrogen with 5 times its volume of water, placed in a sealed test tube, and stored at 4 C until analyses for CO, $^{51}$Cr radioactivity, and myoglobin concentration could be performed. Evidence was obtained that significant quantities of CO were not lost in handling of the muscle specimen.

Carbon monoxide was extracted from the diluted muscle homogenate by bubbling 100% $O_2$ through the vigorously stirred specimen, collecting this gas in a 250-ml tonometer, and analyzing it in an infrared meter. This procedure was repeated until no further CO was detectable. The infrared meter can detect 0.0006% CO in gas or 0.15 $\mu$g CO in muscle specimens (5). In most instances, approximately 60-80% of the total CO was removed during the first...
disintegrations per minute per gram hemoglobin (51Cr/g Hb); b) the CO content of venous blood, expressed as milliliters per gram hemoglobin (CO/g Hb); and c) the 51Cr radioactivity in venous blood, expressed as milliliters per gram hemoglobin (51Crbiop).

Men, in milliliters, was determined from measurements of a) the 51Cr radioactivity in venous blood, expressed as disintegrations per minute per gram hemoglobin (51Cr/g Hb); and because endogenous CO production cannot influence the ratio of CO bound to other ferroproteins is insignificant (8). The quantity of CO bound to hemoglobin in the biopsy specimen was determined by subtracting the venous CO from the total venous CO. In order to determine the ratio of CO bound to the transferrin and myoglobin, we first determined the quantity of CO bound to transferrin and then calculated the quantity of CO bound to myoglobin in the biopsy specimen. 

The quantity of CO bound to myoglobin at the time of biopsy should equal the total CO in the sample minus that bound to hemoglobin, assuming that the quantity of CO bound to other ferroproteins is insignificant (8). The quantity of CO bound to hemoglobin in the biopsy specimen, in milliliters, was determined from measurements of a) the 51Cr radioactivity in venous blood, expressed as disintegrations per minute per gram hemoglobin (51Cr/g Hb); b) the CO content of venous blood, expressed as milliliters per gram hemoglobin (CO/g Hb); and c) the 51Cr radioactivity in the biopsy specimen in disintegrations per minute (51Crbiop).

\[
\text{ml CO bound to hemoglobin} = \frac{\text{51Crbiop}}{\text{51Cr/g Hb}} \times \frac{\text{CO/g Hb}}{\text{Hb}} (1)
\]

The myoglobin content of the specimen (Mb) was measured spectrophotometrically (24) and MbCO was computed as follows:

\[
\text{MbCO} = \text{CO bound to myoglobin, in ml/(Mb × 1.34)} \times 100 (2)
\]

Mb is in grams. The factor 1.34 converts grams myoglobin into the capacity of myoglobin to bind CO (in milliliters). MbCO is expressed in percent saturation.

**Blood 14CO experiments.** In seven experiments we studied the effect of arterial PO2 on the partition of CO between blood and muscle by measuring blood 14CO radioactivity. We added 14CO to the rebreathing system gas and allowed the isotope to be adsorbed and mixed completely in the body stores over a period of 1 hr. Without ligating the right deep femoral vein, we inserted a 2-mm (od) polyethylene catheter. Blood drawn through this catheter was assumed to efflux from muscle. In four of the experiments we subsequently ligated the deep femoral vein at the knee in order to decrease the fraction of blood transversing this vein that drained the skin. In all the experiments we set the oxygen tension of the inspired gas at a desired value, kept it constant for 30 min, and then obtained samples of arterial and deep femoral blood. These samples were analyzed for PO2, PCO2, O2 content, and pH. Mixed venous blood was also drawn and analyzed for 14CO radioactivity. We then altered the inspired PO2 and repeated this sequence for a total of two to four “runs,” varying the inspired PO2 within the range 30–600 mm Hg.

Since over 95% of body CO and 14CO is probably found in either blood or muscle, measurements of blood CO should allow one to determine shifts of CO from blood to muscle. Blood 14CO radioactivity (14CO/g) was expressed as disintegrations per minute per gram hemoglobin; influx of 14CO into blood would increase this unit, and efflux decrease it. Changes in venous hematocrit should not alter 14CO/g (20). 14CO was used, rather than making measurements of blood 14CO, since the latter can be influenced by endogenous production.

In three of these experiments we administered 14CO in addition to 14CO at the beginning of the experiment. We skinned the left thigh and biopsied the hamstring muscle. This procedure enabled us to compare blood 14CO/g changes with MbCO measurements.

**Simultaneous arterial and deep femoral venous blood measurements.** In two additional experiments we made measurements every 30–60 sec of HbCO in arterial blood and in deep femoral venous blood while the oxygen tension in the inspired gas was decreasing at a rate of 5–7 mm Hg/min. This was accomplished by clamping the O2 inlet and letting the PO2 in the rebreathing gas decrease at the desired rate. Deep femoral blood was drawn at a rate of approximately 5–6 ml/min.

