Oxygen Transport and 2, 3-Diphosphoglycerate (DPG)

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Following early descriptions of the effect of pH and temperature on the oxygen dissociation curve, relatively little attention was paid to factors which changed the relationship between oxygen tension and content of human blood. The classic report of Valtis and Kennedy1 in 1954 concerning the increased affinity of hemoglobin for oxygen in stored blood, and the effect on gas exchange produced by transfusion of such blood, received minimal attention for more than a decade. In 1967, Benesch and Benesch,2 and Chanutin and Curnish3 simultaneously described the decrease in oxygen affinity of hemoglobin produced by 2, 3-diphosphoglycerate (DPG). Prior to this, there was no known intraerythrocytic function of this organic phosphate which has a concentration inside the red cell approximately equal to that of hemoglobin (5 mMoles/l).

The decreased oxygen affinity of hemoglobin produced by DPG is manifested by a shift to the right of the dissociation curve, i.e., the oxygen tension required to reach a given oxygen saturation is greater than normal. Since a shift of the oxygen dissociation curve results from a change in the relative affinity for oxygen but does not alter the basic sigmoid shape of the curve, a shift in the curve can be described in terms of the P50 (the oxygen tension necessary to half-saturate blood). Thus, a decrease in hemoglobin affinity for oxygen results in a right-shifted curve and increased P50. On the other hand, increased oxygen affinity shifts the curve to the left, and half-saturation of blood is attained with a Po2 less than the normal P50 of 26.5 mm Hg.

The description of the influence of DPG on oxygen affinity of hemoglobin was followed by a series of reports detailing increased DPG levels in patients with congenital heart disease,4 thyrotoxicosis,5 anemia,6,7 and chronic hypoxemia,8 as well as in normal individuals upon ascent to altitude.9 The common denominator in all these conditions is an altered relationship between tissue oxygen demands and oxygen delivery, implying that a right shift of the dissociation curve may be an adaptive mechanism.

**Effects of an Increased P50**

The value of a shift of the dissociation curve is illustrated in Figure 1. Both curves in the figure have equal hemoglobin levels (15.0 gm percent) and total oxygen capacities (20.0 vol percent). The curve on the left has normal oxygen affinity, and an oxygen tension of 26.5 mm Hg results in a binding of 10 out of a possible 20 vol percent of oxygen. The other curve is shifted to the right and has an elevated P50 of 36.5 mm Hg. An arterial Po2 of 90 results in almost complete saturation of hemoglobin in both cases. As blood perfuses the tissues the mixed venous Po2 decreases to 40 mm Hg and 4.5 vol percent of oxygen are released by the normal curve to meet metabolic demands. When the same conditions of oxygen tension are applied to the right-shifted curve, blood has the potential of releasing 7.2 vol percent of oxygen, an increase of 60 percent.

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![Figure 1. The changes in oxygen delivery at normal arterial and venous oxygen tensions produced by a shift of the dissociation curve to the right of the normal position.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21550/ on 06/25/2017)
in oxygen delivery as compared to the normal curve. The pronounced increase in oxygen delivery is the result of the difference in slopes of the two curves between the arterial and venous points. During oxygen uptake in the lungs, the curve with the higher PaO₂ binds less oxygen. However, this loss of oxygen loading is overshadowed by the superior ability of the right-shifted curve to unload oxygen at the venous tension. The net gain is the difference between the decrease in the oxygen loading in the lungs and the increase in oxygen unloading in the tissues.

**Cardiac Disease**

DPG levels are often elevated in cardiac disease, and this appears to provide an important compensatory mechanism in low-output cardiac failure.¹⁰ Since oxygen delivery to the tissues is the product of oxygen extraction from blood and cardiac output, compensation results if the decrease in tissue perfusion is matched by a corresponding increase in oxygen extraction. If this increased oxygen extraction took place without a change in the position of the dissociation curve, venous oxygen tension would drop to a much lower value. This would not be desirable since the pressure at which oxygen is delivered to tissues has an important bearing on oxygen utilization.¹¹ Oxygen released in capillaries must reach cellular mitochondria by a process of diffusion. Since the rate of diffusion is dependent upon the pressure gradient, and not the concentration gradient, release of oxygen at higher tensions enhances oxygen delivery. The PaO₂ in the mitochondria is estimated at approximately 1 mm Hg, and a relatively high pressure gradient is required to deliver oxygen to this site of utilization. This has led to the concept of the "critical PaO₂," the minimum venous PaO₂ which can be tolerated without deterioration of organ function. The mean "critical PaO₂" for tissues is thought to be in the range of 20 to 30 mm Hg although there is a wide range between individual organs.¹²

