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Erythrocyte Metabolism: Interaction with Oxygen Transport

New findings demonstrate that oxygen transport by the erythrocyte is dependent upon its metabolism.

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A remarkable series of studies has recently demonstrated an important relationship between the metabolism and function of the red cell as an organ of gas transport. It is now clear that the nonnucleated mammalian red cell, once thought to be an inert bag of hemoglobin, has an active carbohydrate metabolism which is crucial both to the viability of the cell and to the proper modulation of oxygen transport [for recent reviews, see (*1*)]. This new information cuts across interdisciplinary borders and involves the relatively diverse fields of red cell biochemistry, blood banking, cardiopulmonary and exercise physiology, and comparative biochemistry and physiology.

The major pathways of carbohydrate metabolism in the mature mammalian red cell are illustrated in Fig. 1. The phosphorylated intermediates shown between the enzymatic steps (arrows) comprise most of the important organic phospho compounds of the red cell (*2*); the amounts of those compounds present in greatest quantity in fresh human red cells are circled in Fig. 1. The levels of adenosine triphosphate (ATP) are relatively high for a cell which does not have a mechanism for generating ATP through oxidative phosphorylation. Even more striking, however, is the very large amount of 2,3-diphosphoglycerate (DPG), which is present at a concentration about four times that of ATP. 2,3-Diphosphoglycerate occurs in only trace amounts in other mammalian cell types (*3*), and examination of the DPG shunt (Fig. 1) reveals that it is not a useful pathway for energy (that is, ATP) production. Due to its lack of apparent function, the high concentration of DPG within the red cell has long puzzled red cell biochemists. Another puzzle has been the fact that the oxygen affinity of intact red cells (Fig. 2, curve *B*) is much less than that of purified hemoglobin (Fig. 2, curve *A*). That is, intact red cells release oxygen at an oxygen pressure much higher than that at which hemoglobin solutions release it. Such a decrease in oxygen affinity (a shift of the oxygen dissociation curve to the right) is vital to life, since it allows the red cells to release oxygen in the tissue capillaries at a relatively high oxygen tension. [Note that in curve *B* of Fig. 2, 50 percent of the oxygen would be released at an oxygen pressure (pO_2) of 26.5 mm-Hg, whereas in curve *A* almost no oxygen would be released at that pressure.] It appears now that both puzzles have been resolved: in the presence of physiological levels of DPG and, to a lesser extent, of ATP, the oxygen affinity of purified hemoglobin is greatly decreased, approaching that of intact cells (*4, 5*) (Fig. 2, curve *B*). This phenomenon implies that red cell function is dependent upon the cell's metabolic status.

The first clue to the relationships between metabolic intermediates and the oxygen dissociation curve came from studies by Sugita and Chanutin (*6*) in which they found that a hemoglobin–organic phosphate complex was responsible for a minor hemoglobin component which appeared in the course of free boundary electrophoresis. Further work (*7*) indicated that the addition of DPG and some other phosphorylated intermediates would produce the largest increase in the proportion of this hemoglobin-phosphate complex. Later, Chanutin and Curnish (*4*) and Benesch and Benesch (*5*) reported, almost simultaneously, that the presence of phosphorylated intermediates such as DPG or ATP would produce a concomitant reduction in the oxygen affinity of hemoglobin solutions.

Hemoglobin Binding of DPG

The results obtained by Chanutin and Curnish and by Benesch and Benesch indicated that a combination of hemoglobin with the phosphorylated intermediates of glycolysis was probably responsible for most of the decrease in oxygen affinity of hemoglobin within the red cell. There has been, however, disagreement on certain aspects of the binding of these intermediates to hemoglobin. Benesch and his co-workers (*5, 8*) have consistently reported that, though deoxyhemoglobin binds DPG rather strongly, oxyhemoglobin (under their experimental conditions) will not bind DPG at all. However, other investigators (*9, 10*) have found that oxyhemoglobin has about half the affinity for DPG that deoxyhemoglobin has. In addition, there appear to be at least two sites of binding, one with much stronger affinity than the other. The lower affinity site is presumably of less physiological significance. Chanutin and Hermann (*9*) report that the association constants of DPG with deoxyhemoglobin and oxyhemoglobin (for the high affinity site) are 1.4×10^5 and

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7.0×10^4 liters per mole, respectively. Garby, Gerber, and deVerdier (*10*), using a more physiological system, have also reported that ATP and DPG are bound by oxyhemoglobin approximately half as tightly as by deoxyhemoglobin, and their evidence also indicates that there are at least two binding sites.