**Analytical Methods**

Blood gas tensions were measured with O2, CO2, and pH electrodes (25, 33). Blood oxygen contents were determined with a Van Slyke apparatus (37). Blood HbCO was measured using a method described previously (5) in which CO is extracted from blood and measured with an infrared meter (at ± 0.03% saturation). Blood 14CO radioactivity was determined by the method of Luomanmäki (20) where extracted CO was counted with an ionization chamber (Cary chamber and Cary model 31 electrometer). This method has an error of ±2% of the total radioactivity in blood. The 51Cr radioactivity was measured in a well scintillation counter.

**Mean Myoglobin Oxygen Tension**

Since CO and O2 compete for binding with myoglobin, the PO2 of a solution of myoglobin can be computed from the PCO2 of the solution and the percentage of myoglobin that is bound to CO. The reaction of CO with oxymyoglobin (MbO2) is:

\[
\text{CO + MbO2} \rightleftharpoons \text{MbCO + O2} (3)
\]

This reaction can also be written:

\[
\text{PO2/MbO2} = M_{MB} \frac{\text{Pco}}{\text{MbCO}} (4)
\]

M_{MB} is the relative equilibrium constant. In equation 4, MbO2 and MbCO are concentrations. Equation 4 shows that the ratio PO2/MbO2 can be computed from MbCO, Pco, and M_{MB}. Because the oxymyoglobin dissociation curve is monotonic (it is a rectangular hyperbola (2)), there is a unique PO2 for a given ratio PO2/MbO2.

Consider a living muscle in which the distribution of myoglobin is uneven and PO2 varies throughout the cell. At each point where myoglobin is present the PO2 and Pco should be nearly in kinetic equilibrium with MbCO and MbO2. At each point Pco/MbCO will be proportional to PO2/MbO2. If we sum the ratio Pco/MbCO of all points in the cell and obtain an average value, this will allow...
calculation of an average ratio PO₂/MbO₂, which in turn will allow determination of a mean PO₂ in equilibrium with myoglobin (mPMbo₂).

In the present experiments we computed mPMbo₂ as follows:

**Step 1.** We computed mean Pco in muscle (which we assume is equal to the mean Pco in capillaries in muscle) using the Haldane equation (7):

\[
\text{mPco} = \frac{\text{HbCO} \times \text{mPco₂}}{\text{MbHb} \times \text{mHbO₂}}
\]

where mPco, and mPco₂ are mean capillary values of CO and O₂ tension, and mHbO₂ the mean capillary oxyhemoglobin percent saturation. The mPco₂ was obtained by Bohr integration from measurements of arterial and deep femoral Pco and the dog oxyhemoglobin dissociation curve for the appropriate pH (26). The mHbO₂ was obtained from mPcO₂ and the oxyhemoglobin dissociation curve at an estimated mean capillary pH.

**Step 2.** We computed mPMbo₂/mMbO₂ from MbCO and mPco₂ (equation 4).

**Step 3.** We read off the value of mPMbo₂ from a curve where we had plotted PMbo₂/MbO₂ as a function of Pco₂.

The mPMbo₂ could also be computed using the following relationship which combines steps 1 and 2.

\[
\text{mPMbo₂/mMbO₂} = \frac{\text{HbCO} \times \text{mPco₂}}{\text{MbHb} \times \text{mHbO₂}}
\]

*Figure 1* shows relationships of mPMbo₂ and mPco₂ for given ratios MbCO/HbCO as computed with equation 6.

This figure illustrates that MbCO/HbCO is a function of mPMbo₂ and mPco₂.

**Partition Coefficient of Myoglobin**

It was necessary to measure M_Mb in canine skeletal muscle since there was no previous value available for this species. We prepared and froze solutions of myoglobin in phosphate buffer of pH 8.3 (41). These solutions were shown by starch gel electrophoresis to be free of hemo- globin. The spectrophotometric curves were those of oxy-myoglobin. Two different solutions were made containing 0.194 and 0.246 mg of myoglobin per 100 ml, respectively. The solutions were tonometered at 37°C with gases containing 0.107-2.976 mm Hg Pco and 3.40-31.50 mm Hg Po₂. Since myoglobin is much more unstable at a lower pH, tonometry was performed at pH 8.3; however, M_Mb is reportedly not affected by pH (2, 29). Gas tensions were chosen so that virtually all myoglobin would be combined with either CO or O₂. Each solution was paired, one aliquot being equilibrated with a ratio of CO/O₂ sufficiently high to almost saturate myoglobin with CO. We could therefore determine the quantity of “active” myoglobin (myoglobin that could combine with CO or O₂) and we found that active myoglobin concentrations decreased at rates of approximately 5-10% /hr, probably due to conversion to metmyoglobin. MbCO was measured using the infrared blood method (5) and MbO₂ percent saturation computed as equal to 100 – MbCO. The Pco₂ and Po₂ of the solutions were obtained from analysis of the gas used for tonometry using gas chromatography, the Po₂ of the solutions was also determined with a membrane-covered platinum electrode. We found it necessary to allow 1–2 hr to reach equilibrium, as indicated by equal O₂ tensions in gas and solution.

