EDITORIAL

The Affinity of Hemoglobin for Oxygen

The delivery of oxygen to the cell depends in good measure upon the affinity with which hemoglobin binds oxygen or releases it from erythrocytes for use by the other cells of the body.

The release of oxygen by normal human hemoglobin A, over a range of partial pressures of oxygen, may be represented graphically by the familiar, sigmoid-shaped, oxygen dissociation curve. Numerically, the affinity with which hemoglobin binds oxygen may be expressed by the T50 (p50) value which is defined as the partial pressure of oxygen at which 50% of the hemoglobin is saturated with oxygen at a temperature of 37 C and pH of 7.40. If the affinity with which hemoglobin binds oxygen is increased, the oxygen dissociation curve is shifted to the left and the T50 value is decreased from the normal value of 26 to 27 mm Hg Po2. If the affinity with which hemoglobin binds oxygen is decreased, the T50 value is increased.

In the past, little physiologic significance was attached to variations in the normal position of the oxygen dissociation curve. Within the last several years, however, renewed attention has been paid to mechanisms whereby alterations in the release of oxygen to the tissues from hemoglobin might enhance the organism's responses to hypoxia. This interest was heightened by the discovery that certain intracellular organic phosphate compounds, namely, adenosine triphosphate (ATP) and, particularly, 2,3 diphosphoglycerate (DPG) exert profound effects upon the release of oxygen from hemoglobin.

On oxygenation and deoxygenation the hemoglobin molecule undergoes conformational changes which are associated with binding of certain ligands whereby the combination of one ligand at a particular binding site of hemoglobin further facilitates the binding of other ligands at distant binding sites.

Heme-heme interaction and the hemoglobin binding of protons with its reciprocal effects on oxygen binding are examples of these allosteric effects. Carbon dioxide and certain salts also exhibit heterotropic, allosteric interactions with oxygen which can be demonstrated in dilute solutions of purified hemoglobin. These nonspecific effects depend upon the tetrameric form of a hemoglobin composed of dissimilar hemoglobin chains. In contrast to the myoglobin molecule, there must be more than one binding site on the hemoglobin molecule. In addition, there is evidence to suggest that protons, carbon dioxide, and certain salts bind more firmly to deoxyhemoglobin than to oxyhemoglobin.

DPG acts similarly to these ligands in dilute solutions of hemoglobin. Just as is the case with salts, the addition of DPG to salt free, dialyzed hemoglobin causes a profound decrease in oxygen affinity.\(^1-8\) The concentration of DPG needed to produce similar quantitative effects is much less than the concentrations of, for example, NaCl. Apparently DPG exerts its effects by a specific, stoichiometric combination with the hemoglobin tetramer mostly in the deoxy-configuration. This DPG-deoxyhemoglobin complex is highly resistant to oxygenation, and DPG must be displaced for oxygen to be bound. Presumably DPG is bound to histidine\(^4\) in the central molecular cavity of hemoglobin. This cavity is widened considerably during the conformational changes which accompany deoxygenation.

ATP also will reduce the affinity of hemoglobin for oxygen. However, DPG exists in much higher concentrations in the red cell than does ATP and, therefore, is a more probable source of this moderating effect on the oxygen affinity of hemoglobin.

In summary, DPG acts similarly to other ligands, such as protons and salt anions. It is competitive with these other ligands. Its
effect is dependent upon a tetrameric form of hemoglobin. Like the other ligands it appears to complex primarily with deoxyhemoglobin, and it is very effective in its influence upon oxygen affinity at concentrations which approach those which are found within the red cell.

Rapoport\textsuperscript{5} has reviewed the biochemistry of DPG recently. The regulation of DPG is primarily the consequence of the interrelations of two enzyme systems, namely diphosphoglycerate mutase, which catalyzes the reaction, 1,3 diphosphoglycerate +3 phosphoglycerate $\rightarrow$ 2,3 diphosphoglycerate, and diphosphoglycerate phosphatase which catalyzes the dephosphorylation of DPG. Particularly important is the fact that diphosphoglycerate mutase is strongly inhibited by DPG, and thus DPG can temporarily suspend its own production. In general, increases in pH, inorganic phosphate, nicotinamide adenine dinucleotide, 2 phosphoglycerate, 3 phosphoglycerate, and phosphoenolpyruvate favor increases in DPG concentration. Increases of the last three organic phosphates occur in pyruvate kinase defects of erythrocytes and may explain the observed increase in DPG in the red cells and the decreased affinity of hemoglobin for O\textsubscript{2} of patients with this defect.\textsuperscript{6}

An intracellular control mechanism thus may be summarized if it is assumed that only free DPG is effective as an inhibitor of diphosphoglycerate mutase. When red cells are in the deoxygenated state, most of the DPG is bound to hemoglobin and the concentration of free DPG is low. Thus, 1,3 diphosphoglycerate may be converted to DPG which is then further trapped by the hemoglobin. As the hemoglobin is oxygenated, the DPG-hemoglobin complex is dissociated by oxygenation and the concentration of free DPG increases. The free DPG inhibits the diphosphoglycerate mutase reaction and 1,3 diphosphoglycerate will be converted mainly to the other intermediates of the Embden-Meyerhof pathway (fig. 1).

