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Effect of Temperature on the p_{50} Value for Human Blood

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We investigated the effect of temperature (19, 30, 37, and 43 °C) on the p_{50} value for normal human blood at p_{CO_2} = 5.72 kPa (43 mmHg), at various pHs (range 7.0 to 7.6) and molar ratios of [2,3-diphosphoglycerate]/[Hb_4] (range 0.4 to 2.4). The d(log p_{50})/d(pH) coefficient varied from 0.39 at 19 °C to 0.35 at 43 °C. The relationship between log p_{50} and 1/T (T = degrees Kelvin) was linear under the experimental conditions used, and the d(log p_{50})/d(1/T) coefficient varied between −2138 at pH 7.0 and −2162 at pH 7.6, independent of the concentration of 2,3-diphosphoglycerate. Assuming that the effect of p_{CO_2} on the p_{50} value is the same at 19, 30, and 43 °C as at 37 °C, one can use the reported coefficients to calculate the p_{50} value for normal human blood under conditions of temperature, pH, p_{CO_2}, and 2,3-diphosphoglycerate concentrations prevailing under physiological and pathological conditions. The p_{50} value calculated by empirical equations, taking into account the effect of temperature, correlated well with the values for p_{50} determined experimentally ($y = 0.9774x + 0.453$; $r = 0.998$; n = 60), with an SD of 52 Pa (0.39 mmHg).

Additional Keyphrases: *blood gases · variation, source of · tonometry*

Earlier, we described (*1*) a new method for tonometering small amounts of blood with a known gas phase, which allowed the study of the effect of 2,3-diphosphoglycerate (2,3-DPG), H^+, and CO_2 on the p_{50} value [the p_{O_2} at which hemoglobin (Hb_4) is half saturated with oxygen] for normal human blood at 37 °C. We derived empirical equations and a nomogram, which made it possible to calculate the value of p_{50} from known values of p_{CO_2}, pH, and the [2,3-DPG]/[Hb_4] molar ratio.

Here we extend the study of the effect of 2,3-DPG and pH on the p_{50} value for normal human blood to the temperature range 19 to 43 °C. Whereas the simple effect of temperature on the p_{50} value for normal, fresh, unmodified blood has already been investigated (see *Discussion*), no description of the separate effect of 2,3-DPG and pH on the value of p_{50} at various temperatures has yet been reported. Because the effect of p_{CO_2} on p_{50} can be assumed to be constant within the considered temperature range, and similar to that found at 37 °C, the data we report here allow calculation of the value for normal human blood under various conditions of temperature, pH, p_{CO_2} and 2,3-DPG concentration.

Materials and Methods

Blood samples. About 30 to 40 mL of blood collected from a healthy, nonsmoking man was used for all experiments, which were done within 8 h. When not in use, the blood was stored in an ice bath. Blood pH and 2,3-DPG concentration were varied as already described (*1*).

Flasks. We used 60-mL flasks (*1*) so as to obtain a larger surface of contact between the blood and the gas phase and between the flasks and the tonometer block. We used 0.40 mL of blood for each run.

Tonometer. The tonometer previously described (*1*) was modified to allow blood samples to be equilibrated with the gas phase at temperatures other than 37 °C. Two Plexiglas coils were applied to the side walls, and water was pumped through them from an external water bath. The temperature in the core of the block was measured with a thermal sensor inserted into a thin, deep hole in the block and calibrated against a National Bureau of Standards certified mercury-bulb thermometer. The circulating water at the exit of the tonometer was used to maintain the temperature of the pH electrode (IL 213; Instrumentation Laboratory, Lexington, MA 02173). Heat dispersion was prevented by thermal insulation of tubings.

We selected three operating temperatures: 43, 30, and 19 °C. The time required to tonometer the blood at the various temperatures was determined by equilibrating a blood sample with nitrogen containing CO_2 (63.1 mL/L) and measuring the hemoglobin saturation for oxygen (S_{O_2}) at 5-min intervals.