Garby and deVerdier (*11*) have made estimates of the amounts of bound and free DPG which would exist in oxygenated and deoxygenated human erythrocytes in vivo. They collated the data of Benesch *et al.* (*12*), of Chanutin and Hermann (*9*), and of Garby *et al.* (*10*) (Table 1). It may be seen that there is a large variation in these estimates, most of it perhaps due to differences in experimental design and conditions. It is not yet known what influence states of intermediate oxygenation have upon the affinity of hemoglobin for DPG. This last question is of critical importance to an understanding of the mechanisms of regulation of DPG levels in vivo.

Although the exact sites of binding of DPG and ATP to hemoglobin are not yet known with certainty, some fairly strong evidence points to a site or sites on the β-chain. Benesch and Benesch (*13*) pointed out that the binding might be to the β-chain, since, although isolated α-chains do not bind DPG at all, isolated β-chains were found to bind ¼ mole of DPG per mole of β-chain. It was suggested that the binding might occur between the two β-chains within the central cavity of the hemoglobin molecule. The entrance to the central cavity of oxyhemoglobin is probably too small to admit the DPG molecule, but, as a result of the 6-angstrom increase in the size of the cavity entrance which occurs in full deoxygenation (*14*), the 9-angstrom DPG molecule could easily be accommodated by deoxyhemoglobin. Furthermore, Muirhead *et al.* (*14*) note a region, in their three-dimensional Fourier synthesis of human deoxyhemoglobin, which differs from the oxyhemoglobin and "probably represents a phosphate or sulphate ion present in deoxy- and absent in oxyhemoglobin." This region is very near the histidine residue (discussed below), which may be responsible for a part of the binding of DPG to deoxyhemoglobin. The binding of DPG between the β-chains of deoxyhemoglobin would help explain what appears to be the partial competition between oxygen and DPG.

As far as we know, it has not been determined how much of the 6-angstrom shift between β-chains at the entrance to the central cavity that occurs with total deoxygenation also occurs in states of partial deoxygenation. Our recent work indicates that partially oxygenated hemoglobin (50 to 60 percent of saturation) may bind about as much DPG as completely deoxygenated hemoglobin will. This implies that the loss of one of the four oxygen molecules from fully oxygenated hemoglobin may increase the DPG binding affinity as much as complete deoxygenation (the loss of all four oxygen molecules) does. Therefore, relatively small changes in the physiological range of average venous saturation (70 to 80 percent) may have proportionately large effects on the amount of DPG that is bound to hemoglobin within the circulating erythrocyte.

Garby *et al.* (*10*) and Garby and deVerdier (*11*) have further discussed the binding site and have pointed out that data on the *p*H dependence of binding suggest that a histidine residue on the β-chain may be responsible. Binding to this histidine residue, H21 (His^{143}, residue 143 from NH_2 terminus), is particularly likely since hemoglobin F, which has a serine in place of this histidine, binds DPG much less strongly than hemoglobin A does. Fur-

