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THE HUMAN RED CELL: THE RELATIONSHIP OF 2,3-DIPHOSPHOGLYCERATE METABOLISM AND OXYGEN TRANSPORT

by

John Wallace Eaton

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of the requirements for the degree of
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Doctoral Committee:

Professor Frank B. Livingstone, Chairman
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ABSTRACT

THE HUMAN RED CELL: THE RELATIONSHIP OF
2,3-DIPHOSPHOGLYCERATE METABOLISM AND OXYGEN TRANSPORT

by

John Wallace Eaton

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One of the metabolic intermediates of the human red cell, 2,3-diphosphoglycerate (DPG), is present in very high concentration. It appears that a most important function of this intermediate is enhancement and control of the red cell's oxygen carrying abilities.

This thesis reports investigations of: 1) the normal levels of red cell DPG in a large population of adult humans; 2) the relationship of these levels to other physiological parameters; 3) the effects of altitude on levels of red cell DPG and the role of DPG in adaptation to altitude; 4) the effects of variations in levels of red cell DPG on the oxygen dissociation properties of hemoglobin and the oxygen carrying capacity of the red cell.

We have examined the levels of DPG in the red cells of large samples of normal adult Negroes and Caucasians and have found significant sexual and racial differences in these levels. Further, a negative correlation was found to exist between whole blood hemoglobin levels and red cell DPG concentrations. This implied a possible functional relationship between DPG and the oxygenating capacity of the red cell.

Our laboratory studies of red cells with varying DPG content have shown that increases in DPG will shift the oxygen dissociation curve to the right and will increase the rate at which the red cell

gives off oxygen. In accordance with this, humans resident at high altitude had significant elevations of red cell DPG. This is probably the basis of the previously observed shift of the oxygen dissociation curve to the right which accompanies adaptation to life at high altitude. In addition, erythrocyte DPG levels were found to increase in response to prolonged periods of moderate exercise.

The evidence gathered here indicates that DPG is an important regulator of red cell oxygen transport. Differences in erythrocyte DPG levels facilitate short- and long-term adaptations to the hypoxia of altitude and exercise. Changes in DPG concentrations were found to occur within relatively short periods of time, and these changes probably constitute one of the most important mechanisms for the maintenance of respiratory homeostasis.

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INTRODUCTION

All living organisms must maintain biological transport systems which accomplish the selective exchange of materials between the organism and the environment. Respiration, the selective exchange of gases between organism and environment, is one of the most ubiquitous of these transport systems. Among the Vertebrata, respiration takes the form of intake and assimilation of oxygen, and the release of carbon dioxide. Almost all of the oxygen transport in these organisms involves the respiratory protein, hemoglobin.

Hemoglobin is contained within the erythrocyte, a specialized cell which functions principally as a carrier for the hemoglobin. Among the Mammalia, the mature red cell has no nucleus and cannot reproduce itself. The cell is produced in the bone marrow, and all the proteins which it will need during its limited life span must be incorporated during this period of protein synthesis. Aside from the hemoglobin within the red cell (which accounts for about 95% of the protein constituent of the cell contents), there are a number of enzymes, most of which are responsible for the production of the energy needed to keep the cell intact as it flows through the circulatory system. Most of this energy is gained from the process of glycoloysis, through the conversion of glucose to lactic acid (see Figure 1).

The glycolytic conversion of glucose to lactic acid involves the phosphorylation and subsequent dephosphorylation of the compounds formed

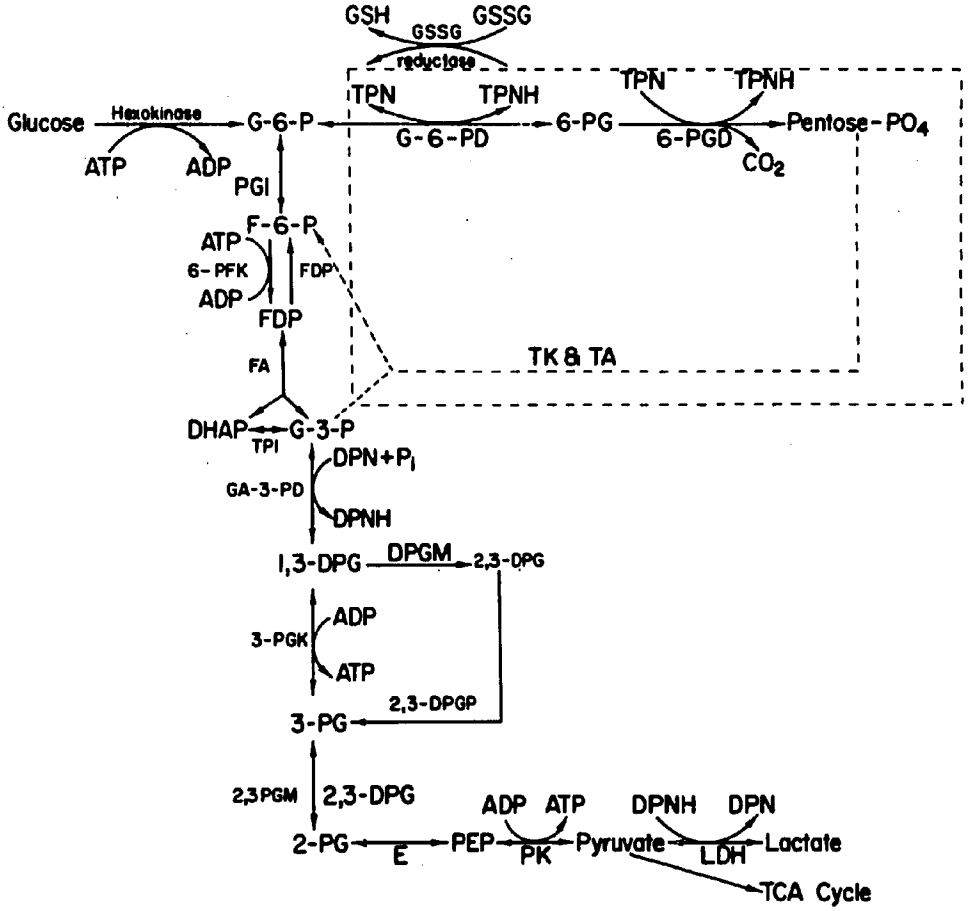


Figure 1.--Glycolysis. Important compounds and enzymes, reading from top to bottom: ATP and ADP = adenosine tri- and diphosphate, respectively; G-6-P = glucose-6-phosphate; GA-3-PD = glyceraldehyde-3-phosphate dehydrogenase; 1,3-DPG and 2,3-DPG = 1,3-diphosphoglycerate and 2,3-diphosphoglycerate; DPGM = diphosphoglycerate mutase; 2,3-DPGP = 2,3-diphosphoglycerate phosphatase; 3-PGK - 3-phosphoglycerate kinase.

from glucose during glycolysis. This process results in a net gain of energy which is subsequently used to help the cell maintain its structural and ionic integrity.

The phosphorylated intermediates of glycolysis are, for the most part, present in only small amounts. However, the red cell does contain relatively high concentrations of two of these intermediates (see Table 1). One of these, adenosine triphosphate (hereafter, ATP), is not a direct conversion product of glucose, but is produced through the energy supplied by the process of glycolysis. ATP is the major energy source of the mature erythrocyte, and is primarily used in: (1) active cation transport (which maintains high potassium and low sodium levels within the red cell) (Post, et al., 1960); (2) the phosphorylation of glucose and its conversion products; (3) (probably) the maintenance of cell shape through a contractile protein (insensitive ATPase) located within the cell wall (Nakao, et al., 1961). A proper level of ATP is known to be of critical importance in the survival and proper functioning of the red cell within the circulation.

The second of these phosphorylated compounds known to be present in high amounts in the red cell is 2,3-diphosphoglycerate (hereafter, DPG). DPG concentration is about four times that of ATP (12 μ moles per gram hemoglobin versus about 3 μ moles per gram hemoglobin). Although ATP is found, in greater or lesser quantity, in almost all of the tissues of the body, DPG is present in significant concentration only in the red cell (Rapoport and Guest, 1941).

In contrast to ATP, DPG is formed from a compound which occurs in the direct conversion of glucose to lactic acid (see again Figure 1).

TABLE 1

CONCENTRATIONS OF SOME OF THE PHOSPHORYLATED INTERMEDIATES OF GLYCOLYSIS IN HUMAN RED CELLS. DATA TAKEN FROM BARTLETT (1959a).

Compound	μ moles of phosphorus per ml. red blood cells
Adenosine diphosphate	0.38-0.49
Adenosine monophosphate	0.01-0.02
Fructose 1,6-diphosphate	0.12-0.24
Fructose 6-phosphate	0.01-0.02
Glucose 1,6-diphosphate	0.38-0.47
Glucose 6-phosphate	0.08-0.10
Adenosine triphosphate	2.70-3.70
2,3-diphosphoglycerate	7.20-10.20

The high levels of DPG present within the red cell have puzzled red cell biochemists for a long time. DPG is formed from 1,3-diphosphoglycerate (through the action of the enzyme diphosphoglycerate mutase), and in the process energy is lost. This conversion involves the exchange of a relatively high energy phosphate bond (the 1 phosphate) for a lower energy bond (the 2). In addition to the energy loss inherent in this transformation, the high concentrations of DPG would seem to constitute extra metabolic baggage for the cell to produce and carry as it performs its primary function of gas transport.

There have been some suggestions as to the reasons for these high intra-erythrocytic levels of DPG. It has been proposed (Prankerd, 1961) that DPG is an energy store, to be used by the red cell for energy in times of metabolic insufficiency or crisis. The 2 bond of DPG is much more stable (less susceptible to random breakage) than the 1 bond of the 1,3-diphosphoglycerate. This stability would, of course, be desirable in a storage product.

Physiological levels of DPG have been found to inhibit several red cell enzymes, including adenosine monophosphate (AMP) deaminase (Askari and Rao, 1968), transaldolase and transketolase (Dische and Igals, 1963), and hexokinase (Dische, 1941). A high level of AMP deaminase activity within the red cell would soon lead to serious depletion of the red cell's levels of adenosine, which is a nucleoside involved in the formation of ATP. Both transaldolase and transketolase are necessary for the return of intermediates formed in the hexose monophosphate shunt to the main glycolytic pathway. A decrease in their activity will probably result in a slowing of the flow of intermediates through the shunt.

Perhaps the most important of the enzymes known to be inhibited by DPG is hexokinase. This enzyme is responsible for the first phosphorylation of glucose, and, therefore, for the initiation of glycolysis. If the activity of hexokinase is slowed or stopped, the level of red cell ATP will drop rapidly, since the process required for its formation is seriously hampered.

The most critical of the functions of DPG, that of influencing the oxygen dissociation properties of hemoglobin, has only recently been hinted at. Work by Benesch and Benesch (1967) and by Chanutin and Curnish (1967) has shown that DPG concentration has a great influence on red cell function in reducing the affinity of hemoglobin for oxygen.

Benesch and Benesch (1967) mistakenly reported that DPG and ATP combine only with deoxygenated hemoglobin in a 1:1 molar ratio. That is, every molecule of deoxygenated hemoglobin is capable of binding one molecule of free DPG. It now appears (Chanutin, person communication; Garby and de Verdier, 1969) that ATP and DPG may be bound to both oxygenated and deoxygenated hemoglobin. The binding constants of oxy- and deoxy-hemoglobin for ATP and DPG are, however, different. Deoxyhemoglobin has about twice the affinity for ATP and DPG (Chanutin, personal communication). Within the normal human red cell, the molar DPG concentration is almost equal to the molar concentration of hemoglobin (Bartlett, 1959a). Therefore, when the red cell is completely oxygenated, there is more free, unbound DPG present. However, when the red cell is completely deoxygenated, much of the free DPG will have been bound to hemoglobin.

Chanutin and Curnish (1967) and Benesch and Benesch (1967) showed that the presence of normal physiological concentrations of DPG will

greatly reduce the oxygen affinity of purified hemoglobin (see Figure 2). The way in which hemoglobin combines reversibly with oxygen is a most critical part of the process of respiration. The combination of oxygen with hemoglobin (and the release of oxygen by hemoglobin) is primarily dependent on the oxygen pressure (pO_2) immediately surrounding the hemoglobin. As the pO_2 drops, the hemoglobin is more likely to give up (and less likely to take on) its oxygen. The red cell has the task of taking up oxygen in the lungs (where the pO_2 is relatively high), and delivering oxygen to the tissues (where the pO_2 is much lower). The affinity of hemoglobin for oxygen at varying oxygen pressures is, therefore, of critical importance in the proper functioning of this respiratory protein. If the oxygen affinity of hemoglobin is very high (that is, if hemoglobin will take up oxygen at relatively low oxygen pressures), oxygenation of hemoglobin in the lungs is extremely rapid and efficient, but little or none of this oxygen will be released in the tissues. On the other hand, if the oxygen affinity of hemoglobin is low, the tissue release of oxygen is enhanced, but oxygenation of the hemoglobin in the lungs may be hindered. It is obvious, therefore, that a proper balance must be maintained between the oxygen pressures in which the hemoglobin must operate and the affinity of the hemoglobin for oxygen.

