

## Adaptive, Genetic, and Iatrogenic Alterations of the Oxyhemoglobin-dissociation Curve

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EVER SINCE Bohr, Hasselbalch, and Krogh<sup>1</sup> described the influence of pH on hemoglobin affinity for oxygen, our understanding of factors influencing the oxyhemoglobin-dissociation curve has steadily advanced. With such understanding, however, have come more questions and greater expectations, including the possibility of controlling tissue oxygen availability by pharmacologic manipulation of the oxyhemoglobin-dissociation curve.

In this presentation, we discuss: the physiologic factors which mediate changes in the oxyhemoglobin-dissociation curve; acquired states in which these factors are modified; the genetic alterations of erythrocytic enzymes and hemoglobin which change oxygen affinity; therapeutic interventions which secondarily influence hemoglobin affinity for oxygen; and the potential role of drug control of the affinity of hemoglobin for oxygen in disease.

The affinity of hemoglobin for oxygen is defined herein by the  $P_{50}$ , which represents the partial pressure of oxygen when 50 per cent of the hemoglobin is bound with oxygen at pH 7.4 and 37 C (fig. 1, curve A). The normal value of  $P_{50}$  is approximately 26 mm Hg. A decrease in the affinity of hemoglobin for oxygen represents a *rightward* shift of the oxyhemoglobin-dissociation curve and is, therefore, represented by an *increase* in  $P_{50}$  (fig. 1, curve B), whereas an increase represents a *leftward shift* of the curve and is represented

by a *decrease* in  $P_{50}$  (fig. 1, curve C). The normal sigmoid shape † of the oxygen-dissociation curve is particularly appropriate for its function. Wide rightward or leftward shifts have little effect on arterial oxygen saturation when  $P_{aO_2}$  is in the normal sea-level range (80 to 100 mm Hg) (fig. 1). Conversely, at the level of  $P_{O_2}$  in the capillaries, a small change in  $P_{O_2}$  is associated with a significant change in oxygen saturation. Therefore, if the arteriovenous oxygen saturation difference remains constant, a rightward shift is accompanied by an increase in mixed venous oxygen tension ( $P\bar{V}_{O_2}$ ) (curve B) and a leftward shift by a decrease in  $P\bar{V}_{O_2}$  (curve C). If  $P\bar{V}_{O_2}$  remains constant, it follows that the arteriovenous oxygen saturation difference increases with a rightward shift and decreases with a leftward shift. Since the rate of oxygen transfer from blood to mitochondria is related to the oxygen-pressure differential, it may be assumed that a rightward shift facilitates this transfer and a leftward shift impairs it.

### Physiologic Factors Affecting the Oxyhemoglobin-dissociation curve

Since Bohr's original treatise on the effect of pH on hemoglobin affinity for oxygen,<sup>1</sup> it has been recognized that  $[H^+]$  is the major

† The sigmoid shape of the normal oxygen-dissociation curve, a result of interaction of the globin chains of hemoglobin, indicates that oxygen affinity changes as oxygen is bound. This characteristic may be expressed empirically by the value of "n" of the Hill equation:

$$n = \left( \frac{1}{\log P_{O_2}} \right) \left[ \log \left( \frac{Y}{1-Y} \right) - \log K \right]$$

where  $P_{O_2}$  is oxygen pressure in mm Hg, Y is oxyhemoglobin fractional saturation, and K is a constant; in whole blood n is 2.6–3.0.

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physiologic factor producing instantaneous changes in the oxygen-dissociation curve. The curve shifts to the right with increasing  $[H^+]$ . At constant  $P_{CO_2}$ , the magnitude of this  $[H^+]$  effect is given by the relation  $\Delta \log P_{50}/\Delta pH = -0.40$ .<sup>2</sup> Thus, a change in pH of 0.10 would change the normal  $P_{50}$  by about 2.5 mm Hg.

While carbon dioxide was originally thought to exert its effect on hemoglobin-oxygen affinity solely via pH, it is now apparent that molecular  $CO_2$  also produces a pH-independent decrease in hemoglobin-oxygen affinity (a shift to the right of the oxygen-dissociation curve, an increase in  $P_{50}$ ) by binding to the N-terminal amino acid residues of hemoglobin as carbamate.<sup>3,4</sup> This is shown, for example, by the increase in Bohr effect of about 25 per cent when pH is changed by varying  $P_{CO_2}$ .<sup>2,5,6</sup>

The effect of temperature on hemoglobin-oxygen affinity is also of considerable physiologic importance, since an increase in temperature produces a rightward shift. Thus, this shift makes more oxygen available at times when a rise in temperature causes an increase in oxygen requirements or when an increase in oxygen requirements (exercise) is accompanied by a rise in temperature. Quantitatively, the relationship is given by the equation:  $\Delta \log P_{50}/\Delta T = -0.024$ .<sup>5</sup> As with pH, the effect of temperature is immediate and varies with the metabolic state.