**RESULTS**

**Muscle Biopsy Experiments**

Muscle MbCO data are listed in Table 1. Single determinations of MbCO had considerable errors (sw ± 17% of total), due to errors in extracting CO from the diluted homogenate. The values in Table 1 are means of triplicate determinations. The ratio MbCO/HbCO is also given for each experiment where MbCO was determined, with both MbCO and HbCO expressed as percent of total pigment bound to CO. This ratio averaged 1.10 ± 0.12 and did not change significantly (P > 0.1) as a function of arterial PO₂ over the range 50–175 mm Hg. When the arterial PO₂ fell below 40 mm Hg (five experiments), MbCO/HbCO increased significantly, (P < 0.01) averaging 1.94 ± 0.20.

**Blood ^14CO Experiments**

Venous ^14CO/g remained constant when arterial PO₂ ranged from 40 to over 700 mm Hg (corresponding to femoral venous blood PO₂ values of 25-70 mm Hg), but decreased markedly when the arterial PO₂ fell below 40 mm Hg (and the deep femoral venous PO₂ below 50–25 mm Hg). Figure 2 illustrates the results of a typical ex-
Table 1. Carboxyhemoglobin and carboxymyoglobin data

<table>
<thead>
<tr>
<th>Exp No</th>
<th>Weight, kg</th>
<th>PaO2, mm Hg</th>
<th>PaCO2, mm Hg</th>
<th>Hb, g/100 ml</th>
<th>Mb, mg/g</th>
<th>HbCO, % Satn</th>
<th>MbCO, % Satn</th>
<th>MbCO/HbCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.2</td>
<td>62</td>
<td>10.23</td>
<td>7.2</td>
<td>17.62</td>
<td>19.2</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16.0</td>
<td>110</td>
<td>13.90</td>
<td>5.7</td>
<td>12.11</td>
<td>13.9</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17.0</td>
<td>74</td>
<td>14.25</td>
<td>4.8</td>
<td>17.50</td>
<td>6.6</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>17.1</td>
<td>50</td>
<td>14.80</td>
<td>5.4</td>
<td>19.10</td>
<td>13.4</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>17.0</td>
<td>68</td>
<td>11.70</td>
<td>5.0</td>
<td>12.96</td>
<td>19.8</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>16.0</td>
<td>62</td>
<td>13.95</td>
<td>5.8</td>
<td>16.30</td>
<td>8.6</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>13.7</td>
<td>14.00</td>
<td>13.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>15.5</td>
<td>13.29</td>
<td>13.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>14.5</td>
<td>16.0</td>
<td>14.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>13.1</td>
<td>90</td>
<td>14.20</td>
<td>5.4</td>
<td>16.50</td>
<td>18.0</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>17.4</td>
<td>12.65</td>
<td>18.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>15.4</td>
<td>19.1</td>
<td>13.00</td>
<td>5.0</td>
<td>25.0</td>
<td>1.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>15.2</td>
<td>13.75</td>
<td>23.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>12.6</td>
<td>172</td>
<td>14.75</td>
<td>4.4</td>
<td>17.40</td>
<td>24.0</td>
<td>1.40</td>
<td></td>
</tr>
</tbody>
</table>

Mean ±SE

Severe hypoxemia experiments

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Weight, kg</th>
<th>PaO2, mm Hg</th>
<th>PaCO2, mm Hg</th>
<th>Hb, g/100 ml</th>
<th>Mb, mg/g</th>
<th>HbCO, % Satn</th>
<th>MbCO, % Satn</th>
<th>MbCO/HbCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>13.7</td>
<td>38</td>
<td>13.8</td>
<td>4.8</td>
<td>18.0</td>
<td>43.0</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>13.7</td>
<td>34</td>
<td>14.0</td>
<td>3.7</td>
<td>19.40</td>
<td>26.2</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>15.5</td>
<td>32</td>
<td>13.3</td>
<td>2.9</td>
<td>15.00</td>
<td>26.5</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>14.3</td>
<td>28</td>
<td>16.0</td>
<td>6.3</td>
<td>14.20</td>
<td>34.9</td>
<td>2.43</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15.2</td>
<td>50</td>
<td>13.7</td>
<td>6.5</td>
<td>18.45</td>
<td>31.0</td>
<td>1.68</td>
<td></td>
</tr>
</tbody>
</table>

Mean ±SE

All animals were given carbon monoxide at beginning of study. Measurements were made after the administered CO was completely mixed in the body stores. PAO2 = arterial oxygen tension, PAO2 = venous oxygen tension, [Hb] = blood hemoglobin concentration, [Mb] = muscle myoglobin concentration, HBCO = carboxyhemoglobin, MbCO = carboxymyoglobin. * Measured in muscle biopsy specimens.

In the experiments where both blood 14CO/g and muscle MbCO were measured (experiments 7, 8, and 9) we found that during hypoxemia a 1% decrease in blood 14CO/g was associated with an average increase in MbCO/HbCO of approximately 5%.

