

The TANH-equation modified for the hemoglobin, oxygen, and carbon monoxide equilibrium

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The model of the hemoglobin-oxygen equilibrium represented by the TANH-equation is incorporated in the Oxygen Status Algorithm, a computer program for calculating and displaying the oxygen status and the acid-base status of the blood. In the presence of carbon monoxide it is necessary to take the Haldane equation into account. We here describe the necessary equations and methods for iterative solutions. The validity of the Haldane equation has previously been demonstrated by Zwart *et al.* (J Appl Physiol 1984; 57: 14-20). We have performed a few experiments to confirm this. Like Zwart *et al.* we find a small deviation from the theory, but in the opposite direction, i.e. the measured $p50$ values are slightly higher than predicted.

We conclude that the Haldane equation adequately accounts for the carbon monoxide effect up to 30 % carboxy-hemoglobin, but further studies are needed to confirm or exclude any minor deviation from the Haldane relationship which may be significant at higher carboxy-hemoglobin fractions.

Key words: algorithms; blood; carboxyhemoglobin; computer program; half saturation tension; oxygen status.

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INTRODUCTION

Several times we have been asked how the Oxygen Status Algorithm computer program [1] implements the Haldane equation (Eqn. 1) to account for the effect of carbon monoxide on the hemoglobin oxygen equilibrium, the

equation formulated 80 years ago by the young J.B.S. Haldane on the basis of experimental work carried out together with his father J.S. Haldane [2].

The fundamental assumption was that oxygen and carbon monoxide bind to hemoglobin in simple competition, although carbon monoxide

binds with a much higher affinity than oxygen. The validity of this assumption has been proven experimentally by several authors most recently in 1984 by Zwart, Kwant, Oeseburg, and Zijlstra, Department of Physiology, University of Groningen, in a very careful study with references to all the previous literature [3]. They found only minor deviations from the Haldane equation at high FCO_{Hb} values where the measured pO_2 values were slightly lower than predicted.

Simpler equations have been proposed to account for the effect of carbon monoxide on the $p50$ value only. Zwart *et al.* proposed a linear relationship:

$$p50 = p50^{\circ} + \beta \cdot FCO_{Hb},$$

where $p50^{\circ} = 3.4$ kPa, and $\beta = -3.6$ kPa.

The same year (1984) Rovida *et al.* published a logarithmic relationship [4]:

$$d \lg p50 / d FCO_{Hb} = -0.848, \text{ or}$$

$$d \ln p50 / d FCO_{Hb} = -1.95.$$

Another model of the carbon monoxide hemoglobin interaction is based on the assumption that carbon monoxide binds so strongly to hemoglobin that it occupies the beginning of the oxygen binding curve until the ordinate equals FCO_{Hb} [5]. Beginning at this point the remaining part of the oxygen binding curve describes the relationship between pO_2 and sO_2 in the presence of CO. Calculations are somewhat easier with this "zero point displacement model" than with the Haldane equation, but as Zwart *et al.* pointed out, this model is based on a false assumption and gives a very poor fit to the experimental data.

The purpose of this communication is to describe the equations of the Oxygen Status Algorithm including the Haldane equation, to calculate the effect of CO on the $p50$ value using the Oxygen Status Algorithm, and to provide a few experimental data to test the validity of the Henderson equation.

EQUATIONS OF THE OXYGEN STATUS ALGORITHM

The Haldane equation relates the pO_2 and pCO to the concentrations of O_2Hb and $COHb$, respectively:

$$pO_2 / cO_2Hb = M \cdot pCO / cCOHb, \quad (1)$$

where the Haldane factor, M , is proportional to the difference in Hb-affinity for CO and O_2 . The value of M (≈ 230) is not important for the following calculations.

The relationship between pO_2 and sO_2 , established when O_2 is the only ligand, should therefore be identical with the relationship between $M \cdot pCO$ and sCO , when CO is the only ligand. For the case where both O_2 and CO are present we define two new quantities:

$$pO_2CO = pO_2 + M \cdot pCO, \quad (2)$$

$$sO_2CO = (cO_2Hb + cCOHb) / (cO_2Hb + cCOHb + cHb). \quad (3)$$

We assume that the concentration of total hemoglobin is represented by

$$ctHb = cHb + cO_2Hb + cCOHb + cMetHb. \quad (4)$$

We can then express sO_2CO in terms of the quantities we usually measure, i.e. sO_2 , FCO_{Hb} , and $FMetHb$, where the latter two are fractions of total hemoglobin:

$$sCO = FCO_{Hb} / (1 - FMetHb), \quad (5)$$

$$sO_2CO = sO_2 + (1 - sO_2) \cdot sCO. \quad (6)$$

The Haldane equation can be expressed in terms of these new quantities:

$$M \cdot pCO = (pO_2CO / sO_2CO) \cdot sCO, \text{ or} \quad (7)$$

$$pO_2CO = pO_2 \cdot sO_2CO / (sO_2CO - sCO). \quad (8)$$

We now transform pO_2CO and sO_2CO into two new quantities, x and y :

$$x = \ln(pO_2CO / p^{\circ}), \text{ where } p^{\circ} = 7 \text{ kPa, and} \quad (9)$$

$$y = \ln(sO_2CO / (1 - sO_2CO)). \quad (10)$$

The relationship between x and y is given by the TANH-equation [6], which in the original form related pO_2 and sO_2 rather than pO_2CO and sO_2CO :

$$y = y^{\circ} + x - x^{\circ} + h \cdot \tanh(k^{\circ} \cdot (x - x^{\circ})). \quad (11)$$

The parameters of the equation are the following. The point (x°, y°) represents the point of symmetry of the S-shaped function between x and y . y° is a constant, whereas x° varies with the Hb- O_2 -CO affinity:

$$y^{\circ} = 1.8747, \quad (12)$$

$$x^{\circ} = a + b, \quad (13)$$

where a is the chemical allosteric affinity term and b is the thermal affinity term:

$$a = a_1 \cdot (pH - pH^{\circ}) \quad (a_1 = -0.88) \quad (14)$$

$$+ a_2 \cdot \ln(pCO_2/pCO_2^{\circ}) \quad (a_2 = 0.048)$$

$$+ a_3 \cdot F\text{MetHb} \quad (a_3 = -0.7)$$

$$+ a_4 \cdot F\text{HbF} \quad (a_4 = -0.25)$$

$$+ a_5 \cdot (c\text{DPG} - c\text{DPG}^{\circ}) \quad (a_5 = a_{50} + a_{51} \cdot F\text{HbF})$$

$$(a_{50} = 0.06; a_{51} = -0.02)$$

$$pH^{\circ} = 7.40, \quad pCO_2^{\circ} = 5.33 \text{ kPa},$$

$$c\text{DPG}^{\circ} = 5 \text{ mmol/L}.$$

$$b = (db/dT) \cdot (T - T^{\circ})$$

$$\text{where } db/dT = 0.055, \text{ and } T^{\circ} = 37^{\circ}\text{C}.$$

Several of the coefficients (a_1, a_2, a_3) are based on experimental data from Zijlstra's laboratory in Groningen [7].

The slope factor, k° is a constant, whereas the interaction factor, h , varies with changes in affinity:

$$k^{\circ} = 0.5343, \quad (15)$$

$$h = h^{\circ} + a, \text{ where } h^{\circ} = 3.5.$$

One additional equation is important, the "total oxygen equation":

$$ctO_2 = \alpha O_2 \cdot pO_2 + sO_2 \cdot ceHb, \quad (16)$$

where $ceHb$ is the effective hemoglobin concentration:

$$ceHb = ctHb \cdot (1 - FCOHb - F\text{MetHb}), \quad (17)$$

and αO_2 is the solubility coefficient for oxygen in blood:

$$\ln(\alpha O_2 / \alpha O_2^{\circ}) = - (d\ln \alpha O_2 / dT) \cdot (T - T^{\circ}) + 0.5 \cdot (d^2 \ln \alpha O_2 / dT^2) \cdot (T - T^{\circ})^2. \quad (18)$$

$$\alpha O_2^{\circ} = 0.0105 \text{ (mmol/L)/kPa},$$

$$d\ln \alpha O_2 / dT = -0.0115 / K,$$

$$d^2 \ln \alpha O_2 / dT^2 = 0.00042 / K^2.$$

TYPES OF CALCULATIONS

Equations (1) to (18) relate 12 primary (measurable) variables: $T, pO_2, sO_2, ctHb, FCOHb, F\text{MetHb}, pH, pCO_2, F\text{HbF}, c\text{DPG}, ctHb$, and ctO_2 . Several types of calculations are relevant, but none of them can be performed without resorting to iterative methods:

1) Calculating sO_2 from pO_2

In addition to the pO_2 we need to know $T, FCOHb, F\text{MetHb}, pH, pCO_2, F\text{HbF}$, and $c\text{DPG}$.