Recently, Chanutin and Curmish<sup>7</sup> and Benesch and Benesch<sup>8</sup> have emphasized the role of certain erythrocytic organic phosphates, namely 2,3-diphosphoglycerate (DPG) and ATP, in influencing hemoglobin affinity for oxygen. The normal erythrocyte contains about 15  $\mu\text{mol/g}$  of hemoglobin. While both of these compounds lead to marked rightward shifts in the curve, DPG is far more important quantitatively. In hemoglobin solutions this effect is due to the binding of DPG to hemoglobin,<sup>9</sup> while its effect in erythrocytes also results from a reduction in intracellular pH via Donnan equilibrium.<sup>10</sup> In addition to its effect on the equilibrium of hemoglobin and oxygen, DPG increases the rate of deoxygenation of hemoglobin, both in solutions and in intact cells.<sup>11,12</sup>

The mechanism by which DPG increases is complex. It is dependent mainly on a decrease

in intracellular hydrogen ion concentration, which both promotes synthesis of DPG, especially by enhancement of DPG mutase activity, and decreases DPG degradation by 2,3-DPG phosphatase.<sup>13</sup> The increase in intraerythrocytic pH may be secondary either to an increase in the average amount of deoxyhemoglobin present at constant blood pH, as in anemia, hypoxemia, and cardiac failure, or to an increase in the pH of whole blood.<sup>14</sup>

A decrease in DPG results from acidosis<sup>14</sup> and, to a small degree, from hyperoxia.<sup>13,15</sup> It was formerly thought that DPG was mainly or exclusively bound to deoxyhemoglobin and that this binding led to increased synthesis of DPG. Recent evidence, however, suggests that differences in binding of DPG to deoxyhemoglobin and oxyhemoglobin are rather small and not likely, therefore, to account entirely for the increased DPG levels seen in hypoxic states.<sup>9</sup>

The effect of DPG upon  $P_{50}$  is further complicated by its interrelationship with both pH and  $CO_2$ . At normal DPG levels, effects of pH and  $CO_2$  on oxygen affinity are as described above. In blood depleted of DPG, however, the Bohr effect, measured at constant  $CO_2$  tension, decreases by about 25 per cent.<sup>2,6</sup> When it is measured by  $CO_2$  titration, there is an appreciable increase in the Bohr effect, which results from enhanced carbamate formation.<sup>6</sup> Expressed as  $\Delta P_{50}/\Delta BE$ , this specific effect of  $CO_2$  increases from 0.0022 in normal blood to 0.0052 in DPG-depleted blood. These interrelationships of pH,  $CO_2$  and DPG appear to result from the pH dependency of DPG binding,<sup>6</sup> the pH dependency of carbamate formation,<sup>16</sup> and the probable competition of DPG and  $CO_2$  for binding at the N terminus of the  $\beta$  chain.<sup>4,17,18</sup> The effect of temperature upon  $P_{50}$  is also influenced by DPG, at least in dilute solutions of hemoglobin. Thus, a given change of temperature produces a greater change in  $P_{50}$  in the absence of DPG than in presence of a large excess.<sup>19</sup> Whether this occurs under physiologic conditions in the erythrocyte is unknown.

Organic phosphates other than DPG and ATP have significantly lesser roles in influencing the affinity of hemoglobin for oxygen.<sup>7,20</sup> Their effect is chiefly the result of their influ-