Simultaneous Arterial and Deep Femoral Venous Blood Measurements

Figure 4 shows data obtained in one of the two experiments where arterial and deep femoral venous blood were obtained while the inspired PaO2 was decreasing. Arterial and venous HbCO remained equal as arterial PaO2 fell from 58 to 40 mm Hg, but further decreases in arterial PaO2 resulted in a fall in venous HbCO relative to arterial HbCO. Similar results were found in the other experiment. These experiments provided further evidence that MbCO/
FIG. 4. Simultaneous arterial and deep femoral venous blood measurements. Arterial and deep femoral venous blood samples were collected at 30-sec intervals during and after progressive respiratory hypoxia. When arterial PO₂ fell below 40 mm Hg, the venous HbCO became smaller than arterial HbCO, indicating that CO was diffusing out of circulating blood.

Table 2. CO and O₂ partition coefficient for myoglobin

<table>
<thead>
<tr>
<th>[Mb], mg/100 ml</th>
<th>PCO/Po₂</th>
<th>MbCO</th>
<th>% Satn</th>
<th>MbO₂</th>
<th>% Satn</th>
<th>MMb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.194</td>
<td>0.0083</td>
<td>17.5</td>
<td>82.5</td>
<td>30.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.194</td>
<td>0.0081</td>
<td>14.3</td>
<td>85.7</td>
<td>21.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.194</td>
<td>0.0049</td>
<td>10.2</td>
<td>90.0</td>
<td>23.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.246</td>
<td>0.0085</td>
<td>72.0</td>
<td>28.0</td>
<td>30.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.246</td>
<td>0.236</td>
<td>82.0</td>
<td>18.0</td>
<td>24.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.246</td>
<td>0.183</td>
<td>81.5</td>
<td>18.5</td>
<td>24.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.246</td>
<td>0.041</td>
<td>47.0</td>
<td>53.0</td>
<td>21.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SE 24.9 ± 1.5

Tonometry was performed at pH 8.3 and 37°C. [Mb] = myoglobin concentration, MbCO = carbamoyxymyoglobin, MbO₂ = oxy-myoglobin, MMb = partition coefficient. MbO₂ was determined as 100 – MbCO (correcting for inactive myoglobin).

FIG. 5. Arterial and mean myoglobin oxygen tensions obtained in 11 runs in four experiments performed at normal HbCO.

then blood HbCO was in the normal range, so we did not have to worry about the uncertainty in correction for effects of CO on the oxymyoglobin dissociation curve. In runs where ^4ÇO/g did not change from the value found at normal arterial Po₂, MbCO/HbCO was assumed to be 1.10, the mean value found in the muscle biopsy experiments. In runs where arterial Po₂ was less than 35–40 mm Hg and blood ^4ÇO/g decreased, we computed MbCO/HbCO from the decrease in ^4ÇO/g using the relationship that 1% decrease in blood ^4ÇO/g was equivalent to an increase in MbCO/HbCO of 5%, as noted above. In computing âPmbo₂ we used values of 25 for MMb and 208 for MMb (29).

In four experiments where Paₐ₀ ranged from 35 to 490 mm Hg and blood ^4ÇO/g did not vary from that found at normal arterial Po₂, âPmbo₂ ranged from 3.7 to 5.5 mm Hg. The âPmbo₂ averaged 4.7 ± SE 0.3 mm Hg in the “runs” where Paₐ₀ was 67–120 mm Hg. As the arterial Po₂ decreased from 490 to 35 mm Hg, âPmbo₂ decreased approximately 1.5–2 mm Hg. When Paₐ₀ was less than 35 mm Hg and ^4ÇO and ^4ÇO shifted from blood into muscle, the computed ratio MbCO/HbCO increased to 1.71–3.28 and âPmbo₂ ranged from 0.5 to 1.5 mm Hg. These data are plotted in Fig. 5.

Discussion

In the present study we have, for the first time, made direct measurements of the distribution of carbon monoxide in blood and resting skeletal muscle and determined the effects of changes in arterial oxygen tension. These data have allowed us to compute the mean oxygen tension in equilibrium with myoglobin in hamstring muscle at different arterial oxygen tensions. We found that âPmbo₂ averaged 4.7 mm Hg at ambient arterial oxygen tensions and that there was only a 1–1.5 mm Hg decrease in âPmbo₂ as arterial oxygen tensions were decreased from 490 to 40 mm Hg. At arterial tensions below 40 mm Hg, âPmbo₂ decreased to values as low as 0.5 mm Hg. In considering the implications of these data we shall first discuss the assumptions and errors in the calculation of âPmbo₂.

The principal assumptions that we have made are as follows:

The Po₂ and PCO are in kinetic equilibrium with MbO₂ and MbCO at every point in the cell when myoglobin is present. This assumption is strongly supported by the very fast rates of reaction and dissociation of O₂ and CO and myoglobin (10, 21). This assumption should be correct even if gas tensions at a given point are changing, although tensions probably remained nearly constant at a given point in our steady-state experiments. Millikan (21, 22) has estimated that spectrophotometric MbO₂ measurements reflect Po₂ with a delay of less than 0.01 sec.