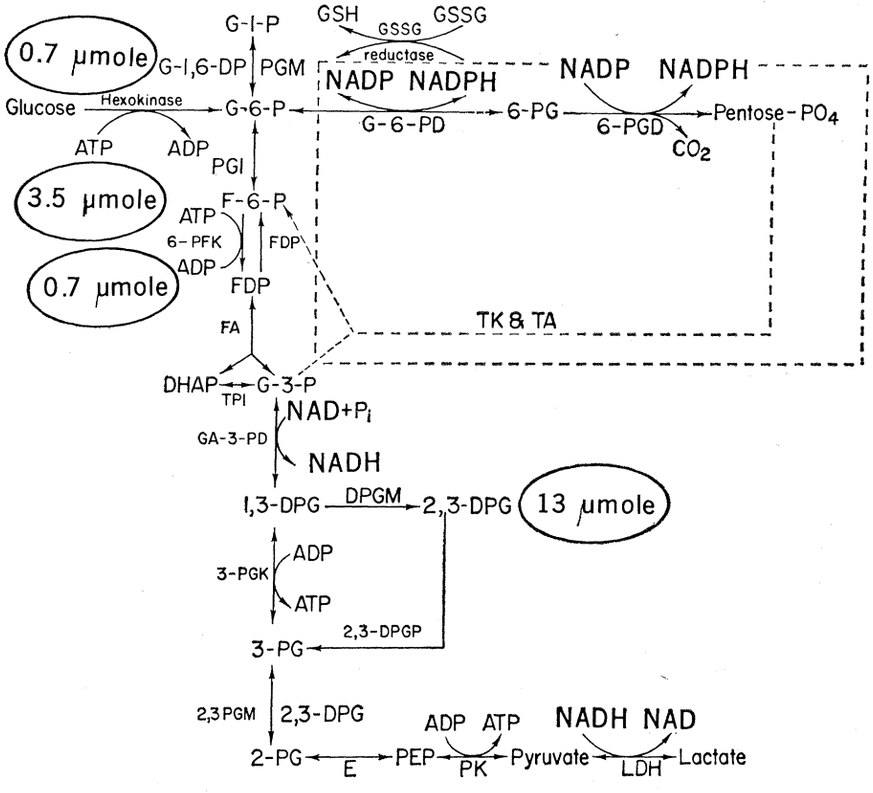

Fig. 1. The major pathways of carbohydrate metabolism in mature mammalian red cells. The arrows represent enzymatic steps. The circled numbers indicate amounts, in micromoles per gram of hemoglobin, of phosphorylated intermediates present in highest concentrations in human erythrocytes. Abbreviations: ATP and ADP, adenosine triphosphate and diphosphate, respectively; G-6-P, glucose 6-phosphate; NADP and NADPH, oxidized and reduced nicotinamide adenine dinucleotide phosphate, respectively; GSSG and GSH, oxidized and reduced glutathione, respectively; G-6-PD, glucose-6-phosphate dehydrogenase; 6-PG, 6-phosphogluconate; 6-PGD, 6-phosphogluconate dehydrogenase; TK, transketolase; TA, transaldolase; F-6-P, fructose 6-phosphate; FDP, fructose, 1,6-diphosphate; DHAP, dihydroxyacetone phosphate; G-3-P, glyceraldehyde 3-phosphate; NAD and NADH, oxidized and reduced nicotinamide adenine dinucleotide, respectively; P_i, inorganic phosphate; 1,3-DPG, 1,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; PEP, phosphoenol pyruvate; PK, pyruvate kinase; LDH, lactate dehydrogenase; PGI, phosphohexose isomerase (also called PHI); 6-PFK, 6-phosphofructokinase; FA, fructoaldolase; TPI, triosephosphate isomerase; GA-3-PD, glyceraldehyde-3-phosphate dehydrogenase; DPGM, diphosphoglycerate mutase; 2,3-DPGP, 2,3-diphosphoglycerate phosphatase; 3-PGK, 3-phosphoglycerate kinase; 2,3-PGM, 2,3-phosphoglycerate mutase; E, enolase; PGM, phosphoglycerate mutase; G-1-P, glucose 1-phosphate; G-1,6-DP, glucose 1,6-diphosphate.

ther, hemoglobin Hiroshima, which is probably a single substitution ($His^{143} \rightarrow$ Asp), has a high oxygen affinity, perhaps reflecting a lack of affinity for DPG. Bunn and Briehl (*15*) have more recently suggested that DPG binding to hemoglobin may also involve the NH_2-terminal valine of the β-chain. It may be that DPG binds to His^{143} of one β-chain and to the terminal valine of the other. The question of which residue (or residues) is responsible for the binding of DPG has not yet been completely settled.

Several other factors, aside from DPG concentration and the state of hemoglobin oxygenation, strongly influence the binding of DPG to hemoglobin (*10, 12*). An increase in *p*H above about 7.4 will reduce the binding affinity, and a decrease in *p*H below 7.4 will increase binding. It has also been reported that high ionic strength will decrease binding, and at low ionic strengths the binding of DPG to hemoglobin is very strong and no longer dependent on the state of oxygenation. The binding is also temperature-dependent (*12*), being much stronger at low temperatures. It appears that any change in the environment of the circulating erythrocyte which affects either the *p*H or the level of deoxygenated hemoglobin will affect the binding of DPG and, possibly, of other intermediates. These factors may therefore have a modulatory effect in vivo on the concentrations of red cell metabolic intermediates. The physiological importance of variations in ionic strength and temperature to DPG binding remains to be evaluated.

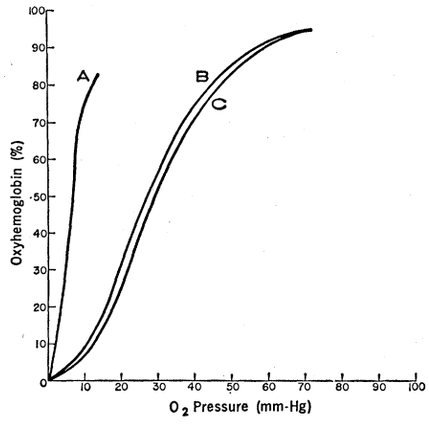

Fig. 2. Oxygen dissociation curves of (*A*) purified human hemoglobin, "stripped" of DPG; (*B*) normal human erythrocytes (or purified hemoglobin in the presence of physiological levels of DPG); and (*C*) erythrocytes from a typical hypoxic individual. Each of these curves is a representation of the amounts of hemoglobin (in percentage of the total) which will be saturated with oxygen at the given oxygen pressures. These curves are generally constructed by equilibrating hemoglobin or whole red cells with gas mixtures of varying oxygen tensions, and then determining the percentage of saturation of the hemoglobin. The oxygen affinity of intact red cells (curve *B*) is much less than that of purified hemoglobin (curve *A*); this is called a "right shift" of the oxygen dissociation curve. The position of the curve is often defined in terms of its P_{50}—that is, the pressure of oxygen required to half-saturate with oxygen. Curve *B* has a P_{50} of 26.5 mm-Hg; curve *C* has a P_{50} of 29.5 mm-Hg, which can be attained by increasing the DPG concentration of the erythrocytes by about 15 percent.

