

Simulation of continuous blood O₂ equilibrium curve over physiological pH, DPG, and Pco₂ range

ROBERT M. WINSLOW, MICHELE SAMAJA, NANCY J. WINSLOW,
LUIGI ROSSI-BERNARDI, AND RICHARD I. SHRAGER

Center for Infectious Diseases, US Department of Health and Human Services, Atlanta, Georgia 30333; Centro di Fisiologia del Lavoro Muscolare del CNR and Cattedra di Chimica e Biologica, University of Milan, Milan, Italy; and Laboratory of Statistical and Mathematical Methodology, National Institutes of Health, Bethesda, Maryland 20205

WINSLOW, ROBERT M., MICHELE SAMAJA, NANCY J. WINSLOW, LUIGI ROSSI-BERNARDI, AND RICHARD I. SHRAGER. *Simulation of continuous blood O₂ equilibrium curve over physiological pH, DPG, and Pco₂ range.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54(2): 524-529, 1983.—We analyzed 56 O₂ equilibrium curves of fresh human blood, each from 0 to 150 Torr P_{O₂}. The data were collected over ranges of values for the 2,3-diphosphoglyceric acid-to-hemoglobin concentration ratio [DPG]/[Hb] of 0.2-2.7, for pH of 7.0-7.8, and for Pco₂ of 7-70 Torr. Each curve was characterized according to the Adair scheme for the stepwise oxygenation of Hb, and the resulting constants (a_1, a_2, a_3, a_4) were analyzed to allow the simulation of the entire O₂ equilibrium curve under any conditions of [DPG]/[Hb], pH, and Pco₂ in the specified range. This analysis provides a powerful tool to study the affinity of Hb for O₂ within the red blood cell and to predict the shape of the O₂ equilibrium curve in various physiological and pathological states. Other attempts to predict blood O₂ affinity have considered only P₅₀ (the P_{O₂} at one-half saturation with O₂) or have provided too little data for continuous simulations.

hemoglobins; blood oxygen affinity; Adair equation; 2,3-diphosphoglycerate; carbon dioxide; pH; Bohr effect

THE POSITION of the whole-blood O₂ equilibrium curve (OEC) is usually represented by the partial pressure of O₂ at which hemoglobin (Hb) is half-saturated (P₅₀). Many physicochemical conditions within the red blood cell determine, in concert, its actual value. However, the principal effectors that vary under physiological conditions to regulate the P₅₀ are 2,3-diphosphoglyceric acid (DPG), H⁺, and CO₂ (2, 4, 11). Nomograms and equations have been developed to estimate P₅₀ from known values of Pco₂, pH, and the [DPG]/[Hb] molar ratio (13).

For two important reasons, we extended our work to a description of the entire OEC over a broad range of P_{O₂}. First, the allosteric effectors of O₂ affinity, DPG, H⁺, and CO₂ react differently with deoxy- and oxyhemoglobin and, therefore, influence the shape in addition to the position (P₅₀) of the OEC. Second, the P₅₀ is a somewhat artificial index for physiological considerations. Under normal conditions (37°C, pH 7.4, Pco₂ 40 Torr) we have found the mean P₅₀ to be 26.7 ± 1.7 Torr (23), whereas in resting humans at sea level arterial P_{O₂} is 90 Torr and mixed venous P_{O₂} is 40 Torr, placing P₅₀ outside the operating range.

Many previous systematic studies of the effects of the allosteric regulators of Hb-O₂ affinity have been done at low Hb concentration (8, 18-20) and therefore are complicated by tetramer-dimer dissociation (9). Such dissociation further affects the shape and position of the OEC, and, therefore, the data do not apply directly to the conditions within the red blood cell. Furthermore, the data that are available from studies on whole blood or concentrated Hb solutions (2, 4, 7) do not encompass ranges of [DPG]/[Hb], pH, or Pco₂ to allow a complete description of physiological functions.

In this report, we present a description of the entire OEC for P_{O₂} 0-150 Torr, pH 7.2-7.8, Pco₂ 7-70 Torr, and [DPG]/[Hb] 0.2-2.7 mol/mol. We have used a method for precisely measuring the OEC under controlled conditions (23) to obtain data for a wide range of pH, Pco₂, and [DPG]/[Hb] molar ratio. The Adair equation (1) was used as a model to characterize the OEC, and the parameters $a_1, a_2, a_3,$ and a_4 were obtained by methods we have previously described (24). The a 's were then reduced to simple empirical functions of the three effectors so that an OEC can be reconstructed under any set of physiological conditions likely to be encountered.

