

OXYGEN AFFINITY IN THE BLOOD OF SHEEP

MICHELE SAMAJA¹ and LUCIANO GATTINONI²

*Clinical Hematology Branch¹, and Laboratory of Technical Development²,
 National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20014, U.S.A.*

Abstract. The oxygen affinity in the blood of adult Dorset sheep with HbB was studied by determining several oxygen equilibrium curves under different conditions of pH and P_{CO_2} by a method that allowed strict control of pH, P_{CO_2} , and HCO_3^- concentration over the entire curve. Nomograms and equations were derived that allowed the estimation of the oxygen saturation in a sample of blood in the range 0 to 100%, either from pH, P_{CO_2} and P_{O_2} , or from P_{O_2} and known P_{50} . The advantage of this approach is that no assumptions about the shape of the oxygen equilibrium curve need be made (*i.e.* the Hill parameter, n , is not required to be constant), since the entire curve was measured.

Extracellular pH	Oxygen dissociation curve
Intracellular pH	P_{50}

Recently, sheep have become widely used in laboratory studies. The determinations of many respiratory parameters require the knowledge of the entire oxygen equilibrium curve (OEC). The equations and nomograms for human blood are frequently used due to the lack of data on the blood of sheep. However, the concentration of some important molecules, such as 2,3-diphosphoglyceric acid (2,3-DPG), is different in the blood of the two species, and differences in the blood oxygen affinity between sheep and humans are, therefore, expected.

In this paper we report the equations and nomograms for the determination of the P_{50} in the blood of adult Dorset sheep, as a function of pH and P_{CO_2} *, and for the calculation of the hemoglobin (Hb) oxygen saturation, as a function of the measured P_{O_2} and the P_{50} obtained under different conditions of pH and P_{CO_2} .

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Present addresses:

¹ Cattedra di Enzymology, University of Milan, Via Celoria 2, 20133 Milan, Italy

² Institute of Anesthesiology, University of Milan, Via F. Sforza 35, 20122 Milan, Italy

* The 2,3-DPG level in the sheep is very low and has no effect on the oxygen affinity of sheep blood (Blunt and Huisman, 1975).

Materials and methods

Heparinized whole blood (50 units/ml) from three adult, healthy Dorset sheep was used. All the experiments were carried out within 12 hours of blood collection. When not immediately used, the blood was kept refrigerated at 0 °C in an ice bath. No significant changes in the oxygen affinity were detected at the end of this period among the three sheep studied. The electrophoretic pattern of the Hb showed the presence of HbB only. The average total Hb concentration for the sheep studied was 10.3 ± 0.5 g/100 ml.

INTRACELLULAR pH

The method described by Enoki *et al.* (1972) for the measurements of intracellular pH was modified as follows. One ml of well mixed blood was placed into an IL 237 tonometer* and equilibrated with a selected O₂-CO₂ mixture**, which was saturated with water vapor at 37 °C. At each P_{CO₂} value, a range of blood pH, *i.e.* the extracellular pH (pHe), was achieved by adding 1 M NaHCO₃ or 1 M lactic acid to the blood. Acid or base was added after 5–10 min to tonometry, so that the formed CO₂-HCO₃⁻ buffer prevented hemolysis or methemoglobin formation, which may be caused by sudden changes in pH. At the end of tonometry (15 min), pHe was measured by an IL 213 pH meter*. The sample was then anaerobically transferred by a gas-tight syringe*** into two small centrifuge tubes and sealed with a drop of paraffin oil. After a 3-min centrifugation, the red cells were separated from the plasma by aspiration. The packed red blood cells were frozen and thawed twice to ensure complete hemolysis. The pH of the lysed solution, *i.e.* the intracellular pH (pHi), was measured at 37 °C. All the measurements were made at least in duplicate on fully oxygenated blood.

