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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[COHb] coefficient is 0.00848, 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_{O_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 glycosylated hemoglobins] have been recently defined (*1–3*), allowing the prediction of p_{50} within ±0.39 mmHg (SD), with two limitations: the presence of hemoglobins with altered affinities for oxygen, and the presence of carboxyhemoglobin (COHb), which is known to affect the oxygen affinity in blood (*4*).[5] 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 absorptivity 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_{O_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 A4, 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 ranges 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_{O_2} values much lower than p_{50} (*10*), the following equation represents the

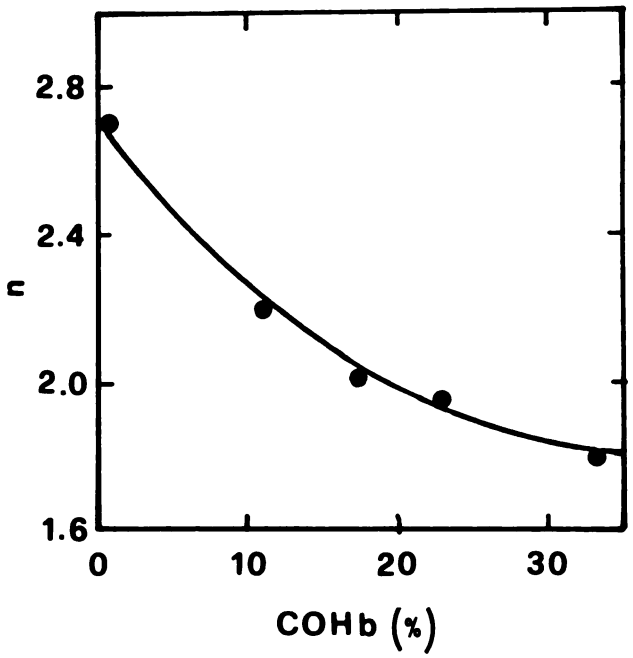

Fig. 1. Hill n value vs % COHb (data fitted to an empirical second-order polynomial: $y = (7.32 \times 10^{-4} \times x^2) - (5.19 \times 10^{-2} \times x) + 2.735$; RMS error = 8.4×10^{-2}

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

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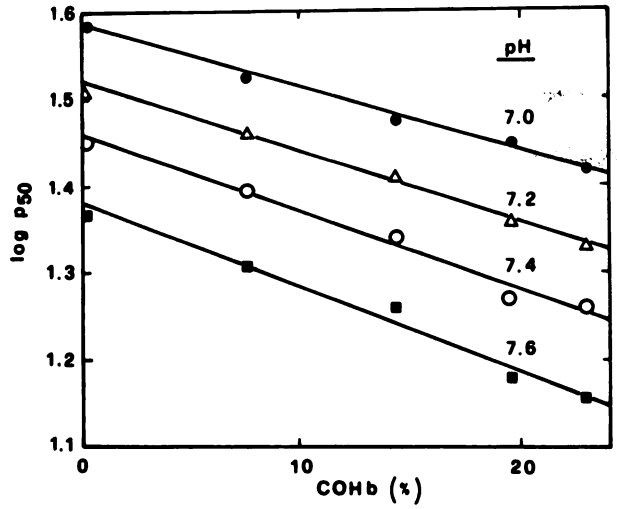

Fig. 2. Linear relationship between log p_{50} and % COHb at various pH values

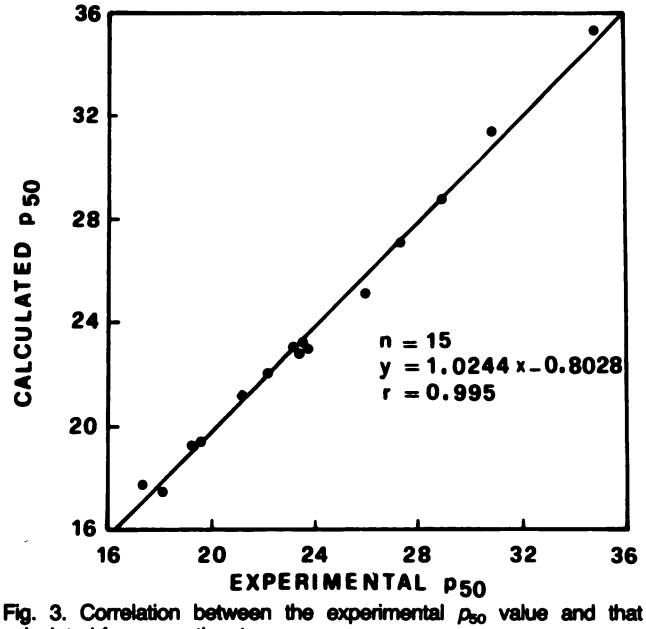

Fig. 3. Correlation between the experimental p_{50} value and that calculated from equation 1

effect of COHb on human blood p_{50} at 37 °C:

$$\log p_{50_x} = \log p_{50_0} - (0.00848 \times x) \quad (1)$$

where x is the percentage of COHb in the sample and p_{50_0} is the p_{50} value of a sample containing no COHb.