METHODS

Blood samples. Heparinized blood from two healthy male nonsmoking donors was obtained by venipuncture. Concentrations of Hb, carboxyhemoglobin, and methemoglobin in whole blood were measured with the use of a Microblood analyzer, kindly loaned by Carlo Erba Strumentazione, Milan, Italy. [DPG] was measured using kits obtained from Calbiochem. When no alterations of [DPG] were required blood was kept on ice at 4°C until use, no more than 4 h after collection. The two subjects were representative of a group of 38 normal individuals studied in a previously reported survey (23) using the same techniques.

Alteration of [DPG] and pH. To increase [DPG], red blood cells were washed in isotonic saline and incubated at 37°C in isotonic media containing inosine, pyruvate, and phosphate at various ratios for 90 min (3) as described previously (14). To decrease [DPG], cells were incubated in isotonic saline at 37°C for various times up to 12 h, depending on the degree of depletion required.

Incubations were followed by recombination of washed red blood cells with their native plasma. To alter the pH of the final suspension, small quantities (up to 10 μ l/ml) of either 4.45 M lactic acid or 10 M NaOH were added to the plasma. In these instances, plasma samples were equilibrated to the final P_{CO₂} before red blood cells were added to avoid extremes of pH and consequent hemolysis.

OEC determinations. Samples of blood (2.5 ml) were deoxygenated in an IL 237 tonometer (Instrumentation Laboratories, Lexington, MA) for 20 min using N₂ containing 1, 5.6, or 10% CO₂. Gases were obtained from Lif-O-Gen, Cambridge, MD, and compositions were specified to \pm 0.01%. Blood OEC's were measured as previously described (23). During the oxygenation, the P_{CO₂} and pH of the blood were maintained at the starting values. Reproducibility of the measurement was reported previously (23).

A total of 56 OEC's were measured at different pH, P_{CO₂}, and [DPG]/[Hb] ratios. The conditions were chosen to encompass the range of variation expected under physiological conditions for the three effectors (Fig. 1). At least three OEC's were determined at different pH for each set of P_{CO₂} and [DPG]/[Hb] ratio.

Data analysis. As discussed previously (23, 24) the major source of error in this method is calibration of the O₂ electrode. A separate method, which does not depend on PO₂ measurement, for the precise determination of P₅₀ only was devised (13). Briefly, the method consists of equilibrating small samples of blood with gas mixtures of known O₂-CO₂-N₂ composition in sealed glass tonometer bottles. At the end of the equilibration period Hb saturation with O₂, pH, and DPG are measured. If the PO₂ is chosen close to the expected P₅₀ value, the Hill equation can be used to calculate P₅₀ from the known PO₂ and measured saturation. This technique was used to measure P₅₀ over a wide range of pH, [DPG]/[Hb], and P_{CO₂}, and a nomogram was constructed to relate the variables. On the assumption that any discrepancy in P₅₀ observed using the two methods was due to the O₂ electrode calibration in the continuous method, all PO₂ values were adjusted by the ratio P₅₀ (P₅₀ nomogram)/P₅₀ (continuous OEC). In any case, the discrepancies were always small (<2 Torr).

Experimental data. The Adair equation in its form for the present work is

$$Y = \frac{a_1P + 2a_2P^2 + 3a_3P^3 + 4a_4P^4}{4(1 + a_1P + a_2P^2 + a_3P^3 + a_4P^4)} \quad (1)$$

where Y is the fractional saturation of Hb with O₂ and P is the partial pressure of O₂. This equation is a convenient representation of the entire OEC, since the four constants (α 's) can be used to determine Y at any P.

The corrected data sets were transferred to a Decsystem-10 digital computer (Digital Equipment, Maynard, MA) for analysis of the Adair parameters as described previously (24). In this analysis, 100 experimental points were used for each OEC, and the α 's were constrained to positive values. P₅₀ was computed as a Newton-Rapheson solution of the Adair equation by finding P such that Y(P) = 0.5. The value n_{max} was found from the slope of the function Y(P)/[1-Y(P)] on a log-log plot by finite differences.