Measurement of S_{O_2}. The method for measuring S_{O_2} previously reported (*2*) was modified as follows. We used an anaerobic, stainless-steel, 1-mL cuvette, with an oxygen electrode in contact with the liquid, similar to that described (*2*) but without optical windows. After filling the cuvette with sodium tetraborate buffer (*2*), we added 10 μL of blood to the buffer, and determined the amount of deoxygenated hemoglobin as described (*2*). We then added to the blood–buffer solution 5 μL of 0.5 mol/L $K_3Fe(CN)_6$ dissolved in 2.75 mol/L H_3PO_4, to oxidize the oxyhemoglobin, and measured the p_{O_2} of the solution. The oxygen capacity was calculated by use of the following equation:

$$O_2 \text{ capacity} = \alpha \times \Delta p_{O_2}/(v_c - v_f) \quad (1)$$

where v_c is the volume of the cuvette, v_f is the volume of the $K_3Fe(CN)_6$–H_3PO_4 solution, Δp_{O_2} is the p_{O_2} difference before and after addition of the oxidant, and α is the solubility coefficient of oxygen in aqueous solutions. The value of α at the required temperature was that of Roughton and Severinghaus (*3*). The S_{O_2} of the blood sample was calculated from:

$$S_{O_2} = (O_2 \text{ capacity} - \text{deoxygenated Hb})/O_2 \text{ capacity} \quad (2)$$

Calculation of the p_{50}. The p_{O_2} in the blood sample at the end of tonometry was calculated as previously described (*2*). Because in separate experiments at t = 19, 30, and 43 °C the value of the Hill coefficient (n) was 2.7 ± 0.2, the p_{50} value was calculated from the p_{O_2} and S_{O_2} by the Hill equation, assuming n = 2.7 (*4*). This correction was applied only if S_{O_2} fell between 40 and 60%. If the S_{O_2} was outside this range, the measurement was repeated at a more suitable p_{O_2}.

Other measurements. At the end of the tonometry, the concentrations of hemoglobin, methemoglobin, and carboxyhemoglobin were measured as described (*1*). The concentration of 2,3-DPG was measured once every three to four runs. We calibrated electrodes for pH measurement at each temperature, using appropriate temperature-related values

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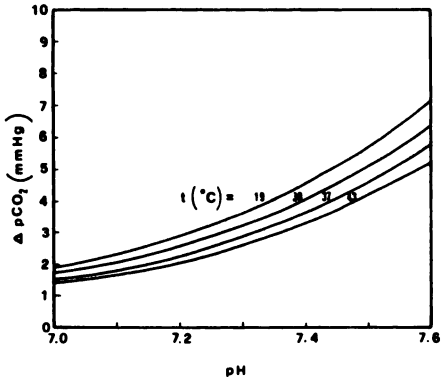

Fig. 1. Decrease in the p_{CO_2} in the flask (volume = 60 mL) after the addition of 0.4 mL of CO_2-free blood, as a function of temperature

for National Bureau of Standards certified phosphate buffers (*5*).

P_{CO_2} *values.* The concentration of CO_2 in the gas phase was the same for all the experiments; it derived from mixing the same binary gas mixtures (6.31% CO_2/balance nitrogen, and 6.34%/balance air), giving a theoretical p_{CO_2} of 6.16 kPa (46.3 mmHg)[3] (*1*). Because we used closed tonometry flasks, the effective p_{CO_2} of the blood sample was somewhat lower (5.72 kPa, about 43 mmHg), but was nearly the same for all temperatures investigated. Small differences in the p_{CO_2} were due to the different extent to which gaseous CO_2 was dissolved in the liquid phase and to temperature-linked changes in the pK value for carbonic acid (*6*). Figure 1 shows how temperature changes affected the value of p_{CO_2} of the blood sample.

Results

Tonometry. The special tonometric flasks used allowed a wide surface of contact between the blood sample and the aluminum block of the tonometer. The temperature gradient between the blood and the block never exceeded 0.05 °C in the investigated temperature range. Decreasing the temperature always increased the oxygen affinity of blood; consequently, the time to equilibrate a blood sample with a gas varied from a minimum of 15 min at 43 °C to a maximum of 45 min at 19 °C.