First calculate the affinity parameters, a and b (Eqn. 14), to find the position of the TANH-function. Then calculate sCO (Eqn. 5) and make a guess of $M \cdot pCO$, based on the value for sCO . Then calculate pO_2CO (Eqn. 2), which leads to sO_2CO via the TANH-equation (Eqns. 10 and 11). Now calculate a new value for $M \cdot pCO$ from pO_2CO, sO_2CO , and sCO (Eqn. 7). Continue this iteration until the difference between two successive values of $M \cdot pCO$ is less than a given limit. Then finally calculate sO_2 from sCO and the last sO_2CO value (Eqn. 6).

2) Calculating pO_2 from sO_2

In addition to the sO_2 we need to know $T, FCOHb, F\text{MetHb}, pH, pCO_2, F\text{HbF}$, and $c\text{DPG}$.

First calculate the affinity parameters, a and b (Eqn. 14). Calculate the 'measured' sO_2CO from $sO_2, FCOHb$, and $F\text{MetHb}$ (Eqns. 5 and 6). Then make a guess of a temporary pO_2CO (preferably choose the point of symmetry of

the TANH-function) and calculate a temporary sO_2CO from the TANH-equation (Eqns. 10 and 11). The difference between the temporary sO_2CO and the 'measured' sO_2CO allows the calculation of a new temporary pO_2CO using a fast Newton-Raphson procedure. The procedure is iterated until the difference between the temporary sO_2CO and the 'measured' sO_2CO is less than a given limit. Finally $M \cdot pCO$ is calculated (Eqn. 7) and subtracted from pO_2CO to give pO_2 (Eqn. 2).

3) Calculating pO_2 from ctO_2

In addition to ctO_2 we need to know T , FCO_{Hb} , $FMetHb$, pH , pCO_2 , $FHbF$, $cDPG$, and $ctHb$.

First calculate a and b (Eqn. 14). Make a guess of a temporary pO_2CO (point of symmetry) and use this for calculating a temporary sO_2CO and a temporary ctO_2 (Eqns. 10, 11, 17). On the basis of the difference between the temporary and the original ctO_2 calculate a new temporary pO_2CO . This can be done with a fast Newton-Raphson procedure. Continue until the temporary ctO_2 matches the original ctO_2 within a given limit. Finally $M \cdot pCO$ is calculated and subtracted from pO_2CO to give pO_2 .

4) Calculating $cDPG$ from pO_2 and sO_2

In addition to the pO_2 and sO_2 we need to know T , FCO_{Hb} , $FMetHb$, pH , pCO_2 , and $FHbF$.

First calculate the 'measured' pO_2CO and sO_2CO (Eqns. 8, 6). Then guess a temporary value for $cDPG$ (e.g. 5 mmol/L) and calculate a temporary value for parameter a (Eqn. 14). Use this value and the 'measured' pO_2CO to calculate a temporary sO_2CO (Eqns. 10, 11). The difference between the temporary and the 'measured' sO_2CO allows the calculation of a new temporary parameter a , using a Newton-Raphson iteration procedure. Iteration continues until the temporary and the 'measured' sO_2CO matches within a given limit. $cDPG$ is calculated from the final value of parameter a (Eqn. 14).

The last type of calculation is the most interesting from a practical point of view in cases

when both pO_2 and sO_2 are measured (with a blood gas analyzer and a hemoximeter). Once $cDPG$ is calculated it is possible to calculate the $p50$ as a measure of the hemoglobin oxygen affinity using calculation type 2.

EXPERIMENTAL

Heparinized blood was sampled from an arm vein after 3 minutes of stasis and fist clenching to reduce the sO_2 to a value somewhat below 50 %. Pure carbon monoxide gas was added in increments with careful mixing in between until FCO_{Hb} approached 40 %.

A Radiometer ABL500 was used to measure pH , pCO_2 , and pO_2 , and a Radiometer OSM3 to measure sO_2 , FCO_{Hb} , $FMetHb$, and $ctHb$. The Oxygen Status Algorithm was used to calculate $cDPG$. The $cDPG$ values were converted to standard $p50$ values, i.e. $p50$ values referred to $pH=7.40$, $pCO_2=5.33$ kPa, $FMetHb=0$, and $FCO_{Hb}=0$:

$$\ln(p50_{std}/p50^{\circ}) = 0.06 \cdot (cDPG - cDPG^{\circ}),$$

where $p50^{\circ}=3.58$ kPa, and $cDPG^{\circ}=5$ mmol/L.