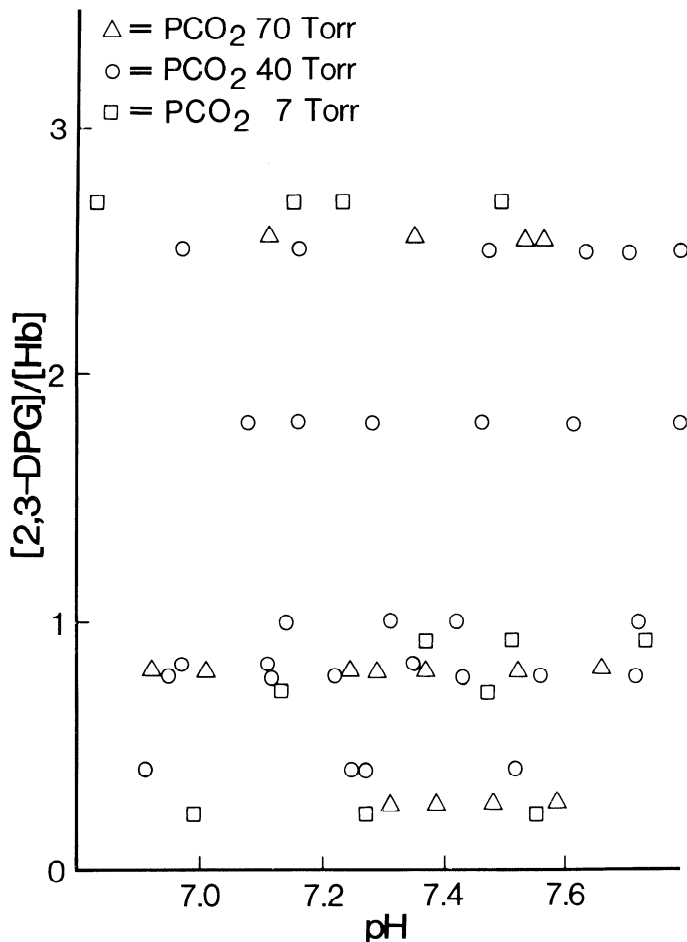


FIG. 1. Conditions under which O₂ equilibrium curves were measured. P_{CO₂} = 7, 40, and 70 Torr.

RESULTS

Experimental data. Table 1 lists the α 's determined for all the OEC's analyzed, along with the P₅₀, n_{max}, and residual sum of squares for each determination. The correlation coefficient for the relationship between P₅₀ using the continuous method and that predicted from the P₅₀ nomogram was 0.99835. Since the nomogram is considered to be more precise in PO₂, we assume that the major source of error is the PO₂ measurement in the continuous method. Although this assumption has not been fully substantiated by experimentation, it will suffice for the purpose of the present analysis.

The logarithms of the Adair constants are apparently a linear function of pH under all conditions investigated. Examples of these relationships are shown in Fig. 2. The behavior of the α 's as functions of [DPG]/[Hb] ratio at constant pH is not so simple as shown in Fig. 3. This result is not surprising, since the effect of DPG on O₂ binding to Hb within the red blood cell is complex and includes a sizeable intracellular pH effect at [DPG]/[Hb] > 1 (14). Moreover, the effects of DPG and CO₂ are highly interactive (11).

Analysis of Adair parameters. To establish the relationship between the Adair parameters and the various allosteric effectors, the parameters were related to [DPG]/[Hb] molar ratio and P_{CO₂} at pH 7.0 and 7.8 according to the following procedures.