Modulation of Hemoglobin Function by DPG

The regulatory effect of DPG upon hemoglobin function is not restricted to the gross adjustment by DPG of the oxygen affinity of hemoglobin of red cells. Both the considerable quantitative variation in the levels of red cell DPG among healthy people and the elevations in the levels of erythrocyte DPG in various hypoxic conditions significantly influence oxygen transport. Relatively high concentrations of red cell DPG will produce a corresponding shift of the oxygen dissociation curve to the right, which will in turn improve oxygen delivery, as discussed above (see Fig. 2).

A considerable number of data now available indicate modulation of hemoglobin function by quantitative variation in the levels of erythrocyte DPG among normal individuals. The first such study involved the demonstration of a significant negative correlation between red cell DPG levels and whole blood hemoglobin levels in normal men and women (*16*). This finding has been amply confirmed in both normal and anemic subjects and in animals (*17–20*). Although such correlations do not prove a cause-and-effect relationship, they are consistent with either of two hypotheses (based on the supposition of a causal relationship): (i) Individuals with levels of erythrocytic DPG which are on the high side of normal will maintain low hemoglobin levels, since a relatively low number of circulating red cells may provide adequate oxygen delivery in the tissues, and vice versa. Thus, the erythropoietic response, and the resulting level of hemoglobin, are influenced by the multitude of factors which affect oxygen delivery, including DPG levels. This hypothesis makes DPG levels a relatively predetermined or primary, factor in a given environment, with hemoglobin levels secondarily determined. (ii) In individuals with low hemoglobin levels, mechanisms operate to raise DPG levels to achieve adequate oxygenation, and vice versa. This hypothesis implies that DPG levels respond to differences in hemoglobin levels.

Of course, the two hypotheses are not mutually exclusive, and there is evidence from certain conditions that either variable is adjustable, with the final DPG-hemoglobin balance depending on a number of covarying factors. In genetic abnormalities of erythrocyte glycolysis in which DPG levels are low, hemoglobin levels may be abnormally high (*21*). On the other hand, defects leading to abnormal hemoglobin levels are characterized by reciprocal DPG responses. In polycythemia produced experimentally in rats, erythrocytes have low DPG levels (*20*), and in anemias of various etiologies the DPG levels are high (*18, 22, 23*). Thus, when one variable is fixed, the other may vary reciprocally; this suggests that the blood is programmed for adequate oxygen delivery through adjustments of both the number of circulating red cells and the DPG levels of the cells.

Implicit in the above concepts is the assumption that physiological variation in DPG levels significantly affects oxygen transport. This assumption is supported by two types of data. Rorth (*24*) has shown a good correlation between P_{50} (a measure of the position of the oxygen dissociation curve, explained in the legend to Fig. 2) and DPG levels, within a sample of normal individuals. Further, Eaton *et al.* (*23*) have shown a positive correlation between DPG levels and oxygen delivery by fresh whole blood in vitro.

The relation between DPG levels and erythropoietic response in normal unstressed individuals may be considered a "fine tuning" mechanism for regulating oxygen delivery. However, there is also strong evidence that an increase in DPG concentrations of circulating erythrocytes is a valuable "coarse adjustment" mechanism for improving oxygen delivery under conditions in which it is grossly inadequate. Hurtado's group first reported that the oxygen dissociation curve for people living at a high altitude was shifted significantly

to the right as compared with that for people of the same ethnic origin living at sea level (*25*). This right shift of the dissociation curve at high altitude has been amply confirmed (*26–28*), but until recently the mechanism has not been understood. Within the last 3 years, two independent sets of studies have shown that the DPG levels of the red cells of people living at a high altitude are higher than those of people living at sea level, and that this accounts for the observed decrease in the oxygen affinity of hemoglobin (*17, 22, 26, 29*). The DPG response is completed after about 24 hours at high altitude, with a half-time of 6 hours (*26*). The change in hemoglobin oxygen affinity closely parallels the change in DPG concentrations (*26*). At an altitude of 10,200 feet (3000 meters) (*17*), there is an increase of about 10 percent in DPG concentrations, relative to concentrations at sea level. At an altitude of 15,000 feet the increase is about 20 percent (*26*) (Table 2).

