

*THE RELATIONSHIP BETWEEN RED CELL
2,3-DIPHOSPHOGLYCERATE AND LEVELS OF
HEMOGLOBIN IN THE HUMAN**

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The central role of 2,3-diphosphoglycerate (DPG) in the metabolism and function of most mammalian red cells is only beginning to become apparent. 2,3-Diphosphoglycerate, a glycolytic intermediate present in high concentration only in the red cell, has been said to function as a storage source for phosphate.¹ It may also act as a metabolic regulator, since DPG has been found to exert significant inhibitory effects on several red cell enzymes, such as transaldolase, transketolase,² and hexokinase.³ Most recently, the findings of Benesch and Benesch,⁴ Benesch *et al.*,⁵ and Chanutin and Curnish⁶ show that DPG (and adenosine triphosphate (ATP), which is quantitatively less important) combines reversibly with deoxyhemoglobin, and, in normal concentrations, greatly decreases the oxygen affinity of hemoglobin, shifting the oxygen dissociation curve to the right. Although this shift increases the oxygen pressure required for oxygenation of the hemoglobin, unloading can occur at higher tissue oxygen tensions. Although both sets of authors⁴⁻⁶ have emphasized the importance of this effect, Benesch *et al.*⁵ stated that variations in levels of red cell DPG within the normal human range will have little or no influence on the oxygen affinity of the whole blood.

Part of the stimulus for the present study derives from observations which suggest that pathological variations in human red cell DPG may significantly effect oxygen transport. An inherited abnormality of pyruvic kinase in which the activities of this enzyme were elevated about twofold was present in a family reported by Zurcher *et al.*⁷ Individuals affected with this disorder had levels of red cell ATP about twice normal and levels of DPG about one-fourth normal. Although both ATP and DPG can decrease the oxygen affinity of hemoglobin, DPG is quantitatively much more important, since its normal concentration in the human red cell is three to four times that of ATP. The authors noted that affected individuals had an unexplained elevation of hemoglobin and hematocrit values of about 20 per cent. We now think it likely that the reason for this increase in blood values was a significant lessening of oxygen unloading in the tissues of affected individuals, due to the decrease in the amount of DPG in the red cell. The resulting tissue anoxia has been compensated by erythrocytosis.

Secondly, a recent abstract⁸ dealt with the levels of DPG in the erythrocytes of individuals homozygous for sickle hemoglobin. The oxygen dissociation curve of intact red cells in sickle cell anemia, compared to that of normal cells, has been known to be shifted to the right. However, this shift is no longer apparent when studies are performed on dialyzed hemoglobin. These authors found that the erythrocytes of individuals homozygous for sickle hemoglobin have significantly higher levels of DPG; this probably accounts for the lower oxygen affinity of the intact cells.

The literature, then, suggests that large variations in the content of DPG in pathological red cells may be related to significant changes in oxygen transport of whole blood *in vivo*. We thought it possible that the effects of physiological variation of DPG concentration in the red cell would also be apparent in normal individuals, particularly as reflected by their hemoglobin levels. Therefore, the purposes of the present work were (1) to define the amount of variation in red cell DPG to be found within a large sample of normal Negro and Caucasian males and females, and (2) within this sample, to test for a correlation between the level of DPG and other blood values, such as hemoglobin.

Materials and Methods.—Blood samples from males were obtained from 200 healthy adult Negroes and Caucasians (100 each) resident in Southern Michigan State Prison, Jackson, Michigan. One hundred adult females (59 Caucasians and 41 Negroes) were bled at the University of Michigan University Hospital Outpatient Laboratory. These were women having pre-employment physical examinations or being treated for a variety of minor, nonhematological disorders. In all cases, the heparinized blood samples were kept iced until processing, which took place within a maximum of 6 hr after the samples were taken. Control studies employing storage of heparinized samples at 4°C for 6 hr showed that the decrease in DPG content of whole blood for this period was negligible. Heparin was used as the anticoagulant instead of acid citrate-dextrose (NIH formula A) because the latter, while excellent for ATP preservation, was found not to preserve DPG adequately. The techniques for the preparation of red cell extracts with trichloroacetic acid, and for removal of trichloroacetic acid with ether, were as previously published.⁹

All assays of DPG were done with a modification of the chromatropic acid method of Bartlett,¹⁰ and subsamples of each group were checked with a modification of the more specific enzymatic method of Krimsky¹¹ (details of these methods will be reported elsewhere). Hemoglobin and hematocrit determinations and red cell counts were performed by standard hematological techniques.

