Carboxyhemoglobin and Oxygen Affinity of Human Blood

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We determined normal human blood \( p_{50} \) at various pH values (range 7.0 to 7.6) as a function of the proportion of carboxyhemoglobin (COHb) in total hemoglobin, from 0 to 23%. The \( d(\log p_{50})/d[\text{COHb}] \) coefficient is 0.00648, independent of pH and 2,3-diphosphoglycerate. The derived equation allows the calculation of \( p_{50} \) as a function of COHb with an approximation of ±0.54 mmHg (about 72 Pa), and can be combined with other calculations (Clin Chem 27:1856–1861, 1981; Clin Chem 29:110–114, 1983) to predict \( p_{50} \) under any condition of pH within the range 7.0–7.6, ratio of [2,3-diphosphoglycerate] to [total hemoglobin] (range 0.3–2.5), \( p_{CO_{2}} \) (range 20–90 mmHg), temperature (range 19–43 °C), and COHb (range 0–23%).

Exact relationships between human blood \( p_{50} \) (the \( p_{CO_{2}} \) at which hemoglobin is half-saturated with oxygen) and the main factors affecting hemoglobin function [pH, \( p_{CO_{2}} \), the ratio between concentrations of 2,3-diphosphoglycerate (2,3-DPG) and hemoglobin, temperature, and glycylated hemoglobin] have been recently defined (1–3), allowing the prediction of \( p_{50} \) within ±0.53 mmHg (SD), with two limitations: the presence of hemoglobin with altered affinities for oxygen, and the presence of carboxyhemoglobin (COHb), which is known to affect the oxygen affinity in blood (4). The purpose of the present work is to extend the reported equations to include the alterations of the oxygen affinity in blood related to the presence of clinically relevant (up to 23%) proportions of COHb in blood, as has been reported, e.g., in heavy smokers (5) and in some patients with chronic lung diseases (6).

Materials and Methods

Blood samples. Fresh blood from a nonsmoking donor (M.S.) whose oxygen affinity parameters were within the normal range (1, 2) was collected in sodium heparin and stored in ice. All experiments were performed within the next 8 h. The blood was partly saturated with CO by tonometry for 10 min with 1 to 3 mL of gaseous CO, introduced via a gas-tight Hamilton syringe, in a 100-mL sealed flask containing 5 mL of oxygenated blood at ice temperature. The flask was then opened and the blood was equilibrated with air for about 30 min to remove excess CO. The total hemoglobin concentration and the concentrations of COHb and methemoglobin (metHb) were determined spectrophotometrically, with use of previously reported absorption coefficients (7). We measured the 2,3-DPG concentration with kits provided by Boehringer Biochemia.

Hill coefficient. For each of the five COHb proportions considered (range, 0 to 33%), we filled as many as four tonometry flasks (1) with gas at an appropriate \( p_{CO_{2}} \) (so that the measured oxygen saturation would fall within the range 0.3 to 0.7), a \( p_{CO_{2}} \) of 45 mmHg, and the rest N\(_2\). The hemoglobin saturation with oxygen was determined after 25 min of tonometry at 37 °C, according to the method described (8). Blood sample pH and [2,3-DPG]/[Hb] ratio were left unchanged. We calculated the Hill coefficient, n, as the slope of the regression line for the experimental data plotted as proposed by Hill (9).

Determination of the \( p_{50} \) value. The \( p_{50} \) value was determined as described previously (1), at five COHb proportions (range 0 to 23%) and at least four pH values in the range 7.0 to 7.6 for each COHb value. 2,3-DPG was kept at the value normally found in vivo and tonometry temperature was kept constant at 37.3 °C. To measure pH, we used an IL 1302 gas analyzer (Instrumentation Laboratory, Paderno Dugnano, Milano, Italy). By introducing appropriate Hill n values and hemoglobin concentrations in equations 1 and 4, respectively, we changed our previous calculations (1) to account for the presence of COHb in the blood sample.

Results

The Hill n value decreases with increasing COHb (Figure 1). The plots of log \( p_{50} \) vs pH—i.e., the Bohr effect—were consistently linear under all the conditions studied (r values from 0.980 to 0.996). Plots of log \( p_{50} \) vs COHb at various pH values were linear, with a mean constant slope of −0.00848 (SD 0.0011) (Figure 2). Therefore, because the dependence of \( p_{50} \) on CO is known to be unaffected by 2,3-DPG (10), and because CO\(_2\) affects oxygen affinity only at \( p_{CO_{2}} \) values much lower than \( p_{50} \) (10), the following equation represents the

\[ COHb (\%) \]

\[ 1.6 \]

\[ 2.0 \]

\[ 2.4 \]

\[ 2.8 \]

\[ 0 \]

\[ 10 \]

\[ 20 \]

\[ 30 \]

Fig. 1. Hill n value vs % COHb (data fitted to an empirical second-order polynomial: \( y = (7.32 \times 10^{-4} \times x^2) + (5.19 \times 10^{-3} \times x) + 2.735; \) RMS error = 8.4 \times 10^{-2}

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5 Nonstandard abbreviations: Hb, hemoglobin; COHb, carboxyhemoglobin; metHb, methemoglobin; and 2,3-DPG, 2,3-diphosphoglycerate.

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the time required for tonometry (25 min). At present, the $p_{50}$ value can be either predicted or compared with the experimental value, to learn whether an observed apparently abnormal $p_{50}$ is accounted for by abnormal values for pH, $pCO_2$, 2,3-DPG, temperature, and/or COHb, or whether other factors such as hemoglobins with altered oxygen affinity or other currently unknown cofactors of hemoglobin oxygenation must be considered. Table 1 shows the printout of a FORTRAN IV subroutine summarizing the equations reported here and in our previous papers (1, 2). A similar program, which can be run on a Texas TI-59 (210 program steps), is available upon request to the authors.

References