Since $cDPG$ is presumably constant during the experiment, $p50_{std}$ should be constant, independent of the FCO_{Hb} value. Thus any change in $p50_{std}$ would indicate a deviation from the Haldane equation. Two experiments were performed on different days on blood from the same subject (one of the authors).

RESULTS

Fig. 1 shows the theoretical effect of increasing FCO_{Hb} on the $p10$, $p50$, and $p90$, based on the Haldane equation and the Oxygen Status Algorithm. This is compared with the three other approaches for calculating the CO effect: a linear change in $p50$, a logarithmic change in $p50$, and a zero-point displacement.

Fig. 2 shows the experimental data. Both experiments revealed a small rise in $p50_{std}$ with increasing FCO_{Hb} . While the pO_2 remained almost constant during the experi-

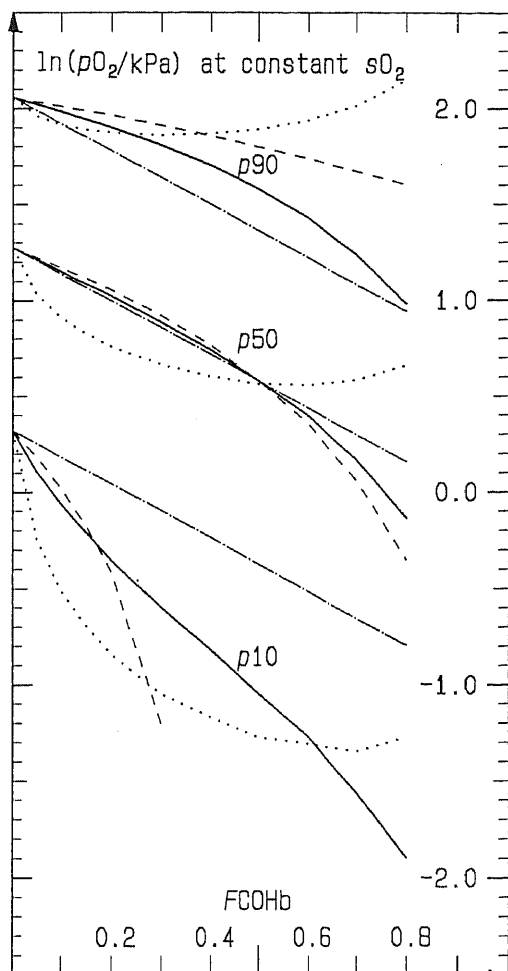


FIG. 1. The effect of carbon monoxide on the pO_2 at sO_2 levels of 10, 50 and 90 %, symbolized by p_{10} , p_{50} , and p_{90} . The heavy lines indicate the theoretical relationship according to the Haldane equation, the dashed straight lines a simple logarithmic relationship: $d \ln pO_2 / dFCOHb = -1.45$, the dashed curves a simple linear relationship: $dpO_2 / dFCOHb = -3.6$ kPa. The bowl-shaped dotted curves illustrate the "zero point displacement" model. Notice that while a simple logarithmic or linear relationship may give a good approximation to the theoretical change in p_{50} up to a $FCOHb$ level of about 50 %, none of the "simple" approaches provide a good approximation at all sO_2 levels.

ments (2.2 kPa in the first, 3.2 kPa in the second), the sO_2 values increased from 25 to 50 % in the first and from 43 to 63 % in the second experiment.