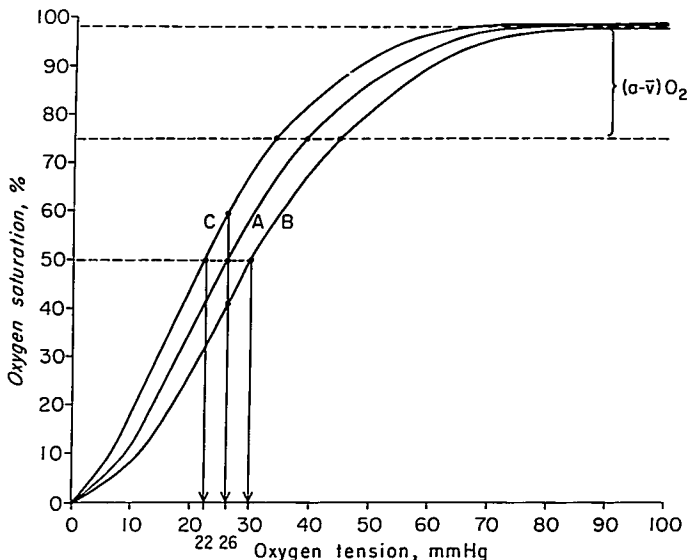


FIG. 1. Normal (A), rightward-shifted (B) and leftward-shifted (C) oxyhemoglobin-dissociation curves, showing normal, decreased, and increased affinity of hemoglobin for oxygen, respectively. A normal resting arteriovenous oxygen difference can be maintained only by an increase in  $P\bar{V}_{O_2}$  (mixed venous oxygen tension) in the case of a rightward shift (curve B) or by a decrease in  $P\bar{V}_{O_2}$  in the case of a leftward shift (curve C) if cardiac output remains unchanged.

ence on intracellular pH.<sup>10</sup> However, increases and decreases in inorganic phosphate increase and decrease the erythrocytic DPG level,<sup>21, 22</sup> and may underlie the effect of vitamin D on DPG synthesis.<sup>23</sup> No study has yet related a vitamin D deficiency to a DPG decrease.

Ionic strength can also affect the oxygen-dissociation curves of hemoglobin solutions to an important degree. In general, there is a rightward shift with increasing salt concentration which is somewhat dependent upon the species of ions present.<sup>19, 24, 25, 26</sup> The effects of changes in salt concentration on  $P_{50}$  of intact cells are complicated by the concomitant osmotic changes in corpuscular hemoglobin concentration. An effect of hemoglobin concentration on hemoglobin-oxygen affinity in hemoglobin solutions has also been demonstrated.<sup>19, 27</sup> Recent work has shown a small

effect on hemoglobin-oxygen affinity of osmotically-induced changes in mean corpuscular hemoglobin concentration (MCHC)<sup>28, 29</sup>; in this situation, the effect of MCHC on  $P_{50}$  is appreciably exaggerated when DPG is depleted and absent when DPG is increased.<sup>28</sup> Whether this effect is due primarily to the change in hemoglobin concentration, ionic strength, or another variable is unclear. Indeed, in hemoglobin solutions the coefficient  $\Delta P_{50}/\Delta[Hb]$  appears to increase with increasing concentrations of DPG.<sup>30</sup>

The magnitude of  $P_{50}$  change associated with simultaneous changes in pH, temperature, DPG, and hemoglobin concentration remains to be defined.

Hormonal influences also affect hemoglobin affinity for oxygen. Thyroid hormone increases DPG synthesis (therefore, decreases hemo-

globin-oxygen affinity), in a hemoglobin-free enzyme preparation, during incubation with intact cells, and in patients with hyperthyroidism.<sup>31, 32</sup> While the physiologic role of the thyroid hormone-induced  $P_{50}$  increase in thyrotoxicosis, and its importance in euthyroid homeostasis, are not yet clear, the presence of such a thyroid- $P_{50}$  interaction suggests that thyroid hormone may be a regulator of hemoglobin affinity for oxygen in the normal state.

Experiments in rabbits have suggested that cortisol and aldosterone can decrease hemoglobin-oxygen affinity. These compounds produce an effect when injected, but not when incubated with erythrocytes.<sup>33</sup> McConn,<sup>34</sup> however, has recently reported an effect on DPG and  $P_{50}$  of incubation of ACD blood with methylprednisolone.

Cell age also influences  $P_{50}$ . Young erythrocytes have less oxygen affinity, while older cells have greater affinity. This effect has been generally,<sup>35, 36, 37</sup> but not uniformly,<sup>38</sup> attributed to a decline in DPG with cell age, presumably a result of decreasing activity of glycolytic enzymes.

A comprehensive molecular model which accounts for many of the above physiologic effects, based on crystallographic studies and correlated with chemical findings, has recently been suggested by Perutz.<sup>18</sup>

#### Alterations of Hemoglobin-Oxygen Affinity in Health and Disease

As indicated above, a rightward shift in the oxygen-dissociation curve allows release of a given amount of oxygen with less decrease in oxygen pressure. While this adaptation would appear advantageous to the organism, and the converse situation disadvantageous, there is little direct evidence indicating the relative physiologic importance of the shifts.