OXYGEN EQUILIBRIUM CURVES

OEC's on the whole blood were determined at 37 °C by the method of Rossi-Bernardi *et al.* (1975). Two ml of blood were deoxygenated for 20 min in a tonometer using nitrogen-carbon dioxide gas mixtures, which were saturated with water vapor at 37 °C and which contained 2, 5.6, and 10% carbon dioxide. Samples were equilibrated at four different pHe's at each P_{CO₂} value. At the end of the tonometry, the pHe of the sample was measured, and the total Hb, carbon-monooxyhemoglobin (HbCO), and methemoglobin (metHb) concentration and the

* Instrumentation Laboratory, Lexington, MA.

** Lif-O-Gen, Cambridge, MD, accuracy = $\pm 0.02\%$.

*** Hamilton Co., Reno, NV.

oxygen capacity were determined by a Micro Blood Analyzer.* The sample was then anaerobically transferred into the cuvette for the complete OEC determination at constant pH and P_{CO_2} at 37 °C. The OEC was considered complete when the oxygen capacity of the blood was reached. This usually occurred at a P_{O_2} above 200–250 mm Hg. The metHb concentration never exceeded 1.5% at the end of the run.

The raw data on the OEC's were then processed according to Winslow *et al.* (1977). This procedure uses the step-wise oxygenation model proposed by Adair (1925) as the fitting equation. The n_{max} (maximum slope of the OEC when plotted as proposed by Hill, 1910) and the four Adair parameters were estimated.

Results and discussion

INTRACELLULAR pH

Intracellular pH measurements showed a linear relationship between ΔpH ($\text{pHe} - \text{pHi}$) and pHe . As observed in human blood (Gattinoni *et al.*, 1975), there was no change in ΔpH at constant pHe when the blood was equilibrated at different P_{CO_2} values. 2,3-DPG is absent in the blood of sheep, and ΔpH , therefore, seems to be a function of pHe only:

$$\Delta\text{pH} = 0.1287 \times \text{pHe} - 0.8247 \quad (1)$$

(Root mean square error is 0.75×10^{-2} pH units)

This formula is valid at 37 °C for oxygenated blood. However, it can also be virtually valid for deoxygenated blood, since the pH gradient across the red cell membrane in deoxygenated blood is close to the value found in fully oxygenated blood (data not shown), although not the same as pointed out by Takano *et al.* 1976, for human blood.

OXYGEN EQUILIBRIUM CURVES

Experimental plots of the OEC's are shown in fig. 1. Table 1 reports the Bohr factors for both intracellular and extracellular environment calculated by the least square rule. We carried out a series of mathematical and empirical considerations in order to relate pH, P_{CO_2} , and $\log P_{50}$. pHe is used in all the following calculations, since its determination is much easier than is that of pHi .

By analyzing the Bohr effect, the following equation occurs:

$$\log P_{50} = \text{slope} \times \text{pHe} + \text{intercept} \quad (2)$$

* Carlo Erba Strumentazione, Milan, Italy. See Rossi-Bernardi *et al.*, 1977, for further details.

TABLE I
Values of the Bohr factor ($\Delta \log P_{50}$, ΔpH)

	P_{CO_2} (mm Hg)		
	14	39	70
Intracellular	-0.42 ± 0.004	-0.35 ± 0.004	-0.30 ± 0.005
Extracellular	-0.37 ± 0.013	-0.31 ± 0.014	-0.26 ± 0.017