The work of Benesch and Benesch (1967) and of Chanutin and Curnish (1967) has demonstrated that, in the absence of DPG and ATP, the oxygen affinity of hemoglobin is tremendously increased (see Figure 2). This means that, though oxygenation in the lungs would be complete as previously mentioned, delivery of the oxygen to the tissues in the absence of DPG and ATP would be impossible. Since the concentration

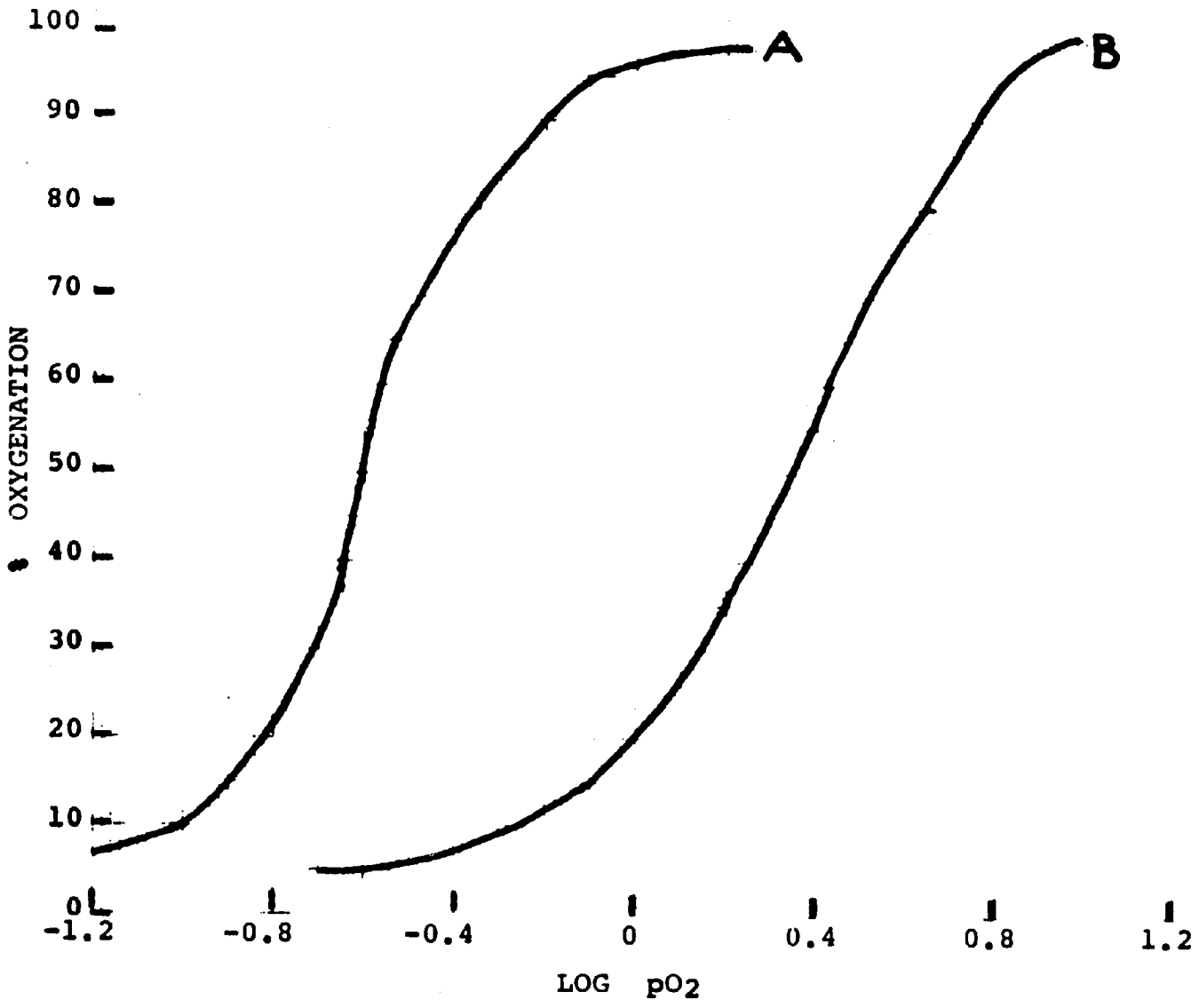


Figure 2.--The oxygen dissociation curves of purified human hemoglobin (curve A) and purified human hemoglobin with 1 mole DPG added per mole hemoglobin (curve B). Note the great shift to the right (representing reduced affinity for oxygen) in the hemoglobin solution with added DPG. (pO_2 = oxygen pressure; % oxygenation = the amount of the total hemoglobin which is carrying oxygen.) Data taken from Benesch, et al. (1968).

of DPG in the red cell is about four times that of ATP, DPG is much the more important of the two compounds with regard to this phenomenon. The authors just cited showed that as the level of DPG in a hemoglobin solution reaches near-physiological levels, the reduction in the oxygen affinity of hemoglobin begins to level off. In their experimental systems (with dilute solutions of hemoglobin) variation of DPG levels around normal physiological values (i.e. 4.5×10^{-3}M) seemed to produce little change in the oxygen affinity of the hemoglobin. Benesch, et al. (1968) were led to conclude from this that "Variations in organic phosphate level in the physiological range would thus have little effect on the oxygen affinity." They do add, however, that reduction of DPG concentration to 3/4 that of normal would have serious effects on the oxygen affinity of hemoglobin.

Since the role which DPG plays in governing the oxygen affinity of hemoglobin had previously been unsuspected, there were no studies in the literature which would directly confirm or contradict the idea that normal physiological variation in DPG had no significant influence on the oxygen transport capacity of hemoglobin. There were, however, indirect evidences that this might not be the case.

One indication was a report by Zurcher, et al. (1965) of a family in which some of the members had an inherited condition characterized by twice normal levels of pyruvic kinase (PK). Accompanying this unusually high PK activity were ATP levels which were twice normal, and most importantly, DPG levels which were only 1/4 normal. This meant that, though ATP was higher than normal, the sum of ATP and DPG was about half that usually found. The authors also mentioned, without explanation, that the affected individuals had whole blood hemoglobin levels which

averaged 20% above normal. In light of the present knowledge of the effects of ATP and DPG on oxygen affinity, it would seem likely that the reason for this elevation in hemoglobin values was a serious inability of the hemoglobin to unload its oxygen in the tissues. This restriction of tissue delivery of oxygen would result in a significant anoxia which, in turn, would presumably stimulate heightened manufacture of red cells (erythropoiesis) and increase the hemoglobin level significantly.

Secondly, a recently published abstract reported that DPG levels in individuals homozygous for hemoglobin S were significantly higher than those of normals (Charache, et al., 1968). It was previously reported that the oxygen dissociation curve of hemoglobin S homozygotes was shifted to the right (Becklake, et al., 1955). However, when hemoglobin from these individuals was dialyzed (purified through selective removal of small, non-protein constituents), the dissociation curve of hemoglobin S returned to the position of that of hemoglobin A (Schruefer, et al., 1962). This was strong indication that the reason for the shift of the hemoglobin S dissociation curve to the right was an increase in the level of red cell DPG.

Shifts in the oxygen dissociation curve were also known to be present in people who were adapted to life at high altitudes (Aste-Salazar and Hurtado, 1944; Hurtado, et al., 1956). The curves of residents at altitude are shifted somewhat to the right, just as the curves of individuals homozygous for hemoglobin S are shifted. However, the underlying mechanism for this shift was completely unknown. In light of the accumulating evidence, it was thought likely that adaptation to altitude might involve an increase in the levels of red

cell DPG. This would explain the decreased oxygen affinity of the blood of high altitude residents.

It was probable, therefore, that changes in concentrations of DPG in either direction were potentially capable of causing accompanying shifts in the oxygen dissociation properties in vivo. It remained to be seen, however, whether variations in DPG concentration within normal individuals would also accompany differences in blood values and in the oxygen dissociation characteristics of normal red cells.

The studies which are reported here were aimed at answering the following questions:

1. What are the normal levels of DPG in human red cells, and how much variation exists between individuals?

2. Are there significant sexual and racial differences in the concentration of red cell DPG?

3. Does normal variation in the concentration of red cell DPG and ATP significantly affect the oxygenating capacity of the blood?

   a. Are DPG concentration and normal blood values (such as whole blood hemoglobin, hematocrit, and red cell count) correlated?

   b. Is there a significant relationship between level of erythrocyte DPG and ATP and the extent of oxygenation and deoxygenation of hemoglobin?

   c. Is it possible that changes in the levels of red cell DPG are responsible for the frequently reported shift to the right in the oxygen dissociation curve of individuals living at high altitude?

LOW ALTITUDE POPULATION STUDY

Introduction

The low altitude population study was designed primarily to define the normal levels of red cell DPG in Caucasians and Negroes of both sexes. It was also to determine the limits of variation in DPG levels and whether or not there were significant correlations between an individual's blood values and red cell DPG content. The existence of such a correlation would be indirect evidence in favor of the hypothesis that even normal variations in DPG concentration have a significant influence on the functioning of hemoglobin *in vivo*.

The work of Benesch and Benesch (1967) and of Benesch, et al. (1968) clearly demonstrated that DPG levels below 75% of normal would seriously impair the unloading of oxygen from the hemoglobin, by shifting the oxygen dissociation curve to the left. Although low DPG concentrations would permit rapid and complete alveolar oxygenation of the hemoglobin, unloading would be difficult or impossible, since the oxygen pressure in the tissues might be higher than that required for the initial oxygenation. Low DPG levels would result in abnormally high oxygen saturation in the venous blood, reflecting a lack of efficient oxygen transport. It was of interest, therefore, to determine whether any individuals in the large low altitude sample had DPG levels below this critical 75% level.

Previous work *in vitro* (cited earlier) indicated that increasing the DPG level above physiological levels (that is, above a 1:1 molar

ratio with the hemoglobin) had negligible effect on the shape or position of the oxygen dissociation curve. This suggested that the distribution of values for red cell DPG concentration in a large sample might be skewed somewhat toward the higher levels. On the same line, a correlation between DPG concentration and blood values such as hemoglobin, hematocrit, and red cell count, would be expected to be particularly strong in those individuals with DPG levels below a 1:1 molar ratio with hemoglobin.

Materials and Methods

Blood samples were obtained from a total of 300 individuals. The males in the sample were 200 Negro and Caucasian prisoners (100 of each) resident in Southern Michigan State Prison, Jackson, Michigan. These blood samples were received in lots of 30 or 40, the two races always being equally represented. One hundred adult females of both races were bled at the University of Michigan University Hospital Outpatient Laboratory. These were women who were having pre-employment physical examinations or who were being seen for a variety of minor, non-hematological disorders.

The blood samples were drawn into "Vacutainers" which contained sufficient dry sodium heparin to prevent clotting. In all cases, the heparinized blood samples were kept at 2 to 4°C until processing, which took place within a maximum of six hours after the samples were taken. Control studies employing storage of heparinized samples at 4°C for six hours showed that decrease in DPG content of whole blood within this period was not detectable.

Hemoglobin and micro-hematocrit measurements were made according to standard hematological techniques. Red blood cell counts were carried out on a Coulter Counter.

The red cells from 10 ml. of whole blood were washed three times in at least two volumes of cold 0.145M NaCl. Three ml. of these washed, packed red cells were precipitated with two volumes of cold 6.7% trichloracetic acid (TCA). Except where indicated, this TCA extract was used without further treatment.

All assays of DPG were done with a modification of the chromotropic acid method of Bartlett (1959b). One hundred lambda of the TCA extract previously described was added to 4 ml. of 0.01% chromotropic acid (4, 5-dihydroxy-2,7-naphthalene-disulfonic acid) dissolved in concentrated sulfuric acid. This mixture was heated at 100°C for 135 minutes, to accomplish complete hydrolysis of the phosphates and combination of the chromotropic acid with the glycerates. The chromotropic acid-glycerate complex formed a greenish-blue color which was then read in a spectrophotometer (Beckman D.U.) at 695 milli-microns. Along with each group of samples, at least five controls were run, which contained 100 lambda of 2mM DPG (cyclohexylammonium salt). Samples and controls were read against a blank which contained only the chromotropic-sulfuric acid mixture and 100 lambda of distilled water. The mean optical density (OD) of the control samples was then used to compute the amount of DPG in the extracts as follows:

$$\frac{\text{OD sample}}{\text{mean OD control}/\mu\text{ mole DPG}} = \mu\text{moles DPG/100 lambda of sample}$$

This dividend times the volume of the TCA extract yielded μ moles of DPG per 3 ml. of packed cells. Then:

$$\frac{\mu\text{moles of DPG per 3 ml. packed cells}}{\text{hemoglobin content (of 3 ml. packed cells in grams \%)}} = \mu\text{moles DPG/gram hemoglobin}$$

An enzymatic technique for the assay of DPG was also tried on subsamples of the low altitude population. The technique was a modified form of that reported by Krimsky (1965). Although the results with this enzymatic technique tend to confirm the values obtained with chromotropic acid assay, the reproducibility of the enzymatic technique was so poor that neither it nor the results obtained will be reported in detail here.

All Negro males were typed for glucose-6-phosphate dehydrogenase and hemoglobin, using starch gel electrophoresis. This electrophoresis was carried out according to the technique of Shows, et al. (1964).

Results

The negative correlation which had been expected between red cell DPG concentration and whole blood hemoglobin was found in all groups studied (adult Negro and Caucasian males and females) (see Table 2 and Figure 3). The level of significance of this correlation varied from $p < 0.1$ in the Negro female to $p < 0.005$ in the Caucasian female. A low negative correlation between DPG and hematocrit was also found in three of the four groups. There were, however, no significant correlations between DPG and red cell count in any of the groups.

The means and standard deviations of levels of red cell DPG in the four groups studied are shown in Table 3. A considerable amount of variation was found within all groups tested (ranging from two- to almost three-fold). The mean DPG of each group differs from those of the others at at least the $p < 0.05$ level. There is a large difference between races, whether the sexes be considered collectively or separately (see Figure 4). The direction of the difference in the means between the

TABLE 2

CORRELATIONS BETWEEN LEVELS OF RED CELL DPG AND HEMOGLOBIN, HEMATOCRIT, AND RED BLOOD CELL COUNT IN ADULT NEGRO AND CAUCASIAN MALES AND FEMALES

Group	Number	DPG vs. Whole Blood Hemoglobin	DPG vs. Hematocrit	DPG vs. Red Cell Count
Caucasian males	100	-0.229 ($p < 0.05$)	-0.184 ($p < 0.1$)	-0.076 (N.S.)*
Caucasian females	59	-0.405 ($p < 0.005$)	-0.270 ($p < 0.1$)	-0.198 (N.S.)
Negro males	100	-0.267 ($p < 0.01$)	-0.185 ($p < 0.1$)	+0.002 (N.S.)
Negro females	41	-0.264 ($p < 0.10$)	-0.123 (N.S.)	+0.026 (N.S.)

* N.S. indicates non-significant at the 0.10 level.

