Edited by Daniel Hershey

Blood Oxygenation

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Proceedings of the International Symposium on Blood Oxygenation, held at the University of Cincinnati, December 1-3, 1969

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INTRODUCTION

Under the broad heading of blood oxygenation there may be specific areas of study, such as the kinetics of the oxygen-hemoglobin reaction, diffusion of gases through the red cell, blood preservation, blood chemistry, oxygen electrode design and the design and evaluation of artificial blood oxygenators. Blood oxygenation is of interest to many disciplines including physicians, chemists, physicists, biologists, physiologists and engineers.

The International Symposium on Blood Oxygenation was organized in order to bring together the people working in the various areas of blood oxygenation. This multidiscipline meeting was held at the University of Cincinnati on December 1, 2 and 3 of 1969. It was jointly sponsored by the U. S. Army Medical Research and Development Command and the University of Cincinnati. Participants came from Australia, England, Israel, Italy, Japan and the United States. There were 122 persons registered for the Symposium.

From the nature of the discussion during the meeting, it seemed apparent that the participants were benefiting from the contacts with colleagues in other disciplines. The result was a significant contribution to the present fund of knowledge of blood oxygenation and an enhancement of the future work.

The papers presented at the Symposium are contained in BLOOD OXYGENATION. This book should be a valuable reference source for those working in the field. It can also serve as a convenient starting point for those about to embark on work in the area of blood oxygenation. With a little imagination, an instructor may also be able to adapt the material to serve as a textbook.

Daniel Hershey

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CONTENTS

FACTORS DETERMINING THE VELOCITY OF GAS UPTAKE BY INTRACELLULAR HEMOGLOBIN

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This paper will consider some of the factors which affect the rate at which red cells can take up oxygen and carbon monoxide. A considerable part of it will be devoted to carbon monoxide and there are several reasons for this. Carbon monoxide combines with hemoglobin at the same site as does oxygen, both ligands attaching to the iron atom of the porphyrin group. The slow rate at which CO dissociates from carboxyhemoglobin causes it to have an affinity for hemoglobin approximately two hundred times as great as the affinity of oxygen. This enables CO in small concentrations to displace oxygen from hemoglobin thus reducing the oxygen carrying capacity of the blood, and is largely responsible for the toxicity of carbon monoxide. The tight binding of CO to hemoglobin has made it of great interest to physiologists and it has been widely used experimentally both as a means of producing hypoxemia and for measuring lung diffusing capacity. Another useful property of CO is that it combines with hemoglobin less rapidly than does oxygen. This enables one to measure some of the CO reaction rates more easily and accurately than the corresponding oxygen reaction rates. From these measurements we can draw general conclusions on the factors affecting gas uptake by the cells.

The original methods for measuring the rapid reactions of hemoglobin were published in 1923 by Hartridge and Roughton and much of our present knowledge is due to the work of Roughton and co-workers. The subject of gas uptake rates by red cells has been reviewed in the last ten years by Roughton (29) and by Forster (7) and so the present paper will concentrate on more recent work and draw attention to certain unsolved problems.

It is divided into sections as follows:

SYMBOLS AND ABBREVIATIONS

The symbols used in the text and equations are standard (8, 29) but for convenience are restated here.

The velocity constants for the four successive stages of oxygenation of the hemoglobin tetramer are given by the symbols k'_1, k'_2, k'_3, k'_4, and the constants for the deoxygenation of the tetramer by k_4, k_3, k_2, and k_1. Throughout the use of the prime (') indicates an association velocity constant and its absence indicates a dissociation velocity constant. For the reactions of CO with hemoglobin ℓ is used instead of k. In cases where the overall rate of gas combination with reduced hemoglobin is measured, the symbol k' or ℓ' is used without a subscript.