Myoglobin is a specific indicator of Po₂ and the physical constants we have used in computing âPmbo₂ are correct for myoglobin in a living cell. MMb does not vary as a result of changes in pH, osmolarity, protein concentration, myoglobin concentration (2, 29), or the presence of reduced myoglobin (2, 29). We cannot, however, exclude the possibilities that myoglobin is altered significantly during extraction and purification and that in vitro studies and measurements of MMb may differ from those of myoglobin in vivo.

The PCO in capillary blood that is in diffusion equilibrium with muscle Pco is in kinetic equilibrium with mean POT, HbO₂, and MbCO in capillary blood. This statement implies that as Po₂ and HbO₂ decrease in blood flowing in a
muscle capillary, Po2 and HbCO are in kinetic equilibrium with HbO2 and Po2 at every point in the capillary blood. As the Po2 and HbO2 in capillary blood decrease, the affinity of hemoglobin for CO increases and CO diffuses from plasma into erythrocyte and reacts with hemoglobin. This assumption therefore implies that diffusion of CO into erythrocytes and rates of chemical reaction of CO with hemoglobin are infinitely fast, an assumption that is not rigorously correct since the rate of reaction of CO with erythrocytes is partially limited by diffusion into the cell and its rate of reaction with hemoglobin (32). However, it can be shown that the above assumption is not critical to the calculation of MbCO2 due to the relatively small change in Pco in blood traversing muscle capillaries. In our steady-state experiments there should have been no net flux of CO between blood and muscle, and the total increase in CO bound to hemoglobin should have equaled that lost from plasma. It is possible to estimate the average Pco gradient between plasma and erythrocytes and thereby approximate the effects of diffusion and CO reaction rates. The mean Pco gradient during transit in a peripheral capillary can be estimated by dividing the mean rate of CO uptake by \( \theta \) (32). (This neglects diffusion within plasma.) As calculated with the Haldane equation, the average difference in CO tension between arterial and venous blood is approximately 8 \( \times 10^{-4} \) mm Hg (at a HbCO of 0.8% saturation) and approximately 9.5 \( \times 10^{-9} \) ml of CO would enter the erythrocytes in 1 ml of blood with a hematocrit of 50%. \( \theta \) is taken to be 3 \( \times 10^{-7} \) ml/(sec \times mm Hg \times ml blood) (32), corresponding to a Po2 of approximately 40 mm Hg. If we take an average transit time of 1 sec, the average CO gradient is computed as 3 \( \times 10^{-7} \) mm Hg or approximately 0.003 of the total change in Pco in capillary blood. Some CO probably diffuses out of arterial segments of capillaries through muscle into venous segments of capillaries, increasing the quantity of CO entering erythrocytes in the distal portions of capillaries and giving a higher Pco gradient between plasma and erythrocyte than we calculated. However, the calculated plasma-erythrocyte CO gradient is so small that it seems unlikely that plasma or red cell Pco varies significantly from that in equilibrium with hemoglobin.

There is, in addition, experimental support for the concept that CO remains in near equilibrium with hemoglobin in peripheral capillaries. The CO diffusing capacity of the placenta has been measured (18) by estimating the CO gradient between maternal and fetal blood from calculations of Pco in maternal and fetal capillaries with the Haldane equation. This method assumed that CO is in near equilibrium with HbCO, HbO2, and Po2 in both maternal and fetal capillaries. The finding that placental diffusing capacities were similar whether determined with CO diffusing from maternal to fetal blood, or in the opposite direction, was taken as evidence that this method of calculating CO gradients did not introduce large error.

It should be noted that there are errors in computing \( M_{\text{Hb}} \), that will influence the computed \( M_{\text{Pco}} \), as will be discussed in a later section.

\( M_{\text{Hb}} \) is constant. Evidence is available that \( M_{\text{Hb}} \) may vary slightly when HbCO + HbO2 approaches 100% saturation or 0% saturation (13, 30). However, our calculations of \( M_{\text{Pco}} \) were made under conditions where HbCO + HbO2 ranged from 60 to 80%. Although questioned in the past (1), the concept that the partition coefficient for the blood reaction remains constant in the presence of changes in pH seems to have gained acceptance (28).

**Error in Computing \( M_{\text{Pco}} \)**

There are several sources of error in the calculation of \( M_{\text{Pco}} \) in addition to error that may result if the assumptions listed above are not correct.

**Values of MbCO/HbCO obtained in muscle biopsy experiments were used in computing \( M_{\text{Pco}} \) in 14CO experiments.** The two groups of experiments were similar except that HbCO and MbCO were elevated in the muscle biopsy experiments. This should not cause error in applying the data to experiments where blood HbCO is in the normal range since the partition of CO between blood and extracellular vascular tissue does not change as blood HbCO is increased from 1% to over 50% saturation (19, 20). We cannot exclude the possibility that there may have been physiological variation in MbCO/HbCO in different experiments and that use of a mean value may have caused error. In recent experiments, however (unpublished data), we have not found significant differences in MbCO/HbCO in several different muscles.