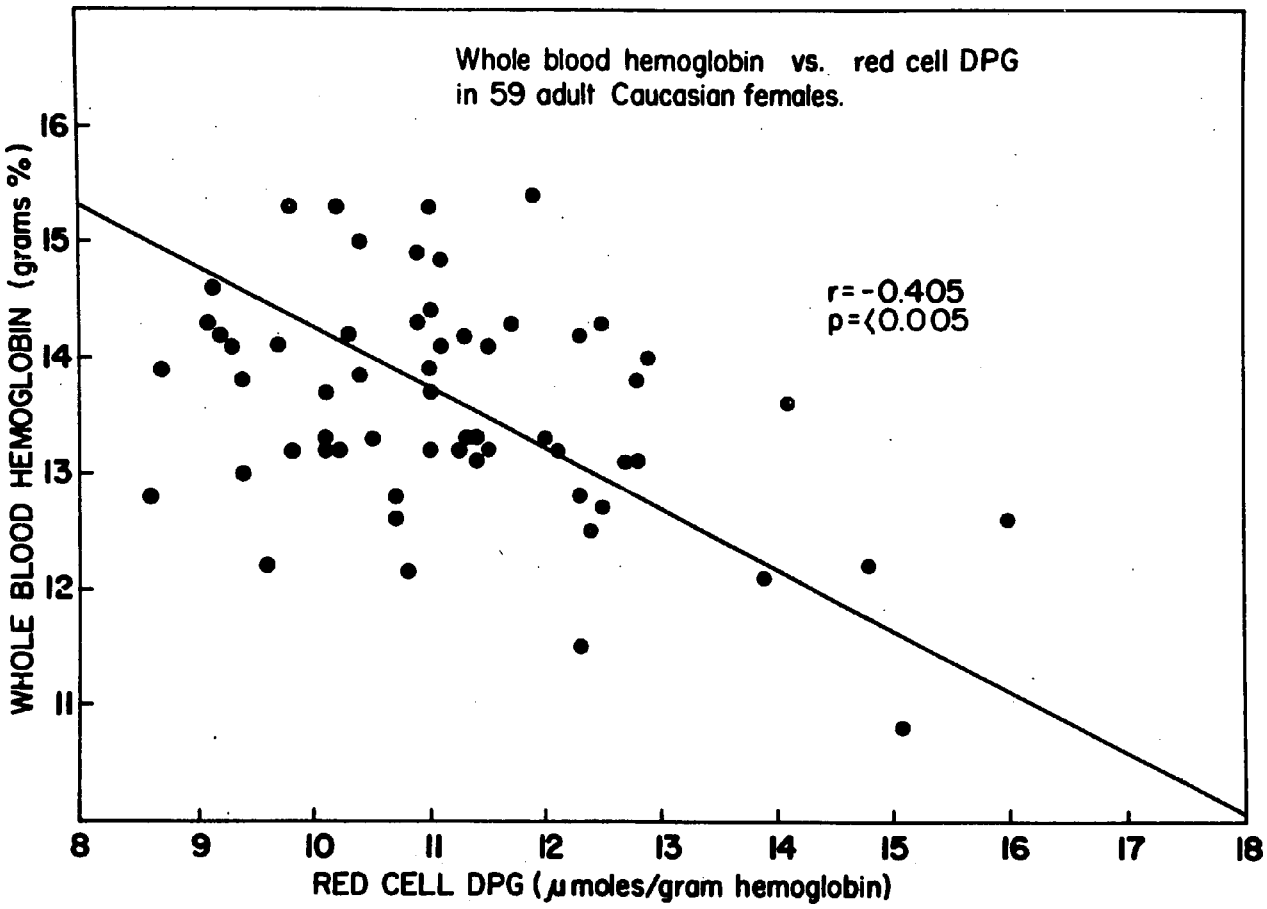


Figure 3.--Scatter diagram showing the negative correlation between whole blood hemoglobin and red cell DPG levels.

TABLE 3

LEVELS OF DPG IN THE RED CELLS OF HUMANS AT LOW ALTITUDE

Group		Number	Erythrocyte DPG Levels[a] Mean (±1 SD)	Range
I.	Caucasian males	100	12.02 (±1.92)[b]	8.50-15.86
II.	Caucasian females	59	11.21 (±1.57)[b]	8.59-16.01
III.	Negro males	100	13.22 (±2.08)[b]	6.78-17.31
IV.	Negro females	41	14.13 (±2.23)[b]	10.89-21.82

[a]DPG values expressed in μmoles per gram of hemoglobin.

[b]Significance levels of difference 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, $p < 0.05$.

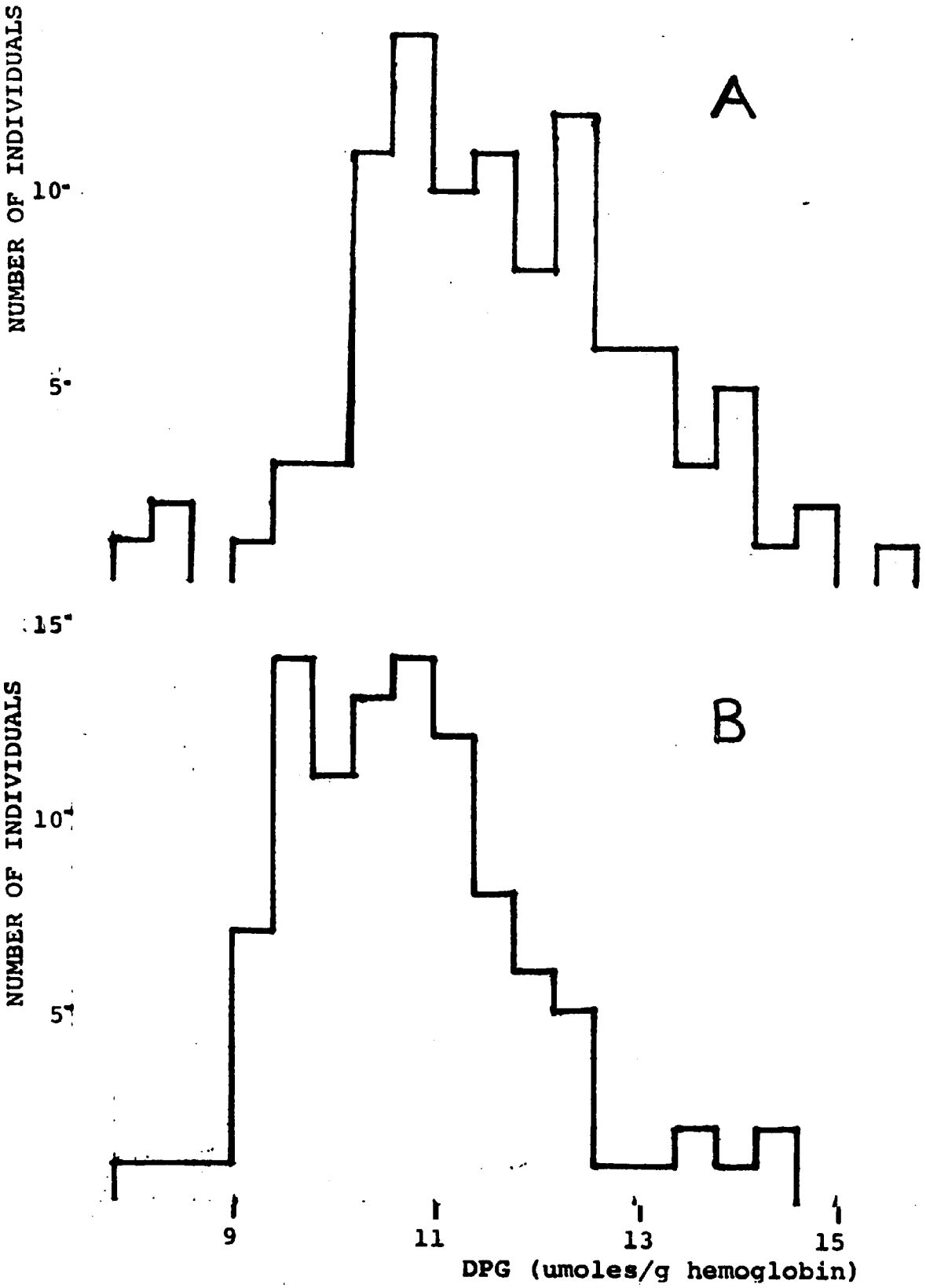


Figure 4.--Frequency distributions of red cell DPG values for 100 Negro males (A) and 100 Caucasian males (B).

sexes in the two races is different. Among the Caucasians the male is higher than the female ($p < 0.01$), while among the Negroes, the female is higher than the male ($p < 0.05$).

As previously mentioned, all Negro males were typed for G-6-PD and hemoglobin. In this sample of 100, 15 were found to be G-6-PD deficient, 9 were heterozygous for hemoglobin S, and 3 were heterozygous for hemoglobin C. Neither of the groups heterozygous for hemoglobins S and C were different in levels of red cell DPG. However, the G-6-PD deficient individuals were found to have mean levels of DPG somewhat higher than the normal mean(13.90 μmoles/gram hemoglobin vs. 13.22 μmoles/gram hemoglobin, $t = 1.168$, which is not significant). This difference was so slight that exclusion of the G-6-PD deficients from the statistical analysis did not affect any of the levels of significance previously mentioned.

Discussion

Perhaps the most important result of the population survey was the finding of significant negative correlations between red cell DPG content and the whole blood hemoglobin levels in all groups studied. 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 "r" values for the correlation between DPG level and whole blood hemoglobin concentration are too low to enable accurate prediction of one value given the other (the square of the "r" being a measure of the amount of variation of one variable accounted for by variation in the other). However, as pointed

out later in the paper (see General Discussion and Implications), a low correlation coefficient is to be expected between variables which may be influenced by so many other physiological parameters. The negative correlation with DPG level is less strong for hematocrit and almost non-existent for red cell count. This may indicate that these correlations are secondary to that of hemoglobin with DPG.

As previously mentioned, the work of Benesch, et al. (1968) led them to conclude that normal variations in the physiological range in red cell DPG content would have little effect on oxygen affinity. However, an inverse relationship between hemoglobin and DPG seems to exist throughout the entire range of the distributions, indicating that DPG level may be functionally related to the ability of the red cell to deliver oxygen. The negative hemoglobin/DPG correlation is probably due to two major considerations which appear to have been overlooked by Benesch.

First, Benesch and Benesch (1967) and Chanutin and Curnish (1967) were working with dilute solutions of hemoglobin (0.3 grams %, or about 1/100 that which normally occurs in the human red cell). They were, however, using physiologically normal concentrations of DPG. As mentioned in the Introduction, the DPG will combine with deoxygenated hemoglobin more readily than with oxygenated hemoglobin. In these previous experimental systems, because of the extremely dilute hemoglobin solutions employed, the molarity of the free, unbound DPG will vary only slightly, due to there being relatively few hemoglobin molecules available to participate in DPG binding.

In the normal red cell, however, the DPG and hemoglobin concentrations are about equal (on a molar basis). Therefore, when the hemoglobin has

released 50% of its oxygen, the molarity of free DPG will have decreased about 25%, a change which the data of both Benesch and Benesch (1967) and Chanutin and Curnish (1967) show to be of great importance in affecting the oxygen dissociation curve. This being the case, rather small differences between individuals in the concentration of red cell DPG may be magnified in proportion to the level of deoxygenation of the red cell. It is probable, therefore, that slight variations in DPG content of the red cell may seriously affect the oxygen dissociation properties (and therefore the oxygen transport properties) of the red cell in vivo.

A second consideration of possible relevance to the relationship of DPG and hemoglobin concentrations in vivo is that variation in DPG concentration has an effect on the rate of hemoglobin oxygenation and deoxygenation. Both groups of authors previously mentioned were working with equilibria obtained 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 phosphates (and evidence that this is the case will be presented in Section IV of this manuscript), then small variations in physiological levels of DPG between individuals may, after all, have very important ramifications. Preliminary evidence presented later indicates that higher levels of DPG will lead to slower oxygenation and more rapid deoxygenation.

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 (see Table 4).

With reference to the first possibility, it is known that DPG levels are increased in anemias of several types (Brewer and Eaton, unpublished observations). A possible mechanism for this is the greater venous desaturation of the blood which is characteristic of most anemic states. Since this will increase average desaturation of the blood, greater amounts of DPG will be bound. Homeostatic mechanisms within the red cell may maintain the levels of free DPG at a constant level, and with an increase in bound DPG, the total content of DPG is increased. This same mechanism, operating in a more subtle degree, would then help 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 erythropoesis, would be partially determined by the concentration of red cell DPG.

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

TABLE 4

THE RELATIONSHIP BETWEEN RED CELL DPG CONCENTRATION AND WHOLE BLOOD HEMOGLOBIN LEVEL:

HYPOTHESIS I: THE LEVEL OF WHOLE BLOOD HEMOGLOBIN DETERMINES THE ERYTHROCYTE DPG (+ATP) LEVEL.

↓HEMOGLOBIN---------↑DESATURATION---------↑AVERAGE AMOUNT OF DEOXYHEMOGLOBIN-BOUND DPG--------

↑TOTAL DPG (+ATP?)

OR

↑HEMOGLOBIN---------↓DESATURATION---------↓AVERAGE AMOUNTS OF DEOXYHEMOGLOBIN-BOUND DPG-------

↓TOTAL DPG (+ATP?)

HYPOTHESIS II: THE CONCENTRATION OF ERYTHROCYTE DPG (+ATP) DETERMINES THE LEVEL OF WHOLE BLOOD HEMOGLOBIN.

↓DPG (+ATP)---------↓DESATURATION---------↑ERYTHROPOESIS---------↑WHOLE BLOOD HEMOGLOBIN

OR

↑DPG (+ATP)---------↑DESATURATION---------↓ERYTHROPOESIS---------↓WHOLE BLOOD HEMOGLOBIN

HYPOTHESIS III: A COMBINATION OF I AND II.

The levels of DPG in the Negro were found to be 15 to 20% higher than in the Caucasians. This contrasts with previous findings regarding ATP levels, in which Caucasians were found to have significantly higher erythrocyte ATP than Negroes (Brewer, 1967). Although at present we cannot be sure of the functional significance or cause of these differences, there is at least one possible contributing factor. The previously mentioned work on pyruvic kinase abnormality by Zurcher, et al. (1965) indicates the existence of a possible reciprocal relationship between levels of ATP and DPG. The work of Zurcher, et al., 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 PK deficiency, it has been reported that the reverse of this situation obtains (Keitt, 1966). That is, red cell ATP is decreased at least 30 to 40% and DPG is increased two- to three-fold. Although these are admittedly 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.

Brewer and Powell (1965) have reported a strong positive relationship between the levels of red cell ATP in humans and the initial development of parasitemia in infections of falciparum malaria. That is, the higher the red cell ATP levels of the subject, the less time is required for the parasite to reach a given level of infection (in this case, 1000 parasites per cubic millimeter of blood). Recently, Eaton and Brewer (1969) have reported finding this relationship also present in a group of rhesus monkeys which were infected with P. cynomolgi. It may be speculated that a part of the increase in DPG level in Negroes may have resulted from selection by malaria. Malaria would tend to selectively decrease red cell

ATP levels, and ATP and DPG seem to be inversely correlated in some of the disorders mentioned. Since both DPG and ATP affect the oxygen binding by hemoglobin, it is probably important to maintain the sum of the two compounds.