Measurement of S_{O_2}. The new method to measure S_{O_2} was fast and simple, requiring only one calibration before the analysis, i.e., calibration of the oxygen electrode with the value of atmospheric p_{O_2}. The rate of oxidation of hemoglobin was high (>99% in 20 s), owing to the acid environment (pH ~6) and the high concentration of $K_3Fe(CN)_6$, which increased the pseudo-first order rate (*7*).

We checked this method against the previously described method (*2*), measuring S_{O_2} on 111 samples equilibrated at p_{O_2} values between 0 and 20 kPa (0 and 150 mmHg). The differences between the results obtained by the new method and those obtained by the reference method were not statistically significant (0.28 ± 0.80 S_{O_2} units, $t = -3.64$, 110 df). The reproducibility of the method was checked by determining the S_{O_2} on 10 samples equilibrated at the same p_{O_2}. Results (mean ± SD) were quite comparable with those of the previously reported method: S_{O_2} = 54.4 ± 0.6 (CV 1.1%) vs 54.6 ± 0.7 (CV 1.2%), respectively, for the comparison method and for the new method.

Effect of temperature. We made 60 determinations of the p_{50} value: at five molar ratios of [2,3-DPG]/[Hb_4] 0.4, 0.6, 0.8, 1.9, and 2.4), at three temperatures (19, 30, and 43 °C), and in the pH range 7.0 to 7.6. To determine the Bohr factor, d(log p_{50})/d(pH), for each constant value of 2,3-DPG and temperature, we determined four p_{50} values (Figure 2). The relationship between log p_{50} and pH was linear in the range of temperature values and [2,3-DPG]/[Hb_4] ratios investigated. The Bohr effect, d(log p_{50})/d(pH), decreased slightly for increasing temperatures (from 0.39 ± 0.08, mean ± SD, at 19 °C to 0.35 ± 0.03 at 43 °C), confirming previous studies (*8–10*). Figure 3 shows the effect of 2,3-DPG on log p_{50} at various temperatures. Data at 37 °C were obtained from equations previously reported (*1*). The Van't Hoff isochores were obtained for the extreme values of pH (Figure 4). The relationship between log p_{50} and 1/T (T = degrees Kelvin) was linear under all the conditions used. The average (±SD) slopes of the lines were −2138 ± 57 at pH 7.0, and −2162 ± 67 at pH 7.6. The difference between the slopes at pH 7.0 and at pH 7.6 was not significant ($t = -0.63$, 7 df), but a small, although statistically poor, trend toward steeper lines could be seen for increasing concentrations of 2,3-DPG.

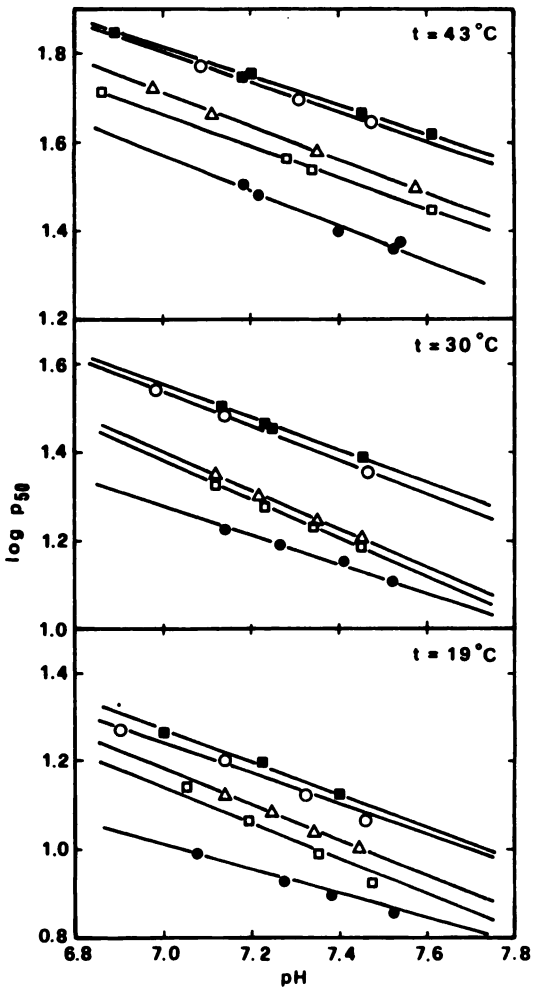

Fig. 2. Plots of log p_{50} vs pH, i.e., the Bohr effect, at p_{CO_2} = 43 mmHg (5.72 kPa) and five [2,3-DPG]/[Hb_4] molar ratios: approximately 2.4 (■), 1.9 (○), 0.8 (△), 0.6 (□), and 0.4 (●)
The regression lines shown are calculated by least-squares analysis

Our data, obtained from fresh unmodified blood, are in good agreement with those obtained by other authors under similar conditions. Figure 5 shows a comparison with the most recent data reported in the literature (*10–12*).