We have also measured MbCO/HbCO at normal levels of HbCO and obtained values not significantly different from those obtained in the present study.

**Effects of error in MbCO/HbCO.** Computed \( M_{\text{Pco}} \) is sensitive to error in MbCO/HbCO. We used an average value of MbCO/HbCO found in the muscle biopsy experiments in computing \( M_{\text{Pco}} \) in runs where Po2 was greater than 40 mm Hg. The best evidence that MbCO/HbCO is constant at Po2, greater than 40 mm Hg under the conditions of our experiments includes the data obtained from the simultaneous arterial and deep femoral venous blood measurements and the finding that blood \( ^4\text{CO} \) remained constant. The muscle biopsy experiments also support this finding; however, on the basis of these alone we could not exclude small changes in MbCO/HbCO over this range of Po2. The mean MbCO/HbCO in those experiments where Po2 was greater than 40 mm Hg could have been in error by \( \pm 0.12 \) which could produce an error in \( M_{\text{Pco}} \) of 1 mm Hg. In the severe hypoxemia experiments, error in MbCO/HbCO was probably larger, since we computed this ratio from the shift of \(^4\text{CO}\) out of circulating blood. The value used, however, is not as critical, and error in \( M_{\text{Pco}} \) expressed as mm Hg should be very small.

**Errors in computing \( M_{\text{Pco}} \) and \( M_{\text{Pco}} \).** We have concluded that only small errors result from the assumption that erythrocyte Pco is in equilibrium with mean HbO2, HbCO, and Po2 in capillary blood. There are however, several additional sources of error.

**Error in computing \( M_{\text{Pco}} \).** We could arise due to error in our measurement of venous Po2. We have considered that computed values of \( M_{\text{Pco}} \) represent values of mean myoglobin Po2 in the hamstrings muscles, where values of MbCO/HbCO were available. However, our calculation of \( M_{\text{Pco}} \) was based on measurements made on blood draining many different leg muscles. It is possible that \( M_{\text{Pco}} \) in blood effluxing from hamstrings muscle is different.
from that in deep femoral blood. Since one of our primary goals in these experiments was to avoid dissecting or otherwise disturbing the preparation, we have had to accept this error in estimating \( m \text{PCO}_2 \) of hamstring muscles, as well as possible error due to blood in the deep femoral vein effluxing from nonmuscular structures.

As computed with the Bohr integration technique, \( m \text{PCO}_2 \) is probably closer to plasma \( \text{PO}_2 \) than to intraerythrocyte \( \text{PO}_2 \) in equilibrium with \( \text{HbO}_2 \). This may cause an error of approximately 1 mm Hg (9, 16). The major error in computing \( m \text{PCO}_2 \) probably results from effects of uneven blood flow or ratios of oxygen extraction to blood flow (\( \text{VO}_2/\text{Q} \)) in different areas of muscle.

Fortunately, errors in computing \( m \text{PCO}_2 \) and \( m \text{PCO}_2 \) have a relatively small effect on computed \( m \text{Pmbo}_2 \). For example, if we assume \( m \text{PCO}_2 \) to be equal to venous \( \text{PO}_2 \), as have other workers studying muscle respiration (34, 35), \( m \text{Pmbo}_2 \) would decrease less than 1 mm Hg in most of our experiments. If hamstring muscle \( m \text{PCO}_2 \) varies 10 mm Hg from that estimated from blood draining the entire limb, \( m \text{Pmbo}_2 \) would be in error by less than 0.5 mm Hg.

In order to estimate the small effect of nonuniform \( \text{VO}_2/\text{Q} \) we have made calculations of \( m \text{Pmbo}_2 \) in two compartments with different \( \text{VO}_2/\text{Q} \). Compartment A has \( \text{VO}_2 \) per unit blood flow twice that in compartment B, but \( \text{Q} \) is equal in the two compartments. Arterial blood is assumed to have a \( \text{Pa}_02 \) of 70 mm Hg, giving \( \text{HbO}_2 \) of 90% and \( \text{O}_2 \) content of 18 ml/100 ml. If we first consider the whole muscle, venous blood from the muscle has a \( \text{Pa}_02 \) of 40 mm Hg, \( \text{HbO}_2 \) of 75%, and \( \text{O}_2 \) content of 15. The \( \text{Pmbo}_2 \) of the whole muscle was calculated to be 59 mm Hg and \( m \text{Pmbo}_2 \) to equal 4.7, assuming \( \text{MbCO}/\text{HbCO} \) of 1.1. Looking at each compartment, \( A \) extracted 0.4 ml \( \text{O}_2 \) per milliliter of blood and had a venous \( \text{Pa}_02 \) of 44 mm Hg, \( \text{HbO}_2 \) of 70%, \( \text{O}_2 \) content of 14 ml/100 ml, and \( m \text{PCO}_2 \) of 52 mm Hg. Compartment \( B \) extracted 0.02 ml \( \text{O}_2 \) per milliliter and had a venous \( \text{Pa}_02 \) of 54, \( \text{HbO}_2 \) of 80%, \( \text{O}_2 \) content of 16 ml/100 ml, and \( m \text{PCO}_2 \) of 62 mm Hg. If we assume that \( \text{MbCO}/\text{HbCO} \) is equal in the two compartments, \( m \text{Pmbo}_2 \) in compartment \( A \) is 4.2 mm Hg and in compartment \( B \), 4.8 mm Hg, giving an average \( m \text{Pmbo}_2 \) of 4.5. This is very close to the value 4.7 mm Hg computed for the muscle as a whole.

