Oxygen Equilibrium Curve of Normal Human Blood and Its Evaluation by Adair’s Equation

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Oxygen equilibrium curves of fresh, normal human blood have been measured by new methods which allow the control of pH, Pco2, and 2,3-diphosphoglycerate and which yield higher accuracy at the extremes of saturation than was possible previously. The curve determined by these techniques lies slightly to the right of the standard curve of Roughton et al. (Roughton, F. J. W., Deland, E. C., Kernohan, J. C., and Severinghaus, J. W. (1972) in Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status (Astrup, P., and Rorth, M., eds) pp. 73-83, Academic Press, New York). The greatest difference is at low oxygen saturation, probably owing to the fact that the latter data were obtained under conditions which would lead to depletion of cellular 2,3-diphosphoglycerate. The range of p50 (oxygen pressure at half-saturation) values for four normal subjects was 28.3 mm Hg to 29.0 mm Hg.

Adair’s stepwise oxygenation scheme has been used to analyze the curves with the result that a1 = 0.1514 x 10^-1 (± 10%) mm^-1; a2 = 0.9723 x 10^-3 (± 8%) mm^-2; a3 = 0.1703 x 10^-5 (± 50%) mm^-3; a4 = 0.1671 x 10^-6 (± 2%) mm^-4 for the best of four data sets. Because these constants are very sensitive to changes in the shape of the oxygenation curve, this analysis is much more useful than p50 values in the investigation of the various allosteric effectors of the function of hemoglobin within the red cell.

Although a number of theoretical questions remain in regard to the details of hemoglobin-oxygen interactions, a reasonably clear overall picture can be presented (for example, see Ref. 1). This understanding is possible because of parallel studies of the functional and structural properties of hemoglobin solutions and have led to a description of the influence of various effectors (principally 2,3-diphosphoglycerate, H+, and CO2) on the equilibrium and kinetic reactions. A major contribution to this field has been the development of methods for the precise measurement of the hemoglobin-oxygen equilibrium curve, especially at extremely high and low oxygen saturation (2). In particular, evaluation of equilibrium data in terms of the generalized Adair equation (3) allowed Imai and co-workers to quantify the effects of H+, 2,3-DPG, and temperature, and to compare the properties of various hemoglobin derivatives (4-7). The principal value of the Adair analysis is that it is sensitive to changes in the shape of the oxygen equilibrium curve.

Whole blood is a far more complex system than is hemoglobin in solution, and to date, most investigations into the regulation of the blood oxygen equilibrium curve have dealt with p50 values, measures of the position of the curve. Quantitation of the roles of the various allosteric effectors in whole blood has been retarded because of a lack of methods by which the precise whole blood oxygen equilibrium curve could be obtained. Roughton et al. (8) evaluated the blood oxygen equilibrium data according to Adair’s generalized formula (3):

\[ Y = \frac{a_1 p + a_2 p^2 + a_3 p^3 + a_4 p^4}{4(1 + a_1 p + a_2 p^2 + a_3 p^3 + a_4 p^4)} \]  

where Y is fractional saturation and p, oxygen pressure. The experiments, however, were performed before the importance of 2,3-DPG was fully appreciated and their methods were so laborious that preservation of this compound was impossible.

The abbreviations used are: 2,3-DPG, 2,3-diphosphoglycerate; Hb, hemoglobin; MetHb, methemoglobin; COHb, carboxyhemoglobin.
In order to investigate the in vivo regulation of hemoglobin oxygen affinity in normal red cells and in pathological states, we have sought a method which would yield data of precision equal to that possible for pure hemoglobin. The method described by Rossi-Bernardi, et al. (9) allows strict control of pH and pO2 during the measurement and can be done quickly enough so that 2,3-DPG concentration is maintained. We have previously described its application in the study of blood from subjects with sickle cell disease and some of its shortcomings (10). Although it is quite reliable in the middle range of saturation, it does not yield data of sufficient precision at extremes of oxygenation to permit analysis in terms of the Adair equation. Therefore, a mixing method has been employed to explore these critical regions of saturation. We present here the method and the results of our analysis of oxygen equilibrium curves for normal, fresh human blood.