Results.—The correlation that we had hypothesized might exist between DPG and blood values was indeed present. Table 1 shows the correlations between DPG and the various blood values for Caucasian and Negro males and females. The strongest of these are the inverse correlations between DPG and whole blood hemoglobin. This correlation is higher in Caucasian females than in the other three groups, but it is significant (at the levels shown in parentheses) for all groups studied.

The mean, standard deviation, and range for the DPG values of the four racial and sexual groups are shown in Table 2. The range of values in both races was

TABLE 1. Correlations between level of red cell DPG and hemoglobin, hematocrit, and red blood cell count in adult Negro and Caucasian males and females.

Group	No.	DPG vs. whole blood hemoglobin	DPG vs. hematocrit	DPG vs. red cell count
Caucasian males	100	-0.229 (<i>p</i> = 0.05)	-0.184 (<i>p</i> = 0.1)	-0.076 (N.S.)*
Caucasian females	59	-0.405 (<i>p</i> = 0.005)	-0.270 (<i>p</i> = 0.1)	-0.198 (N.S.)
Negro males	100	-0.267 (<i>p</i> = 0.01)	-0.185 (<i>p</i> = 0.1)	+0.002 (N.S.)
Negro females	41	-0.264 (<i>p</i> = 0.1)	-0.123 (N.S.)	+0.026 (N.S.)

* N.S. indicates nonsignificant at 0.05 level.

TABLE 2. Levels of DPG in human red cells.

Group	No.	Erythrocyte DPG Levels*	
		Mean (± 1 sd)	Range
I. Caucasian males	100	12.02 (± 1.92)†	8.50–15.86
II. Caucasian females	59	11.21 (± 1.57)†	8.59–16.01
III. Negro males	100	13.22 (± 2.08)†	6.78–17.31
IV. Negro females	41	14.13 (± 2.23)†	10.89–21.82

* DPG values expressed in μ moles per gram of hemoglobin.

† Significance levels of differences in means ("t" test): I vs. II, $p = 0.01$; I vs. III, $p = 0.001$; I vs. IV, $p = 0.001$; II vs. III, $p = 0.001$; II vs. IV, $p = 0.001$; III vs. IV, $p = 0.05$.

found to be about twofold or greater. The mean level of DPG in Negroes of corresponding sexes was higher than in Caucasians ($p = 0.001$). The differences between the sexes in the two races are not in the same direction. The mean of the Caucasian male is significantly higher than that of the female ($p = 0.05$). There was no significant difference in the levels of DPG in individuals heterozygous for hemoglobin S compared to Negroes homozygous for hemoglobin A.

Discussion.—The most important result of this study is the finding of significant negative correlation between DPG and whole blood hemoglobin levels. This inverse relationship suggests that variation in the amount of red cell DPG from one individual to another may be causally related to the physiological variation in hemoglobin levels among individuals.

The work of Benesch *et al.*⁵ led these authors to conclude that "... variations in organic phosphate levels in the physiological ranges would thus have little effect on the oxygen affinity." However, they did point out that "... a decrease to less than three-fourths of the normal concentration should lead to a sharp decrease in the ability of hemoglobin to unload oxygen." Indeed, our results do indicate that the normal range of DPG rarely extends below three fourths of the mean level (Table 2). However, an inverse relationship between hemoglobin and DPG seems to exist throughout the entire range of the distributions. The fact that a relationship such as this does exist may be related to the effects of DPG concentration on the rate of hemoglobin oxygenation and deoxygenation.