Figure 3 shows the correlation between predicted (calculated from equation 1) and experimentally measured p_{50} values. The p_{50} value normalized with respect to COHb can be predicted with an SD of 0.54 mmHg.

The proportion of metHb never exceeded 1% in any of the studies.

Discussion

The correction factor reported here, d(log p_{50})/d[COHb], is higher than hitherto reported: 0.006 (*4, 5*) and 0.007 (*10, 11*). In this study, particular care was devoted to the preparation of partially CO-saturated blood samples. The described method represents somewhat the "in vivo" situation, where hemoglobin equilibrates with a low concentration of CO and the ligand is distributed uniformly among the hemes in the erythrocytes. In separate experiments, fully CO-saturated blood mixed with CO-free blood before tonometry yielded essentially the same results (not shown), indicating that the redistribution of CO within and between cells occurs within the time required for tonometry (25 min).

Table 1. Printout of a FORTRAN Subroutine to Calculate p_{50} from pH, p_{CO_2}, [2,3-DPG]/[Hb], Temperature, and COHb

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      SUBROUTINE P50(PH,PCO2,DPG,T,COHB,P50)
C     NORMALIZE FOR PH, PCO2 AND [2,3-DPG]/[HB]
      G=ALOG10(DPG)
C     FIND LOG P50 AT PH 7.0
      A=(-0.69117E-3*PCO2 + 0.3365)*G +
        (0.3598E-3*PCO2 + 1.599)
C     FIND LOG P50 AT PH 7.6
      B=(-0.138E-2*PCO2 + 0.3607)*G + (0.9089E-3*PCO2
        + 1.36)
C     INTERPOLATE AT THE DESIRED PH
      P50LOG=((PH-7.)*(B-A))/0.6 + A
C     NORMALIZE FOR TEMPERATURE
      TK=T + 273.        ! NEED ABSOLUTE TEMPERATURE
      P50LOG=P50LOG - 2149.*((1./TK) - (1./310.))
C     NORMALIZE FOR COHB (%)
      P50LOG=P50LOG - 0.00848 * COHB
C     END OF CALCULATIONS
      P50=10.**P50LOG
      RETURN
      END
```

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, p_{CO_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

1. Samaja M, Mosca A, Luzzana M, et al. Equations and nomogram for the relationship of human blood p_{50} to 2,3-diphosphoglycerate, CO_2, and H^+. *Clin Chem* 27, 1856–1861 (1981).

2. Samaja M, Melotti D, Rovida E, Rossi-Bernardi L. Effect of temperature on the p_{50} value for human blood. *Clin Chem* 29, 110–114 (1983).

3. Samaja M, Melotti D, Carenini A, Pozza G. Glycosylated haemoglobins and the oxygen affinity of whole blood. *Diabetologia* 23, 399–402 (1982).

4. Roughton FJW, Darling RC. The effect of carbon monoxide on the oxyhemoglobin dissociation curve. *Am J Physiol* 141, 17–31 (1944).

5. Goldsmith JR, Landaw SA. Carbon monoxide and human health. *Science* 162, 1352–1359 (1968).

6. Hebbel RP, Eaton JW, Modler S, Jacob HS. Extreme but asymptomatic carboxyhemoglobinemia and chronic lung disease. *J Am Med Assoc* 24, 2584–2586 (1978).

7. Rossi-Bernardi L, Perrella M, Luzzana M, et al. Simultaneous determination of the hemoglobin derivatives, oxygen content, and oxygen saturation in 10 μL of whole blood. *Clin Chem* 23, 1215–1225 (1977).

8. Samaja M, Rovida E. A new method to measure the haemoglobin oxygen saturation by the oxygen electrode. *J Biochem Biophys Meth* 7, 143–152 (1983).

9. Hill AV. The possible effect of aggregation of the molecules of haemoglobin on its dissociation curve. *J Physiol (London)* 40, 4–7 (1910).

10. Okada Y, Tyuma I, Ueda Y, Sugimoto T. Effect of carbon monoxide on equilibrium between oxygen and hemoglobin. *Am J Physiol* 230, 471–475 (1976).

11. Hlastala MP, McKenna HP, Franada RL, Detter JC. Influence of carbon monoxide on hemoglobin-oxygen binding. *J Appl Physiol* 41, 893–899 (1976).