**EXPERIMENTAL PROCEDURE**

**Hematological Measurements**—All blood samples were drawn less than 2 h (and usually less than 20 min) before their use. The blood was mixed with heparin (100 units/ml) and immediately chilled to 4°C. Measurement of Hb, MetHb, and COHb was by a modification of the method of Rossi-Bernardi et al. (11). Well mixed, whole blood (10 μl) was mixed with 1.0 ml of a Sterox/borate solution (10 mm Na₂B₄O₇, 0.01% [v/v] Sterox, pH 9.14). The optical density was measured at 405, 565, and 620 nm with a Cary 118C spectrophotometer (Varian). The extinction coefficients for methemoglobin, methemoglobin, and CO-hemoglobin at these wavelengths (11), their relative concentrations were determined. 2,3-DPG was measured according to Nygaard and Rahr (12) using kits from Sigma.

**Calculation of Oxygen Electrode**—The oxygen electrode (model 203355, Instrumentation Laboratories, Lexington, Mass.) was mounted in the apparatus described previously (9). It was fitted with Teflon membrane 5937, from Yellow Springs Instruments. Yellow Springs, Ohio, for pO2 less than 150 mm Hg, and polypropylene membrane (19010 Instrumentation Laboratories) for pO2 above 150 mm Hg. The electrode was a modified version of that described by Hahn et al. (13). It contained 1.0 M KH₂PO₄, 0.1 M NaCl and was titrated to pH 10.2 with solid NaOH.

Gas mixtures were obtained from Lifogen Inc. (Cambridge, Md.) and their compositions were certified nominally to ±0.02%. The gases were humidified at 37°C and then directed into the chamber of the reaction cuvette. The output from the oxygen electrode was calibrated by equilibrating water with O₂/N₂ mixtures of varying composition. Blood samples were equilibrated with selected gases in an II model 237 tonometer (Instrumentation Laboratories, Lexington, Mass.) for 20 to 30 min each. The output from the electrode was then checked when the cuvette was flushed with equilibrating gas and the blood separately. The results of these procedures were used to determine the true pO2 of blood.

**Middle Oxygen Equilibrium Curve**—The middle portion of the blood oxygen equilibrium curve was measured as described previously (10). Two milliliters of blood were deoxygenated for 30 min at 37°C in an II 237 tonometer in the presence of 5.6% CO₂ (balance N₂). During oxygenation of a 1-ml sample by the slow addition of hydrogen peroxide (Parke-Davis) in the presence of catalase (Sigma), pO2 was monitored with an II 17026 CO₂ electrode fitted with a Teflon membrane (Beckman, 7794SD). pH and pO2 were held constant by the addition of 6.4 M NaOH from a micrometer syringe. By this method, the pO2 could be maintained between ±0.2 mm Hg and pH between ±0.01 unit. The total amount of NaOH required for this procedure was recorded.

**Mixing Experiments**—A sample of blood was divided into two portions, one of which was equilibrated with 94.4% N₂, balance CO₂. The second sample was subjected to the same centrifugation to remove the red cells and 0.4 M NaOH was added to the separated plasma to bring the NaOH concentration up to that at the end of the H₂O₂ run. After equilibration of the plasma with 94.4% O₂ (balance CO₂) for 5 min, the red cells were added back and equilibration was continued. Both samples were equilibrated with their respective gases for a period of 30 min. A cuvette was loaded with 1 ml of oxygenated blood with a gas-tight syringe (Hamilton, Reno, Nev.). Deoxygenated blood was taken up in a 1-ml gas-tight syringe containing a small magnetic stirrer. The cells were kept in suspension by gently moving a magnet along the side of the syringe. Small aliquots (2 to 5 μl) were added to the oxygenated blood through a 26 gauge needle, using a micrometer syringe drive. After each addition the pO2 decreased quickly and then plateaued at a new level. When the pO2 was stable, the next increment was added.