Calculating p_{50} *values at various temperatures.* Because the slopes of the lines shown in Figure 4 are practically the same with respect to pH and 2,3-DPG concentration, the following equation may be applied to describe the data:

$$d(\log p_{50})/d(1/T) = -2149 \pm 56 \quad (3)$$

[3] 1 mmHg ≃ 133 Pa.

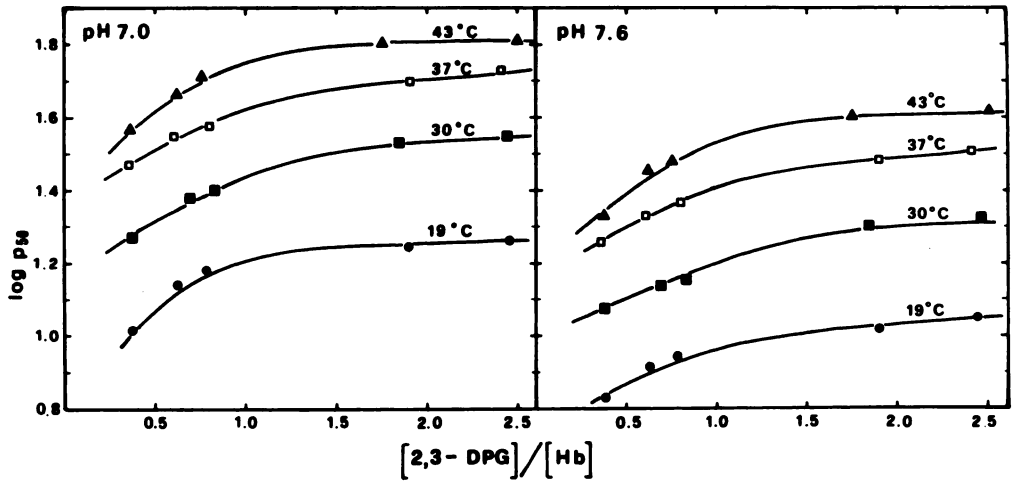

Fig. 3. Effect of 2,3-DPG on log p_{50} at the two extremes of the pH range and at four temperatures: (▲) 43, (□) 37, (■) 30, and (●) 19 °C

This equation can be used to calculate the p_{50} value of human blood at any temperature in the range 19 to 43 °C, when its value at another temperature in the same range is known, and it is valid at p_{CO_2} = 43 mmHg (5.72 kPa), in the pH range 7.0 to 7.6, and in the [2,3-DPG]/[Hb_4] molar ratio range 0.4 to 2.5. The p_{CO_2} correction from 43 mmHg to any other value of p_{CO_2} can be estimated at any temperature from 19 to 43 °C by using as a first approximation the previously described nomogram (*1*). Figure 6 shows the correlation between the experimental p_{50} value and that obtained by the combined use of the nomogram (*1*) and equation 3. The p_{50} value was calculated with an SD of 52 Pa (0.39 mmHg).

Discussion

The increase in the oxygen affinity of human blood with decreasing temperature was first described in 1909 (*13*). Since then, many investigations on its effect on the oxyhemoglobin dissociation curve in blood have been reported, but without full consideration of all the factors later discovered to affect the oxygen affinity of whole blood. More recently, some authors have investigated the effect of temperature on the p_{50} value, the Bohr effect, and the Hill coefficient of normal human blood (*10–12*). However, in these reports the effect of 2,3-DPG was not investigated at various temperatures. To study the separate effects of the known allosteric regulators of the oxygen affinity of blood at different temperatures, we have adapted our recently reported method (*1*), which is suitable for the rapid determination of the p_{50} value under defined conditions of pH, p_{CO_2}, and 2,3-DPG concentration.