To describe the rate at which CO replaces oxygen from the fully saturated tetramer, Roughton (1945) introduced the symbol m' defined by the equation

$$\frac{d[COHb]}{dt} = m' \frac{[CO][O_2Hb]}{[O_2]} \qquad (1)$$

The value of m' is not constant but varies with the ratio $[CO]/[O_2]$ and thus is referred to as a pseudoconstant rather than as a constant. Its relation to the more fundamental constants is expressed by the equation

$$m' = \frac{k_4 \ell_4}{4 k'_4 \left\{ 1 + \frac{\ell'_4 [CO]}{k'_4 [O_2]} \right\}} \qquad (2)$$

Roughton, Forster, and Cander (27) introduced the term m'_∞ to describe the rate of the replacement reaction when the ratio $[CO]/[O_2]$ is negligible, for example when lung diffusing capacity is measured with low concentrations of CO. From equation (2) it follows that

$$m'_\infty = \frac{k_4 \ell'_4}{4 k'_4} \qquad (3)$$

Symbols such as ℓ', k'_4 without the subscript "c" refer to reactions of hemoglobin in solutions. In describing the velocities of uptake of oxygen and CO by reduced red cells the symbols k'_c and ℓ'_c are used and m'_c is used for the rate of CO replacing O_2 in cells. The definition of k'_c is

$$\frac{d[O_2Hb]}{dt} = k'_c [O_2][Hb] \qquad (4)$$

$[O_2]$ being the concentration of oxygen in the fluid immediately surrounding the red cells, and the reverse reaction again being neglected. Similar equations apply for ℓ'_c and m'_c.

The use of expressions for the apparent velocity constants for gas uptake by cells has certain limitations. The rate must be measured as soon after the reactants are mixed as possible. This is because the equations of Roughton describing the combined processes of diffusion and chemical reaction into the cells are not valid except at zero time (29). The reverse reaction must also be neglected in order to solve the equations; the error introduced by neglecting it is minimized by measuring the rate at zero time and, in the case of CO, by the inherent slowness of the dissociation reaction.

All the combination velocity constants referred to in this paper are second order and are expressed in units of "liters per millimole per second" which is equivalent to "per millimolar per sec" abbreviated as mM^{-1} sec^{-1}. The dissociation velocity constants, and m', m'_∞ and m'_c are first order and are expressed in units of sec^{-1}.

MODEL OF THE RED CELL

The factors affecting red cell reaction rates have usually been analyzed using one of Roughton's models of the red cell. It has not

so far been possible to consider the red cell as a biconcave disk (its form in mammals) but equations are available for the uptake rate of gases by a sphere of equivalent volume (26), by a membraneless layer of hemoglobin (25) and by a layer of hemoglobin bounded by a membrane whose permeability to gases can take different values. Of these, the last mentioned, described by Nicolson and Roughton (22) is the most useful.

In this model the cell is regarded as being a sheet of infinite extent, and of thickness equal to the average half-thickness of the red cell. The gas is regarded as entering through the membrane from one side only. The thickness of the layer is expressed by the symbol b (in cm) and the concentration of hemoglobin is taken as being that in the red cell, normally approximately 20 mM (hemoglobin millimolarity throughout is stated with reference to concentration of the monomer, this being the unit which combines with one molecule of gas). At 37°C the diffusion coefficient of oxygen in this concentration of hemoglobin is 7.1×10^{-6} cm^2 sec^{-1} and for CO the value is 7.6×10^{-6} cm^2 sec^{-1} (15, 19, 29). The diffusion coefficient (D) is taken as having a temperature coefficient of 2.5% per degree centigrade.

The permeability of the red cell membrane to the gas is expressed as λ where λ is the ratio of the permeability of the membrane to the permeability of the cell interior. It can be expressed in terms of the diffusion coefficients as follows

$$\lambda = \frac{D_2}{b_2} \Big/ \frac{D}{b} = \frac{D_2 b}{D b_2} \tag{5}$$

where D_2 is the diffusion coefficient of the gas in the red cell membrane, b_2 is the thickness of the red cell membrane, D and b are as defined above. A high value for λ indicates high membrane permeability.