**Anemia**

The DPG-mediated shift of the dissociation curve is a particularly effective compensatory mechanism in anemia. In Figure 2, a normal dissociation curve and a curve constructed from the observations of Bromberg and Jensen¹⁰ in sickle cell patients depicts the efficacy of a right-shifted dissociation curve in anemia. Although the oxygen capacity of the anemic blood is reduced to one-half the normal value, the “effective anemia” is less than would be expected from the absolute level of hemoglobin. The decreased oxygen affinity of hemoglobin permits the delivery of 3.3 vol percent of oxygen at the same venous tension which is present in the non-anemic state. This oxygen delivery represents a 47 percent increase over the amount which would be delivered (4.5 vol percent ÷ 2 = 2.25 vol percent) if the reduction in hemoglobin concentration were not accompanied by a shift of the dissociation curve. This degree of compensation is similar to that reported by Torrance et al.¹³ It seems likely that this adaptive mechanism is largely responsible for the observed minimal changes in cardiac output in mild and moderate anemias.¹⁴ From the standpoint of conservation of body resources, maintenance of tissue oxygen delivery by a shift of the dissociation curve is far more economical than a corresponding increase in cardiac output. This leads to the concept of "functional anemia"—anemia which is characterized by the impairment of oxygen delivery rather than by the reduction in the absolute level of hemoglobin. In their report of hemoglobin Seattle, a hemoglobin variant with a marked right shift, Stamatoyannopoulos et al¹⁵ have suggested that the increased efficiency of oxygen delivery permits a lower level of circulating hemoglobin to provide adequate tissue oxygenation. This concept is reinforced by the low levels of erythro-

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*In illustrating the advantages of the right-shifted dissociation curve, we have assumed that venous oxygen tension remains constant during stress. While this is not necessarily true, it permits quantitation of the advantages of the curve-shift. In all probability the adaptation to decreased oxygen delivery also involves some decrease in venous oxygen tension.*

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**Figure 2. Oxygen delivery in anemia as compared to normal circumstances.** Hemoglobin concentration and oxygen capacity for the normal curve are 13.0 gm percent and 20.0 vol percent. The hemoglobin concentration and oxygen capacity in the example of anemia are 7.5 gm percent and 10.0 vol percent. The value of P₅₀ = 34.0 in an anemia of 7.5 gm percent is taken from the data of Bromberg and Jensen.¹²
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poietin measured in their patients. However, final evaluation of this hypothesis awaits further data since patients with hemoglobin Seattle have a hemolytic component which may also affect the hemoglobin concentration.

EXERCISE

The usefulness in oxygen delivery of hemoglobin with a decreased oxygen affinity is further illustrated by the data of Oski et al. in two patients with congenital erythrocytic enzyme deficiencies which produce abnormal DPG concentrations and PsO's. A patient with hexokinase deficiency, which results in a low DPG concentration and a left-shifted curve, was compared to a patient with pyruvate kinase deficiency, which is accompanied by an elevated DPG concentration and a right-shifted curve. At the highest exercise level attained, the patient with the right-shifted curve met tissue metabolic demands predominantly by increasing oxygen extraction; cardiac output rose only 50 percent. In contrast, at the same level of oxygen consumption, the patient with the left-shifted curve increased the arteriovenous oxygen difference to a lesser degree and more than doubled cardiac output to meet tissue needs.

PULMONARY DISEASE

In the preceding discussion, we have assumed normal oxygen loading, i.e., normal pulmonary function resulting in normal PaO. In the presence of abnormal oxygen loading, the situation is changed (Figure 3). The net gain in oxygen delivery is the difference in oxygen content of the two curves at the arterial and venous points. With hypoxemia, the arterial tension moves toward the steeper portion of the dissociation curve and, as noted in the figure, eventually reaches a point where the gain in oxygen unloading is balanced by the loss in oxygen loading in the lungs.