Also in this regard, it might be noted that the Negroes in our sample of two hundred males had significantly lower blood values than the Caucasians (hemoglobin and hematocrit - $p < 0.001$ for both). Apparently, this difference has not previously been reported. The lower value for mean hemoglobin level in the Negro male may be causative (or, indeed, perhaps reflective) of the increased levels of red cell DPG.

In a purely functional vein, we might propose the following: Since the best correlation with hemoglobin is with the sum of the two major phosphorylated intermediates (ATP and DPG), it would seem likely that a reduction in one would occasion an increase in the other, since the hemoglobin requires a certain level of (i.e., sum of) phosphorylated intermediates within the red cell to function properly. Therefore, if the ATP level were reduced in the red cell, for whatever reason, we might expect the level of DPG to be (homeostatically or evolutionarily) adjusted upward to meet this deficit.

In summary, the low altitude population studies showed that:

1. There is a significant inverse correlation between levels of red cell DPG and whole blood hemoglobin.

2. There are large inter-individual differences in levels of red cell DPG.

3. Significant sexual and racial differences exist in levels of red cell DPG.

HIGH ALTITUDE POPULATION STUDY

Introduction

Man's physiologic adaptations to the hypoxia of high altitude have received a great deal of attention. Most studies of these adaptations, however, have focused on the organs of circulation and respiration. One exception to this emphasis has been the work of Aste-Salazar and Hurtado (1944) which showed that a definite shift to the right of the oxygen dissociation curve occurs in the blood of individuals normally acclimatized to high altitude (Figure 5).

As previously mentioned, a shift of the oxygen dissociation curve to the right would mean that somewhat higher oxygen pressures would be required for satisfactory oxygenation in the lung. However, the right shift will favor fuller release of the bound oxygen as the red cell passes through the tissues. Therefore, if the impedance of oxygen uptake in the lung is proportionately less than the enhancement of delivery to the tissues, this shift of the oxygen dissociation curve to the right might well be functionally important in adaptation to hypoxia.

The results which were obtained during the low altitude population studies gave strong indication that DPG and the respiratory functioning of hemoglobin were related. In view of the accumulated evidence, it was considered quite possible that an increase in the concentration of red cell DPG might account for at least a portion of the shift to the right in the oxygen dissociation curve of high altitude residents.

Further, it has been known for some time that a significant proportion (5-10%) of the adult population is incapable of making satisfactory

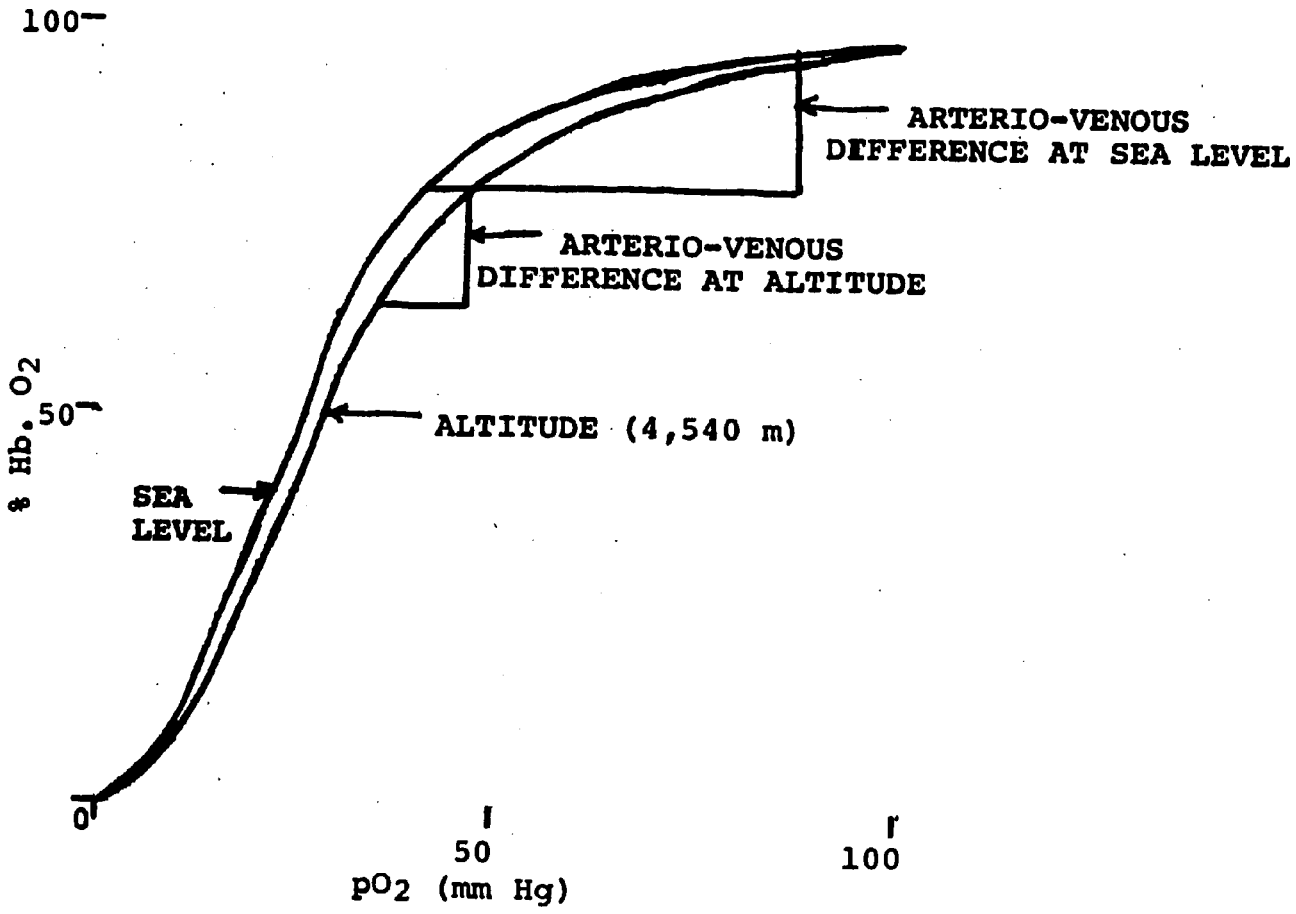


Figure 5.--The positions of the oxygen dissociation curves in residents of sea level and altitude (4,540 m), showing the drop in pO_2 and percent saturation in each (from Hurtado, 1964).

adjustment to altitudes of 10,000 feet, and will develop an excessive polycythemia (often with hematocrits well above 60 volumes per cent). Excessive polycythemia of this sort is one of the major symptoms of individuals affected with chronic mountain sickness (Monge and Monge, 1966). Chronic mountain sickness (or Monge's disease) is frequently characterized by peptic ulcer and severe cardiovascular disorders. It has been observed that the arterial oxygen saturations of people affected with chronic mountain sickness are lower than normal (Monge and Monge, 1966). No fully adequate explanation for this disorder has yet been offered. It was felt that an unsatisfactory adjustment of the levels of red cell DPG in these individuals might be involved.

The high altitude study was designed, therefore, to determine: 1) whether high altitude residents had significantly elevated levels of red cell DPG; 2) whether the inverse correlation between DPG and hemoglobin which had been found at low altitude was also present at high altitude; and, 3) whether the DPG levels in individuals affected with excessive polycythemia were abnormal.

Materials and Methods

The subjects used in this study were residents of the area of Leadville and Climax, Colorado (elevations 10,200 and 11,300 feet respectively). Most of these subjects who volunteered to donate blood samples worked at the Climax Molybdenum Company in Climax. There are relatively few females in the sample because most of the employees of this mining company are male. Patients with excessive polycythemia were referred to us by local physicians (all individuals with hematocrits

over 58 volumes per cent for men and over 53 volumes per cent for women are considered excessively polycythemic at this altitude). The usual therapy for polycythemia at high altitude is occasional phlebotomy which will temporarily reduce the hematocrit value to acceptable levels. It is known that DPG levels (and ATP levels) are elevated in young red cells (Bernstein, 1959), a great number of which will be produced following phlebotomy. Because of this, we chose polycythemics who had not been recently bled, in order to avoid the confounding influence of this factor.

All laboratory manipulations were performed as reported in the previous section on low altitude populations. The red cell extracts, however, were stored frozen for an average of seven days. Control studies done previously indicated that no detectable change in DPG level occurs during this period of storage.

Results

As had been hypothesized, there was a significant elevation of red cell DPG in individuals adapted to high altitude (see Table 5). This elevation amounted to about 6.4% in the males ($p < 0.01$) and 19.7% in the females ($p < 0.001$). The hemoglobin and hematocrit values were, of course, higher than those of the low altitude population (Table 6). To check whether the elevation in DPG levels was an artifact caused by such factors as lower amounts of hemoglobin per cell, the DPG levels were also calculated on a per cell basis. The results of these calculations (Table 5) demonstrate that DPG is also elevated significantly when expressed on a basis of μmoles of DPG per

TABLE 5

THE LEVELS OF RED CELL DPG IN ADULTS RESIDENT AT LOW ALTITUDE AND IN NORMALS AND POLYCYTHEMICS AT HIGH ALTITUDE

	Number	DPG per Gram Hemoglobin (± 1 S.D.)[a]	DPG per 10^{10} Red Blood Cells (± 1 S.D.)[b]
Ann Arbor:			
I. Males	100	12.02 (±1.92)	4.12 (±0.385)
II. Females	59	11.21 (±1.57)	3.64 (±0.547)
Leadville:			
III. Normal males	119	12.79 (±2.22)	4.65 (±0.710)
IV. Normal females	34	13.16 (±3.40)	4.37 (±0.936)
V. Polycythemic males	44	16.23 (±3.22)	5.61 (±1.220)
VI. Polycythemic females	5	16.49 --	5.26

[a]For DPG levels expressed in μmoles per gram hemoglobin, significance of differences between means (Student's "t" test): I vs. III, $p < 0.05$; II vs. IV, $p < 0.001$; III vs. V, $p < 0.001$.

[b]For DPG levels expressed per 10^{10} red blood cells, significance of differences between means (Student's "t" test): I vs. III, $p < 0.01$; II vs. IV, $p < 0.01$; III vs. V, $p < 0.01$. Based on subsamples of twenty for groups I through V.

TABLE 6

MEAN BLOOD VALUES OF LOW ALTITUDE (ANN ARBOR, MICHIGAN) AND HIGH ALTITUDE (LEADVILLE, COLORADO) CAUCASIAN POPULATIONS

Group	Number	Whole Blood Hemoglobin (grams %) (± 1 S.D.)	Hematocrit (± 1 S.D.)
Ann Arbor males	100	15.22 (±1.14)	46.67 (±2.81)
Leadville males	119	18.02 (±1.35)	51.74 (±3.54)
Leadville polycythemic males	44	19.79 (±1.79)	60.03 (±4.84)
Ann Arbor females	59	13.56 (±0.94)	42.40 (±2.44)
Leadville females	34	15.79 (±1.61)	46.24 (±3.24)
Leadville polycythemic females	5	19.32 (±1.57)	57.80 (±5.58)

10^{10} red cells. This is an indication that the DPG increase at altitude is not a secondary manifestation of variation in mean cell hemoglobin.

The results of DPG assay on samples from 59 male and female polycythemics were 27% higher than high altitude normals, and for female polycythemics, 25% higher (Table 5).

The negative correlation between whole blood hemoglobin and DPG levels, which had been previously found in the low altitude population, was also present in all groups of this high altitude sample (Table 7).

Discussion

The increase in levels of red cell DPG in high altitude residents is almost certainly a factor in the shift to the right of the oxygen dissociation curve at high altitude. It has been previously pointed out that this shift to the right will result in greater oxygen unloading in the tissues. It might seem at first glance that the increases in DPG level which have been found are insufficient to explain the shift in the curve. However, as the cell progressively desaturates, it has the effect of magnifying what were previously small differences in molarity of free DPG.

For example, let us consider two red cells, one of which has a DPG/hemoglobin ratio of 1:1, and the other 1.2:1 (20 per cent higher than the first). Now, if both cells are desaturated to 50%, and if equivalent amounts of free DPG are accordingly bound, the differences in free DPG will be on the order of 20%. The work of Benesch and Benesch (1967) has shown differences of this magnitude to be of importance in affecting the position of the oxygen dissociation curve.

TABLE 7

THE CORRELATIONS OF RED CELL DPG CONCENTRATION WITH WHOLE BLOOD HEMOGLOBIN LEVEL IN ADULTS RESIDENT AT LOW AND HIGH ALTITUDES

Group	Number	Correlation of DPG with Whole Blood Hemoglobin
Ann Arbor males	100	-0.229 ($p < 0.05$)
Leadville males	119	-0.217 ($p < 0.05$)
Leadville polycythemic males	44	-0.345 ($p < 0.02$)
Ann Arbor females	59	-0.405 ($p < 0.005$)
Leadville females	34	-0.671 ($p < 0.001$)
Leadville polycythemic females	5	--

Although the average level of venous desaturation is about 70% in humans at low altitude, the amount of desaturation varies greatly with the part of the body through which the red cell happens to pass. For example, the blood which passes through the coronary artery is regularly desaturated to 30% (Grover, personal communication). In this case, even small differences in DPG concentration would be very important in dictating the amount of oxygen delivered by the cell. Further, these small differences may have large effects on cardiac function. In sum, then, small differences in the level of erythrocyte DPG are magnified in proportion to the level of deoxygenation of the red cell.

A second, and very important consequence of small differences in DPG level may be a significant change in the rate of deoxygenation and oxygenation of whole blood. The experimental evidence in this regard will be presented in the third section of this paper. Increases in DPG level may result in the delivery of even more oxygen than would have been predicted on the basis of the shift of the curve itself.

The elevation of red cell DPG levels in high altitude residents may be explained by the greater average levels of desaturation. At low altitude, arterial saturation is generally about 97%, whereas at altitudes of 10-11,000 feet, the saturation is depressed to about 90% (Weil, et al., 1968) (see again Figure 5). Since the mixed venous saturation will also be somewhat lower than at low altitude, the average level of desaturation is depressed at least 10%. Because deoxy-hemoglobin has a much greater affinity for free DPG, we would expect that the average amount of bound DPG at altitude would be greater than at sea level. If the amount of unbound DPG is controlled within fairly narrow limits by certain of the enzymes of glycolysis, then the total DPG will

increase as more DPG becomes bound. In the case of most normals examined at altitude, the calculated increase in desaturation is sufficient to explain the elevation of the red cell DPG levels.

After the submission of the above results for publication (Eaton, et al., 1969), a paper by Lenfant, et al. (1968) appeared which confirms the elevations of red cell DPG in long term residents at altitude. Their work also indicates that increases in the concentration of red cell DPG are associated with the shift of the oxygen dissociation curve to the right during the early stages of acclimatization of humans to altitude.

