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THE PREDICTION OF HUMAN BLOOD OXYGEN EQUILIBRIUM CURVE UNDER VARIOUS CONDITIONS OF pH, Pco_2 AND 2,3-DPG *

PRÉDICTION DE LA COURBE D'ÉQUILIBRE DE L'OXYGÈNE SANGUIN CHEZ L'HOMME POUR DIFFÉRENTES VALEURS DE pH, Pco_2 ET 2,3-DPG

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ABSTRACT : We have analysed 56 continuous oxygen equilibrium curves of fresh human blood, each from 0 to 150 Torr Po_2. The data were collected over a range of: values of the [2,3-DPG]/[Hb] ratio of 0.2-2.7, of pH 7.0-7.8, and Pco_2 7-70 Torr. The generalized Adair equation for the stepwise oxygenation of hemoglobin was fit to the data for each curve, so that the curve could be reproduced using the equation and the four constants a_1, a_2, a_3 and a_4. The behaviour of the constants in response to variations of pH, Pco_2 and 2,3-DPG was characterized by empirical relationships, and a set of equations was developed which could estimate the Adair constants for the physiological range of these variables. Previous attempts to describe the effects of physiological conditions on the oxygen transport properties of red cells have dealt mainly with P_{50}, the Po_2 at one-half saturation, or have provided too little data for continuous simulations. Moreover, the mathematical descriptions of variations in the oxygen equilibrium curve have usually used the Hill equation which assumes that the shape of the curve is invariant. Recent evidence has shown this assumption to be incorrect, and the use of the Adair equation eliminates the need for such simplifying assumptions. This analysis provides a powerful tool to predict the shape of the oxygen equilibrium curve in various physiological and pathological states, as well as to study the affinity of hemoglobin for oxygen within the red cell. The equations we have developed can easily be incorporated into large programs for computations in acid-base and oxygen exchange properties of blood and for modelling gas exchange in man.

Blood pH ; 2,3-DPG ; gas exchange ; oxygen equilibrium curves ; oxygen transport.

We have previously reported [5, 6] several equations to predict human blood P_{50} (the Po_2 at which hemoglobin is half-saturated with oxygen) from pH, Pco_2, the [2,3-DPG]/[Hb] ratio and temperature, on the assumption that the slope of the oxygen equilibrium curve (OEC) is always constant in the middle portion, also for different values of the factors. The reported equations are useful for a fast rough estimation of the oxygen affinity of blood, and particularly to assess if the apparently abnormal P_{50} value of a given blood sample can be explained with abnormal values for pH, Pco_2, the [2,3-DPG]/[Hb] ratio and temperature, or if other factors, such as abnormal hemoglobins, impaired intra-

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cellular pH and presence of unknown hemoglobin effectors, are involved. An example of this « normalization » procedure has been reported in the case of diabetic patients, with high levels of glycosylated hemoglobins [4]. The P_{50} is however a somewhat artificial index for some physiologic considerations when hemoglobin oxygen saturations (So_2) much higher or lower than 50 % may occur, and therefore not only the position, *i.e.* the P_{50}, but also the shape of the OEC must be considered.

In order to gain insight into the behaviour of the blood OEC as a function of the hemoglobin allosteric effectors, 56 OEC were measured at 37 °C under different preset conditions of pH, Pco_2, and the [2,3-DPG]/[Hb] ratio, chosen to encompass their physiological range of variation, by a method which allowed the precise determination of the OEC with strict control of these parameters [10]. The OEC were then analysed in terms of the stepwise model for the oxygenation of hemoglobin, as proposed by Adair [1], and the equilibrium constants were determined for each condition :

$$\mathrm{Hb} + \mathrm{O_2} \Leftrightarrow \mathrm{Hb(O_2)} \qquad K_1 = \frac{[\mathrm{Hb(O_2)}]}{[\mathrm{Hb}]\,[\mathrm{O_2}]} \tag{1}$$

$$\mathrm{Hb(O_2)} + \mathrm{O_2} \Leftrightarrow \mathrm{Hb(O_2)_2} \qquad K_2 = \frac{[\mathrm{Hb(O_2)_2}]}{[\mathrm{Hb(O_2)}]\,[\mathrm{O_2}]} \tag{2}$$