As in the case of people who live at high altitudes, decreased hemoglobin oxygen affinity is known to occur in most patients with hypoxemic diseases such as anemia and cardiopulmonary disorders (*30*). The DPG effect also seems to underlie the right shift of the dissociation curve in these disorders; recent data have demonstrated an increased level of DPG in the erythrocytes of most patients with these diseases (*18, 19, 22, 23*) (Table 2). Elevation of DPG concentrations is quite striking in severe anemia, being often twice the normal mean or higher. Patients differ considerably in this respect; some, particularly those with pulmonary disease, may not have elevated levels of DPG. The reasons for these differences are not understood. It is possible that hypoxic patients who do not have a good DPG response may eventually be helped by therapy aimed at raising the levels of red cell DPG.

A right shift of the hemoglobin oxygen dissociation curve during strenuous exercise, beyond that attributable to the decrease in blood *p*H and the rise in blood temperature, has been noted by several investigators (*31*). As in the case of the other hypoxic and hypoxemic conditions, the cause of the decrease in hemoglobin oxygen affinity has not been known. Data have been reported (*32*) which indicate that elevations of DPG may occur during strenuous exercise. Much of this earlier work, however, was carried out with an assay (based on color development with chromatropic acid) which was somewhat nonspecific. More recent work, with specific assays, has shown very little increase in DPG with exercise; this suggests that some other substance in the blood, perhaps another glycerate, may have been responsible for at least part of the response reported (*32*).

The observations discussed in this section suggest a highly attractive hypothesis: that DPG not only grossly decreases the hemoglobin oxygen affinity of erythrocytes but plays a role in the sensitive modulation of hemoglobin function in health and disease. However, it should be made clear that modulation of oxygen transport, and, in fact, modulation of hemoglobin function itself, are complex subjects with many factors involved in addition to those we are considering here. For example, cardiac output and ventilatory rate and capacity will markedly affect oxygen transport. Hemoglobin oxygen affinity will be affected by carbon dioxide levels, by the acid-base status of blood, by temperature, by phosphorylated intermediates other than DPG, and by various ions. Thus, in considering the role of DPG in the modulation of hemoglobin function, we must not forget that DPG is interacting with many other variables in the regulation of oxygen transport. Nonetheless, variation in the levels of DPG appears to be a factor which can explain many of the previously puzzling data on changes in hemoglobin oxygen affinity.

There is also evidence of an unknown curve-shifting agent which may act in very short periods of time. Shappell *et al.* (*33*) have reported slight but consistent shifts of the dissociation curve to the right as the blood circulates through the heart during anginal episodes. As yet there is no explanation of the etiologic factor in this rapid shift of the curve.

In the next section we consider the physiologic ramifications of changes in hemoglobin oxygen affinity.

Physiological Relevance of Red Cell Metabolic Variation

Data of Rorth (*24*) have shown a good correlation between P_{50} and DPG levels—in other words, a rightward shift of the dissociation curve with increases in DPG levels. Until now, we have more or less tacitly assumed that the rightward shift of the dissociation curve in hypoxic states enhances oxygen delivery. The validity of this assumption is crucial in evaluating the importance of DPG variation in maintaining respiratory homeostasis. However, very few data are available on the relationship between hemoglobin oxygen affinity and oxygen delivery. As an initial approach to this problem we have studied the rate at which whole fresh blood of normal individuals and of patients with various hypoxic disorders will release oxygen in an apparatus called a "rogeometer" (for "rate of gas exchange" plus "-ometer") (*22, 23, 28, 34*). In this apparatus, fresh oxygenated blood is pumped at a standard rate through a length of gas-permeable (Silastic) tubing enclosed in a nitrogen atmosphere, and the amount of oxygen released is measured. The diameter of the Silastic tube is greater by two or three orders of magnitude than that of a small capillary, but it can be shown theoretically that the rate of oxygen release by the red cell will be the driving force controlling the steepness of the oxygen diffusion gradient despite the long diffusion distance (*23*).

A significant positive correlation was detected, in the fresh blood of normal women, between the rate of desaturation in the rogeometer and the levels of red cell DPG; moreover, the rate of desaturation of whole blood of females was significantly greater than that of males (*22, 23*). Also, there was a negative correlation between the level of whole blood hemoglobin and the rate of oxygen release (*22, 23*). The mean rate of desaturation was significantly increased in patients with anemia and pulmonary disease. In many of the anemic patients the decrement in the amount of oxygen transported by the blood due to the lowered levels of hemoglobin was almost completely compensated by the increase in oxygen delivery per unit volume of blood. Of course, it should not be assumed from this that individuals with anemia have completely overcome their oxygen transport difficulties. It is obvious that under stress, such as that of exercise, a normal individual will have much greater reserve oxygen transport capacity on which to draw.