Many investigators have described alterations of the dissociation curve in a variety of

![Figure 1](http://circ.ahajournals.org/)

\textsuperscript{1} Phosphoenolpyruvate

\textsuperscript{2} Pyruvate Kinase

\textsuperscript{3} Diphosphoglycerate Mutase

\textsuperscript{4} DPG Phosphatase

\textsuperscript{5} Rapoport\textsuperscript{5}

\textsuperscript{6} An intracellular control mechanism thus may be summarized if it is assumed that only free DPG is effective as an inhibitor of diphosphoglycerate mutase. When red cells are in the deoxygenated state, most of the DPG is bound to hemoglobin and the concentration of free DPG is low. Thus, 1,3 diphosphoglycerate may be converted to DPG which is then further trapped by the hemoglobin. As the hemoglobin is oxygenated, the DPG-hemoglobin complex is dissociated by oxygenation and the concentration of free DPG increases. The free DPG inhibits the diphosphoglycerate mutase reaction and 1,3 diphosphoglycerate will be converted mainly to the other intermediates of the Embden-Meyerhof pathway (fig. 1).

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clinical states associated with hypoxia or an increased demand of tissues for oxygen. Although a change in the affinity of hemoglobin for oxygen might be a homeostatic mechanism of physiologic importance, there is, as yet, no direct proof that oxygen delivery to the cell is affected by changes in the normal oxygen dissociation curve.

The rapid decrease in the affinity of hemoglobin for oxygen occurring in subjects exposed to high altitude hypoxia\(^7\) has been confirmed by Lenfant and associates\(^8\) who have demonstrated an increase in DPG in the red cells of these subjects. Inverse correlations between the level of DPG and the levels of hemoglobin have been noted in patients with anemia of various types.\(^9\) Presumably the proportion of deoxyhemoglobin is increased, thus more DPG is bound to hemoglobin with the subsequent release of inhibition of DPG formation in the red cell and the shift of the oxygen dissociation curve to the right. Those hemoglobinopathies associated with heterogeneous hemoglobin chains and anemia have increased DPG in the red cell and an increased T\(_{50}\) value. Those hemoglobinopathies associated with identical hemoglobin chains, however, do not exhibit allosteric changes, and mechanisms such as the Bohr effect and the effects of DPG are not operative. The hemoglobins exhibit a marked left shift of the oxygen dissociation curve.

Valeri and Fortier\(^10\) observed an increase of erythrocyte DPG levels in patients with red cell mass deficits. They point out the possible diagnostic significance of this finding.

It is known that the oxygen dissociation curve is shifted to the left, and the level of erythrocyte DPG is markedly reduced in blood stored in ACD solution. Fortier and associates\(^11\) have demonstrated that the level of DPG in transfused cells begins to rise within 24 hours after transfusion. This group has demonstrated that the rate of restoration of DPG in vitro using glucose, inorganic phosphate, and inosine was similar to the rate of restoration in vivo. These observations suggest that the recipient's environment provided substances, in addition to glucose, for the rapid restoration in vivo of the transfused red cell DPG level.

Acidosis decreases and alkalosis increases the erythrocyte DPG level,\(^12\) but these effects may take some hours. Recently Bellingham and co-workers\(^13\) have noted that the sudden correction of acidosis caused a marked increase in hemoglobin affinity for oxygen which did not improve until some 10 hours after administration of bicarbonate. Rapoport\(^5\) has suggested that DPG levels also are affected by plasma inorganic phosphate levels.

It appears that in clinical conditions associated with hypoxia when the relative proportion of deoxyhemoglobin may be increased, the oxygen dissociation curve shifts to the right, and the T\(_{50}\) values, and whenever measured, the DPG in the red cell increase. An exception to this is the recent report\(^14\) of a rapid increase in T\(_{50}\) value in coronary-sinus blood of patients with angina pectoris who did not exhibit changes in DPG, ATP, or pH of red cells. This suggests that other factors besides the organic phosphates, or pH may affect the oxygen dissociation curve under physiologic conditions.

Ultimately the significance of abnormal variations in the position of the oxygen dissociation curve depends on the critical oxygen tension at which cellular enzymes may work. Chance and associates\(^15\) have identified several cellular enzyme systems with oxygen affinities which might be influenced by a shift of the oxygen dissociation curve to the right.

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Addendum

Attesting to the widespread interest in this subject has been the recent publication of the extensive review by Bunn and Jandl.\(^16\)

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Oxyhemoglobin Dissociation Curve

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