$$\mathrm{Hb(O_2)_2} + \mathrm{O_2} \Leftrightarrow \mathrm{Hb(O_2)_3} \qquad K_3 = \frac{[\mathrm{Hb(O_2)_3}]}{[\mathrm{Hb(O_2)_2}]\,[\mathrm{O_2}]} \tag{3}$$

$$\mathrm{Hb(O_2)_3} + \mathrm{O_2} \Leftrightarrow \mathrm{Hb(O_2)_4} \qquad K_4 = \frac{[\mathrm{Hb(O_2)_4}]}{[\mathrm{Hb(O_2)_3}]\,[\mathrm{O_2}]} \tag{4}$$

The equilibria (1-4) can be expressed by the following equation :

$$Y = \frac{K_1P + 2\,K_1K_2P^2 + 3\,K_1K_2K_3P^3 + 4\,K_1K_2K_3K_4P^4}{4\,(1 + K_1P + K_1K_2P^2 + K_1K_2K_3P^3 + K_1K_2K_3K_4P^4)} \tag{5}$$

Now, let :

$$a_1 = K_1 \tag{6}$$
$$a_2 = K_1 * K_2 \tag{7}$$
$$a_3 = K_1 * K_2 * K_3 \tag{8}$$
$$a_4 = K_1 * K_2 * K_3 * K_4 \tag{9}$$

Equation (5) may be rewritten :

$$Y = \frac{a_1P + 2\,a_2P^2 + 3\,a_3P^3 + 4\,a_4P^4}{4\,(1 + a_1P + a_2P^2 + a_3P^3 + a_4P^4)} \tag{10}$$

The method for fitting the experimental data to equation 10 is described elsewhere [10]. Although the Adair model is in many instances inadequate to describe the complex interactions between hemoglobin and its ligands, since many factors such as the dimer-tetramer equilibrium and the unequivalence of the hemoglobin chains are not considered, the OEC can be nevertheless reproduced in the range 0 to 150 Torr Po_2 with sufficient accuracy ($< \pm 0.5$ % So_2) from the values of the four constants a_1, a_2, a_3 and a_4, which were determined for each OEC.

In order to reproduce an OEC under any condition of pH, Pco_2 and the [2,3-DPG]/[Hb] ratio, it becomes necessary to establish some relationships, even empirical, between the Adair constants and the various effectors :

— 1. The plots of log a_i *vs* pH, *i.e.* the Bohr effect, are linear under all the investigated conditions of Pco_2 and the [2,3-DPG]/[Hb] ratio. Only the two extreme pH values (7.0 and 7.8) were therefore selected for further calculations, being the value of a_i at any intermediate pH easily interpolated ;

— 2. The values of log a_i interpolated at pH 7.0 and 7.8 in the preceding step were fit to second order equations of the type :

$$\log a_i = w\,(G)^2 + y\,(G) + z \tag{11}$$

where G = [2,3-DPG]/[Hb]. One set of (w, y, z) was calculated for each Adair constant, for each Pco_2 and both pH ;

— 3. In turn, w, y, and z were fit separately to other second order equations of the type :

$$w,\ y,\ \text{and}\ z = A\,(Pco_2)^2 + B\,(Pco_2) + C \tag{12}$$

Since there is a different set of (A, B, C) for each w, y, and z value, and for each 2 pH and each 4 a, this makes a table of 72 coefficients (table I, see also [9]), which describes an OEC from 0 to 150 Torr Po_2 under any condition of pH (range 7.0 to 7.8), Pco_2 (7 to 70 Torr) and [2,3-DPG]/[Hb] ratio (0.2-2.7).

An OEC can be calculated as it follows (the flowchart for computer simulation is shown in figure 1) : 1) calculate the sets of (w, y, z) using equation 12, the selected value for Pco_2 and the coefficients A, B, C shown in table I. In total, equation 12 should be used 24 times, *i.e.* the number of the lines in table I ; 2) calculate log a_i (one value for each a_i and for each of the two pH) using equation 11, the selected value for G and the appropriate set (w, y, z) calculated in the preceding step ; 3) since log a_i is linear function of pH (the Bohr effect), the following linear interpolation calculates log a at the selected pH :

$$\log a_{pH} = [(pH\ 7.0) \times (\log a_{pH\ 7.8} - \log a_{pH\ 7.0})]/0.8 + \log a_{pH\ 7.0} \tag{13}$$

4) now that the 4 Adair constants are known, one out of the So_2 or the Po_2 when the other is known can be calculated by equation 10.