It should be stressed that Benesch *et al.*⁵ were working with equilibria reached after prolonged exposure to a given oxygen pressure. Physiologically, of course, oxygenation and deoxygenation of hemoglobin must take place at an extremely rapid rate. If the rate of equilibration of hemoglobin with oxygen is related to concentrations of organic phosphate, then small variations in physiological levels of DPG between individuals may, after all, have very important ramifications. Preliminary evidence from our laboratory indicates that variations in levels of DPG have significant effects on the rate of oxygenation—that is, the higher the levels of DPG, the slower the rate of oxygenation.

The negative correlation reported here does not necessarily prove a causal relationship between DPG and hemoglobin. However, since there may be a causal relationship, it is worthwhile to consider the two possibilities: (1) that the level of whole blood hemoglobin (and its average saturation with oxygen) is affecting the amount of DPG in the red cell, or (2) that the content of DPG in the cell is affecting the level of whole blood hemoglobin.

With reference to the first possibility, it is known that DPG levels are increased

in anemias of several types.¹² A possible mechanism for this is greater venous desaturation of the hemoglobin (characteristic of most anemic states), resulting in increased amounts of hemoglobin-bound DPG. (Only deoxygenated hemoglobin binds DPG.) Homeostatic mechanisms within the red cell may maintain the levels of *free* DPG at a constant level; with an increase in bound DPG, the total content of DPG is increased. This same mechanism, operating in a more subtle degree, would then also explain the negative correlation between DPG and hemoglobin within the "normal" range.

The second possible explanation of the negative hemoglobin-DPG relationship is that if the level of DPG in the red cell is relatively fixed, that level may determine the oxygen-carrying efficiency of the hemoglobin (higher levels would result in more efficient oxygen unloading in the tissues), with the result that a given amount of hemoglobin would have varying oxygenating capacity depending on the level of red cell DPG. In this case, the whole blood hemoglobin levels maintained through erythropoiesis would be partially determined by the concentration of red cell DPG.

Of course, the two possibilities discussed above are not mutually exclusive, and it may be that the negative correlation between DPG and hemoglobin results from the operation of both factors.

The levels of DPG in the Negro were found to be 15–20 per cent higher than in the Caucasian. This contrasts with previous findings regarding ATP¹³ in which Caucasians were found to have significantly higher levels of erythrocyte ATP than Negroes. Although at present we cannot be sure of the cause or the functional significance of these differences, there is at least one possible contributing factor. The previously mentioned work on pyruvic kinase abnormality by Zurcher *et al.*⁷ indicates the existence of a possible reciprocal relationship between levels of ATP and DPG. The work of Zurcher *et al.*⁷ suggests that enhancement of the activity of the lower glycolytic pathway may result in the elevation of ATP and the lowering of DPG. Further, in cases of pyruvic kinase *deficiency*, it has been reported^{14, 15} that the reverse of this situation obtains. That is, red cell ATP is decreased at least 30–40 per cent and DPG is increased two- to three-fold. Although these admittedly are abnormal conditions, they suggest that changes in enzyme activities of at least one area of the glycolytic pathway may change the balance and absolute amounts of ATP and DPG.

Finally, as the hemoglobin within the red cell is alternately oxygenated and deoxygenated, the amount of unbound DPG will vary, since only deoxygenated hemoglobin is capable of binding DPG. When the hemoglobin within the cell is oxygenated, there will be a comparatively greater concentration of free DPG, and vice versa upon deoxygenation. One implication of this rapid cyclic binding and release of DPG by hemoglobin in the course of circulation is that, in all probability, the activities of various portions of the glycolytic pathway, and perhaps glycolysis itself, will have a marked cyclic variation. We might expect that the fully oxygenated cell would initiate glucose metabolism at a lower rate (due to DPG inhibition of hexokinase) and would have a less active pentose phosphate shunt (due to DPG inhibition of transaldolase and transketolase). This implies that the net rate of glycolytic activity in the red cell may be dependent upon a

balance between rapid variations in levels of metabolic activity as the cell moves through the circulation. The effect of oxygen tension on relative binding of DPG together with the effect of unbound DPG on hexokinase activity offer an explanation for the Pasteur effect in red cells, i.e., a decrease in glucose consumption with increasing oxygen tension.¹⁶

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