Error in \( M_{\text{Mb}} \) and \( M_{\text{Hb}} \). Possible error also arises due to uncertainty in values used for \( M_{\text{Mb}} \) and \( M_{\text{Hb}} \). \( M_{\text{Mb}} \) was assumed to be 208. This value was recently determined on human hemoglobin solutions using gas chromatography, and it had a standard error of only 2% (28). There is evidence that dog \( M_{\text{Hb}} \) is identical to or similar to that found in man (1). As determined in the present study, \( M_{\text{Hb}} \) had an error (SE) of approximately 7–8%. Our average value of 23 was larger than that found by Theorell (36) using horse myoglobin at 37°C, but smaller than those found by Antonini (2) with horse myoglobin 20°C.

We conclude that our estimate \( m \text{Pmbo}_2 \) at normal arterial \( \text{PO}_2 \) may be in considerable error, the major source being error in \( \text{MbCO}/\text{HbCO} \). If the mean \( \text{MbCO}/\text{HbCO} \) varied 2% this could produce an error of 1.5–2 mm Hg in \( m \text{Pmbo}_2 \) at a given \( m \text{PCO}_2 \). Maximal error in \( m \text{Pmbo}_2 \) resulting from uncertainty of \( m \text{PCO}_2 \) and \( m \text{PCO}_2 \) is estimated to be less than 0.5 mm Hg, and from error in \( M_{\text{Mb}} \) or \( M_{\text{Hb}} \), approximately 1 mm Hg. If all of these sources of error were additive, \( m \text{Pmbo}_2 \) might be in error as much as 3 mm Hg; but since it is likely that some of the errors might be in opposition, our average \( m \text{Pmbo}_2 \) is probably accurate within 1–2 mm Hg, and the normal mean \( \text{PO}_2 \) in equilibrium with myoglobin appears to be in the range 2.5–6.5 mm Hg. All of the sources of error would probably produce relatively consistent error at varying values of \( m \text{PCO}_2 \); therefore, the extent of change in \( m \text{Pmbo}_2 \) with change in arterial \( \text{PO}_2 \) found here should be valid. We consider that our data support the idea that changes in intracellular \( \text{PO}_2 \) are very small over the range of arterial \( \text{PO}_2 \) of approximately 300–40 mm Hg. Our finding of a marked decrease in \( m \text{Pmbo}_2 \) when arterial \( \text{PO}_2 \) was below 40 mm Hg is not influenced by the above errors.

**Significance of Our Calculated \( m \text{Pmbo}_2 \)**

The values of \( m \text{Pmbo}_2 \) found in the present study are estimates of the mean intracellular oxygen tension in equilibrium with myoglobin in hamstring muscle. A more precise interpretation would depend on the location of myoglobin in the cell. It should first be pointed out that \( m \text{Pmbo}_2 \) is influenced principally by muscle fibers that contain the largest quantities of myoglobin. Since recent studies have suggested that myoglobin may be bound to myosin (11, 14), our values of \( m \text{Pmbo}_2 \) may reflect the mean \( \text{PO}_2 \) of myosin. The possibility that myoglobin is distributed throughout cytoplasm has not been excluded. There may be very large variations in the \( \text{PO}_2 \) that is in equilibrium with myoglobin in different portions of the cell, and therefore large variations in \( \text{MbCO} \) within the cell. The finding of a relatively low value for \( m \text{Pmbo}_2 \) suggests that myoglobin may be localized in close proximity to mitochondria.