TABLE 1. O_2 equilibrium curve parameters

OEC No.	[DPG]/[Hb]	pH _e	P ₅₀ , Torr	log a ₁	log a ₂	log a ₃	log a ₄	n _{max}
<i>P</i> CO ₂ = 7 Torr								
L073	0.22	6.99	22.8	-1.932	-2.642	-4.169	-5.375	2.48
L074	0.22	7.27	18.1	-1.564	-2.536	-3.660	-5.010	2.33
L075	0.22	7.74	11.5	-1.287	-2.213		-4.190	2.46
N072	0.73	7.26	27.6	-1.614	-2.796		-5.638	2.46
N071	0.73	7.68	19.9	-1.730	-2.567		-5.119	2.55
2208	0.86	7.36	27.4	-1.666	-3.177		-5.131	2.76
2206	0.86	7.73	19.5	-1.883	-2.604		-5.131	2.57
2207	0.86	7.51	24.0	-1.604	-2.845		-5.405	2.71
H072	2.70	6.83	58.4	-1.724	-4.186		-6.876	3.09
H073	2.70	7.15	46.1	-1.476	-3.569		-6.406	2.84
H074	2.70	7.22	45.6	-1.830	-3.337		-6.511	2.89
H075	2.70	7.48	36.3	-1.710			-6.108	2.85
<i>P</i> CO ₂ = 40 Torr								
0528	0.40	6.91	33.5	-2.210	-2.971	-5.052	-6.129	2.26
0523	0.40	7.26	25.1	-1.990	-2.721	-4.355	-5.622	2.44
0527	0.40	7.27	24.8	-1.996	-2.886	-4.380	-5.672	2.36
0524	0.40	7.52	20.6	-2.295	-2.796	-4.151	-5.379	2.53
2004	0.80	6.95	39.9	-1.842	-3.286		-6.295	2.71
2023	0.80	7.12	34.8	-1.812	-3.070		-6.063	2.57
2005	0.80	7.22	31.9	-1.848	-3.177		-5.979	2.60
2003	0.80	7.43	26.8	-1.740	-2.879		-5.680	2.66
2021	0.80	7.56	24.1	-1.750	-2.833		-5.442	2.70
2022	0.80	7.72	21.1	-1.670	-2.706		-5.206	2.68
N403	0.83	6.97	38.6	-1.807	-3.086		-6.253	2.46
N404	0.83	7.11	36.3	-1.625	-3.310		-6.086	2.81
N401	0.83	7.35	29.8	-1.824	-2.967		-5.827	2.54
0518	1.00	7.14	36.7	-1.866	-3.101		-6.161	2.55
0516	1.00	7.31	31.6	-1.785	-2.967		-5.900	2.54
0515	1.00	7.42	29.0	-1.660	-2.907		-5.728	2.56
0521	1.00	7.72	22.4	-2.076	-2.699		-5.361	2.62
0826	1.80	7.08	45.9	-1.833	-3.644		-6.520	2.96
0827	1.80	7.16	43.0	-1.903	-3.426		-6.429	2.81
0823	1.80	7.28	38.3	-1.854	-3.331		-6.229	2.82
0828	1.80	7.46	33.5	-1.708	-3.688		-6.049	2.81
0824	1.80	7.61	29.2	-2.133	-3.072		-5.818	2.85
0602	2.50	6.97	56.2	-1.818	-4.076		-6.870	2.94
0601	2.50	7.16	47.8	-1.889	-3.866		-6.638	2.91
0604	2.50	7.47	35.8	-1.660	-3.433		-6.072	2.95
0603	2.50	7.63	32.8	-1.592	-3.839		-5.930	2.87
0605	2.50	7.70	30.3	-1.527	-3.896		-5.807	2.94
0606	2.50	7.80	27.7	-1.785	-3.136		-5.682	2.94
<i>P</i> CO ₂ = 70 Torr								
L703	0.27	7.31	21.8	-1.449	-3.536	-4.144	-5.345	2.61
L705	0.27	7.39	23.0	-1.533	-3.457	-4.245	-5.451	2.63
L702	0.27	7.48	19.7	-1.538	-3.146	-4.165	-5.169	2.71
L704	0.27	7.59	20.1	-1.775	-2.967	-4.237	-5.240	2.69
2117	0.80	6.93	41.8	-1.979	-3.289		-6.406	2.63
2118	0.80	7.01	39.3	-2.040	-3.195		-6.307	2.61
2116	0.80	7.24	32.3	-1.955	-3.059		-5.967	2.66
2112	0.80	7.29	31.7	-1.642	-3.199		-5.936	2.53
2113	0.80	7.37	29.9	-1.839	-3.032		-5.815	2.72
2114	0.80	7.52	26.6	-2.123	-2.917		-5.658	2.74
2115	0.80	7.66	23.9	-1.730	-3.042		-5.450	2.84

pH_e, extracellular pH. See text for explanation of all other abbreviations.

1) The value of log a_i was fit, using the least-square method, to a second-order equation

$$\log a_i = w(G^2) + y(G) + z \quad (2)$$

where G = [DPG]/[Hb]. The values of w, y, and z were estimated for each a and Pco₂ at pH 7.0 and 7.8. Thus each a_i can be empirically estimated at pH 7.0 and 7.8 as a function of G.

2) At each pH and G value, w, y, and z are functions of Pco₂ only and can be related according to another second-order equation

$$w, y, \text{ or } z = A(Pco_2)^2 + B(Pco_2) + C \quad (3)$$

From the relation of w, y, and z to Pco₂ for each G, values of A, B, and C were further determined by least-squares analysis.

We have avoided the use of subscripted variables, for clarity, but it is understood that there is a different set of A, B, and C for each set of w, y, and z and for each two pH's and each of four a's, making 3 × 2 × 4 = 24 sets of A, B, and C in all. Saturation under all pertinent conditions is now specified by these 72 coefficients.

The values of all required A's, B's, and C's are given in Table 2.