The average value for d(log p_{50})/d(t °C) = 0.0229, as determined on fresh, unmodified blood, is comparable (Table 1) with the values reported by other authors (*10–19*) under similar conditions. However, we found the coefficient d(log p_{50})/d(1/T) more suitable to express the effect of temperature, the slopes of the lines of Figure 4 being practically independent of the concentration of 2,3-DPG and of pH. We did not investigate the effect of p_{CO_2}, and thus we did not calculate the d(log p_{50})/d(p_{CO_2}) coefficient. However, CO_2 is known to affect physiologically the oxygen affinity of blood, mainly through the alkaline Bohr effect. In the presence of 2,3-DPG, the effect of CO_2 is greatly decreased, because 2,3-DPG and

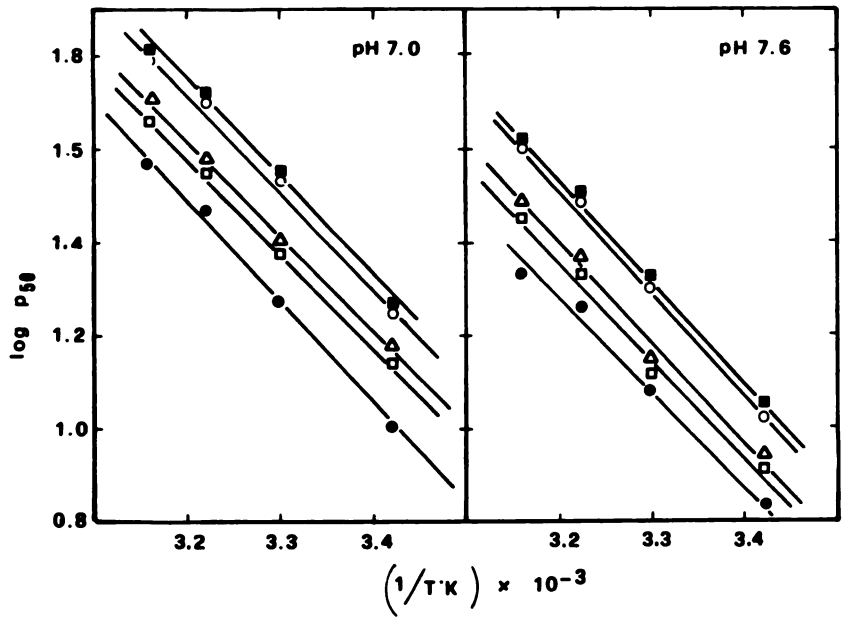

Fig. 4. The Van't Hoff isochores at pH 7.0 and 7.6, and five molar ratios of [2,3-DPG]/[Hb_4] labeled as in Fig. 2
The correlation coefficients varied between 0.995 and 0.999

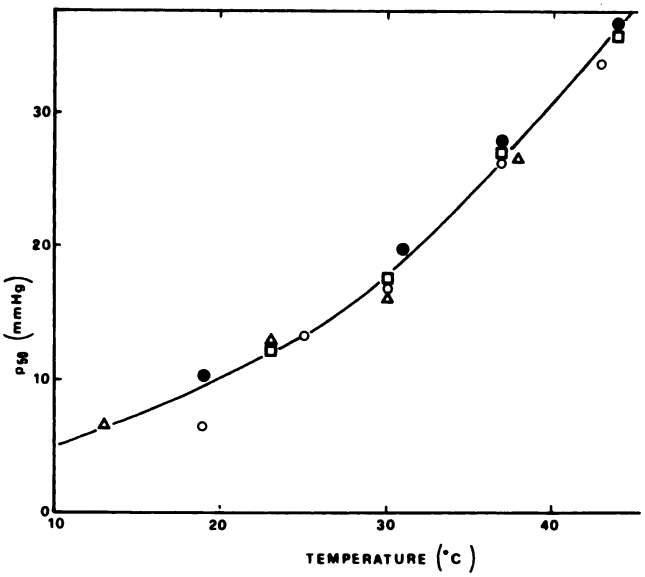

Fig. 5. Comparison of our data with the most recent data available in the literature under similar conditions