The slopes and the intercepts are related to P_{CO_2} as follows:

$$\text{slope} = 0.1902 \times 10^{-2} \times P_{CO_2} - 0.3916 \quad (3)$$

$$\text{intercept} = -0.126 \times 10^{-1} \times P_{CO_2} + 4.4527 \quad (4)$$

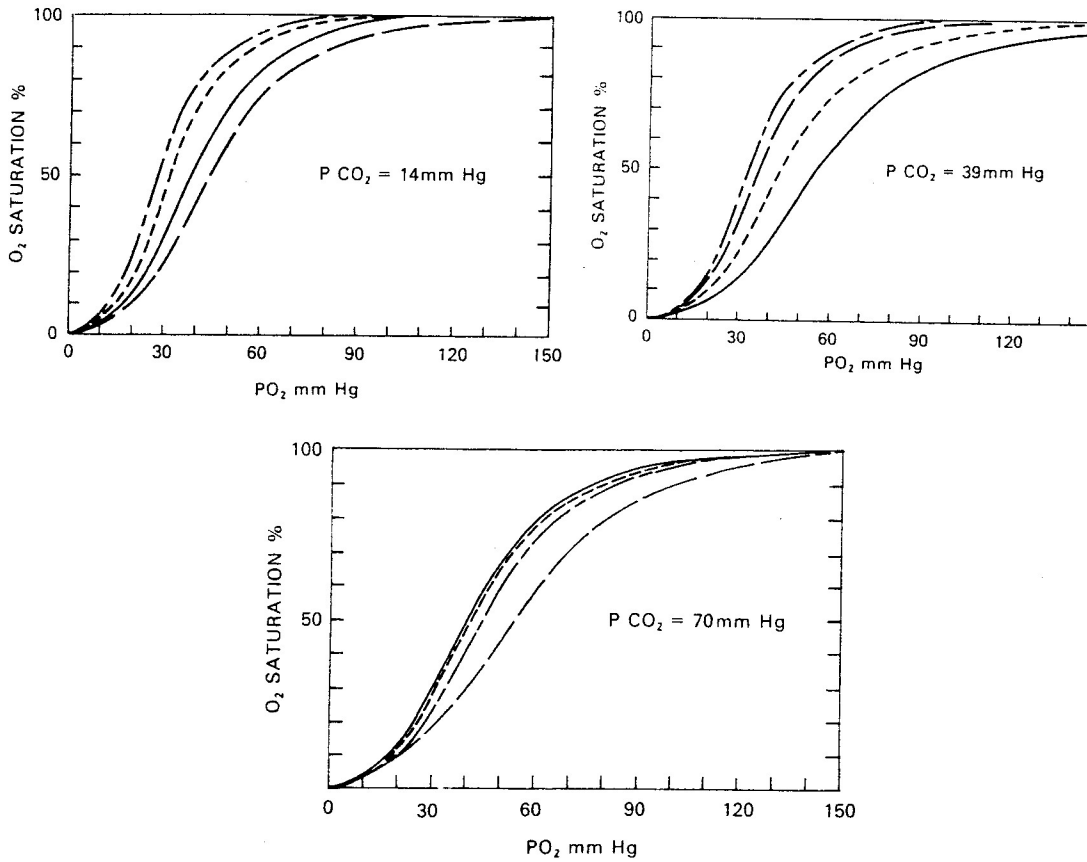


Fig. 1. Experimental OEC's at $P_{CO_2} = 14$ mm Hg (a), 39 mm Hg (b), and 70 mm Hg (c), at four pH's.

The parameters were estimated by the least square rule. Therefore, by combining equations 3, 4, and 2 we obtained:

$$\log P_{50} = -0.0126 \times P_{CO_2} - 0.3916 \times \text{pHe} + 0.00192 \times \text{pHe} \times P_{CO_2} + 4.4527 \quad (5)$$

The $\log P_{50}$ at a given pH and P_{CO_2} can also be estimated from fig. 2. Meschia *et al.* (1961) reported a great variability in the position of the OEC within the same type of sheep that was related to factors such as weight of the animal, age, and type of hemoglobin. The largest error in calculating the oxygen saturation by the presently described procedure, therefore, occurs in the measurement of P_{50} . It should be noted that fig. 2 refers to adult Dorset sheep blood containing HbB, at a hemoglobin concentration of 10 g per 100 ml of blood.