The two sexes show unequal increases in levels of red cell DPG, the female having a much greater elevation than the male in comparison to low altitude populations. This greater increase on the part of the female is so far unexplained. It is interesting that the female at high altitude seems much less susceptible than the male to excessive polycythemia. Only 5 of the 49 individuals with excessive polycythemia whom we were able to find were female. Further, those females who do develop this condition tend to be past reproductive age. All of the five females with polycythemia whom we studied were post-menopausal, and most reported a fairly recent onset of the disorder.

The relatively extreme elevations of red cell DPG in people with chronic mountain sickness cannot now be completely explained. Certainly, a portion of this elevation may be due to greater average levels of desaturation as discussed above. However, in many cases an increase in desaturation level is definitely insufficient as an explanation for the rise in DPG. For example, 21% of the polycythemic males in our sample had DPG levels at least 50% above the mean for normally adapted males. Elevations of this degree would require that arterial saturations be on

the order of 40 to 50%. However, even at much higher altitudes (4,450 meters), the arterial saturation does not drop below 60% in people with excessive polycythemia (Monge and Monge, 1966). This means that other mechanisms beside a simple increase in desaturation are probably responsible for the elevations of red cell DPG levels found in some of the sample of polycythemics.

It is possible that the extreme elevation of DPG in people with excessive polycythemia is contributory or causative. It was mentioned earlier that a shift of the oxygen dissociation curve to the right was desirable if it resulted in greater oxygen release. For this greater release, the amount of decrease in arterial saturation must be less than the amount of increase in venous desaturation. In other words, the blood must be able to release a greater amount of oxygen although it is starting out with less. The adjustment which in normals at altitude produces an arterial saturation of about 90% seems quite satisfactory. However, some of the polycythemics in Leadville have been found to have arterial saturations between 70 and 80%. It may be that this low starting point results in insufficient oxygenation of the tissues. One reason for this arterial unsaturation may be the high levels of red cell DPG.

The most frequent explanation of polycythemia at high altitude has been hypoventilation, such that the blood going through the pulmonary beds is not exposed to a satisfactory oxygen tension (see, for example, Hurtado, 1964). In support of the possibility that excessively high levels of red cell DPG may decrease arterial desaturation, it may be noted that several authors (e.g., Rucknagel and Witkop, 1959) have shown that there is significant arterial desaturation in patients homozygous

for hemoglobin S. The average arterial saturation in these individuals is about 83% (as opposed to 97% in normals). Part of this relative desaturation may be due to inhibition of pulmonary oxygenation, caused by a shift of the dissociation curve too far to the right. In view of the evidence available at present, the extraordinary elevation of red cell DPG is as likely to be causal of excessive polycythemia at altitude as is hypoventilation.

In summary, the high altitude study showed that:

1. The levels of red cell DPG were elevated in both sexes of residents of high altitude.

2. The negative correlation between whole blood hemoglobin and DPG levels which had been found at low altitude was also present in the high altitude sample.

3. Individuals affected with excessive polycythemia (a major symptom of chronic mountain sickness) were found to have extreme elevation of red cell DPG. This elevation may be causal or contributory in the pathogenesis of excessive polycythemia.

DPG, ATP, AND OXYGEN TRANSPORT: IN VITRO EVIDENCE

Introduction

The results just reported imply a specific and central role for DPG (and ATP) in the control of oxygen transport. There had not been any previous work aimed at strengthening the connection between the levels of glycolytic intermediates and the gas transport function of the red cell. To this end, an apparatus was designed which would allow measurement of the rate of oxygen transport by red cells of different individuals in a controlled environment.

Previous studies of the oxygen transport capacity of blood have usually employed oxygen dissociation data. Oxygen dissociation curves are constructed through exposure of the blood to a series of oxygen tensions. The per cent saturation of the blood after equilibration with each gas mixture is measured, and the points derived are combined to produce an "oxygen dissociation" curve (see, for example, Figures 2 and 5). Since these curves are based on static equilibria reached between hemoglobin and a series of different oxygen tensions, the results are not directly relevant to the physiologic situation.

As the red cell passes through the alveolae of the lungs, its time of transit is extremely short (on the order of 780 milliseconds according to the calculations of Roughton, 1945). Therefore, the time available to the cell to reach equilibrium is quite limited. Indeed, equilibrium is not reached. Arterial blood normally has an oxygen saturation of about 96%, although if it were in equilibrium with the

alveolar gas, the saturation would be 100%. Likewise, as the red cell passes through the capillaries, equilibrium between the tissue oxygen pressure and that within the red cell is not reached.

The only previous work which measured the rate of oxygen delivery of whole blood employed very involved apparatus (Baumberger, et al., 1967). These authors reported that significant differences existed between individuals in the rates at which oxygen was delivered by whole blood. The rates of oxygen delivery at different starting saturations were measured in a number of subjects. Above about 80% starting saturation, the differences between individuals were insignificant. Below 80% saturation, however, the inter-individual differences increased progressively. This finding agrees well with the idea that differences in red cell DPG and ATP levels are magnified as desaturation becomes greater. In fact, Baumberger, et al. (1967) state that "While no definite explanation has been found for the observed individual differences in oxygen delivery rate, variations in the erythrocytes themselves would appear to be a decisive factor."

It seemed likely that measurement of the rate of oxygen transport by whole, fresh blood might maximize information on differences between individuals, and might perhaps enable definition of some of the factors behind these differences. Therefore, an apparatus was designed to answer the following questions:

1. Is it possible to demonstrate significant differences between individuals in the rates at which oxygen delivery occurs?

2. Are these differences in rate correlated with blood values and/or with the sum of ATP and DPG within the red cell?

3. Are the differences in rate of deoxygenation greater or smaller than corresponding differences in points of equilibration of whole blood with certain oxygen tensions?

Materials and Methods

A. Rate of desaturation:

The apparatus which was constructed to measure rates of oxygen transport took advantage of a relatively new, gas-permeable tubing called "Silastic"^{R} (see Figure 6). This tubing allows a free interchange of gasses.

Freshly drawn heparinized blood from 12 adult men and 8 adult women was placed in a tonometer and equilibrated with 95% oxygen and 5% CO_2 for thirty minutes. The blood was then withdrawn from the tonometer (the resultant oxygen saturation being 100% in all cases) into a 10 ml. glass syringe which contained a magnetic stirring bar. The syringe was then mounted on a constant-flow infusion pump (adjusted to deliver about 1.0 ml. every three minutes), and a magnetic stirring device was placed over it to keep the erythrocytes in suspension. The blood was then pumped through gas-impermeable tubing into a coil of Silastic tubing (approximately 150 cm. long, 0.635 mm. in diameter) which was contained in a gas-tight chamber. The chamber was perfused with a gas mixture of 95% nitrogen and 5% CO_2, at a flow rate of about 2 cubic feet per hour. As the whole blood passed through the nitrogen environment, it lost some of the oxygen previously bound to the hemoglobin.

The blood which left the chamber was collected under mineral oil in a cuvette, and the oxygen saturation was read in a AO reflectance-type oximeter.

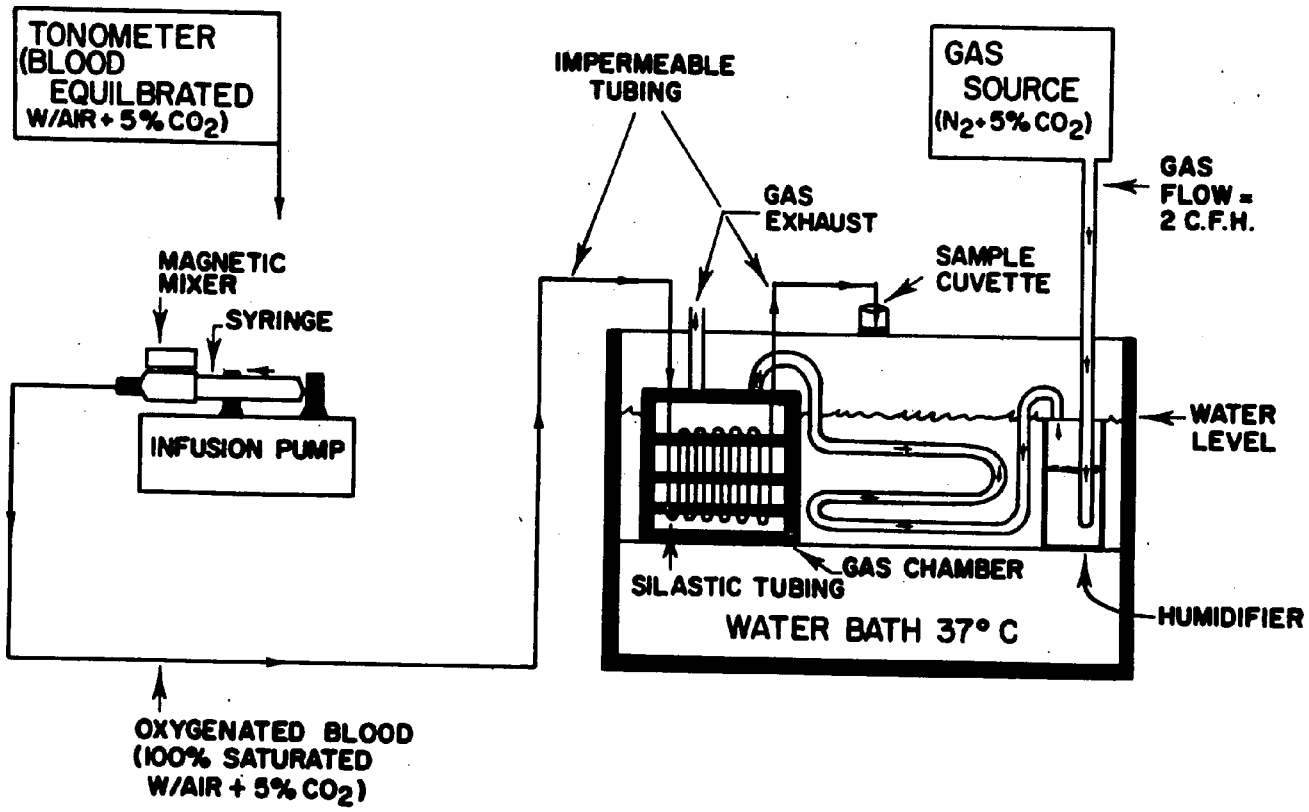


Figure 6.--The apparatus for measuring the rate of oxygen release by whole blood. See text for explanation.

It should be stressed that the rate of oxygen release by the whole blood is only one of the parameters reflected in the resultant desaturation after passage through the rate measuring apparatus. The steepness of the gradient in oxygen tension between the interior of the red cell and the exterior of the silastic tubing is probably related to the amount of oxygen which the whole blood unloads. For example, if the dissociation curve is left-shifted (toward greater oxygen affinity), the gradient will be less steep, and less oxygen will be unloaded than if the curve were right-shifted (in the direction of lesser oxygen affinity). Also, factors such as the diffusibility of the oxygen released by the red cell through the plasma are probably of importance in controlling the absolute amount of oxygen unloaded by any given sample of whole blood. It is not possible, therefore, to state that the rate of release of oxygen by the red cell is the only variable being measured in this apparatus.

Since both ATP and DPG have been found to have significant influence on the oxygen combining capacities of hemoglobin, both substances were assayed in the small group reported in these experiments. A quantity of the blood which passed through the chamber was collected for ATP and DPG assay, and for determination of hemoglobin and hematocrit. The whole procedure, from withdrawal of the blood until the extract for DPG and ATP was made, lasted about 45 minutes. Assays of ATP and DPG, done before and after this experimental manipulation indicated that neither compound decreased during this 45 minute period. The assay results reported here are those from samples precipitated after exit from the apparatus.

The assay of ATP was done according to Brewer (1967), and the assay of DPG was done as described earlier in this paper (see Low Altitude

Population Studies, materials and methods), both with the following exceptions: 1) Only 0.5 ml. of whole blood was precipitated, using 2 ml. of 6.7% TCA. 2) Since the plasma does contribute some color to the chromotropic acid assay, 0.5 ml. of plasma was also precipitated at the same time to serve as a blank for the whole blood extract. Following the assay, the color contributed by the plasma was subtracted from that of the whole blood extract. 3) Since the quantities being used were so small, the volume of extract was not measured, but was assumed to be the sum of the volumes used (i.e., 0.5 ml. whole blood + 2 ml. TCA solution = 2.5 ml.). This assumption resulted in mean ATP and DPG values about 15% higher than those reported for the population studies. This difference is not important, since comparability between subjects in this group and in the first group was not required.

Materials and Methods

B. Equilibration study:

Oxygen dissociation curves (based on the static equilibria which are reached between various gas mixtures and whole blood or hemolysates) of normal people are of a characteristic sigmoid shape (see, for example, Figure 5). The shape of the dissociation curve varies only slightly between individuals. Because of this lack of significant variation in shapes of curves, the p50 (the oxygen tension at which 50% of the hemoglobin is saturated with oxygen) is normally estimated by interpolation between two oxygen tension/saturation measurements which are above and below 50% saturation. In and of itself, the p50 is no more

meaningful than any other part of the dissociation curve, except insofar as it occurs in an area of the curve in which small changes in oxygen pressure produce relatively large changes in hemoglobin saturation.

In the present study, it was decided to choose an oxygen tension which would give saturations in the area of 40 to 50%. The whole, freshly drawn heparinized blood was equilibrated with 2.65% oxygen, 5% CO_2, and 92.35% nitrogen. The resultant oxygen saturation was measured as previously described (Materials and Methods, Section IV, Rate Study). Since the equilibrations were all done at ambient room temperature (which was recorded each time), the saturations were corrected to 37°C using a temperature/saturation correction nomogram (Dittmer, 1961). ATP and DPG measurements and determinations of whole blood hemoglobin and hematocrit were also made as described in the previous section.