The equations of Nicolson and Roughton predict two slowing factors which cause the rate at which reduced cells take up the gas to be less than the rate at which hemoglobin solution combines with the gas. To express these factors a term w is introduced, w being defined by the equation

$$w = b\sqrt{\frac{k'[Hb]}{D}} \tag{6}$$

where [Hb] is the initial concentration of reduced hemoglobin inside the red cells. For the CO reaction k' is substituted for k' in equation 6.

The first slowing factor is that attributable to the process of simultaneous diffusion and chemical reaction in the substance of the red cell. It predicts that the cell reaction will be slowed, when compared to the solution reaction, by a factor of $(\tanh w)/w$ so that in a hemoblogin layer without a membrane

$$\frac{k_c'}{k'} = \frac{\tanh w}{w} \tag{7}$$

The second slowing is caused by a barrier to gas diffusion at the cell membrane and slows the reaction by a further factor of $1/[1 + (w/\lambda) \tanh w]$. Thus the combined equation relating cell and solution rates is

$$\frac{k_c'}{k'} = \frac{\tanh w}{w} \cdot \frac{1}{1 + \frac{w}{\lambda} \tanh w} \tag{8}$$

Similar equations describe the slowing of the reaction with carbon monoxide.

The use of these equations to analyze some recent data is described in the subsequent sections.

THE IMPORTANCE OF RED CELL SIZE

In applying equation 8 to the analysis of the reaction rate a value has to be inserted for b, the mean half-thickness of the red cell. Mammalian erythrocytes are normally non-nucleated biconcave circular discs, except for those of camel and llama which are ovoid rather than circular. Thus there is no obvious value to use for half-thickness. Roughton (25) took the value of 0.7 microns in the case of the sheep; this was a compromise between half-thicknesses at the thinnest and thickest parts and made some allowance for gas entering at the disc edge. This value has been used for half-thickness of the sheep cell in other publications (22, 31) and also for the half thickness of the human red cell (6). However, the mean corpuscular volume (MCV) of the erythrocytes in these two species differs considerably. Sheep MCV is 33 cubic microns and human MCV is 90 cu. microns. Of the common mammals the goat has the smallest MCV, 20 cu. microns, while high values are found in lower forms, particularly in amphibia (1).

It was therefore of interest to investigate whether the size of the cells affected the velocity of gas uptake. In the case of oxygen uptake by reduced cells there was a considerable effect. Holland and Forster (11) found k'_c for the goat to be more than double the value found for k'_c in the dog and in the rabbit (MCV of each approximately 60 cu. microns) and in general found a fair