Prediction of the consequences of shifts in oxygen dissociation on cellular oxygen would require definition of oxygen diffusion *in vivo*, determination of average and maximal distances through which oxygen must diffuse, and definition of the oxygen affinities of the various subcellular respiratory systems. While quantitative data are not available, Chance has reasoned that the oxygen affinities of the microsomal and perhaps the peroxisomal systems,

but not the cytochrome systems, are such that oxygen availability might be affected by oxygen tension changes in the range provided by shifts of the curve.<sup>39</sup> In addition, he has reported evidence of impaired cytochrome respiration in tumor tissue *in vivo* when reduced oxygen tension and the geometry of the system together impede cellular oxygen supply. Thus, when arterial oxygen saturation was reduced, a reduction of pyridine nucleotides was observed in cells remote from blood vessels, while those nearby received adequate oxygen. Thus, it is conceivable that a shift in the oxygen-dissociation curve might alter oxygen availability under like circumstances.

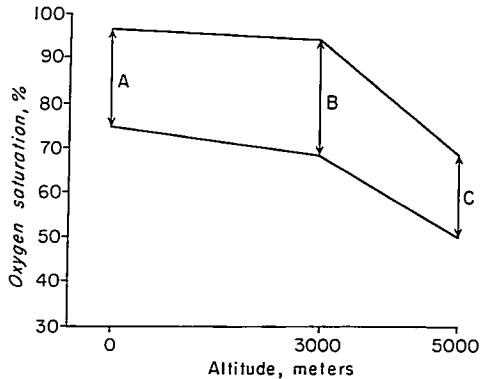
In addition to this theoretical evidence for the importance of changes in hemoglobin-oxygen affinity, at least two other arguments may be set forth. First, changes in the blood hemoglobin level, as described below, do occur in response to shifted oxygen-dissociation curves, indicating the sensitivity of at least one indicator tissue to such changes in oxygen tension. Second, in health and disease changes in hemoglobin-oxygen affinity which usually appear appropriate for the circumstances occur in a wide variety of animals and man. However, more data describing the effect of  $P_{50}$  on oxygen availability are needed. Changes in hemoglobin-oxygen affinity occurring in health and in various abnormal states are summarized below.

Acute changes in pH,  $CO_2$ , and temperature have immediate effects on hemoglobin-oxygen affinity, as described above. These factors appear to permit increased oxygen release in exercise.<sup>40</sup>

Acute acidosis improves oxygen release for the first several hours, although this is eventually offset by a decline in DPG due to the effect of reduced pH on cellular metabolism. Conversely, acute alkalosis, as in early endotoxin shock,<sup>41</sup> would impair oxygen delivery via the Bohr effect. With sustained acidosis and alkalosis, however, compensatory decreases and increases in DPG, respectively, partially compensate for the pH changes.<sup>42</sup>

Sudden correction of acidosis of more than a few hours' duration results in an appreciable leftward shift of the *in-vivo* oxyhemoglobin-

FIG. 2. Systemic arteriovenous oxygen differences at sea level (A) 3,000 meters (B) and 5,000 meters (C), corrected for increase in DPG only. Note that the right-shifted oxyhemoglobin-dissociation curve allows a greater arteriovenous oxygen difference at 3,000 meters (B) and therefore can be considered "adaptive," while the arteriovenous oxygen difference at 5,000 meters is less, the decrease in  $P_{aO_2}$  having impaired oxygen loading sufficiently to preclude an adaptive role of the rightward shift.



dissociation curve which may impair oxygen availability. This appears especially relevant to therapy for diabetic ketoacidosis.<sup>43</sup>

Within 24 to 48 hours after exposure to high altitude, there is a rightward shift in the oxyhemoglobin-dissociation curve<sup>44</sup> which is mediated by an increase in DPG, seemingly caused by an increase in pH and a decline in  $P_{CO_2}$ . The degree to which this rightward shift is beneficial depends in part on the altitude, as a rightward-shifted curve also decreases oxygen loading when  $P_{aO_2}$  is appreciably reduced<sup>45</sup> (fig. 2). Thus, at altitudes above 4,000 meters, it affords no advantage. The fact that volunteers given acetazolamide (Diamox) did not have increased DPG on ascent to high altitude,<sup>46</sup> yet experienced fewer symptoms,<sup>47</sup> indicates the importance of acid-base changes in relation to altitude.