Additional studies should be made, with experimental approaches more closely approximating physiological conditions. The significance of the rogeometer data is that they indicate that changes in DPG levels, and hence

in hemoglobin oxygen affinity, correlate well with oxygen delivery under standardized in vitro conditions, and thus provide support for the hypothesis that changes in DPG levels of the red cell are involved in respiratory homeostasis.

Erythrocyte Glycolysis and DPG Levels

There are two general ways in which the nonnucleated red cell might bring about changes in the concentrations of a glycolytic intermediate such as DPG. It might do this by (i) changing the overall rate of glycolysis (glucose consumption), and by (ii) making alterations within the glycolytic pathway—for example, increased production or decreased degradation of DPG. Of these two possibilities an overall change in glycolytic rate seems to be of greater importance, since there is no evidence of a major decrease in the levels of other intermediates (such as ATP) when DPG levels increase in various hypoxic conditions. If the increase in DPG levels was associated with a constant rate of glycolysis, we would expect some decrease in the concentration of red cell ATP, since increased *production* of DPG would be at the expense of the ATP-generating step, 1,3-DPG → 3-PG (see Fig. 1), and decreased *degradation* of DPG would reduce the amount of substrate passing through the ATP-generating step PEP → pyruvate. We would hypothesize, then, that modulation of hemoglobin function by changes in DPG levels is directly related to modulation of the glucose consumption of the cell.

This hypothesis is in keeping with the known observation that glucose consumption by erythrocytes is normally less than maximal, which permits modulation by the relief of inhibition. The normal physiological rate of glucose consumption by erythrocytes is only one-fourth to one-third the rate which can be obtained in hemolyzates. Further, the hypothesis predicts that a negative correlation will exist between glucose consumption by erythrocytes and hemoglobin levels within a sample of people, since the people with the highest hemoglobin levels have the lowest DPG levels and should require the least glucose consumption to maintain normal oxygen transport through the DPG mechanism. Of course, it is difficult to evaluate in vivo glucose consumption by erythrocytes from in vitro data. However, in vitro studies have shown a negative correlation between hemoglobin levels and glucose consumption by erythrocytes (*28*). Also, in anemia, where adequate adjustment of hemoglobin levels to combat hypoxia is impossible, and where DPG levels are often twice the normal levels, glucose consumption by erythrocytes is three times the normal rate (*28*).

It is apparent that a further evaluation of the mechanisms whereby hypoxia can stimulate glucose consumption by erythrocytes and increase concentrations of DPG is needed. One possible mechanism which we have studied involves a system in which DPG inhibits the action of hexokinase (*35, 36*), the enzyme which initiates glycolysis and which may be an important rate-limiting enzyme in red cell glycolysis. Inhibition of the action of hexokinase by DPG is relieved by increasing the concentrations of ATP or Mg^{2+} (*36*). This DPG inhibition of the action of hexokinase, modulated by ATP or Mg^{2+}, or by both, seems well suited for feedback control of glycolysis and hence for control of the levels of DPG and ATP. However, further studies of this and other possible mechanisms of glycolytic control are required before we can do more than speculate.

Mechanisms of Environmental Influences on DPG Levels

At least three distinct mechanisms exist that alter the amount of DPG in the cell, and the actual level achieved is probably the culmination of the interaction of these (and possibly of other, unidentified) factors. First, the relative desaturation of hemoglobin in vivo almost certainly is one mechanism affecting DPG levels (*16, 23*) since deoxyhemoglobin binds DPG with a greater affinity than oxyhemoglobin does. Hypoxias in conditions such as anemia, pulmonary disease, exposure to high altitudes, and exercise all result in increased desaturation of hemoglobin in the venous blood and thereby cause an increased binding of DPG during the time the cell is in the venous circulation. The glycolytic mechanism regulating levels of DPG within the cell (which is presumably sensitive only to unbound DPG) will respond with increased synthesis of DPG, and the levels of total DPG will rise. It is probable that this mechanism (the hemoglobin desaturation mechanism) acts to elevate DPG in the ordinary hypoxias in which increased hemoglobin desaturation occurs.