Results

A. Rate of Desaturation:

It was found that there were significant differences (about two-fold) between individuals in the rates of deoxygenation within this controlled environment. One of the most remarkable results of this study was the strong inverse correlation found to exist between whole blood hemoglobin level and the per cent desaturation during passage through the rate apparatus (see Figure 7 and Table 8). This implies that the lower the whole blood hemoglobin, the greater the per cent desaturation which may be expected. In agreement with this, fairly

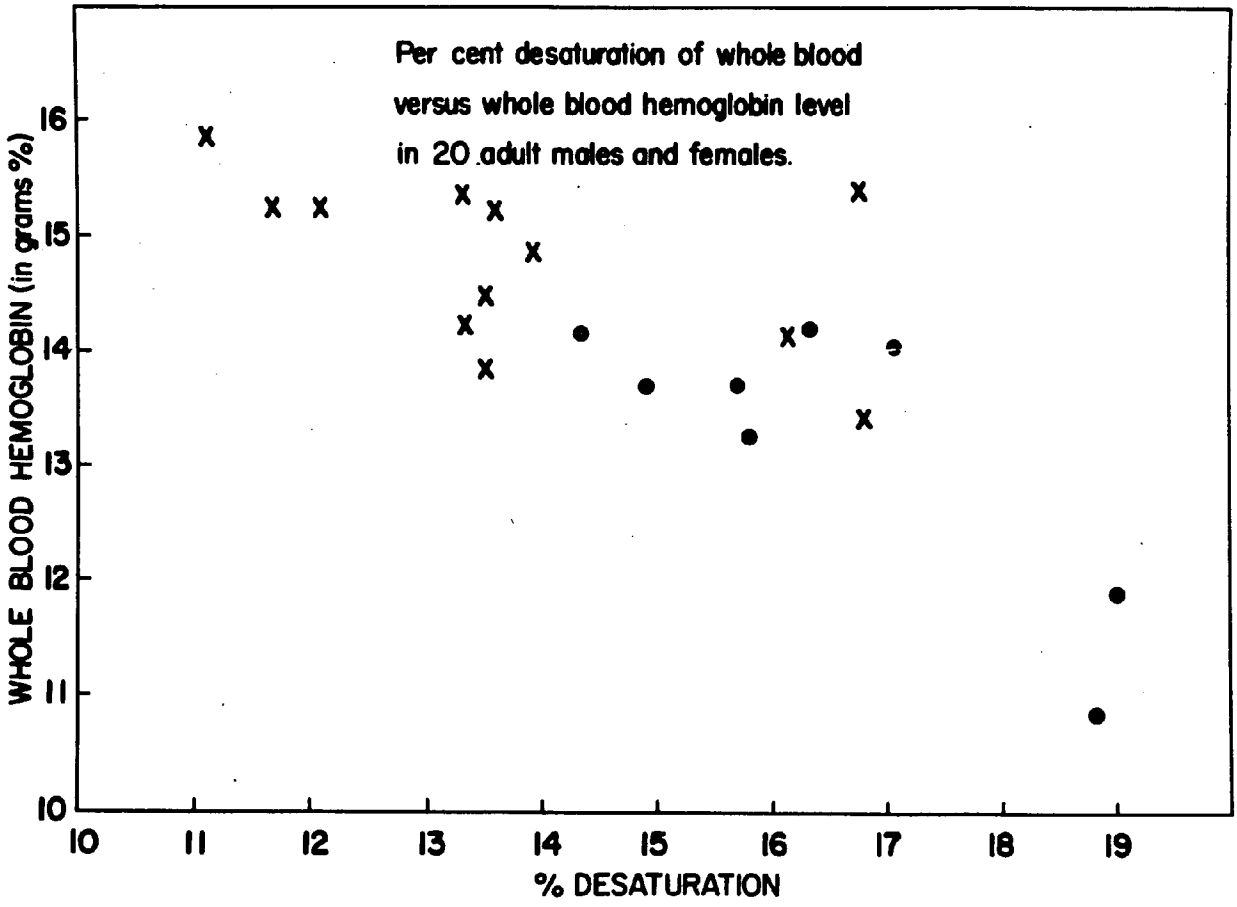


Figure 7.--The relationship between whole blood hemoglobin levels and percent desaturation in the rate measuring apparatus in 12 males (crosses) and 8 females (solid circles).

TABLE 8

THE COEFFICIENTS OF CORRELATION AND p VALUES FOR WHOLE BLOOD HEMOGLOBIN VS. % DESATURATION AND FOR THE SUM OF ATP AND DPG VS. % DESATURATION IN A SAMPLE OF 8 FEMALE AND 12 MALE ADULT CAUCASIANS

		Whole Blood Hemoglobin vs. % Desaturation:		ATP + DPG vs. % Desaturation:	
Group	Number	Coefficient	p Value	Coefficient	p Value
Females	8	-0.816	<0.01	+0.412	N.S.
Males	12	-0.479	<0.10	+0.370	N.S.
Combined sexes	20	-0.763	<0.001	+0.464	<0.05

large sexual differences were present, the female unloading 10 to 20% more oxygen per gram of hemoglobin (see Table 9). This negative hemoglobin/desaturation relationship was later borne out in a larger sample of 20 males and 20 females (Brewer and Eaton, unpublished observations) (see Figure 8).

In view of the surprisingly high correlation found between hemoglobin level and per cent desaturation of the whole blood, it was thought possible that this was an artifact of the experimental technique (i.e., a rate limitation caused either by the permeability of the Silastic tubing or by diffusion through the plasma). In order to control for this possibility, parts of single samples of blood were adjusted to different hemoglobin levels (from about 9 grams per cent to about 20 grams per cent). These different hemoglobin concentrations were then run through the apparatus, and it was found that there was a linear relationship between the concentration of hemoglobin in the sample and the amount of oxygen which the hemoglobin unloaded (see Figure 9). In other words, the per cent desaturation of the hemoglobin was almost exactly the same for all five hemoglobin concentrations used. This ruled out the possibility that diffusion of the oxygen through the plasma or the Silastic tubing was rate limiting, and indicated that the amount of desaturation which took place was a function of the efficiency of the red cell in oxygen release.

In addition to the strong negative correlation found between whole blood hemoglobin and per cent desaturation, a low positive correlation obtained between the sum of red cell DPG and ATP and the per cent desaturation (see Figure 10 and Table 8). This indicated

TABLE 9

DIFFERENCES BETWEEN THE SEXES IN RATES OF DESATURATION (DESATURATION %) AND POINT OF EQUILIBRATION WITH 2.65% OXYGEN (EQUILIBRATION %)

Variable	Sex	Number	Mean (± 1 S.D.)	t Value and Level of Significance[a]
Desaturation % (this report)	female	8	16.54 (± 1.60)	3.31 ($p < 0.01$)
	male	12	13.80 (± 1.80)	
Desaturation % (Brewer and Eaton, unpublished observations)[b]	female	20	26.10 (± 2.90)	2.25 ($p < 0.05$)
	male	20	24.18 (± 2.51)	
Equilibration %	female	7	37.84 (± 3.87)	3.73 ($p < 0.01$)
	male	10	44.02 (± 2.55)	

[a]Student's t test.

[b]The greater level of desaturation in this second study is due to a reduction in the rate of passage of blood through the rate apparatus.

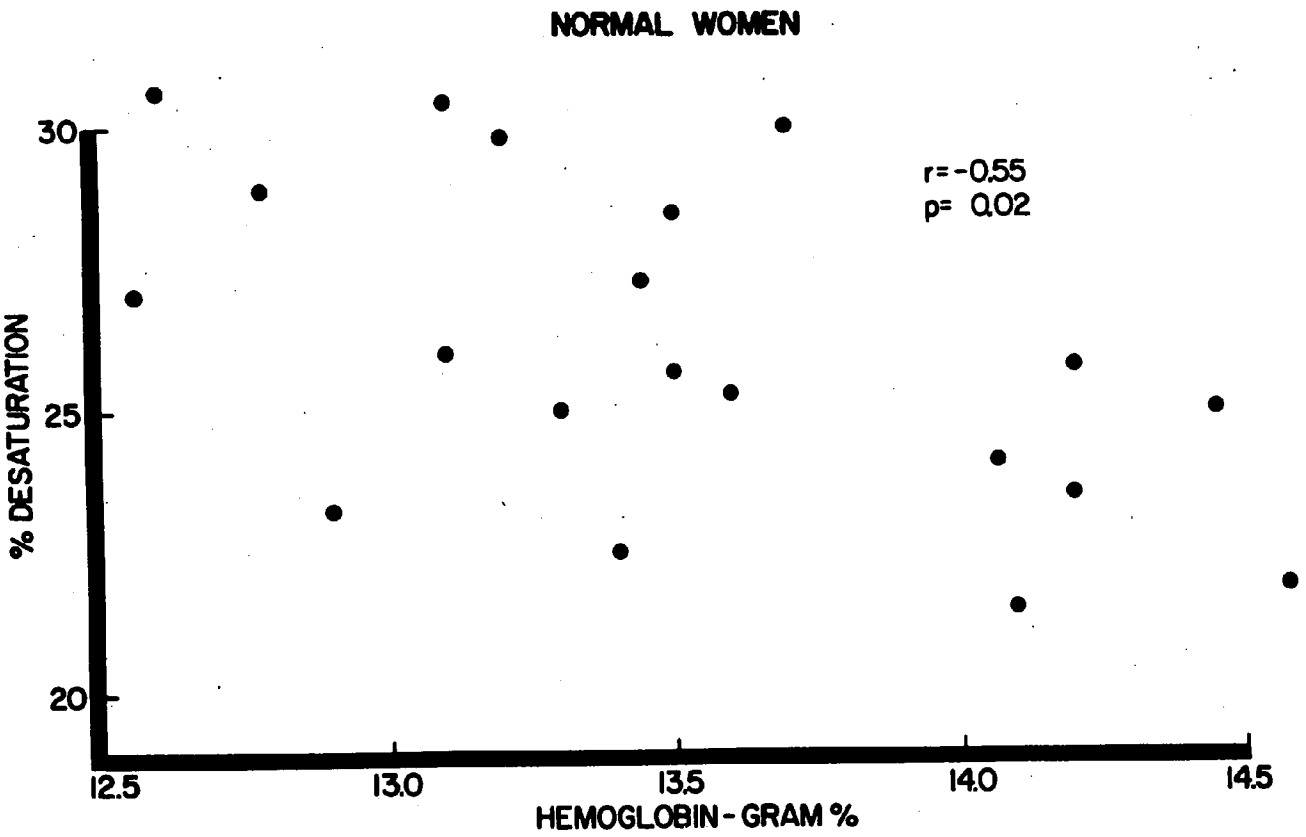


Figure 8.--The inverse relationship between whole blood hemoglobin levels and per cent desaturation in the rate apparatus in 20 adult Caucasian females. Taken from Brewer and Eaton (unpublished observations).

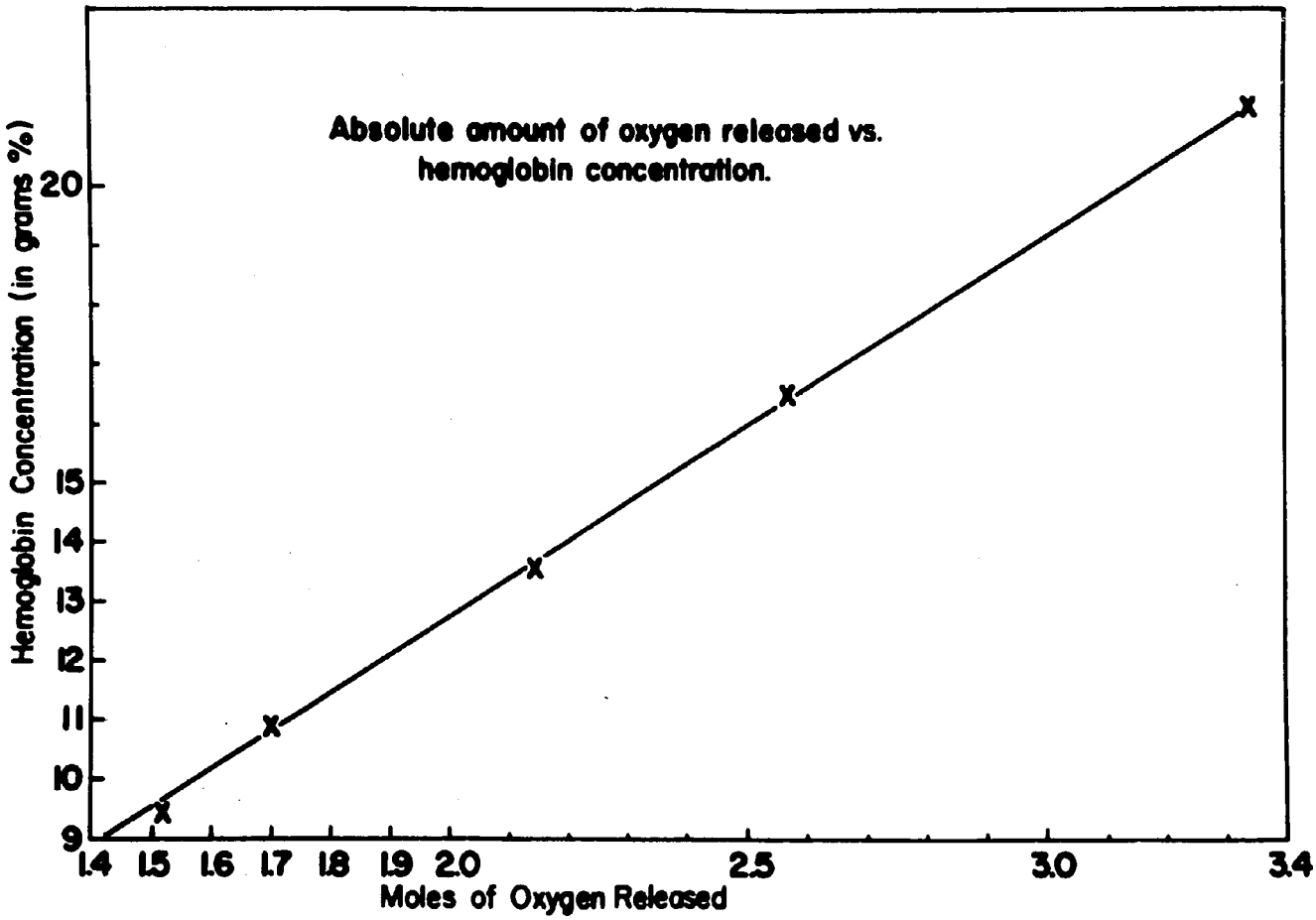


Figure 9.--The linear relationship between hemoglobin concentration and the amount of oxygen released in the rate measuring apparatus. See text for experimental details.