A rightward shift in the oxyhemoglobin-dissociation curve mediated by DPG appears to be a major adaptive response to congestive heart failure, the magnitude of such change being proportional to the severity of heart failure.<sup>48, 49</sup> Such measurements may be useful objective indices of the severity of cardiac disease.

During angina pectoris, a shift of the oxyhemoglobin-dissociation curve occurs within minutes of the onset of pain. This shift, yet unexplained, is unrelated to erythrocytic DPG,

ATP, or pH, MCHC, pH *in vivo*, or lactate production.<sup>50</sup>

A DPG-mediated decrease in hemoglobin-oxygen affinity also occurs in hypoxemia as a result of right-to-left shunts.<sup>51</sup> As at high altitudes, the degree of benefit decreases with severity of hypoxemia. Increases in DPG and  $P_{50}$  also occur in pulmonary disease associated with hypoxemia,<sup>52</sup> but less consistently. At times, leftward-shifted as well as normal curves are encountered, presumably owing to the effects of other factors such as pH,  $CO_2$  and drugs.

A DPG-mediated rightward shift in the oxygen-dissociation curve occurs with anemia of various types.<sup>53, 54, 55</sup> This shift is generally proportional to the severity of the anemia.

Of the endocrinologic disturbances, hyperthyroidism and hypothyroidism are associated with rightward and leftward shifts of the oxygen-dissociation curve, respectively, which tend to revert to normal after treatment.<sup>52, 56</sup> Whether this results chiefly from the direct effect of thyroid hormones on erythrocytic enzymes<sup>51</sup> or is related to the individual's total oxygen requirement is unknown.

Panhypopituitarism is also associated with a low DPG level, despite an appreciable erythrocyte-mass deficit.<sup>57</sup> An increased DPG level is characteristic in anemia, so its absence may be a useful indication of an adaptive decrease in erythrocyte mass.

Hepatic cirrhosis has an associated increased  $P_{50}$  (pH 7.4, 37 C). Whether this represents an adaptive response is not clear.<sup>58, 59, 60</sup> In addition, the mechanism of this shift is still uncertain.

Inhalation of carbon monoxide induces a leftward shift of the oxygen-dissociation curve which probably results from both an effect of carboxyhemoglobin on oxygen affinity and a decrease in DPG.<sup>15, 61</sup> Apparently carbon monoxide inhibits erythrocytic glycolysis, reducing the formation of DPG.<sup>62</sup> Whichever mechanism prevails, the leftward shift probably interferes with  $O_2$  release into the tissues; resultant impairment of fetal oxygenation during pregnancy may help to explain the higher incidence of prematurity and smaller babies among smoking mothers.<sup>63, 64</sup> However, specific data relating impaired unloading of oxygen to the fetus as the mechanism for the problems cited above remain unavailable. It is apparent theoretically, at least, that any leftward shift of the maternal curve might impair oxygen unloading to the fetus.<sup>65, 66</sup> That several generations of mothers with hemoglobin<sub>M</sub> have had no problems with gestation, despite their  $P_{50}$ 's being lower than fetal  $P_{50}$ ,<sup>67</sup> does not minimize this concept, because such individuals have the benefit of a compensatory erythrocytosis, whereas smokers do not. That the presence of carboxyhemoglobin is associated with a decrease in venous  $P_{O_2}$  appears to be established.<sup>68</sup>

Along these lines, it is tempting to speculate that the higher incidence of coronary events in cigarette smokers may be related to the CO-induced leftward shifts of their oxygen-dissociation curves.

Conversion of hemoglobin to methemoglobin, like conversion to carboxyhemoglobin, renders the liganded heme groups inactive and shifts the oxygen-dissociation curve of the remaining normal groups to the left.<sup>69, 70</sup> Methemoglobinemia may arise in normal subjects after exposure to a wide variety of drugs or chemicals, while subjects with congenital methemoglobinemia have either a deficiency of one of the enzymes which maintain hemoglobin in a reduced state or an abnormal hemoglobin particularly susceptible to oxidation.<sup>71</sup>

The factors that influence  $P_{50}$  are listed in table 1.