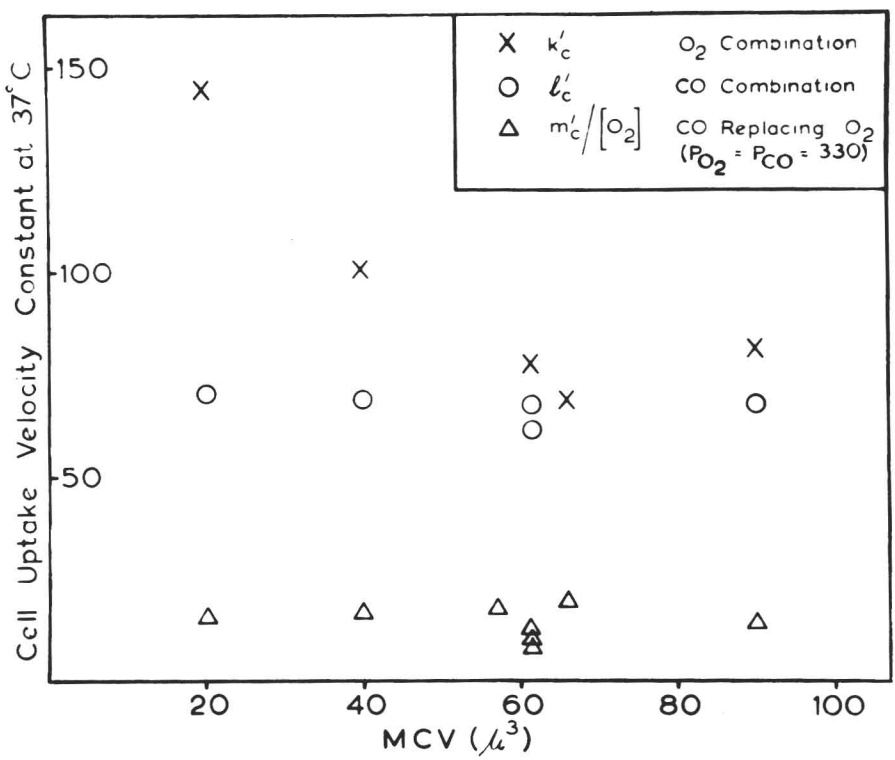

Fig. 1 The effect of cell size on the cell uptake rate constant or pseudoconstant at 37°C. The effect of cell size is greatest for the most rapid reactions. The points for the replacement reaction pseudoconstant σ' cell ($=m'_{cell}/[O_2]$) are from Holland (14) and for the uptake constants for the CO and O_2 reactions the points are from references 10 and 11 respectively. The species represented in the figure are, from left to right; goat, horse, cat, rabbit, dog and adult man. Mean values are shown except in the rabbit where there was considerable intraspecies variation.

relationship between smallness of red cell and rapidity of oxygen uptake (Fig 1). The value of k'_c was also determined for the bullfrog whose erythrocytes are nucleated, ovoid, and biconvex with a value of 680 cu. microns for MCV. Bullfrog k'_c is not shown in Fig 1 but was 19 mM^{-1} sec^{-1}, which is about one-eighth of the value in the goat and one-quarter of the value in man.

We had to consider two possible explanations other than cell size to account for the interspecies differences in oxygen uptake rate. The first was the possibility that the species with smaller cells also had hemoglobin molecules which reacted faster with oxygen. However, this reaction rate in solution was approximately the same in all species where we measured it. Also from equation 8, for a reaction as rapid as this, the cell rate is rather insensitive to changes in the solution rate. Therefore, despite the difficulty in accurately measuring the rate of a reaction as rapid as the combination of oxygen with reduced hemoglobin solution, we felt we had excluded differences in the hemoglobin molecule as a cause of the observed differences in k'_c.

The second possible explanation was that the cell membrane permeability (λ) might be higher in the species with the smaller cells. There is no experimental way of excluding this; in equation 8 the value of b and λ are interdependent, and unless one is known with accuracy, it is impossible to know the other. However, it seems inherently unlikely that membrane permeability would vary inversely with cell size. We therefore consider that the variations found in k'_c represent a cell size effect although the other factors mentioned may well play a part any may be responsible for the irregularities in the graph of k'_c against MCV.

We are not able to say what value should be taken for cell half-thickness. Ponder (23) gives values for the greatest and least thicknesses of the erythrocytes of man, rabbit, and sheep, showing the sheep (MCV = 33 cu microns) to have thicker cells than the rabbit (MCV = 61 cu microns). Holland and Forster (11) found k'_c to be higher in the sheep (137 mM^{-1} sec^{-1}) than in the rabbit (77 mM^{-1} sec^{-1}). Clearly the value taken for b in equation 8 can be at best an approximation giving some expression to the importance of cell size.

When one measures the relative rates of slower reactions one finds less cell size effect (Fig 1). The reaction between CO and reduced hemoglobin solution at 37°C is about one-eighth as rapid as the oxygen reaction. The cell reaction rates are not slowed in proportion but the cell size effect was found to be much less (10). When the much slower two stage reaction of CO replacing oxygen from oxyhemoglobin was studied (14), size effects were found to be minimal (Fig 1, 2).