To obtain the bottom end of the oxygen equilibrium curve, the procedure was reversed: small increments of oxygenated blood were added to the deoxygenated blood in the cuvette. Complete deoxygenation could be checked by the addition of a small amount of sodium dithionite to the blood after the experiment. The rate of mixing in the cuvette was critical. If the rate was too low, pO2 measurement was erroneously slow, possibly due to stagnation of blood at the surface of the oxygen electrode. To avoid this difficulty, the stirring rate was optimized so that the pO2 reading was maximal.

**Solubility Coefficient, α**—When the cuvette contains oxygenated blood and the blood being injected into it is completely deoxygenated, the relationship between total oxygen in the cuvette before and after injection is:

\[
T_v = T_i + \Delta V \left(1 - \frac{V}{V'}\right)
\]

where \(T_v\) is total O₂ concentration before the ith injection, \(T_i\) is total O₂ concentration after the ith injection, \(V\) is the volume of the cuvette, and \(\Delta V\) is the volume of the ith injection. For all \(i\), \(T_i\) is related to \(\alpha\) by:

\[
T_i = (\alpha P/760) + C_i
\]

At very high hemoglobin saturation, \(S_i = S_i^1\), and \(S_i\) is nearly equal and \(S_i^1\) is nearly 1. Therefore, if \(\Delta V\) is not chosen too small, \(\alpha\) may be approximated from the relation:

\[
\lim_{\Delta V \to 0} \frac{760 C_i \Delta V}{P_i \left(1 - \frac{V}{V'}\right)} = \alpha
\]

where \(C_i\) is the oxygen capacity of hemoglobin per unit volume of the syringe.

In fact, an entire curve of approximations to \(\alpha\) can be generated, one point for each \(\Delta V\), and the apparent limit of that curve at large \(P\) can be used as the value of \(\alpha\).

**Calculation of Saturation from Mixing Data**—The general formula for the calculation of the hemoglobin saturation at the ith point is:

\[
S_i = \frac{T_i - D_i}{C_i}
\]

where \(S_i\) is saturation, \(T_i\) is the total oxygen content of the cuvette after the ith addition, and \(D_i\) is dissolved oxygen.

At the ith incremental addition of volume \(\Delta V\) of blood from the syringe where the total oxygen content is \(AV_i\), an equal volume of blood with total oxygen content \(\Delta V_i\) is expelled. Mixing then occurs and the new total oxygen content is \(V T_i\). This is given by:

\[
T_i = VT_i + \Delta V (\frac{T_i^1}{V'} - \frac{T_i^1}{V})
\]

or

\[
T_i = T_i^1 + (T_i^1 - T_i^1)k (1 - V'/V)
\]

Thus, total oxygen concentration is given by Formula 4, dissolved oxygen concentration by \((\alpha P/760)\) and saturation by Formula 3. These equations apply to either the high or low saturation mixing experiments.

**Complete Oxygen Equilibrium Curve**—Since the error in the \(H_2O_2\) curve is least at low saturation, it was always in close agreement with bottom mixing data. However, as described earlier (10), there is uncertainty in determination of the end point of the \(H_2O_2\) saturation curve. To account for this uncertainty, the saturation at 100 mm Hg was determined for each set of data by linear interpolation, and the ratio saturation (100 mm, top data)/saturation (100 mm, middle data) was...
The proportion of the 0\(_2\) taken up by the deoxygenated blood is a given amount. This is because at lower saturation a larger amount of deoxygenated blood must be added in order to reduce \(p_{O_2}\) by a given amount. This is shown in Fig. 2. As saturation decreases larger aliquots are obtained when \(H_2O_2\) is added in steps with sufficient time allowed between steps for full equilibration of \(p_{CO_2}\).

The response time of the \(CO_2\) electrode was quite slow (54 s, 90% response). However, in unpublished control experiments, we have demonstrated that when the oxygenation time is greater than 10 mm, the curve is identical to that obtained when \(H_2O_2\) is added in steps with sufficient time allowed between steps for full equilibration of \(p_{CO_2}\).