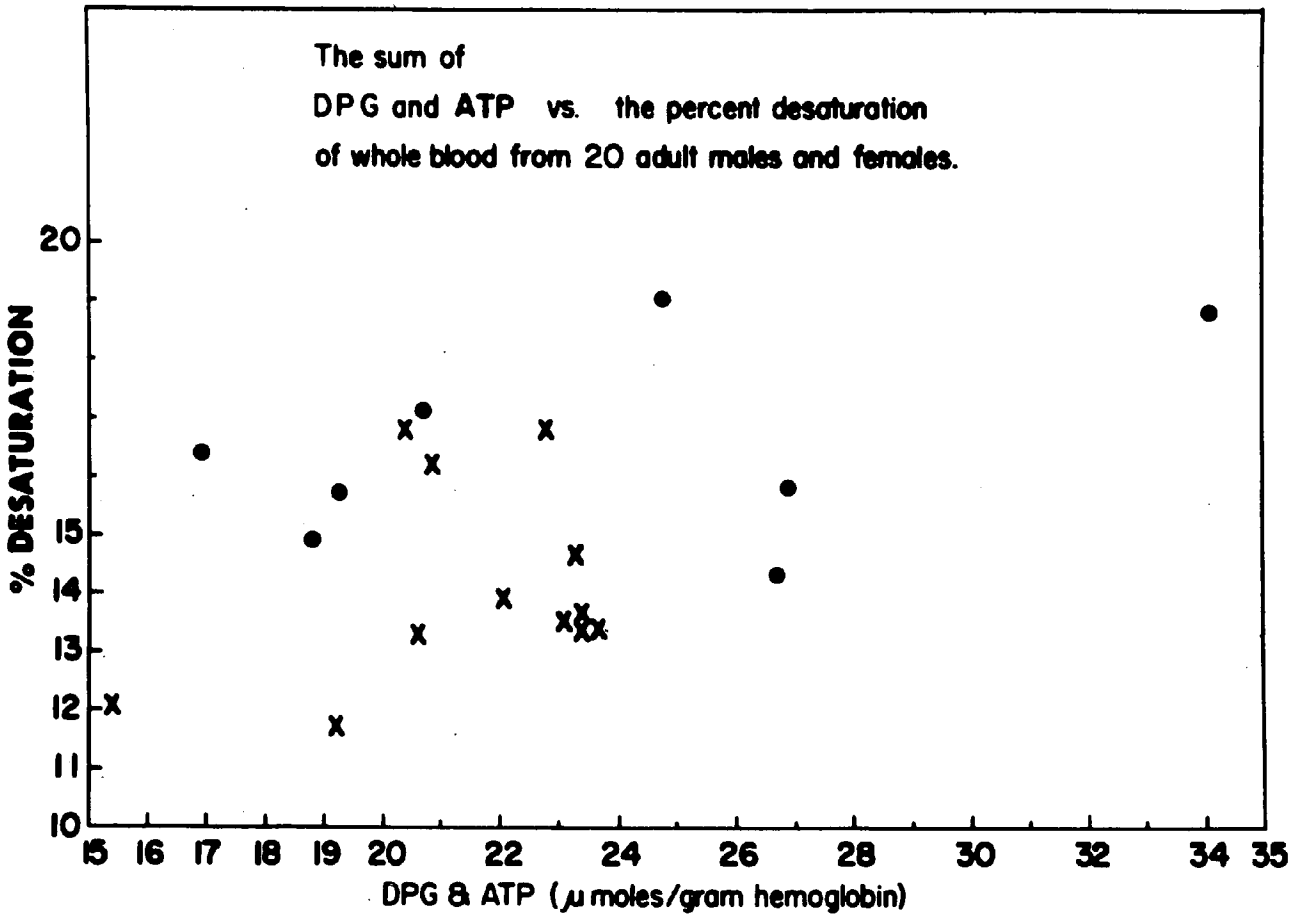


Figure 10.--A scatter diagram showing the relationship between the sum of red cell DPG and ATP and the % desaturation in the rate measuring apparatus in 12 adult males (crosses) and 8 adult females (solid circles).

that the higher the levels of ATP and DPG within the red cell, the greater the per cent desaturation which might be expected to occur in the rate apparatus.

When the results for the sexes in this sample of 20 males and females are considered separately, the correlations (desaturation vs. whole blood hemoglobin and desaturation vs. ATP + DPG) and their levels of significance are decreased or obliterated. More recently, however, Brewer and Eaton (unpublished observations) have examined larger samples (20 of each sex). The results obtained with these samples are shown in Table 10. These results confirm the idea that the sum of DPG and ATP is significantly correlated with the rate of desaturation, and that the rate of desaturation correlates well with the whole blood hemoglobin levels.

Another result of these studies was the lack of any correlation between whole blood hemoglobin level and the absolute amounts of oxygen unloaded. This means that individuals with relatively low hemoglobin concentrations release about as much oxygen as do individuals with high hemoglobin levels.

B. Equilibration study:

Measurements of the equilibration point of whole blood with 2.65% oxygen showed that 1) there is a strong positive correlation between whole blood hemoglobin level and the (corrected) resultant saturation, and 2) the sum of red cell ATP and DPG is negatively correlated with the equilibration saturation. Table 11 summarizes the results of the equilibration study.

TABLE 10

COEFFICIENTS OF CORRELATION BETWEEN % DESATURATION AND THE VARIABLES SHOWN IN 20 ADULT FEMALES AND 20 ADULT MALES (TAKEN FROM BREWER AND EATON, UNPUBLISHED OBSERVATIONS)

Group	DPG	ATP	DPG + ATP	Whole Blood Hemoglobin Levels
Females	+0.446 ($p < 0.05$)	+0.242 N.S.	+0.456 ($p < 0.05$)	-0.553 ($p < 0.02$)
Males	+0.339 N.S.	+0.058 N.S.	+0.502 ($p < 0.05$)	-0.217 N.S.

TABLE 11

THE COEFFICIENTS OF CORRELATION AND p VALUES FOR WHOLE BLOOD HEMOGLOBIN VS. EQUILIBRATION % AND FOR THE SUM OF ATP AND DPG VS. EQUILIBRATION % IN A SAMPLE OF 8 FEMALE AND 12 MALE ADULT CAUCASIANS

Group	Number	Whole Blood Hemoglobin vs. Equilibration %:		ATP + DPG vs. Equilibration %:	
		Coefficient	p Value	Coefficient	p Value
Females	8	+0.351	N.S.	-0.463	N.S.
Males	12	+0.641	<0.05	-0.575	<0.10
Combined	20	+0.735	<0.001	-0.605	<0.01

The correlation between whole blood hemoglobin and equilibration point indicates that those individuals with high hemoglobin levels have oxygen dissociation curves which are relatively left-shifted, and those with low hemoglobin values have curves shifted to the right (much like the differences in oxygen dissociation curves seen between low and high altitude residents). The negative correlation between the sum of red cell DPG and ATP and the equilibration point suggests that high levels of these compounds tend to reduce the oxygen affinity of hemoglobin (i.e., shift the oxygen dissociation curve to the right). The fact that the DPG + ATP sum will affect the point of equilibration is direct contradiction of Benesch, et al.'s statement that normal physiological variation in phosphorylated intermediates would have no effect on the oxygen affinity of hemoglobin. The present results point out the great differences between Benesch, et al.'s nonphysiological experimental design, and that of the present study.

A significant sexual difference was found in the point of equilibration, the females' having lower (right-shifted) saturation values (Table 9). Accordingly, the rate of release of oxygen is greater in the female, and the per cent saturation achieved at a given oxygen tension will be lower than that for the male.

Discussion

The results just reported imply that the red cells of each individual are "programmed" for a certain oxygen affinity and oxygen transport capability, commensurate with the blood values of the individual. The close associations found between whole blood hemoglobin level and both

desaturation rate and equilibration point imply that hemoglobin level may be an important determinant of the level of efficiency for which the red cells of each individual are programmed.

Further support for this possibility comes from earlier studies which show a good correlation between the degree of anemia and the p50 value. Bromberg and Jensen (1967) report that individuals with sickle cell anemia (SS homozygotes) usually have elevated values for p50, averaging about 32 mm. Hg. as compared with their normal mean of 25.2 mm. Hg. Further, these authors found that there was a strong negative correlation between the whole blood hemoglobin level and the p50. That is, those individuals with the lowest hemoglobin values tended to have the highest p50s ($r = -0.71$, $p < 0.01$). The authors offer no explanation for this phenomenon. Later work, however, indicates that the red cells of SS homozygotes contain about 40 to 50% higher concentrations of DPG than those of normals (Charache, et al., 1968). Charache, et al. conclude that there are indications that DPG may play a role in determining the oxygen affinity of hemoglobin within the red cell.

As mentioned previously, there was little or no difference between individuals with widely divergent blood values in the absolute amounts of oxygen unloaded. This is evidence for the efficiency of the programming of each individual's red cells for adequate oxygen delivery. This result indicates the ability of the red cell to make satisfactory homeostatic adjustments to potentially hypoxic situations. It may well be that an (unstressed) individual with a hemoglobin of 11 grams per cent is not necessarily at a respiratory disadvantage. Variations in concentrations of red cell DPG and ATP are a factor in this important homeostatic adjustment. The ways in which DPG and ATP values might vary concomitantly with the hemoglobin levels have already been discussed.

The equilibration point was found to correlate fairly well with both the whole blood hemoglobin level and the sum of erythrocyte ATP and DPG. Interestingly, the r value for hemoglobin versus equilibration point (although positive) is almost exactly that reported by Bromberg and Jensen (1967). The rather high negative correlation between the sum of ATP + DPG and the equilibration point is further indication of the role of these intermediates in influencing the oxygen dissociation characteristics of hemoglobin. This correlation may be stronger than that found in the rate studies since the resultant saturation in the equilibration studies was on the order of 40 to 50%. In the rate studies, the level of saturation was about 85%. As was mentioned earlier, higher levels of desaturation will tend to intensify differences in oxygen dissociation properties which are related to differences in the cell content of DPG and ATP. Later work (Brewer and Eaton, unpublished observations), in which the desaturations obtained were about 30%, gave considerably better correlations between the rate of oxygen release and the DPG and ATP content.

Although the initial sample was quite small, there appear to be significant sexual differences in both the rate of oxygen unloading and in the oxygen/hemoglobin equilibria (see again Table 9). The females in this sample unloaded a significantly greater proportion of the oxygen than did the males, but had depressed equilibration values. The former finding suggests a reason for the female's being able to maintain lower blood values, yet still provide adequate tissue oxygenation.

The differences in the oxygen dissociation curves of these individuals are probably quite small, but the differences in rate of oxygen unloading which have been found are about two-fold. This suggests the

inadequacy of oxygen dissociation curves in the evaluation of the oxygenating capacity of an individuals blood. Preliminary results (based on 13 individuals common to both the rate and equilibration experiments) do indicate that these large differences in rate of oxygen transport are related to smaller variations in the oxygen/hemoglobin equilibria obtained at a constant oxygen pressure.

As was pointed out earlier, the rate of oxygen release is not the only parameter measured during passage of the blood through the apparatus. There are a number of possible mediating factors. However, a good many of these factors (e.g., limitations of oxygen release by the cell due to plasma diffusion) will be less important in vivo.

The techniques with which we have measured the rate of oxygen release are not comparable to those employed by Baumberger, et al. (1967). Our results, though, tend to support their contention (cited earlier) that variations in the red cells themselves appear to be a decisive factor in determining individual differences.

In summary, the laboratory experiments, which were aimed at definition of relationships between the rate of oxygen transport, point of equilibration, and levels of metabolic intermediates, have demonstrated that:

1. There are significant differences between individuals in the rates at which their blood will unload oxygen.

2. These differences in rate of oxygen transport are large relative to differences in the position of the oxygen dissociation curves.

3. The rate of oxygen transport seems well correlated with the level of whole blood hemoglobin, implying a sort of programming of the circulating erythrocyte.

4. The programming seems to be efficient, as judged by the fact that there is no significant correlation between whole blood hemoglobin level and absolute amount of oxygen unloaded in the rate apparatus.

5. There appear to be significant sexual differences in the rates at which oxygen is unloaded by whole blood, suggesting a reason for the female's ability to maintain characteristically lower blood values than the male.

6. Correlations which have been found between levels of red cell DPG + ATP and both the rate of oxygen transport and equilibration point suggest that levels of these metabolic intermediates are responsible for a part of the programming of the red cell for varying levels of oxygen transport efficiency.

GENERAL DISCUSSION AND IMPLICATIONS

In the present work, we have attempted to gather evidence concerning connections between the metabolism of the human red cell and its respiratory function. The majority of the evidence presented here does indicate that the levels of metabolic intermediates (ATP and DPG) do affect the functioning of hemoglobin *in vivo*.

The negative correlation regularly found between whole blood hemoglobin level and red cell DPG concentration implies the adjustment of one variable to maximize the operation of other variables. Although it is assumed that there is a causal relationship underlying the DPG/hemoglobin correlation, it is by no means clear which of the two variables has the greater effect in determining the other.

If we assume that the average level of hemoglobin desaturation is important in controlling the levels of DPG and ATP, then the two intermediates should vary in response to changes in saturation. Differences in saturation could be caused by variations in any of the following parameters: basal metabolic rate; time of circulation (and therefore, cardiac output-total blood volume ratio); efficiency of pulmonary function; small variations in the tissue oxygen tension which the body maintains; and, perhaps even variations in the efficiency of oxygen delivery to the tissues.

On the other hand, it is quite possible that the levels of DPG + ATP strongly influence the concentration of whole blood hemoglobin. In this case, one determinant of whole blood hemoglobin would be the functional efficiency of the red cell in providing adequate oxygen delivery.

It is most probable that both factors, acting in concert, exert an influence on one another. It is apparent, therefore, that many different external variables will act to confound any attempt to define the relationship between whole blood hemoglobin and red cell DPG. Nothing like a 1:1 correlation is to be expected of two variables which are influenced by so many other parameters. The fact that the correlation does appear regularly, and at significant levels is strong evidence in favor of the intimate involvement of metabolic intermediates with the functioning of the red cell.

Variation in DPG and ATP, and therefore in the oxygen delivering capacity of the blood, may be of use in two ways:

1. Adjustment to intra-organismal variation. With this mechanism, it is possible that the effects of unwonted fluctuations in the availability of oxygen (such as those caused by anemia or variation in cardiac or pulmonary function) may be reduced. In a sense, this represents the individual's adaptation to himself.

2. Adjustment to environmental variations which change the availability of oxygen to the organism. The hypoxia of altitude seems to be partially corrected through increases in DPG levels. Also, recent evidence (Brewer and Eaton, unpublished observations on rats) indicates that exposure to hyperoxic conditions will result in a substantial reduction of the amounts of erythrocyte DPG + ATP. Adjustments of this sort are made in response to changes in the physical (gaseous) environment of the organism.