### Effects of Genetic Alterations in Erythrocytic Enzymes and Hemoglobin on the Oxygen-dissociation Curve

Genetically determined deficiencies in certain erythrocytic glycolytic enzymes influence hemoglobin affinity for oxygen by affecting DPG homeostasis. Hexokinase deficiency produces a block early in glycolysis, thereby decreasing synthesis of DPG and increasing hemoglobin-oxygen affinity. Hemoglobin in such patients is variable but uniformly subnormal;  $P_{50}$  in one patient was 19 mm Hg.<sup>72-75</sup> Conversely, pyruvate kinase deficiency results in a marked accumulation of DPG and other glycolytic intermediates up to the enzymatic defect. The resulting  $P_{50}$  is in the range of 38 mm Hg. In this condition the hemoglobin concentration is also variable, and generally subnormal.<sup>72, 76, 77</sup> Both of these disorders are associated with appreciable hemolysis. An alteration in DPG due to a deficiency of DPG mutase has also been reported by Schröter,<sup>78</sup> but data on oxygen equilibrium are not available.

Analysis of a group of hemolytic anemias has suggested that the hemoglobin level is more closely related to  $P_{50}$  than to the degree of hemolysis.<sup>79</sup> Thus, a subnormal hemoglobin level appears appropriate for pyruvate kinase deficiency, while a higher value might be expected for hexokinase deficiency. This may indicate that the degree of hemolysis in the latter disorder precludes such compensation.

Genetically determined alterations of erythrocytic enzymes may also affect hemoglobin function by failure to maintain hemoglobin in the normal reduced state.<sup>71</sup> As described above, the resulting methemoglobin increases the oxygen affinity of the remaining normal hemoglobin.

Genetic abnormalities of hemoglobin structure, generally due to a single amino-acid substitution or deletion at a critical part of the molecule, may be associated with increased or decreased affinity for oxygen.<sup>80</sup> This effect may result from an intrinsic characteristic of the altered molecule, as in hemoglobin<sub>Chesapeake</sub>, or from altered reactivity with DPG, as in fetal hemoglobin.<sup>17, 81</sup> In some hemoglobinopathies a relatively small complement of the abnormal

TABLE 1. Causes of Alterations in Hemoglobin-Oxygen Affinity

Factors That Increase $P_{50}$	Factors That Decrease $P_{50}$
By direct or unknown action Increased temperature Increased $[H^+]$ Increased DPG (and ATP) Increased Hb concentration Increased ionic strength Abnormal hemoglobin Cortisol Aldosterone Pyridoxol phosphate (in Hb solution) <sup>91</sup> Cell age ?	By direct action Decreased temperature Decreased $[H^+]$ Decreased $P_{CO_2}$ Decreased DPG (and ATP) Decreased Hb concentration Decreased ionic strength Abnormal hemoglobin Carboxyhemoglobin Methemoglobin Cell age ?
By increasing DPG in cells Decreased $[H^+]$ Thyroid hormone Erythrocytic enzyme deficiency Cell age Increased inorganic phosphate Inosine Increased sulfate	By decreasing DPG in cells Increased $[H^+]$ Decreased thyroid hormone Erythrocytic enzyme deficiency Cell age Decreased inorganic phosphate

hemoglobin may produce an effect on the oxygen affinity of whole blood beyond that expected for the amount present, presumably by hybridization.<sup>82</sup> Genetically determined abnormalities of hemoglobin function other than or in addition to a simple shift of oxygen dissociation are also recognized. Thus, the normal sigmoid shape of the curve, or reactivity with hydrogen ion, may be changed. Likewise, a mutant hemoglobin may be highly susceptible to oxidation and denaturation; in such cases the normal enzyme system of the cell may not be able to prevent some methemoglobin formation.<sup>80</sup>

Hemoglobinopathies with increased and decreased oxygen affinity appear to limit and to facilitate tissue oxygen delivery, respectively. This results in increased and decreased erythropoietin secretion and consequent erythrocytosis and anemia. It is of interest that the final hemoglobin level is sometimes not as high or low as would be predicted from  $P_{50}$ . These anomalies are explained, however, by the shape of the curve and the fact that oxygen delivery, and presumably erythropoietin elaboration, relate to the upper end of the curve rather than to the midpoint.<sup>80</sup>

While physiologic consequences of these genetically determined alterations in hemoglobin-oxygen affinity and resulting hematocrit ad-

justments are only partially known, patients generally appear healthy. Maximal exercise tolerance might be limited, however, both in anemia, because of the decrease in the oxygen-carrying capacity of the blood,<sup>83</sup> and in erythrocytosis, because of the blood's increased viscosity and increased affinity for oxygen. In addition, an individual whose blood has an increased affinity for oxygen may not adapt normally to high altitudes and may tolerate anemia poorly. One might also expect the fetuses of individuals with left-shifted curves not to withstand intrauterine life. However, the lack of fetal distress due to high hemoglobin-oxygen affinity appears explained by the compensatory erythrocytosis.<sup>67, 84, 85</sup>