△, Astrup et al. (*11*), pH 7.4, p_{CO_2} about 36 mmHg, [2,3-DPG]/[Hb_4] unknown; □, Hlastala et al. (*10*), pH 7.4, p_{CO_2} to neutralize the base excess, [2,3-DPG]-/[Hb_4] = 0.90; ○, Reeves (*12*), pH 7.4, constant CO_2 content, [2,3-DPG]/[Hb_4] = 0.85; ●, present work, pH 7.4, p_{CO_2} = 43 mmHg, [2,3-DPG]/[Hb_4] = 0.80

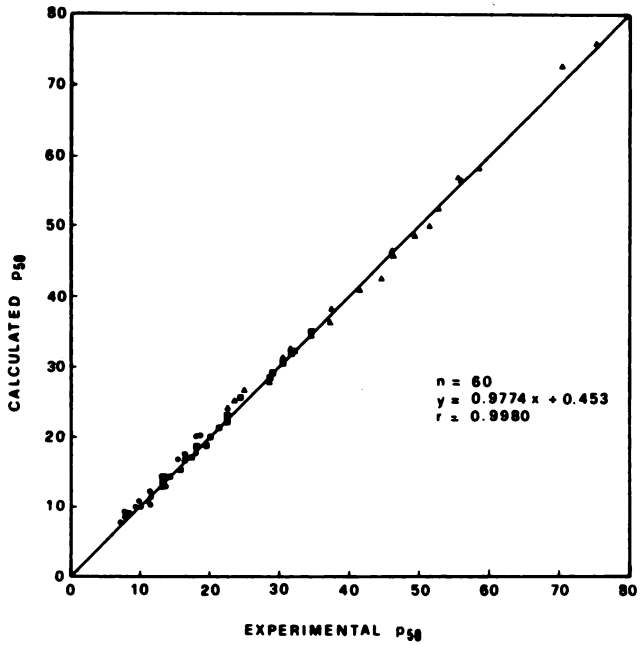

Fig. 6. Correlation between the experimental p_{50} value and that calculated from the combined use of equation 3 and the nomogram (*1*)

Least-squares regression analysis: $y = 0.453 + 0.9774x$, $r = 0.998$, n = 60. Temperatures 43 °C (▲), 30 °C (■), and 19 °C (○)

CO_2 compete for two of the four N-terminal amino groups of hemoglobin (*20*). Moreover, the effect of CO_2 has already been found experimentally not to be significantly affected by temperature changes (*12*). We therefore assumed that the effect of CO_2 (separated from the collateral effect of pH) on the oxygen affinity of blood is small and of the same order of magnitude in the temperature range 19 to 43 °C as is found at 37 °C.

As a result of the present study, one can now determine both the separate and the integrated effect of the three main allosteric regulators of the oxygen affinity of human blood as a function of temperature. In fact, the previously described equations (*1*) can be used to calculate the p_{50} value as a function of the concentration of 2,3-DPG, pH, and p_{CO_2} at 37 °C, whereas the effect of temperature at any value of pH, p_{CO_2}, and 2,3-DPG concentration is described by equation 3. A program for a Texas TI 59 calculator that requires less than 180 program steps is available upon request from the authors for the numerical solutions of the equations. Figure 6 shows the accuracy of this procedure and the homogeneity of the data obtained at three different temperatures.

Table 1. Correction Factor, d(log p_{50})/d(*t* °C), Reported for Normal, Fresh Human Blood

Authors	Year	Factor
Barcroft and King (*13*)	1909	0.0283
Brown and Hill (*15*)	1923	0.0229
Dill and Forbes (*16*)	1941	0.0192
Albers et al. (*17*)[a]	1958	0.0231
Callaghan et al. (*18*)[b]	1961	0.0176–0.0228
Astrup et al. (*11*)	1965	0.0245
Severinghaus (*19*)	1966	0.0230
Hlastala et al. (*10*)	1977	0.0226
Reeves (*12*)	1980	0.0230
Present work	1982	0.0229

[a] Using canine blood. [b] Interpolated from figure.

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