The \( m \text{Pmbo}_2 \) values found in the present study provide insight into the degree of adaptation to arterial hypoxemia that occurs in resting skeletal muscle. The evidence that an increase in functioning capillaries occurs during hypoxemia has been reviewed by others (12, 34, 35). Our finding that \( m \text{Pmbo}_2 \) decreased less than 2 mm Hg as arterial \( \text{PO}_2 \) was decreased from 400 to 40 mm Hg suggests that there are mechanisms which tend to maintain intracellular \( \text{PO}_2 \) at a nearly constant level just above the "critical" \( \text{PO}_2 \) of mitochondria. Whalen (39, 40) recently has come to the same conclusion based on his measurements using intracellular electrodes. Our data indicate that the intracellular \( \text{PO}_2 \) of resting hamstring muscle of the anesthetized dog decreases to approximately 1 mm Hg when the arterial \( \text{PO}_2 \) falls below 35–40 mm Hg and the deep femoral venous \( \text{PO}_2 \) to 20–25 mm Hg. One can speculate that these values for arterial and venous \( \text{PO}_2 \) represent the lower limit of adaptation to hypoxia by this tissue. It is of interest that they are similar to critical arterial and venous oxygen tensions found by other authors who studied respiration in resting skeletal muscle (34, 35) (critical arterial and venous oxygen tensions are defined as the lowest tension where oxygen is not limiting cellular aerobic metabolism).

The shift of CO out of blood into muscle that occurred in our experiments as arterial \( \text{PO}_2 \) decreased below 35–40 mm Hg appears to have resulted from a decrease in intracellular \( \text{PO}_2 \), relative to mean capillary \( \text{PO}_2 \). In Fig. 1,
which showed the theoretical relationship of mean capillary \( P_{co_2} \) to \( mPbo_2 \) for several ratios of MbCO/HbCO, the curves drawn at constant MbCO/HbCO have an inflection point at a \( mPco_2 \) of approximately 35 mm Hg. As \( mPco_2 \) decreases below this point, \( mPbo_2 \) increases, a very unlikely situation. It might be predicted that the distribution of CO between blood and muscle would not remain constant as \( mPco_2 \) decreased below this point. It is interesting that the inflection point in this figure occurs at approximately the same mean capillary \( P_{o_2} \) below which the distribution of CO was shown to change in the present experiments.

**Other Estimates of Intracellular Oxygen Tension in Muscle**

Millikan's ingenious measurements (22) were performed on cat soleus muscle, a muscle chosen because its total hemoglobin content is small (which was important since hemoglobin interfered with the spectrophotometric measurement of myoglobin). In addition, this muscle is relatively thin (2-4 mm), allowing light transmission and only minimal light-scattering artifacts. Millikan was able to calibrate his system by clamping the circulation, which resulted in unloading of \( O_2 \) from myoglobin and an estimate of 0% saturation, or by infusion of cyanide, which inhibited \( O_2 \) consumption and allowed a measurement of 100% MbO2 to be made. Millikan's data appear to indicate that the normal resting MbO2 in his preparation was approximately 75-85%. We computed the average MbO2 from the average \( mPbo_2 \) found in our experiments and obtained a value of approximately 65% (\( P_{o_2} \), 60-110 mm Hg). The cause of this discrepancy is not clear. It could reflect error in our measurement, be a species difference, or result from variation in \( P_{o_2} \) in different muscles. Millikan's preparation did not control temperature, and his higher MbO2 could reflect a lower muscle temperature; in addition, it was necessary for him to place the muscle in an optical apparatus, possibly altering muscle blood flow or respiration. Arterial \( P_{o_2} \) was not determined in his study and could have been greater than ours.

Whalen (39) has developed a very small polarographic electrode which appears to be superior to electrodes used previously. He has obtained a mean \( P_{o_2} \) in frog resting sartorius muscle of 11.8 mm Hg (39) and 6 mm Hg in cat resting gracilis muscle (40). In the latter study, breathing 100% \( O_2 \) increased the mean intracellular \( P_{o_2} \) by an average of 2.1 ± 0.4 mm Hg. His data pertaining to cat muscle are within the limits of experimental error associated with the data obtained in our study. Cater (3) and Kunze (15) obtained higher values in resting skeletal muscle; the reasons for this are not clear. The limitations of tissue \( P_{o_2} \) electrodes have been discussed by others (17).

**GLOSSARY OF TERMS**

- \( \text{MbCO} \) = carboxymyoglobin percent saturation
- \( \text{MbO}_2 \) = oxymyoglobin percent saturation
- \( \text{HbCO} \) = carboxyhemoglobin percent saturation
- \( \text{HbO}_2 \) = oxyhemoglobin percent saturation
- \( M_{eq} \) = equilibrium constant for the reaction of CO with \( \text{HbO}_2 \)
- \( M_{eq} \) = equilibrium constant for the reaction of CO with \( \text{MbO}_2 \)
- \( \text{MbO}_2 \) = mean myoglobin oxygen tension
- \( \text{HbO}_2 \) = mean \( \text{HbO}_2 \) tension in capillary blood
- \( \text{MbO}_2 \) = mean \( \text{MbO}_2 \) in muscle
- \( \text{CO}_2/g \text{Hb} \) = disintegrations per minute per gram hemoglobin in venous blood
- \( \text{CO}_2/g \text{Hb} \) = disintegrations per minute per gram hemoglobin in venous blood
- \( \mu \text{Cr}/g \text{Hb} \) = disintegrations per minute per gram hemoglobin in venous blood
- \( \mu \text{Cr}_{\text{biop}} \) = disintegrations per minute in biopsy specimen

**REFERENCES**