Mixing Experiments - A mixing experiment at high saturation is shown in Fig. 3. It was previously possible, so that unlike Roughton we estimate \(Y\), the solubility coefficient, from the high and middle saturation curves, and its rate of addition. Since error can appear in these values, the data are subject to slightly greater uncertainty than are the mixing experiments. Although this causes little derived from hemoglobin, and a smaller proportion from solution (dissolved \(O_2\) is measured by the oxygen electrode). Thus, when saturation is close to 100\% the drop in \(p_{O_2}\) is nearly linear with the volume of deoxygenated blood added, and allows the estimate of the solubility coefficient \(\alpha\), described under "Experimental Procedures" (Fig. 2B).

An inherent difficulty in the method is that the assumption of 100\% saturation of the oxygenated sample is required. The error introduced by this assumption however, is negligible because as discussed below, computer fit of the data to Adair's scheme does not improve appreciably by varying the saturation value of the oxygenated sample.

A low saturation mixing curve is shown in Fig. 3. It was essential to completely deoxygenate the sample and to calibrate the \(p_{O_2}\) electrode with low \(p_{O_2}\) gas (1\% \(O_2\)). The zero point of the electrode must be checked at the end of the experiment by the addition of dithionite to the blood sample.

Solubility Coefficient, \(\alpha\) - Roughton and co-workers used a similar method to evaluate the solubility coefficient (17). However, improved tonometry allows us to achieve higher \(p_{O_2}\) than was previously possible, so that unlike Roughton we estimate \(\alpha\) at 37\(^\circ\)C for blood directly. Using our method, we find that \(\alpha = 0.0234\) ml/ml atm for normal human blood (range, 0.0230 to 0.0240 for four subjects) under standard conditions a value in good agreement with that reported by Roughton (0.0238). It must be pointed out that this analysis assumes that blood is a homogenous system and will not reflect possible differences between intracellular and extracellular solubility.

Middle Oxygen Equilibrium Curve - In addition to the solubility coefficient \(\alpha\), hemoglobin concentration, and cuvette volume, the measurement of the oxygen equilibrium curve by the \(H_2O_2\) method requires accurate knowledge of \(H_2O_2\) concentration and its rate of addition. Since error can appear in these values, the data are subject to slightly greater uncertainty than are the mixing experiments. Although this causes little

### RESULTS

**Hematologic Measurements** - The subjects from which the blood for the present study was obtained were healthy, nonsmoking Caucasian males. Electrophoretic analysis revealed the presence of Hemoglobins A and A\(_2\). Hemoglobin concentration, hematocrit, and 2,3-DPG concentrations are given in Table I. The method used to measure hemoglobin, CO-hemoglobin and methemoglobin employs the spectrophotometric analysis at three wavelengths of blood which has been lysed, and diluted into Sterox/borate buffer, pH 9.14. Thus all hemoglobin is present as oxyhemoglobin, CO-hemoglobin, or methemoglobin.

The oxygen capacity used to calculate saturation has taken into account the very small amounts of CO-hemoglobin and methemoglobin present.

**Electrodes** - The voltage output from the \(p_{O_2}\) electrode is linear with \(p_{O_2}\) up to 1 atm. The output in blood is somewhat less, and care must be taken to calibrate the electrode before precise work can be undertaken, especially at high \(p_{O_2}\). In Fig. 1 the calibration of the electrode is described. The modified electrolyte described under "Experimental Procedures" gave a response (10 s, 90% response) which was much faster than that obtained with commercially available solutions (33010, IL or S-4064, Radiometer). Moreover, long "conditioning" periods in the appropriate \(p_{O_2}\) ranges were used were not necessary.

The present experiments were performed at constant \(p_{CO_2}\) (40 mm Hg). In the case of the \(H_2O_2\) curves, \(p_{CO_2}\) was held constant by titration of the \(H^+\) liberated during oxygenation with \(NaOH\). The response time of the \(CO_2\) electrode was quite slow (54 s, 90% response). However, in unpublished control experiments, we have demonstrated that when the oxygenation time is greater than 10 min, the curve is identical to that obtained when \(H_2O_2\) is added in steps with sufficient time allowed between steps for full equilibration of \(p_{CO_2}\).