Simulation of the OEC. The calculation of the four Adair parameters from known values of pH, [DPG]/[Hb], and Pco₂ can be obtained by using the following steps. 1) For pH 7.0 and 7.8, calculate w, y, and z from Eq. 3 and the selected Pco₂. 2) Using the values of w, y, and z obtained in step 1, calculate the four log a's at pH 7.0 and 7.8 from Eq. 2 and the selected [DPG]/[Hb] ratio. 3) Since log a's are linear functions of pH at each [DPG]/[Hb] and Pco₂ condition (Fig. 2), the log a's at the selected pH are calculated by linear interpolation. 4) The a_i's are substituted in Eq. 1 to compute Y at any P and, hence, to generate the entire OEC. 5) Evaluation of P at any Y can conveniently be accomplished using the Newton-Raphson method of successive approximation. To obtain a solution for P to an error <0.1 Torr, three to four iterations are usually required.

An example of a FORTRAN IV program for performing all of the above computations is available on request.

Error analysis. The use of the Adair model to fit the experimental OEC using our method of curve fitting has been discussed extensively (23, 24). Winslow et al. (23) demonstrated that the deviation of the directly measured saturation from that estimated from the Adair parameters was 0.5% on the range of Po₂ 5-100 Torr. This magnitude of discrepancy is routinely observed using the present methods, and we accept the Adair simulation as an accurate representation of the real OEC. The ±0.5% deviation is certainly within the limits of our ability to measure saturation using the present experimental techniques.

To estimate the magnitude of the discrepancy between the experimental OEC and that calculated using the present algorithms, simulations were made for each set of conditions for the OEC's listed in Table 1. Then, at each 10% increment of saturation from 10 to 90%, saturations were computed using both observed and simulated a's. For example: 1) Find P_{obs} at Y_{obs} = 10% from an experimental OEC. 2) Using that p_{obs}, find Y_{calc} using the equations. 3) Find an error, Y_{obs} - Y_{calc}. These errors were computed for all the OEC's, and the standard deviations were calculated for them. The result is shown in Fig. 4, which illustrates that under all conditions encompassed by the experimental conditions, the saturation can be found from Po₂ to a maximum standard deviation of -1.2 to +2.0% saturation. The analysis also shows that the error in saturation estimation is greatest at around 50%.

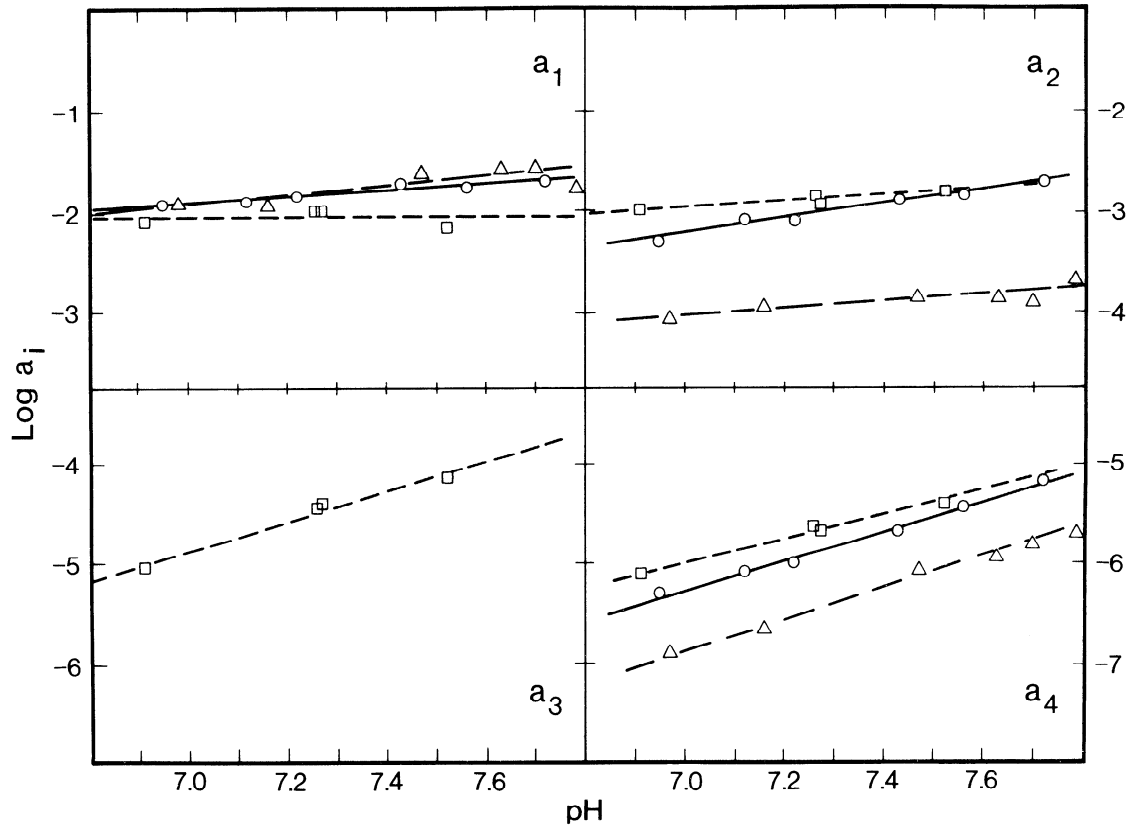


FIG. 2. Relationship of $\log a_i$ vs. pH for representative O₂ equilibrium curves: P_{CO₂} is 40 Torr, [DPG]/[Hb] ratios are 0.4 (□), 0.8 (○), and