ADAIR ANALYSIS

Raw data of the OEC's have been fitted to the Adair equation in the form:

$$Y = \frac{a_1 P + 2a_2 P^2 + 3a_3 P^3 + 4a_4 P^4}{4(a_1 P + a_2 P^2 + a_3 P^3 + a_4 P^4)} \quad (6)$$

where Y is the fractional saturation of hemoglobin for oxygen and P is the P_{O_2} . a_1 , a_2 , a_3 , and a_4 are the four composite constants that obey Adair's step-wise oxygenation scheme, and their values were estimated as described by Winslow *et al.* (1977). As in human blood, a_3 was so small that it could be assumed to be zero. Moreover,

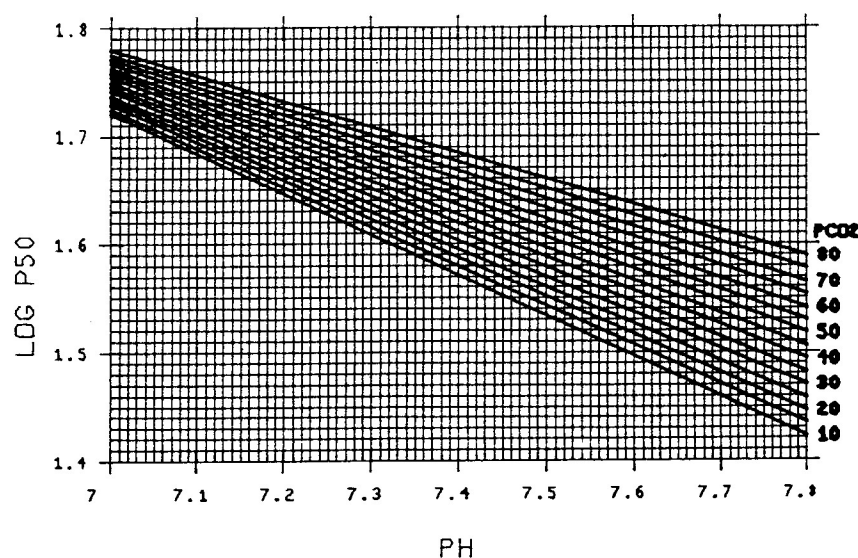


Fig. 2. Relationship among $\log P_{50}$, pH, and P_{CO_2} in the blood of the adult Dorset sheep with HbB.

we found that CO_2 does not affect these constants since the general shape of the curve remains the same, but affects only the relative position of the OEC.

The purpose of the following considerations was to achieve some reliable methods to estimate the oxygen saturation in the full range at a given P_{O_2} and P_{50} . Since a_1 , a_2 , and a_4 were a linear function of pHe (data not shown), and since pHe was a linear function of $\log P_{50}$, the following first order equations could be set up (see also fig. 3):

$$\log a_1 = 0.6968 \times \log P_{50} - 1.117 \quad (7)$$

$$\log a_2 = 0.7473 \times \log P_{50} - 5.207 \quad (8)$$

$$\log a_4 = 3.955 \times \log P_{50} + 0.0238 \quad (9)$$

By substituting equations 7, 8 and 9 in equation 6, the oxygen saturation of blood can be determined at any P_{O_2} from known P_{50} . These calculations can be combined with equation 5 to give the P_{50} and the oxygen saturation at any pH , P_{CO_2} , and P_{O_2} in the physiological range. It has been possible to set up a very simple computer program and/or a subroutine which performs these calculations, since no complicated mathematical functions or branchings are involved.

The oxygen saturation at a given P_{50} (range 20 to 60 mm Hg) and P_{O_2} (range 0 to 150 mm Hg) can also be obtained from fig. 4, which shows a number of EOC's, each of which has been traced by keeping the P_{50} constant and substituting the appropriate value of a_1 , a_2 , a_3 , and a_4 , as calculated from equations 7, 8, and 9, in equation 6.