**Mixing Experiments** - A mixing experiment at high saturation is shown in Fig. 2. As saturation decreases larger aliquots of deoxygenated blood must be added in order to reduce \(p_{O_2}\) by a given amount. This is because at lower saturation a larger proportion of the \(O_2\) taken up by the deoxygenated blood is...
FIG. 2. The top of the blood oxygen equilibrium curve, obtained by the mixing method. The numbers in A represent the volumes of the small increments of deoxygenated blood which were added to the larger volume of oxygenated blood (1000 µl). In B the solubility coefficient, α, is estimated. As hemoglobin saturation approaches 100% the apparent α approaches a limiting value which is estimated by linear extrapolation to infinite pO2 (1/pO2 = 0). In this experiment, α = 0.0234 ml/ml atm. In C the final calculated data points are shown with the Adair curve obtained by fitting (see "Appendix").

FIG. 3. The bottom of the blood oxygen equilibrium curve obtained by the mixing method. The data were obtained by the reversal of the procedure outlined in Fig. 2. The raw data (A) were subjected to the analysis given under "Experimental Procedures" and the final calculated points are shown in B with the Adair curve.

error in the estimation of pO2, large uncertainty can be expected in the determination of the upper end of the curve. Thus, at 150 mm Hg, the end of the oxygenation run, saturation commonly ranges from 90 to 96%. One method of dealing with this problem by curve fitting has been described (10). Here, however, we assume that the blood samples in the middle and upper experiments achieve the same saturation at pO2 of 100 mm Hg and a normalization is carried out as outlined under "Experimental Procedures." Minor variation in the saturation at 100 mm Hg appears to be random, however, and Fig. 4 shows an example in which the data from each saturation range agree well without the normalization procedure.

This result indicates that mismatching of high and low saturation mixing curves observed by others previously (18, 19) was due to experimental error, rather than to "hysteresis" in the oxygenation reaction.

The Complete Curve—In experiments with subject RW, saturation in the middle oxygen equilibrium curve was 96.44% at 100 mm Hg, while in the top end mixing curve it was 97.03%. Therefore, the middle oxygen equilibrium curve saturations were multiplied by 0.9703/0.9644 to obtain a final middle data set. No adjustments were required to match the bottom and middle data. The complete curve was then obtained by joining the bottom, middle, and top portions (Fig. 5, Table II).
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Fig. 4. Top, middle, and bottom portions of the oxygen equilibrium curve for subject MS. This figure demonstrates the extension of the curve which is possible by the mixing method: the continuous curve is least reliable at its extremes.

Fig. 5. The complete oxygen equilibrium curve for subject RW. The data for the three saturation ranges were joined and the resulting complete curve was subjected to the fitting procedure described under "Appendix." The data points (○) and the Adair fit (—) are shown.

Fig. 6. A comparison of the curve of Roughton et al. and that of subject RW. The curves were drawn from Formula 1 and the parameters of Table II. The largest deviation occurs at low saturation. In spite of the large differences in \( a_3 \) and \( a_4 \), the two curves are nearly equivalent at high saturation.