#### Effects of Therapeutic Intervention on the Affinity of Hemoglobin for Oxygen

The use of massive blood replacement and exchange transfusion for conditions such as cardiac surgery, severe bleeding, hemolytic disease of the newborn, and hepatic coma is common. It is well established that erythrocytes lose DPG progressively with increasing hemoglobin-oxygen affinity during storage.<sup>36, 86-89</sup> Following transfusion, the half-recovery time for DPG is four hours; it takes several days to reach its final level.<sup>90, 91</sup> Thus, trans-

fusion of massive amounts of blood may impair oxygen delivery.

Despite the use in cardiac surgery of blood which has not been stored more than three days,  $P_{50}$  is often less than 20 mm Hg post-operatively<sup>92</sup>;  $P_{50}$  may take as long as seven days to reach normal levels and as long as 13 days to reach the final level rightward of normal. A value of 20 mm Hg or less represents an appreciable leftward shift for the postoperative patient, because patients with congestive heart failure usually have  $P_{50}$ 's greater than 30 mm Hg. Other things being equal, such a change would necessitate either a marked increase in cardiac output or a reduction in the average venous oxygen tension, with possible cellular anoxia. The effect of this decrease may in fact be potentiated by inadequate perfusion in the early postoperative period owing to depressed myocardial function.

Oxygen availability during cardiopulmonary bypass may also be questioned, because hypothermia, certain acid-base changes, and a progressive decline in DPG together will increase hemoglobin-oxygen affinity *in vivo*. It is difficult to estimate the net result of these changes in view of reduced metabolism, altered blood flow distribution, and other variables.<sup>90</sup> Nevertheless, it is clear that hypothermia as a technique has generally proved effective.<sup>93, 94</sup>

Exchange transfusion of the newborn involves removal of cells containing fetal hemoglobin that has a leftward-shifted oxygen-dissociation curve. These cells are then replaced with normal adult cells with hemoglobin that has a normally-positioned curve. This procedure improves oxygen unloading<sup>95</sup>; any effect on oxygen loading should be minimal. Indeed, such treatment may be useful for respiratory distress syndrome.<sup>96</sup> It is of interest that intrauterine transfusion for erythroblastosis fetalis produces no obvious fetal impairment,<sup>97</sup> suggesting that the decrease of hemoglobin-oxygen affinity *in utero* does not impair placental oxygen transport to an important degree.

As described above, the sudden correction of acidosis, as in treatment of metabolic acidosis, produces an immediate increase in hemoglobin-oxygen affinity, with a possible decrease

in cellular oxygen availability. Likewise, rapid induction of alkalosis, as in hyperventilation, has been associated with deleterious arrhythmias, a situation in which the attendant increase in hemoglobin-oxygen affinity, in addition to other established factors, may play some part.<sup>98</sup>

Rightward shifts of the oxygen-dissociation curve have been attributed to certain volatile anesthetics.<sup>99</sup> However, a recent study suggests that these shifts may be artifacts resulting from the effects of the anesthetics on the oxygen electrodes.<sup>100</sup>

Hyperoxia has been associated with a small DPG-mediated decrease in  $P_{50}$ .<sup>12, 13</sup>

#### Potential Role of Drugs to Control the Affinity of Hemoglobin for Oxygen in Disease

As described above, little information about the effects of changes of oxygen affinity on oxygen availability in the intact organism is currently available. Assuming that such effects can be demonstrated, there are a number of situations in which a change in oxygen affinity might be desirable. However, it should be realized that chronically-maintained alterations in hemoglobin-oxygen affinity, as in hemoglobinopathies, would lead to alterations in the circulating hemoglobin level. Unless prevented in some other way, these hemoglobin adjustments would negate any benefit provided by the shifted curve.

As has been described, massive or exchange transfusion of stored blood represents one circumstance where modification of oxygen affinity may be useful. This would appear particularly appropriate in situations where there is already evidence of impaired oxygen supply to one or more organs.