In addition, the efficacy of a decreased oxygen affinity varies from one tissue to another. While a mixed venous oxygen content is helpful in the study of overall oxygen exchange, this provides no information concerning individual organs. Oxygen extraction under normal circumstances varies from as little as 1.7 vol percent in the kidney to 13.0 vol percent in the heart. As a result of this variation, the effect of a shift in the dissociation curve is not the same in every tissue. For example, under the same conditions of arterial PsO and oxygen affinity present in Figure 3, the influence of the curve shift on oxygen delivery to different tissues is shown in Figure 4. Despite the fact that the curve shift produced no change in overall oxygen delivery, individual organs have net gains or losses. The kidney fares better with a right-shifted curve in this case, receiving 2.0 vol percent instead of 1.7 vol percent of oxygen with this same arteriovenous tension difference. However, the heart is at a disadvantage since it receives only 10.6 vol percent instead of 13.0 vol percent. Thus, the value of a shift in the dissociation curve not only varies from patient to patient, but also from tissue to tissue in the individual patient.

Oxygen delivery to individual tissues is not only a function of the shift of the dissociation curve but is

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also dependent upon arterial oxygen tension. The influence of arterial P\textsubscript{O\textsubscript{2}} on the gain or loss in oxygen delivery accompanying a shift of the dissociation curve is shown in Figure 5. The change in oxygen delivery due to the increase in P\textsubscript{S\textsubscript{0}} (26.5 to 36.5 mm Hg), plotted on the ordinate, is relatively small for the kidney and variation of arterial P\textsubscript{O\textsubscript{2}} has a minimal effect. Oxygenation of the brain is enhanced by the curve shift at high P\textsubscript{O\textsubscript{2}}'s, but this advantage decreases with the development of arterial hypoxemia. The heart has an oxygen extraction eight times that of the kidney, yet benefits only slightly more from the curve shift when arterial P\textsubscript{O\textsubscript{2}} is normal. With increasing hypoxemia this advantage is rapidly lost, and in this example oxygen delivery at a P\textsubscript{O\textsubscript{2}} of 56 is the same as that obtained with a normal dissociation curve. With increasing hypoxemia, cardiac oxygenation suffers due to the right shift of the dissociation curve. This theoretical example underscores the complexity of oxygen delivery when a shift of the dissociation curve is accompanied by arterial hypoxemia.

The increase in P\textsubscript{S\textsubscript{0}} of 10 mm Hg in Figure 5 would accompany a twofold increase in DPG. Although this DPG change may seem large, such changes are observed clinically. In addition, similar curve shifts are frequently encountered in patients with acute respiratory failure due to changes in hydrogen ion concentration. The same shift in the dissociation curve present in Figure 5 could also be produced by a decrease in arterial pH to 7.1 without any concomitant changes in DPG concentration. The effects of increases in DPG and hydrogen ion concentrations are additive and can markedly alter the position of the dissociation curve.

Wide variations in DPG levels and P\textsubscript{S\textsubscript{0}}'s have been observed in pulmonary diseases, ranging from levels below normal\textsuperscript{17,18} to above normal\textsuperscript{9,17} This variation may result from different degrees of arterial hypoxemia and tissue oxygen extraction. Factors which control DPG synthesis in these patients are presently unclear. Most likely, red cell DPG production is dependent upon the internal erythrocytic environment and, therefore, DPG concentration would react to a weighted mean of conditions in all tissues rather than to gas exchange in a single organ.

**MECHANISM OF DPG EFFECT**

The shift of the dissociation curve produced by DPG apparently is the result of two separate mechanisms. The first involves the direct binding of DPG to the hemoglobin molecule. DPG binds more readily to deoxyhemoglobin than to oxyhemoglobin.\textsuperscript{19} The binding sites\textsuperscript{*} are located near the central cavity of the molecule, and DPG fits into this cavity when hemoglobin is in the deoxygenated state.\textsuperscript{21} With oxygenation, the conformation of the hemoglobin molecule changes and the central cavity closes so that DPG binding is more difficult.\textsuperscript{21} Binding of DPG tends to "hold" hemoglobin in the deoxygenated conformation; the increased oxygen tension required to saturate blood is a manifestation of these events.

The second mechanism is related to the intracellular synthesis of DPG and its inability to cross the erythrocyte membrane. This produces a large transmembrane gradient of the markedly negative DPG anion. The presence of high concentrations of intracellular DPG alters the Donnan equilibrium, causing a decrease in intracellular pH.\textsuperscript{22} This decrease in pH shifts the dissociation curve to the right despite the lack of a demonstrable change in plasma pH. Thus, DPG appears to decrease oxygen affinity directly by binding to hemoglobin and indirectly by its effect on intracellular pH.

**CONTROL OF DPG CONCENTRATION**

Although the mechanism of the DPG effect and its influence on gas exchange are understood to a reasonable degree, the processes which control the level of intracellular DPG concentration remain unclear. In the red cell, anaerobic glycolysis serves...