In a separate series of experiments, we noted that Hb type affects the position and not the shape of the OEC of sheep blood. This could mean that, once P_{50} or any other point of the OEC has been established by any method at a fixed pH and

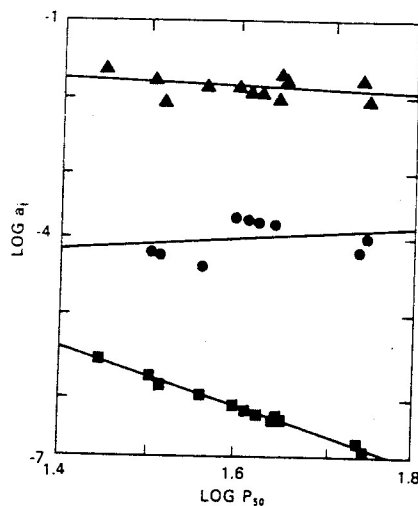


Fig. 3. Behavior of the four Adair constants as a function of $\log P_{50}$. The root mean square errors are 0.116, 0.258, and 0.036 for a_1 , a_2 , and a_4 , respectively, when data points are fitted to a straight line.

P_{CO_2} , the nomogram of fig. 4 and equations 6–10 are still valid, and one can extrapolate all the OEC from that P_{S_0} .

Conclusions

The main advantage of the described method for the determination and analysis of the OEC is represented by the fact that no assumptions on the slope of the curve (*i.e.* Hill's coefficient) need be made since the entire curve was traced and analyzed. We believe that this method is preferable to those that use Hill's formula as a mathematical model (for an example, see the work of Rossing and Cain (1966) on the blood of dogs). Very high oxygen saturations ($> 90\%$) usually occur in normal laboratory practice, and the calculations proposed by Hill are, therefore, not reliable because they are correct only in the range of saturation from 20 to 80%. Instead, Adair's equation correctly represents the curve over the entire oxygen saturation range, and it is also reliable at very low and very high oxygen saturations.

We also showed that pH and P_{CO_2} affect only the position but not the shape of the OEC in the blood of sheep. Therefore, once the P_{S_0} has been established, either via the nomogram of fig. 2 or equation 5 or via any experimental method, it is possible to trace the entire curve regardless of the conditions under which the curve should be traced.

As a comparison with previous data from the literature, Baumann *et al.* (1975) showed that the P_{S_0} in sheep blood was 41.8 mm Hg at $P_{CO_2} = 40$ mm Hg and $pH_i = 7.2$. We found a P_{S_0} of 44.1 mm Hg under the same conditions. The intracellular Bohr factors at $P_{CO_2} = 40$ mm Hg were -0.29 in the work of Baumann *et al.* and -0.35 in this study.

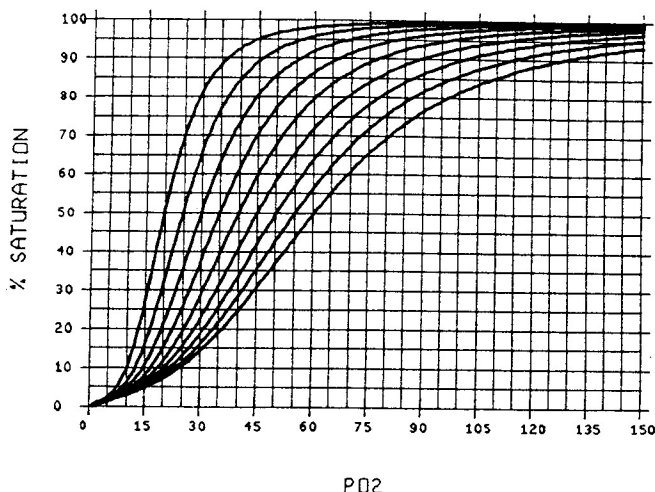


Fig. 4. Theoretical OEC determinations. See text for explanation.

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