Table II

| \( P_H \) (mm Hg) | Saturation | \( P_H \) (mm Hg) | Saturation | \( P_H \) (mm Hg) | Saturation |
|------------------|------------|------------------|------------|------------------|------------|
| 0.32             | 0.0016     | 17.75            | 0.2256     | 49.79            | 0.8064     |
| 0.61             | 0.0040     | 19.83            | 0.2517     | 53.44            | 0.8329     |
| 1.32             | 0.0064     | 19.83            | 0.2779     | 57.42            | 0.8593     |
| 1.82             | 0.0088     | 20.91            | 0.3041     | 62.89            | 0.8855     |
| 2.24             | 0.0112     | 22.07            | 0.3302     | 69.71            | 0.9116     |
| 2.68             | 0.0136     | 23.15            | 0.3565     | 80.16            | 0.9370     |
| 3.09             | 0.0161     | 24.14            | 0.3826     | 100.91           | 0.9609     |
| 3.49             | 0.0185     | 25.39            | 0.4090     | 140.48           | 0.9758     |
| 3.84             | 0.0209     | 26.39            | 0.4354     | 163.35           | 0.9815     |
| 4.22             | 0.0233     | 27.38            | 0.4618     | 194.66           | 0.9860     |
| 4.87             | 0.0281     | 28.63            | 0.4882     | 233.99           | 0.9885     |
| 5.50             | 0.0329     | 29.87            | 0.5146     | 277.34           | 0.9904     |
| 6.11             | 0.0376     | 31.12            | 0.5410     | 328.72           | 0.9944     |
| 7.11             | 0.0472     | 32.53            | 0.5675     | 381.30           | 0.9962     |
| 8.00             | 0.0566     | 33.85            | 0.5940     | 436.29           | 0.9975     |
| 8.77             | 0.0660     | 35.10            | 0.6206     | 492.88           | 0.9991     |
| 9.21             | 0.0701     | 36.10            | 0.6471     | 524.59           | 0.9995     |
| 11.20            | 0.0958     | 38.17            | 0.6737     | 555.89           | 0.9998     |
| 12.61            | 0.1217     | 39.91            | 0.7002     | 589.21           | 0.9999     |
| 14.02            | 0.1476     | 41.74            | 0.7268     | 622.19           | 1.0000     |
| 15.26            | 0.1736     | 43.81            | 0.7534     |                |            |
| 16.59            | 0.1996     | 46.30            | 0.7800     |                |            |

Table III

| Adair parameters, whole blood | \( a_1 \times 10^4 \) | \( a_2 \times 10^5 \) | \( a_3 \times 10^5 \) | \( a_4 \times 10^5 \) |
|------------------------------|------------------|------------------|------------------|------------------|
| Roughton*                    | 0.218 ± 6%       | 0.912 ± 8%       | 0.0038 ± 22%     | 0.247 ± 16%     |
| Roughton*                    | 0.237 ± 24%      | 0.925 ± 20%      | 0.273 ± 100%     | 0.253 ± 4%      |
| RWc                          | 0.151 ± 10%      | 0.972 ± 8%       | 0.170 ± 60%      | 0.167 ± 2%      |
| MS                           | 0.194            | 0.645            | 0.142            | 0.161           |
| FA                           | 0.151            | 0.857            | 0.177            | 0.142           |
| SC                           | 0.183            | 0.691            | 0.162            | 0.168           |

* From Ref. 1. pH 7.4, RH 40, 37°.
*b Data of Ref. 1, recalculated according to the procedure of this report.
*c Errors are for 95% confidence limits.
Analysis According to Adair's Equation — The fit of the data to Adair's equation is summarized in Table II and shown in Fig. 5. This procedure as outlined under "Appendix" is rapid, efficient, and highly reproducible. Excellent agreement for the parameters among four subjects was found, but the results differed consistently with the earlier data of Roughton and coworkers (17) (Table III, Fig. 6). The errors in the values of \( a \) were determined by systematically varying the value of \( a \) and then fitting the other 3 \( a \)'s to the data set. In this way, the residual sum of squares is observed to be a parabolic function of the value of \( a \), and the 95% confidence limits could be found using the \( f \) test. Analysis for one data set is given in Fig. 7.

**DISCUSSION**

The regulation of the oxygen affinity of hemoglobin within red cells is complex. Much is already known about the qualitative mediation of this regulation, but much remains to be learned about its precise quantitation. About aberrations in pathological states and about possible therapeutic intervention. As a first step toward a quantitative understanding we have attempted to describe the complete curve under conditions which very closely resemble those found in vivo. The advantages of our methods over those previously available are the following. (a) MetHb and COHb are not present in appreciable amounts, and their quantities are known. (b) 2,3-DPG is preserved. (c) The entire curve is obtained on the same sample, within a few hours of its collection. (d) \( pH \) and \( P_{CO2} \) are constant throughout the curve, so that no "corrections" need be made for the Bohr effect. (e) Use of the properly calibrated oxygen electrode in the high and low ranges of saturation is more precise than older gasometric methods. (f) An analysis of the solubility coefficient \( (a) \) can be done on each experiment. (g) Analysis of the data according to Adair’s scheme is possible.