\*DPG binding involves the terminal valine amino acid residues of the beta chains of hemoglobin; it is likely that the beta 143 histidine groups are also involved.\textsuperscript{20}
as the primary energy source. In normal glycolysis, 1, 3-diphosphoglycerate is directly converted to 3-phosphoglycerate. In the erythrocyte an alternate pathway between these two compounds exists whereby the 1, 3-diphosphoglycerate is converted to 2, 3-diphosphoglycerate (DPG), and then to 3-phosphoglycerate. This alternate route in the glycolytic scheme provides a means of synthesizing DPG, and is the source of the high concentration of DPG present inside the red cell. The factors which control the pathway used, and hence, the intracellular concentration of DPG, are not completely understood.

The first suggested control mechanism stemmed from the observation that DPG binds preferentially to deoxyhemoglobin. Synthesis of DPG from 1, 3-diphosphoglycerate is accomplished through a reaction mediated by DPG mutase; the activity of this enzyme is inhibited by its product, DPG. It was reasoned that the presence of large amounts of deoxyhemoglobin would bind free DPG, relieving the inhibition of DPG mutase, and resulting in increased DPG synthesis. While this occurs in vitro, this mechanism does not appear to be the dominant factor which controls DPG in vivo.

A more promising possibility is the increased DPG synthesis associated with increase in pH. Changes in DPG have been correlated with pH both in patients with acid-base disturbances, and in normal individuals with deliberate alterations in acid-base status. Lenfant, Torrance and Reynafarje have provided the most convincing evidence for control of DPG by pH in their studies of normal subjects abruptly taken to high altitude. The increase in DPG found in these individuals is accompanied by hypoxemia and alkalosis. When the alkalosis is prevented by administration of acetazolamide prior to ascent, both plasma pH and DPG remain unchanged despite the presence of hypoxemia. Gerlach et al. in similar experiments noted an increase in DPG when rats were exposed to 11 percent oxygen. Addition of 5 percent CO₂ to the inspired gas prevented alkalosis secondary to hyperventilation, and the increase in DPG did not occur even though the experimental hypoxemia persisted.

Astrup has speculated that the observed correlation of DPG with hypoxemia may result from an accompanying intracellular change in pH. Deoxyhemoglobin is a weaker acid than oxyhemoglobin, and increased amounts of the former would raise intracellular pH without significant change in plasma pH. The increased intracellular pH resulting from an increased deoxyhemoglobin/oxyhemoglobin ratio, rather than the deoxyhemoglobin per se, may cause increases in DPG concentration.

Other factors, such as congenital glycolytic enzyme deficiencies, affect DPG levels. Snyder and Reddy have recently published evidence indicating that the increase in DPG concentration occurring in thyrotoxicosis is a result of the direct effect of thyroid hormone on the enzyme DPG mutase. The control of DPG synthesis and degradation is complex and involves many factors; with our present knowledge it appears that intracellular pH has the strongest influence in this control.

**Acid-Base Disturbances**

The influence of pH upon DPG concentration plays an important modifying role in oxygen delivery in patients with acid-base disturbances. In alkalosis, the dissociation curve is shifted to the left as a result of the pH change (Bohr effect). The alkalosis also causes an increase in intracellular DPG concentration which, in turn, results in a shift in the dissociation curve to the right. This latter shift mediated by DPG nullifies the shift produced by the pH change so that the net result is a normal in vivo dissociation curve. Since the Bohr effect is an immediate process, the P₅₀ decreases with the onset of alkalosis, but within 24 hours DPG concentration increases, and the P₅₀ measured at the patient's pH returns to normal. In this case a change in DPG concentration does not compensate for a primary defect in oxygen delivery, but rather insures normal oxygen delivery in the face of an acid-base disturbance which might otherwise compromise this delivery. In acidosis, the changes in pH and DPG are opposite in direction to alkalosis, but similarly maintain a normal in vivo dissociation curve.

The P₅₀ is normally measured at pH 7.4 so that the DPG effect can be assessed without the complication of changes in pH. In terms of oxygen delivery to the tissues, however, the P₅₀ at the patient's pH rather than at pH 7.4 is the determining factor. This concept is particularly important in patients with abnormal acid-base status.