The regression of the experimental P_{50} values on those calculated by the use of the described procedure is satisfactory (y = 1.013 X — 0.201 ; r = 0.9968). The maximum error in the estimation of So_2 from Po_2 is in the range — 1.2 % to 2 % under all the encompassed conditions, and certainly within the limits of our ability to measure Po_2 or So_2 with the presently available techniques.

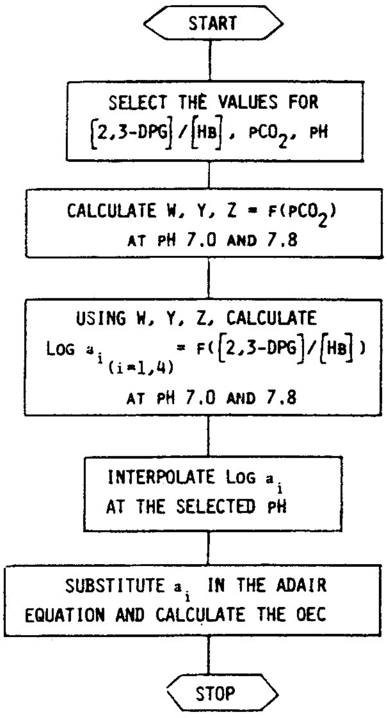

Fig. 1. — Flowchart for the calculation of the Adair constants and the determination of the oxygen equilibrium curve (OEC) for whole normal human blood.

In this report, we did not attempt a physical interpretation of the reactions of hemoglobin with H^+, CO_2 and 2,3-DPG within the red cell. Instead, this model is adequate in other instances, such as to predict the effects of the variation of one or more of the considered factors on the *in vivo* oxygen exchange in normal and hypoxic situations, to implement some other physiologic models, such as the Krogh capillary and the Bohr models, and in general under all the circumstances where the exact knowledge of the position and shape of the OEC is required.

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RÉSUMÉ : Nous avons analysé en continu 56 courbes d'équilibre de l'oxygène sur sang frais humain, pour une Po_2 allant de 0 à 150 Torr. Nos résultats portent essentiellement sur les paramètres rapport [2,3-DPG]/[Hb] : 0,2-2,7, pH : 7,0-7,8 et Pco_2 : 7-70 Torr. L'équation d'Adair, qui permet l'analyse pas-à-pas de l'oxygénation de l'hémoglobine, a été développée et ajustée aux résultats pour chaque courbe ; on peut ainsi reproduire la courbe en se servant de l'équation et de quatre constantes a_1, a_2, a_3 et a_4. Les relations entre ces constantes et les variations de pH, Pco_2 et 2,3-DPG ne peuvent être qu'empiriquement connues ; nous avons développé une série d'équations, qui permettent d'estimer les constantes d'Adair pour les valeurs physiologiques de ces variables. Les études précédentes des effets des conditions physiologiques sur le transport de l'oxygène par les globules rouges portaient surtout sur P_{50} (Po_2 à la saturation de 50 %) ou ne donnaient que peu de résultats sur les simulations continues. De plus, les descriptions mathématiques des variations de la courbe d'équilibre de l'oxygène utilisent ordinairement l'équation de Hill, qui présuppose une pente constante de la courbe. Des études récentes ont montré que cette hypothèse était fausse. L'équation d'Adair évite d'avoir à recourir à de telles simplifications ; elle fournit un instrument efficace pour prédire la forme des courbes d'équilibre de l'oxygène pour différents états physiologiques et pathologiques, et pour étudier l'affinité de l'hémoglobine pour l'oxygène dans les globules rouges. Les équations que nous proposons peuvent facilement être incorporées à de vastes programmes de calcul de l'équilibre acido-basique et du transport de l'oxygène, ainsi qu'à la modélisation des échanges gazeux chez l'homme.