The curve differs significantly from that reported by Roughton et al. (8) (Table III, Fig. 6). The difference can be explained by the fact that the earlier data were obtained before the full importance of 2,3-DPG was appreciated and the older method of deoxygenation required long periods of time during which the blood was held at 37°C. Furthermore, the earlier data was "corrected" to \( pH \) 7.4 using an assumed Bohr factor. In fact, the Bohr effect is not constant on the entire range of saturation (6, 20, 21). Finally, the earlier data was obtained from blood samples on more than one subject, and the data points were corrected by a factor (observed \( pH \)/26.5).

The curves differ in their position on the abscissa by 2.3 mm Hg at 50% saturation, but the difference is less at high saturation, and greater at low saturation. Thus, they differ not only in their position but in their shape, and it is the latter observation for which the Adair analysis is useful. For example, it is known that 2,3-DPG and \( H^+ \) exert their influence at low saturation (5), suggesting that either reduced 2,3-DPG or increased intracellular \( pH \) (or both) in the older experiments could account for the observed differences.

Furthermore, in our experiments the Bohr protons are treated as they are released. In methods which employ tonometry of samples with constant \( CO_2 \) but differing \( pH \) and total \( HCO_3^- \) will vary with saturation. The effect of \( pH \) on the oxygen equilibrium curve is well known, but the effect of \( HCO_3^- \) is less well understood (22). Therefore, additional data of the type reported here will be required to fully describe the intracellular effects of \( H^+ \), \( CO_2 \), \( HCO_3^- \), and 2,3-DPG.

We believe that the principal usefulness in the determination of the Adair parameters is the comparison of the shapes of oxygen equilibrium curves and in quantitatively testing the reproducibility of the curves. The parameters have been used in experiments with pure hemoglobin to calculate the equilibrium constants for the successive oxygenation steps.

These can be calculated as follows (4, 23).

\[
\begin{align*}
k_1 &= \frac{1}{4} a_1 \\
k_2 &= \frac{2}{3} a_2 \\
k_3 &= \frac{3}{2} a_3 \\
k_4 &= \frac{4}{a_4}
\end{align*}
\]

Thus, the \( k \)'s will have variances which will be the weighted sum of the co-variances for the \( a \)'s from which they are calculated, and, except for \( k_1 \), will become almost meaningless when the magnitude of the errors is considered (Table III). Nevertheless, Table IV summarizes such calculations. The errors are largest in the values of \( a_2 \) and \( a_4 \) and therefore \( k_2 \), \( k_3 \), and \( k_4 \) will be increasingly less reliable. Therefore, we are not able to attach any physical meaning to the constants of Table III.

The error analysis in Fig. 7 demonstrates that the data can reasonably be described by three parameters, \( a_1 \), \( a_2 \), and \( a_4 \), while the Adair oxygenation scheme (Formula 1) requires four parameters. Therefore, in order to determine the equilibrium constants (\( k \)'s) from the Adair parameters (\( a \)'s) data points of even greater number and precision than those in Fig. 6 would be required. We believe that this is extremely unlikely using currently available methods and that other data, perhaps kinetic, would be required.

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R. M. Winslow, M. Suwenberg, R. L. Berger, R. I. Shrager, M. Luzzana, M. Samaja, and L. Rossi-Bernardi

Page 2331, Equation 1
Numerator should read “a_1p + 2a_2p^2 + 3a_3p^3 + 4a_4p^4.”

Page 2332, Equation 2
Denominator should read “P_{r-1} (V - \Delta V_r) - P_r V.”

Page 2332, Equation 4
“V,” should read “\Delta V_r.”

Page 2335, Table III
“a_1 \times 10^5” should read “a_1 \times 10^5.”

Page 2337, Ref. 22
“Kreuger” should read “Kreuzer.”

Page 2337, Appendix
In the third line below Equation 8, references should read “Roughton et al. (8) and Imai (41.”

In Equations 12 and 13, “e” should read “e.”

In Equation 14, “y,” should read “y.”

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.