Patients with normovolemic hypotension, such as those with septic or cardiogenic shock, might profit from reduction of hemoglobin-oxygen affinity. Although acidosis, which is often present, would provide immediate improvement in oxygen affinity *in vivo*, this effect would diminish rapidly as DPG decreased.<sup>14</sup>

Congestive heart failure is ordinarily associated with an increased peripheral extraction of oxygen, especially during exercise, which tends



to decrease venous oxygen tension. While this is partially corrected by a rightward shift of the curve,<sup>42</sup> a further shift would seem useful in patients with moderate failure. In patients with severe heart failure who also manifest arterial desaturation and considerable rightward shifts, however, further rightward shifts would only encroach upon oxygen loading, negating any advantage of oxygen unloading. Similar reasoning applies to congenital heart disease with right-to-left shunt and to pulmonary disease associated with arterial desaturation, where there is already a right-shifted curve.<sup>45</sup>

Patients who have localized tissue hypoxia, such as angina pectoris, might also benefit from changes in hemoglobin-oxygen affinity. Such a contention is perhaps supported by the frequency of worsening of angina, with development of anemia. Cerebrovascular insufficiency might also be improved by a shift in the oxygen-dissociation curve. Available data suggest that the relatively small change in oxygen tension provided by a shift of the curve would not affect oxygen delivery in normally-perfused brain cells,<sup>101</sup> and that such a shift might be beneficial when blood flow is compromised. The temporary improvement of intellect in patients with severe cerebrovascular disease after administration of oxygen at high pressure is of interest in this connection.<sup>102</sup>

Several agents can alter the affinity of hemoglobin for oxygen, usually by producing changes in DPG. Possible approaches include alteration of oxygen dissociation by systemic administration of drugs and modification of homologous or autologous blood *in vitro*, with subsequent transfusion.

Several methods for maintenance or restoration of DPG in stored blood have been proposed. These include storage of CPD, which produces a lesser decrease in DPG during storage,<sup>88, 103, 104</sup> storage in a new artificial medium,<sup>105</sup> and supplementation of media with adenine and inosine, either during storage or prior to transfusion.<sup>106</sup> Recently, Duhm *et al.* and Oski *et al.* have reported total normalization of DPG and  $P_{50}$ , as well as appreciable increases to above normal of both, following incubation with inosine, inorganic phosphate, and pyruvate.<sup>89, 107</sup> Methylprednisolone has

also been reported to decrease the oxygen affinity of stored blood.<sup>34</sup> While these approaches have been applied to stored blood, those capable of increasing DPG to above normal could be applied *in vitro* to the patient's own blood, with subsequent transfusion.

Alteration of DPG *in vivo* is another approach. Thus, Pollock *et al.* have reported some changes in DPG and  $P_{50}$  in monkeys given inorganic phosphate, pyruvate, and inosine intravenously.<sup>108</sup> Methylene blue is another nontoxic compound reported to alter DPG.<sup>109, 110</sup> There is evidence that sulfate also affects DPG *in vitro* and *in vivo*,<sup>111</sup> and therefore might be useful pharmacologically. Thus, it seems that some combination of these drugs, their chemical congeners, or other agents may prove capable of altering DPG *in vivo*, with resulting effects on hemoglobin-oxygen affinity.

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### Obstetrics

**NEONATAL RESPIRATORY DISTRESS** The author proposes that hyaline membrane disease, hyaline membrane-like disease without membranes, lobar opacification, some cases of intra-alveolar pulmonary hemorrhage, and small areas of atelectasis in the lungs of newborn infants are all variants of one syndrome caused by aspiration of maternal blood in amniotic fluid during delivery. Although no evidence is presented, certain clinical observations, heretofore unexplained, appear to be accounted for by the theory: 1) In premature-twin delivery, the second twin is *in utero* longer than the first twin, has a greater chance to aspirate blood, and, in fact, does have a higher incidence of respiratory distress than the first twin. 2) In an isolated instance of a triplet delivery, the smallest twin, delivered with membranes around the head, had no respiratory distress; the next twin had slight respiratory distress; the last twin, the largest, had marked respiratory distress. This finding formed the basis for the report. 3) The peak incidence of respiratory distress in newborns occurs in infants with body weights between 1.0 and 1.5 kg, with gradual decreases in those lighter and those heavier. In the lighter group, most alveoli are needed to sustain life; if any alveoli malfunction because of aspiration of maternal blood, death occurs before respiratory distress can develop. Some infants in this group are too weak to aspirate during delivery; this also reduces the incidence of respiratory distress in this group. In the heavier, more mature group, there is greater pulmonary reserve, and the incidence of respiratory distress also decreases. (Pender, C. B.: *Respiratory Distress in Multiple Births and Premature Infants*, *Am. J. Obstet. Gynecol.* 112: 298-299, 1972.)