Hypothesis: Changes in the O₂ Dissociation Curve and Sickling: A General Formulation and Therapeutic Strategy

By Ernest Beutler

THE RELATIONSHIP between oxygen tension and the sickling phenomenon has been recognized for over 45 years¹ and has become central to our thinking about sickle cell disease. It has been emphasized that sickling of red cells depends to a large degree upon the absolute concentration of reduced sickle hemoglobin.² In the past two decades many therapeutic strategies have been explored in the management of sickle cell disease. These include the formation of methemoglobin,³ carboxyhemoglobin,⁴ alkalinization,² and most recently, carbamylation of hemoglobin with cyanate⁵ or with carbamyl phosphate.⁶ The treatments enumerated have in common the fact that they produce a left shift in the oxygen dissociation curve. When such an experimental therapeutic agent is shown to diminish the amount of sickling observed at a given partial pressure of oxygen, the question arises whether the observed effect is due to a direct effect of the experimental agent upon sickle hemoglobin, or whether the decreased sickling is due merely to the lower concentration of reduced hemoglobin present at a given pO₂ level. Recently, it has been implied that effect of cyanate on sickling was due to decreases in the proportion of deoxyhemoglobin present at any particular pO₂.⁷,⁸

Our own studies on the viscosity of partially deoxygenated hemoglobin solutions has demonstrated that even at a constant per cent saturation of sickle hemoglobin with oxygen, cyanate produces a marked decrease in viscosity.⁹ Indeed, we have found that all alterations of hemoglobin that lead to a left-shifted dissociation curve produce a decrease of sickling at a given per cent saturation, while all modifications that cause a right shift of the dissociation curve produce an increase of hemoglobin viscosity at a fixed per cent saturation.⁹⁻¹¹ Similar findings with respect to 2,3-DPG were recently reported by others.¹² Some of our previously published data and newer findings are summarized in Table 1.

It seems worthwhile to speculate about the reason for this consistent effect based on current concepts of hemoglobin function. To understand our view of this general phenomenon it is important to distinguish between the percentage of hemoglobin which is in the oxy-conformation, on the one hand, and the percentage saturation of the hemoglobin solution with oxygen, on the other. Monod et al.,¹³ in presenting models to explain the action of allosteric enzymes, called attention to the fact that

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the sigmoid oxygen dissociation curve of hemoglobin could well be explained on the basis of two different conformations of the hemoglobin molecule. Although the details of the mechanism are probably more complex than the Monod model because the subunits of hemoglobin are not identical and other models such as that proposed by Koshland may be more appropriate, the fact that hemoglobin exists in different conformations has been put on firm experimental ground by the elegant studies of Perutz who has demonstrated a structural basis for the existence of hemoglobin in a "tense" or "relaxed" state. The tense state, sometimes referred to as the "deoxy-" conformation, has a relatively low affinity for oxygen and is constrained by a series of salt bridges and by 2,3-DPG. The relaxed state, sometimes referred to as the "oxy-" conformation, has a relatively high affinity for oxygen. The binding of oxygen by hemoglobin tends to break the restraints that hold it in the "tense" conformation and convert it to the high affinity form. But oxygen is apparently only one of a great many factors that affect the equilibrium between the two states of hemoglobin. Any agent that tends to stabilize the hemoglobin in the "deoxy-" (tense) conformation (such as 2,3-DPG or hydrogen ions) should produce a right shift in the oxygen dissociation curve by having stabilized the molecule in the low affinity conformation. Conversely, it is reasonable to suppose that agents that produce a leftward displacement of the oxygen dissociation curve tend to do so by shifting the equilibrium between the two forms of hemoglobin at any given per cent saturation toward the high affinity ("oxy-" or relaxed) form. Thus, a solution of hemoglobin which is 50% saturated with oxygen could have quite different proportions of its molecules in the high- and low-affinity states, depending on factors such as the presence or absence of 2,3-DPG carboxamidation of amino groups, or pH (Fig. 1). If it is not the oxygen itself, but the proportion of molecules in the tense (low affinity; "deoxy-") versus the relaxed (high affinity; "oxy-") conformation, rather than the oxygen content of the sample that determines the degree of sickling, a very consistent picture emerges. Table I summarizes the results of our studies of sickle hemoglobin viscosity at a fixed per cent saturation with oxygen. It is apparent that all conditions known to produce a right shift in the dissociation curve, therefore presumably to increase the number of molecules in the low-affinity state at any given per cent saturation, increased sickle hemoglobin viscosity. The converse is true of conditions which produce a leftward displacement of the dissociation curve. This is not to say that other mechanisms may not also play a role. Steric hindrance, weakening of hydrophobic bonds, and other factors may also influence the aggregation of hemoglobin molecules.
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This interpretation of the data regarding agents that cause a displacement of the oxygen dissociation curve leads us to conclude that the use of modalities to shift the dissociation curve to the left should exert a favorable influence on the sickling phenomenon. Although reservations have been expressed about the therapeutic effectiveness of a strategy which may lower tissue pO₂, this in itself is not a serious objection to the use of such therapeutic approach. Patients who inherit unstable hemoglobins with left-shifted dissociation curves are able to manage very well indeed. One must remember that the main clinical problems in sickle cell disease derive not from tissue hypoxia itself, but from the altered rheologic properties of the patient's blood. Thus experimental approaches to sickling that produce a leftward displacement of the oxygen dissociation curve may well be successful in improving the clinical status of patients with sickle cell disease.

**REFERENCES**


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