2.7 (Δ). Straight lines are determined by least-squares regression. The a_i 's were also linear with pH at P_{CO₂} 7 and 70 Torr and are not shown.

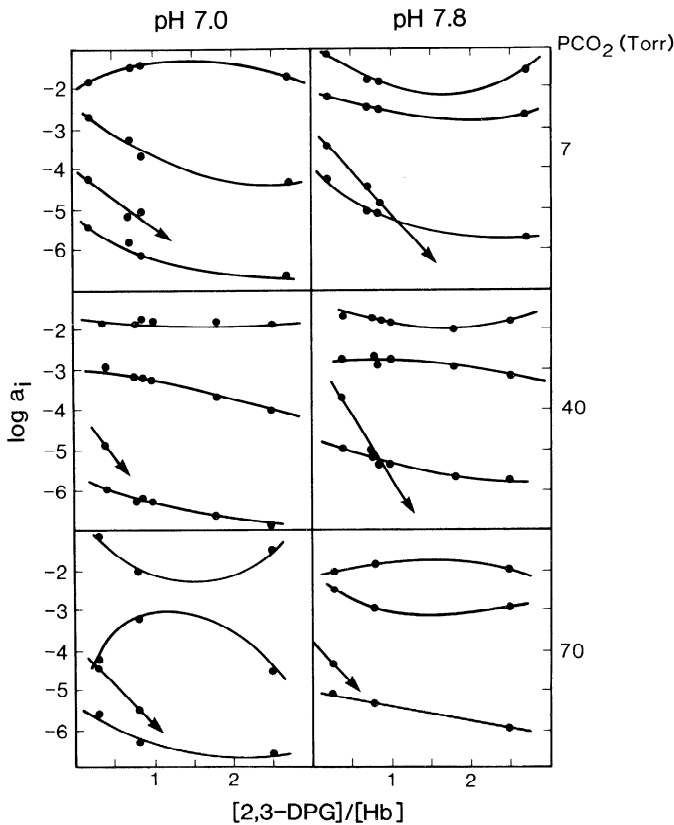


FIG. 3. Relationships of $\log a_i$ vs. [DPG]/[Hb] at different P_{CO₂} and for 2 extremes of pH. Curved lines were determined by least-squares fit to second-order equations as described in text. Under some conditions a_3 tended to negative values, shown by arrows.

P₅₀ estimation. The procedures outlined above were used to calculate P₅₀ for each of the 56 sets of conditions from the experimental OEC's and from the computer model. Figure 5 demonstrates that the correlation is excellent for all conditions studied.

OEC shape estimation. The strength of the present approach is that the shape of the OEC can now be estimated under a variety of physiological conditions. In Fig. 6, we compare the behavior of n (at P₅₀) predicted from the present analysis with that actually found in a previous study (14). The agreement demonstrates that the present method does, indeed, account for these changes.

DISCUSSION

In the past decade, the influences of the allosteric effectors of Hb function, H⁺, CO₂, and DPG, have been well defined. Each of these molecules has different reactivities with the deoxy and oxy conformers of Hb, and they therefore have important effects on the shape as well as the position of the OEC. Before these effects were known, the shape of the OEC was considered to be invariant when its position was defined. The most frequently used equation for the OEC is that described by Hill (5)

$$\log Y = n \log P + C \tag{5}$$

A major drawback of Eq. 5 is that the slope, n , is not constant over the full range of oxygenation. Although the value at P₅₀ may be about 2.6, it decreases to 1 at high

and low saturation (18). Thus, when calculation of saturation from PO_2 (or vice versa) is required, significant errors can occur at extremes of saturation.

Some algorithms and nomograms for calculations based on the OEC use this simple representation, with the implicit assumption that the shape of the OEC does not vary with conditions. One of these is the blood gas calculator of Severinghaus (15, 16). In his original paper describing the use of the calculator (15), he stated these assumptions but gave recognition to the fact that the behavior of the shape under different conditions was unknown.