A second variable known to affect

Table 1. Estimates of the amounts of free and hemoglobin-bound DPG in whole red cells in vivo. [From Garby and deVerdier (*11*)]

	Total DPG concentration						Reference
	4 millimolar		5 millimolar		6 millimolar		
	Free	Bound	Free	Bound	Free	Bound	
Oxyhemoglobin	4.0	0.0	5.0	0.0	6.0	0.0	(*13*)
Deoxyhemoglobin	0.2	3.8	0.5	4.5	1.2	4.8	(*13*)
Oxyhemoglobin	1.5	2.5	2.0	3.0	2.7	3.3	(*9*)
Deoxyhemoglobin	1.0	3.0	1.5	3.5	2.2	3.8	(*9*)
Oxyhemoglobin	3.0	1.0	4.0	1.0	5.0	1.0	(*10*)
Deoxyhemoglobin	2.0	2.0	2.5	2.5	3.0	3.0	(*10*)

Table 2. Elevations of DPG concentrations in erythrocytes in response to hypoxia.

Situation or condition	Number in sample	Approximate elevation above normal (%)	Reference
High altitude			
10,200 feet above sea level	153	10*	(*17*)
14,500 feet above sea level	?	20*	(*29*)
Hypoxic diseases			
Severe pulmonary disease	9	0–100	(*23*)
Nonhemolytic anemia (uremia)	4	50–150	(*23*)
Nonhemolytic anemia (leukemia)	11	20–150	(*23*)
Hemolytic anemia	14	10–100	(*19, 23*)
Thalassemia	5	50– 75	(*19*)
Iron deficiency	11	40– 75	(*19*)
Prolonged exercise	16	0– 25	(*32*)

* "Normal" refers to concentrations at sea level.

DPG levels of erythrocytes is the acid-base status of the cell. Three separate effects of *p*H on DPG levels have been identified, and there may be others as yet unknown. First, *p*H influences the binding of DPG to hemoglobin: with increased *p*H there is decreased binding. This frees a higher proportion of DPG and tends to inhibit synthesis of DPG. Variation in physiological *p*H may also have differential effects on the two enzymes (DPG mutase and DPG phosphatase) directly responsible for synthesis and degradation, respectively, of DPG. However, there are conflicting reports in the literature as to the nature of the *p*H effects on these enzymes (*37*). The third effect of higher *p*H is a stimulation of glycolysis, which tends to raise levels of intermediates such as DPG.

The net effect of *p*H variation on the DPG levels of the intact cell is the culmination of these various effects. Unfortunately, there are at present few data on the relationship between whole blood *p*H and intracellular *p*H. Therefore, we do not know what change in intracellular *p*H will be produced by a given change in whole blood *p*H. However, data collected by Rorth (*38*) indicate that, as whole red cells are incubated in buffers of increasing *p*H, DPG increases, up to about *p*H 7.4, then levels off and begins slowly decreasing with more alkaline conditions. Astrup (*39*) has found, in patients with acid-base disturbances, a good positive correlation between *p*H and DPG levels. Further, when acid-base changes are induced in normal individuals by administration of ammonium chloride (a decrease in *p*H) or of sodium bicarbonate (an increase in *p*H), the DPG levels are decreased or increased, respectively. This makes it seem likely that acid-base changes, as well as the hemoglobin desaturation mechanism, actually operate in vivo to affect DPG levels in many circumstances. Astrup (*39*) has even suggested that red cell *p*H may be the main determinant of DPG levels. He points out that, in anemia and other hypoxias, there is an element of respiratory alkalosis, due to hyperventilation, which may be an important factor in increasing DPG levels. Further, increased hemoglobin desaturation tends to cause an increase in intracellular *p*H; thus, even the effect of hemoglobin desaturation on DPG may be brought about through an effect on red cell *p*H.

However, it appears unlikely that *p*H effects alone can explain the elevation of DPG concentrations in hypoxic conditions. Changes in *p*H after acclimation to high altitudes and in many types of anemia, if they occur at all, are very small. For example, in excessively polycythemic individuals living in Leadville, Colorado (altitude, 10,200 feet), the DPG levels are sometimes twice the normal levels, yet the *p*H of arterial blood was found to be no higher (actually it was slightly lower) than that of control subjects at the same altitude (*28*). Further, in anemia, DPG levels are often twice as high as normal levels. On the other hand, DPG levels are generally less high in pulmonary disease, in which hyperventilation and respiratory alkalosis tend to be greater than in anemia. According to Astrup (*39*), the *p*H mechanism produces a 3.8 percent increase in DPG for every 0.01 unit increase in *p*H. To achieve a twofold increase in DPG, the *p*H would have to increase by more than 0.25 unit, a change not seen either in anemia or in acclimation to high altitude. Perhaps the most striking discrepancy in the positive correlation between *p*H and DPG levels is found in anemia associated with uremia; in this condition some degree of metabolic acidosis is usually present, yet DPG levels are very high (*23, 39*). Astrup (*39*) has attributed the high DPG levels in uremia to high levels of serum phosphorus; nonetheless, this disease points up the inadequacy of the *p*H hypothesis alone for explaining the alterations in DPG levels seen in the various conditions.