No investigation of DPG and the oxygen dissociation curve has been performed in patients with acute respiratory failure. These data are essential to the understanding of problems in oxygen delivery in respiratory failure because it is not possible to project the cumulative effect of acute hypoxemia and acidosis which are often superimposed upon chronic hypoxemia and acidosis. Since changes in DPG produced by acidosis lag behind the actual changes in hydrogen ion concentration, large swings in the position of the dissociation curve may occur in acute respiratory failure.
OXYGEN AFFINITY IN STORED BLOOD

Valtis and Kennedy first called attention to the increased oxygen affinity of stored blood and its possible effects on gas exchange after transfusion. Following the initial reports of the influence of DPG on oxygen affinity, Bunn et al demonstrated that the increased oxygen affinity of stored blood is related to an accompanying decrease in DPG concentration. In these experiments red cell concentrations of adenosine triphosphate (ATP) also showed a slight decrement with storage. Although ATP also affects the oxygen affinity of hemoglobin, its concentration is only one-fifth of the normal DPG concentration so that its influence on oxygen affinity is minimal in comparison to DPG.

The level of DPG decreases rapidly in conventional acid-citrate-dextrose (ACD) preservative with the result that DPG concentration is only one-third of normal after blood has been stored one week. The rapidity of this decrease can be slowed considerably by incorporation of inosine into the preservative. Following storage in ACD preservative, DPG concentration can be returned to normal within 30 minutes by incubation of the cells with inosine, pyruvate and phosphate. These findings, although still in the experimental stage, offer a promising approach to the usefulness of stored blood in conditions which necessitate transfusion of blood possessing a normal oxygen affinity. Most of the alteration of the dissociation curve produced by transfusion of stored blood depleted of DPG is corrected within 24 hours. Using a differential agglutination technique, Valeri and Hirsch demonstrated that the concentration of DPG in transfused cells also approaches normal values within 24 hours after administration. This rapid return of DPG and the corresponding correction of the abnormal dissociation curve indicate that transfusion of blood possessing a normal DPG level is necessary only in those patients who have an immediate, life-threatening defect in oxygen delivery.

EFFECT ON CARBON DIOXIDE EXCHANGE

DPG also has a direct effect on carbon dioxide exchange. The majority of carbon dioxide is carried in blood as bicarbonate, but approximately 5 percent is transported as hemoglobin-carbamate, a compound formed by the direct combination of carbon dioxide with hemoglobin. Hemoglobin-carbamate is oxylabile, i.e., more carbon dioxide can be bound to reduced hemoglobin than to oxygenated hemoglobin. As hemoglobin is deoxygenated in the tissues, carbon dioxide is bound as hemoglobin-carbamate; in the lungs the bound carbon dioxide is released as hemoglobin is oxygenated. As much as 30 percent of total carbon dioxide exchange has been attributed to changes in concentration of this compound.

However, two of the four terminal valine residues of hemoglobin which bind carbon dioxide are also involved in binding of DPG. We have recently demonstrated that the effectiveness of hemoglobin-carbamate in carbon dioxide exchange is reduced by one-half in red cells with normal DPG concentration. Further studies are being undertaken to determine the effect of elevated concentrations of DPG. Although DPG facilitates oxygen delivery, these data suggest that it hinders carbon dioxide exchange.

SUMMARY

Reports from many laboratories have firmly established that control of oxygen affinity, and therefore, the position of the dissociation curve of whole blood, is effected through the concentration of DPG inside the red cell as well as by pH and temperature. An increase in P50 (shift of the dissociation curve to the right) increases the amount of oxygen delivered by blood at normal arterial and venous oxygen tensions. This is a particularly effective mode of compensation in anemic states. In pulmonary disease, many patients have a right-shifted curve. However, with increasing arterial hypoxemia, the value of a right-shifted curve decreases and may even become a liability. Most likely this accounts for the observation of normal or left-shifted curves in some patients with lung disease. Changes in the position of the dissociation curve must be interpreted not only in terms of overall oxygen delivery, but also in terms of oxygen delivery to individual tissues.

DPG shifts the dissociation curve by direct preferential binding to deoxyhemoglobin, as well as by its effect on intracellular pH. Although strongly influenced by intracellular pH, the control of intracellular DPG concentration is still unclear. In acid-base disturbances, changes in DPG concentration tend to counteract alterations in the dissociation curve produced by the acid-base process, preserving the normalcy of the in vivo oxygen dissociation curve. The dissociation curve in the patient, rather than that obtained in vitro under standard conditions, is the determining factor in gas exchange. DPG is depleted in stored blood, but is rapidly regenerated following transfusion so that the resulting temporary left shift of the dissociation curve is probably innocuous except in the acutely-ill patient. While DPG facilitates oxygen transport, it
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may hinder carbon dioxide exchange due to its effect in decreasing the formation of hemoglobin-carbamate.

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