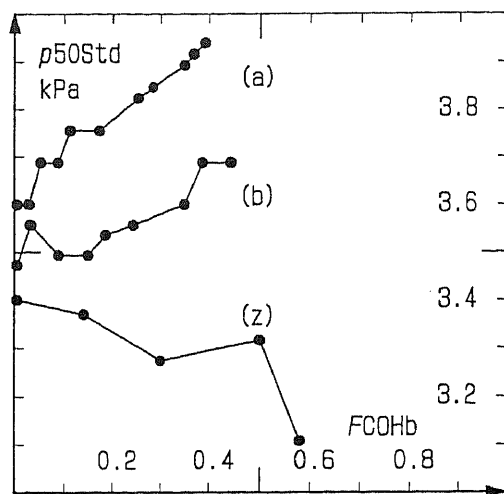


FIG. 2. The effect of carbon monoxide on the "standard p_{50} " value, i.e. p_{50} referred to $FCOHb=0$ on the basis of the Haldane equation (and referred to $pH=7.4$, $pCO_2=5.33$ kPa, $F_{MetHb}=0$). Curves (a) and (b) show the results of our own experiments, curve (z) the results of Zwart *et al.* If the experimental data conformed exactly to the Haldane equation the "standard p_{50} " should be constant independent of the $FCOHb$ level. Notice that our results show a rising tendency opposite to the effect found by Zwart *et al.* Notice also that for $FCOHb$ values less than 30 % any change in "standard p_{50} " is very small, maximally 0.2 kPa.

DISCUSSION

According to the Haldane equation the relationship between p_{50} and $FCOHb$ is neither linear nor logarithmic (see Fig. 1). Thus, a linear approximation for $FCOHb$ values between 0 and 0.1 would give a slope of $\beta = -4.2$ kPa, but between 0 and 0.5 the value would be -3.6 kPa, exactly as found by Zwart *et al.* for this interval. Similar values for a logarithmic relationship (base e) are -1.25 and -1.49, respectively, i.e. much lower (numerically) than the value of -1.95, reported by Rovidia *et al.* who themselves draw attention to the fact that their value is higher than all previous studies, but offer no explanation.

Fig. 1 illustrates that either a linear or a logarithmic relationship may provide a good approximation of the theoretical change in p_{50} up to $FCOHb$ values of about 50 %, but nei-

ther a constant linear nor a constant logarithmic relationship applies to calculate the change in pO_2 at all sO_2 levels. For this purpose the Haldane equation appears to be indispensable.

It is evident from Fig. 1 that the zero point displacement model is inappropriate at all sO_2 levels as already pointed out by Zwart *et al.*

The Groningen group in their study concluded that the Haldane equation correctly predicts the change in pO_2 at all sO_2 levels, except for FCO_{Hb} values above 50 % where they found lower pO_2 values than predicted (see Fig. 2), indicating that CO at high tensions may increase the hemoglobin affinity for oxygen. They found support for this theory by Roughton who found the Hb-CO binding curve somewhat steeper than the Hb- O_2 binding curve [8].

Our limited data does not confirm this. We find that the measured pO_2 values are slightly higher than predicted and increasingly so with increasing FCO_{Hb} . Thus 20 % CO_{Hb} would give a standard $p50$ value 0.1 to 0.2 kPa higher than predicted. This corresponds to a positive bias in the calculated $cDPG$ of 0.3 to 0.7 mmol/L.

The cause of this discrepancy between our results and those from Groningen is not clear. First of all analytical errors must be ruled out. A positive bias on the Clark pO_2 electrode caused by carbon monoxide is unknown to us, and a positive drift of the calibration of the pO_2 electrode during the experiments is not very likely; it would have been detected during the recalibration of the ABL500 after the measurement series. A negative bias on the measured sO_2 values could explain a positive bias in $p50$. However, to explain the curves of Fig. 2, a FCO_{Hb} of 50 % would have to cause negative bias in the sO_2 measurement of 5 %, i.e. an sO_2 value of 45 % instead of 50 %. An error of this magnitude is not very likely, since CO_{Hb} has very little influence on the sO_2 measurement when the sO_2 is either zero or 100 %. Furthermore the highest FCO_{Hb} levels did not affect the $FMetHb$ values, which remained 0.4 - 0.7 % during the whole experiment. So, at present we have no

satisfactory explanation for the discrepancy between our results. Unfortunately we did not increase the FCO_{Hb} above the 50 % level, where Zwart and colleagues found a significant deviation from the Haldane theory.

CONCLUSION

The Haldane equation adequately describes the effect of carbon monoxide on the hemoglobin oxygen equilibrium as stated by Zwart *et al.* in 1984. Although the change in $p50$ with increasing FCO_{Hb} can be described approximately by a simple linear or logarithmic relationship, there is no alternative to the Haldane equation when the effect of carbon monoxide on the whole hemoglobin oxygen binding curve is to be calculated. With a computer program it is no problem to perform the slightly complicated iterative calculations. Further experiments are needed to clarify whether a small deviation from the Haldane equation in one or the other direction remains at high FCO_{Hb} values.

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