The age of the erythrocytes probably has some influence on the levels of DPG, but the details have not yet been worked out. It is known that the very youngest nonnucleated red cells, reticulocytes, have DPG concentrations lower than those of mature red cells. In contrast, the ATP concentrations of reticulocytes are considerably higher than those of mature red cells. The effect of these variations, associated with variation in cell age, on the oxygen dissociation curve of humans has not been studied. We have observed very little effect of hemolytic anemia induced by phenylhydrazine, associated with reticulocytosis (reticulocyte count of 10 to 50 percent) on the dissociation curve of rabbits. Possibly the higher levels of ATP in reticulocytes approximately balance the lower levels of DPG in terms of oxygen dissociation properties of the cell.

Genetic Influences on DPG Levels

Previous work in humans has revealed that the levels of red cell ATP are under hereditary influence (*40*). Aside from studies in unusual families with inherited enzyme defects, no human family studies of DPG levels have been carried out. However, in the hooded strain of rats, a strong hereditary influence on DPG levels in erythrocytes has been detected (*28*). It is quite likely that the levels of DPG are also under genetic control in other species, including man. DPG levels are a quantitative characteristic, and probably result from a combination of genetically determined and environmentally influenced factors. We have discussed above many of the environmental factors that may affect DPG levels. Genetic factors affecting DPG levels may also be quite diverse, ranging from influences on the activities of glycolytic enzymes of erythrocytes to genetic modifications of any of the various features of oxygen transport, such as pulmonary function and cardiac output. In producing relatively good or relatively poor tissue oxygenation, these latter factors may tend to produce lower or higher DPG levels, respectively.

In hooded rats, but not in man, the levels of ATP and DPG in erythrocytes have a strong positive correlation (*28*). Two strains of hooded rats are being developed in our laboratory by genetic selection, one with high levels of ATP and DPG and one with low levels. It is hoped that the physiological importance of DPG and ATP variations can be studied by contrasting animals of the two strains with respect to such characteristics as tolerance to exercise and hypoxia, resistance to malaria, and rate of aging.

Clinical Aspects

The role of DPG in oxygen transport, discussed above, makes DPG of obvious importance in clinical medicine. In hypoxemias of various types we see an elevation of DPG concentration, presumably as a compensatory mechanism to assist in oxygen delivery.

Yet, many questions remain to be answered, and the answers may be of great importance to patients with a variety of diseases. First, why do we see much greater elevation of DPG concentrations in some hypoxic diseases (for example, anemia) than in others

where oxygen transport is as seriously curtailed (for example, pulmonary disease); and, as a corollary, why do we find such differences in DPG response among patients with similar syndromes? Second, are the individuals with very high DPG concentrations better off clinically? Third, are there methods by which we can alter hemoglobin oxygen affinity to obtain therapeutic benefit? Such methods might include, for example, the use of agents which themselves shift the dissociation curve to the right, or which do so by elevating DPG levels.

In addition to the benefits which may be derived in the future from such studies, more immediate application of our present knowledge may be forthcoming in an improved understanding of the functional usefulness of transfused blood. Heretofore, critical evaluation of the acceptability for transfusion of stored blood was based on survival of the red cells in the recipient's circulation, a value of 70 percent survival being set as the lower limit of acceptability. This value is reached, on the average, after about 3 weeks of storage. However, in blood stored longer than a week the level of DPG is quite low, and the oxygen dissociation curve is markedly shifted to the left. It has been demonstrated that it takes 24 hours or longer (*18*) for the DPG levels of the transfused cells to return to normal, during which time the oxygen transport capacity of the cells is presumably not good, yet oxygen must be transported in the circulation. These findings dictate the use of relatively fresh blood in acutely ill patients, and the use of older stored blood for patients with stabilized chronic disease. They also suggest further study of the functional usefulness, not just the survival, of blood after it is transfused.

Summary

Studies of the interactions between the metabolism and the function of the red cell have shown the importance of the red cell's metabolism in contributing to the maintenance of adequate oxygen delivery. Some of the phosphorylated intermediates of glycolysis, especially DPG, are now known to reduce the affinity of hemoglobin for oxygen. Current evidence indicates that this phenomenon is due to the effects of the binding of DPG to the β-chains of deoxyhemoglobin. It appears that increases in red cell concentrations of DPG commonly occur during hypoxia, and that these increases (as well as normal physiological variation) significantly enhance oxygen transport. Artificial manipulation of erythrocyte metabolism may soon prove to be of great clinical usefulness in the treatment of a great variety of disorders which limit oxygen transport.

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