We previously reported that DPG and H^+ have impor-

TABLE 2. Parameters for Eqs. 2 and 3

a_i	pH		A	B	C
a_1	7.0	w	0.6675×10^{-3}	-0.3351×10^{-1}	-0.8114×10^{-1}
		y	-0.2077×10^{-2}	0.1057	0.2550
		z	0.1389×10^{-2}	-0.8233×10^{-1}	-1.522
	7.8	w	-0.1323×10^{-3}	-0.1180×10^{-2}	0.5527
		y	0.3564×10^{-3}	0.8291×10^{-2}	-1.815
		z	-0.257×10^{-3}	-0.1163×10^{-2}	-0.7493
a_2	7.0	w	-0.4558×10^{-3}	0.1002×10^{-1}	0.3322
		y	0.1411×10^{-2}	-0.2945×10^{-1}	-1.643
		z	-0.100×10^{-2}	0.3303×10^{-1}	-2.432
	7.8	w	0.3562×10^{-3}	-0.243×10^{-1}	0.2996
		y	-0.9808×10^{-3}	0.6717×10^{-1}	-1.016
		z	0.5823×10^{-3}	-0.4721×10^{-1}	-1.768
a_3	7.0	w	0.1151×10^{-2}	-0.1023	-1.660
		y	0.2031×10^{-2}	-0.1639	2.278
		z	-0.3756×10^{-1}	0.3432×10^{-1}	-4.632
	7.8	w	-0.7407×10^{-3}	0.5148×10^{-1}	-3.654
		y	0.2855×10^{-2}	-0.2157	2.78
		z	-0.1325×10^{-2}	0.8771×10^{-1}	-4.129
a_4	7.0	w	0.3457×10^{-3}	-0.2257×10^{-1}	0.4241
		y	-0.1019×10^{-2}	0.6913×10^{-1}	-1.774
		z	0.6414×10^{-3}	-0.4939×10^{-1}	-4.796
	7.8	w	0.6947×10^{-4}	-0.1197×10^{-1}	0.5114
		y	-0.3443×10^{-3}	0.5013×10^{-1}	-2.214
		z	0.2638×10^{-3}	-0.3888×10^{-1}	-3.541

See text for explanation of abbreviations.

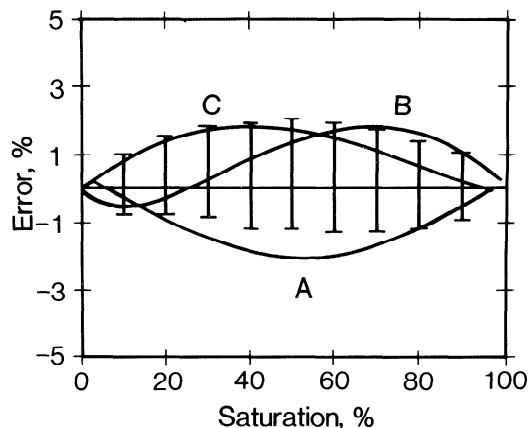


FIG. 4. Deviation of computed from observed O_2 equilibrium curves (OEC). Vertical bars represent ranges of error for all OEC in Table 1. See METHODS for discussion of calculations. Continuous curves represent errors for 3 standard curves (A, B, and C).

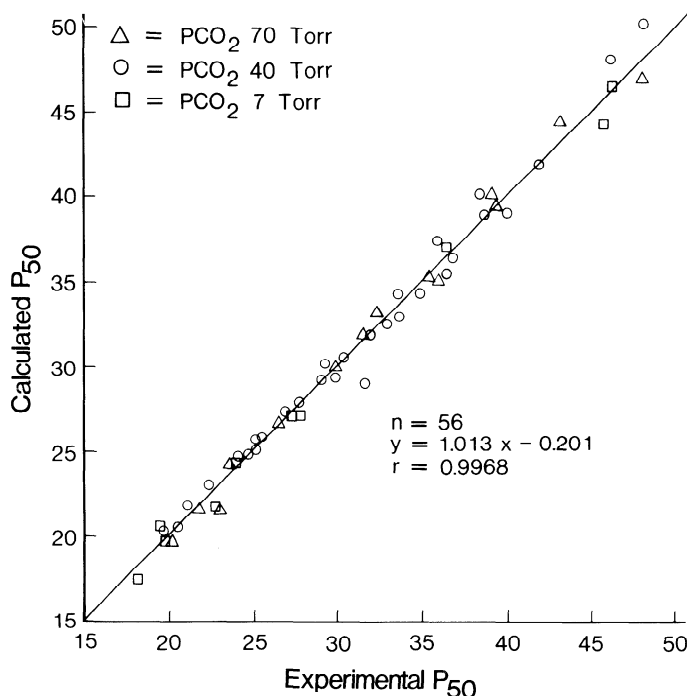


FIG. 5. Correlation between experimental P_{50} and that calculated from Eqs. 2-4 and Table 2. Regression lines are shown. P_{50} , PO_2 at half-saturation with O_2 .

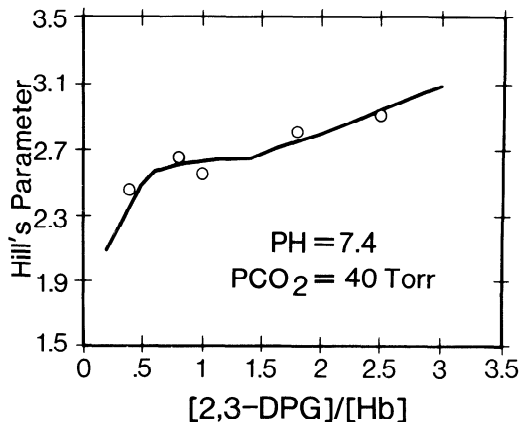


FIG. 6. Effect of $[DPG]/[Hb]$ on shape of OEC. Ability of present simulation method to predict changes in shape of OEC is demonstrated. Hill's parameter (n) has been estimated for present method (continuous curve) and compared with measured values (14) taken from Table 1. Blood conditions are $pH = 7.4$ and $PCO_2 = 40$ Torr.

tant effects on Hill's parameter, n , at constant PCO_2 (14). Furthermore, in whole blood the influence of these effectors is not uniform over the entire range of saturation (6, 21). Neville (12) has pointed out that such changes in the shape of the OEC can affect peripheral O_2 delivery. Others, for example West (22), have attempted to calculate the influence of alterations of shape on physiological functions, but without adequate model data.

No comprehensive attempt to describe the effects of H^+ , CO_2 , and DPG has been reported heretofore, with the exception of the work of Arturson et al. (2). They reported experiments in which OEC's were measured using the Radiometer DCA-1 instrument at three pH values (7.20, 7.40, and 7.60), three values of $[DPG]$ (3, 5,

and 7 mol/l blood), and three values of PCO₂ (20, 40, and 80 Torr).

Several problems with the Arturson et al. data reduce their utility. First, they are cumbersome to use; i.e., data at only a few conditions are available, and linear interpolations must be made where it is known that linear relations do not exist. In contrast, our approach to use the Adair scheme means that continuous functions can be defined which allow calculations at any combination of conditions. Second, in the data of Arturson et al. [DPG] is given as moles of DPG per liter of blood. Without knowledge of [Hb], no regular pattern of effect of DPG can be defined; i.e., the DPG effect is a function of the [DPG]/[Hb] molar ratio (8, 14), and the concentration unit mol/l blood will not account for variations in hematocrit or Hb values in different individuals. Finally, the tables of Arturson et al. (17) cannot be readily adapted to computer programs for continuous determinations.

We have chosen the Adair equation to describe the OEC, because it is based on physicochemical considerations. It probably does not describe the OEC better than the Kelman equation (10) or other empirical relations, but it does allow the possibility of comparing complex cellular phenomena to data obtained from much simpler experiments with purified Hb solutions (4). We have previously described the methods used to obtain the values of α 's and their uncertainties (23, 24).

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An obvious problem with the Adair scheme is the difficulty in estimation of α_3 . This has been discussed extensively in our previous work on this subject (23, 24). Indeed, the OEC can be often described using only three of the Adair parameters (23). The high degree of interdependence among the parameters does not invalidate the Adair scheme, however. Rather, further work should be directed toward obtaining independent estimations of the parameters.

In this report we do not attempt a physical interpretation of the reactions of DPG, H⁺, and CO₂ within the red blood cell. Instead, an initial estimation of the behavior of the Adair parameters is intended only as a starting point for the detailed investigation of the interactions of these effectors with Hb. Still more data are required to continue such an approach, particularly at different temperatures and over a wider range of conditions.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the Department of Health and Human Services.

Address for correspondence: R. M. Winslow, Centers for Disease Control, Public Health Service, US Dept. of Health and Human Services, Atlanta, Georgia 30333.

Address for M. Samaja: Centro di Fisiologia del Lavoro Muscolare del CNR, Via Mangiagalli 32, 20133 Milan, Italy.

Address for L. Rossi-Bernardi, Cattedra di Chimica Biologica, University of Milan, Ospedale San Raffaele, via Olgettina 60, 20132 Milan, Italy.

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