It is not now possible to define the full importance of this mechanism of homeostasis, but some of the evidence gathered (such as

the results from altitude in normals and polycythemics) indicates that it may be of prime importance in certain circumstances.

Sexual dimorphism in blood values, such as hemoglobin and hematocrit, has been repeatedly noted, and there is no doubt that females maintain significantly lower values than males. The reasons for the existence of this difference are still not completely clear. Unfortunately, it is not known if this sexual dimorphism in blood values extends to other species of primates. It might be suggested, however, that the higher blood values of the males provide a greater potential oxygen transport capacity in situations of stress.

Most plains dwelling primates show morphological sexual dimorphism. This may result, in part, from the role of group protector assigned the adult male. Inasmuch as man began as a plains dwelling primate, it might be suspected that at least a rudimentary sexual division of labor (and associated sexual dimorphism) has been present from the Australopithecines onward. In the role of group protector, a male might engage in more prolonged strenuous activities, and would certainly run a higher risk of serious blood loss.

The time required for significant changes in the level of red cell DPG has not yet been exactly defined. Lenfant, et al. (1968) have found that a major part of the increase in levels of DPG will occur within 24 hours after arrival at high altitude. This increase is, of course, accompanied by a shift to the right in the oxygen dissociation curve.

More recently, it has been found that increases in DPG level may occur within 40 minutes. A preliminary study has been carried out

on the influence of moderate exercise on the levels of red cell DPG. Blood samples were drawn from six adult males (between the ages of 24 and 40) immediately before and after 40 minutes of exercise (basketball). Measurement of DPG concentration was done as reported in the Materials and Methods section of part IV of this manuscript. Precipitation of both the pre- and post-exercise samples was carried out within 25 minutes after the samples were drawn. Table 12 shows the results of this study. In spite of the fact that the team lost, red cell DPG was elevated in all individuals sampled. Also, a mild hemo-concentration (as may be seen from the higher hemoglobin concentrations in the after-exercise samples) was produced by this period of exercise. The average elevation of red cell DPG was 50% over that of the pre-exercise level. The meaning of an increase of this magnitude has already been discussed.

The fact that levels of DPG can be changed so rapidly raises some interesting possibilities. Brace and Montagu (1965) have pointed out that running down large herbivores is frequently practiced among the hunting-gathering cultures which inhabit a plains environment. Increases in DPG would certainly enhance respiratory capacity under these conditions, and would be a useful adjunct to other adaptations known to be made to prolonged physical exertion.

If the levels of DPG within the red cell are as flexible as the preliminary evidence indicates, an individual with higher blood values will have a greater range of adjustment to the hypoxic stress of acute blood loss or long-term physical exertion. It may be that the sexual dimorphism in blood values has resulted from selection imposed by relatively infrequent respiratory stress, rather than from small differences in mean level of physical activity. Although the female

TABLE 12

THE INFLUENCE OF 40 MINUTES OF MODERATE EXERCISE ON THE LEVELS OF RED CELL DPG IN SIX ADULT MALES
SAMPLE A = IMMEDIATELY PRIOR TO EXERCISE
SAMPLE B = IMMEDIATELY FOLLOWING EXERCISE
PLASMA CONTROL SAMPLE TAKEN FROM SAMPLE B

Subject	Sample	Whole Blood Hemoglobin	Red Cell DPG (μ moles/gram hemoglobin)
G.B.	A	13.42	9.70
	B	13.85	16.20
C.C.	A	14.94	7.97
	B	15.32	10.67
F.A.	A	14.83	10.65
	B	14.99	16.08
L.R.	A	14.88	8.38
	B	15.26	13.25
P.D.	A	14.18	8.20
	B	14.72	14.30
J.D.	A	16.13	8.07
	B	16.78	9.41

DPG Concentration:

$\bar{X}$ Sample A = 8.83 μmoles

$\bar{X}$ change (B-A) = +4.49 μmoles (+51%)

$\bar{X}$ Sample B = 13.32 μmoles

Significance level: $t = 3.74$, $p < 0.01$.

is more efficient at unloading oxygen (on a per gram of hemoglobin basis), only unstressed individuals have been examined in this regard. It is probable that, following exercise (and the concomitant increases in red cell DPG level), the adult male will have considerably greater oxygen delivery capacity than the female.

In adaptation to the high altitude environment, a great many physiological adjustments to the hypoxia are known to take place:

1. At altitude, there is a relative hyperventilation. Both the frequency and depth of breaths taken is increased (Hurtado, 1964). This slightly increases the availability of oxygen in the lung, and also produces a relative hypocapnia.

2. A marked increase in the volume of blood cleared by the heart within a given time is another characteristic of man's adaptation to altitude. Other cardiac changes which occur frequently include a pulmonary hypertension which is thought to produce more efficient use of all available alveolar space. Surprisingly, there is no increase in the pulse rate of adapted individuals compared to those at low altitude. Also, systolic and diastolic pressures are characteristically lower in high altitude residents.

3. Some adaptations to hypoxia on the tissue level have been reported. For example, the muscle tissue of high altitude residents appears to have a significantly higher concentration of myoglobin, a respiratory protein which functions to store and transfer oxygen which is unloaded by the hemoglobin (Reynafarje, 1962). However, research on these facets of adaptation has been comparatively neglected.

4. Adaptation to altitude is accompanied by a large increase in the amount of hemoglobin per volume of whole blood (largely at the expense of the plasma volume) (see, e.g., Hurtado, 1964). This increase provides the individual with significantly greater oxygen carrying ability. In addition to this change in hemoglobin level, there are also slight increases in the total blood volume (at 10-11,000 feet) (Monge and Monge, 1966). Concomitant with this last, there must be enlargement of pre-existing blood vessels and (probably) a proliferation and enlargement of the capillary beds.

Man's short term adaptations to altitude are centered about one major goal: maintenance of tissue oxygen tensions. All the changes which have been listed (and more not mentioned) aid in this homeostasis. In addition to the adjustments just mentioned, man has resorted to decreasing (instead of increasing) the affinity of his blood for oxygen. Although this decrease makes oxygenation of the red cell in the lung somewhat more difficult (and, as stated before, reduces the arterial oxygen saturation), it enhances the delivery of oxygen to the tissues.

Animals which are evolutionarily adapted to high altitude have apparently lowered the oxygen tensions required for satisfactory metabolism at the tissue level. Because of this, the oxygen pressures at which they unload oxygen from the hemoglobin are also reduced. This sort of evolutionary adaptation to altitude has to be a multi-factorial one, involving concomitant changes of two sorts: 1) lowering of the oxygen pressures which the tissues require and 2) increasing the affinity of the blood for oxygen (that is, shifting the dissociation curve to the left, probably through changes in the hemoglobin molecule itself).

Man, on the other hand, has characteristically resided at altitudes of 5,000 feet or lower in the course of his evolution. Man has probably not lived above 10,000 feet anywhere for longer than 400 generations or so (Monge and Monge, 1966). This appears to have been too little time for high altitude populations to develop and select for the types of adaptations which we find in animals which normally inhabit the higher altitudes.

All of the known physiological changes which occur in man at high altitude are, of course, necessary to completely satisfactory adjustment to life in a low oxygen environment. However, the importance of these changes probably varies a great deal. The shift in the oxygen dissociation curve (and the change in rate and amount of oxygen transport which accompanies it) is undoubtedly among the most important of these. As explained previously, the shift which occurs at altitude functions to allow the unloading of greater amounts of oxygen in the tissues. It does this through producing a relative increase in the change in saturation per unit change in oxygen pressure. Therefore, a smaller change in the arterial-to-venous oxygen pressure will result in greater desaturation of the hemoglobin. This is so because the functional part of the curve (that part on which loading and unloading is occurring) is much more steep than when it is in its normal (low altitude) position. Inspection of Figure 5 will show that if this shift did not occur, the amount of oxygen which the blood could transport would be reduced by at least half. This indicates that, if the shift did not occur, adaptation to altitudes above 10,000 feet or so would probably be impossible.

Of perhaps equal or greater importance than the simple shift to the right is the accompanying effect on the rate of desaturation. A

regression line drawn on the initial sample of 20 adults (see Section III) indicates that an increase of 1 μmole DPG + ATP will increase desaturation by 0.6% within the physiological range (see Table 13). As mentioned before, this effect would be even more marked *in vivo*, at the greater desaturations known to occur in many tissues. These calculations indicate that, if arterial saturation is unchanged, a 10% increase in the level of DPG + ATP will result in an increase of about 6% in the amount of desaturation. If this relationship also obtains *in vivo*, it would indicate that adjustment of the DPG + ATP levels are of signal importance in the reduction of hypoxic stress.

Also, it might be suggested that less energy is involved in maintenance of higher DPG levels than in most of the other known ways of improving oxygen transport. Increases in cardiac output involve considerably higher levels of energy expenditure. Hyperventilation necessitates more frequent contraction of a large portion of the thoracic musculature. In contrast, an increase in the level of red cell DPG, although it requires an initial small energy expenditure, would appear to be much more economical in terms of long range energy use.

The increase in levels of DPG in the red cells of residents of high altitude is, almost certainly, adequate to explain the long noted shift to the right in the oxygen dissociation curve. Fully successful adaptation to life at high altitude for the human, then, involves some elevation of the red cell DPG level with its resulting shift of the oxygen dissociation curve, permitting an adequate unloading of oxygen in the tissues.

TABLE 13

THE RELATIONSHIP BETWEEN INCREASES IN THE SUM OF DPG + ATP AND THE % DESATURATION IN THE RATE MEASURING APPARATUS (IN THE PHYSIOLOGICAL RANGE)

Sum of DPG+ATP	Percent Desaturation	Increase in % Desaturation Per Additional µMole DPG+ATP	Increase in Oxygen Delivery
15.00	20.5		
		0.6	14.7%
20.00	23.5		
		0.6	12.8%
25.00	26.5		
		0.6	11.3%
30.00	29.5		
Total increase in oxygen delivery			38.8%

Included in the clinical ramifications of this work are the following:

1. Individuals who are homozygous for hemoglobin S are frequently subject to crises, during which the red cells sustain a great deal of destruction, presumably due to intra-vascular sickling and subsequent lysis. Because people with hemoglobin SS tend to have higher levels of DPG (accompanied by a significant shift of the oxygen dissociation curve to the right) (Charache, et al., 1968), it is probable that they desaturate to a greater degree. If the amount of desaturation which occurs in these cases could be controlled, it might be possible to alleviate or completely forestall these crises. A lowering of the red cell DPG level would result in less desaturation, and evidence is available which indicates that DPG concentration can be reduced within the circulating erythrocyte. Further support for the possibility that shifting the curve will alleviate sickle cell crises comes from evidence concerning the level of hemoglobin F (foetal) in these individuals. It has been noted that the most healthy of the S homozygotes are those with higher percentages of hemoglobin F (e.g. Bromberg and Jensen, 1967). The reason for this salutary effect has been thought to be the fact that hemoglobin F is not incorporated into the hemoglobin tactoids formed during sickling, thus reducing the effective concentration of hemoglobin S. However, it might also be pointed out that the oxygen dissociation curve of hemoglobin F is well to the left of that of hemoglobins A and S. This should mean that cells with a high percentage of hemoglobin F (i.e. 15 to 20%) will unload significantly less oxygen and will therefore run less risk of intra-vascular sickling. Lowering of the red cell DPG might partially mimic the effect of high hemoglobin F content.

2. As has been previously pointed out, the extreme polycythemia of high altitude, which is usually associated with chronic mountain sickness, may be caused, or at least aggravated, by excessive levels of red cell DPG. These high levels would presumably inhibit oxygenation of the blood in the lung, and therefore limit the supply of oxygen available to the tissues. It is quite possible that a lowering of these levels of DPG might have significant therapeutic value in the treatment of chronic mountain sickness and excessive polycythemia.

3. The efficiency of the blood in oxygen transport may relate to some kinds of coronary disease. The blood usually gives up 70% of its oxygen in transit through the coronary circulation. Desaturation of this sort will greatly magnify differences between individuals in the efficiency of oxygen release. It may be that low levels of DPG may produce a predisposition to coronary disease by reducing the amount of oxygen which would ordinarily be delivered to the heart muscle. Coronary insufficiency (inadequate coronary circulation leading to anginal pain and deficit in coronary function) is usually caused by an ischemia of some sort. An enhancement of the blood's ability to deliver oxygen to this ischemic area might result in improvement of most cases. Artificial elevation of DPG, discussed earlier in this paper, might produce better myocardial oxygenation.

4. Artificial elevation of DPG might also be of use in treatment of acute anemias of many sorts.

5. It has been shown (Bernstein, 1959) that there are great decreases in the levels of DPG during the first week or two of blood storage. These decreases will shift the oxygen dissociation curve strongly

to the left, making the blood inefficient in oxygen transport immediately after transfusion. Restoration of the DPG levels prior to transfusion would make the blood more immediately useful to the patient. This would be especially helpful in cases of severe blood loss which require enhancement of oxygen carrying capacity immediately, and not just within a few hours.

In the course of biological evolution, increasingly higher levels of organization have necessitated greater complexity in control systems. These systems variously involve maintenance of organization and function in the course of growth, development, and adult life. Most of these systems of control involve the production of variable quantities of proteins which subsequently enable the cells to carry out their metabolic work at the required level. However, among the Mammalia, the erythrocyte is anucleate, incapable of reproducing itself, and also incapable of making proteins. Because the red cell cannot make protein, the previously mentioned method of metabolic control is, of course, closed to it. The red cell must, as a result, make use of direct feedback systems of control. DPG, which has been under discussion here, appears to be an important element in the control of erythrocyte metabolism and function.

Evidence has been presented here which indicates that DPG is directly involved in determining the efficiency with which the red cell operates. Other lines of investigation (cited earlier) have implied the importance of DPG (and ATP) as regulators of the red cell's metabolism. Combined, these functions form an impressive list of the ways in which DPG interdigitates with red cell metabolism and function:

1. DPG, through its interactions with hemoglobin, has a powerful regulatory effect on the oxygen transport properties of the red cell.

2. Through its inhibition of transketolase and transaldolase, red cell DPG level may determine the rate of operation of the pentose-phosphate pathway.

3. Hexokinase, the enzyme responsible for initiating glycolysis, is known to be inhibited by physiological levels of DPG.

4. Physiologic levels of DPG have also been shown to inhibit the action of adenosine monophosphate deaminase. This enzyme will cleave AMP and would, if allowed to function at uninhibited levels, probably deplete the red cell's store of adenine quite rapidly. Without adenine, of course, the red cell would have no basis for its requisite high energy compound, ATP.

5. DPG has been found to interact with magnesium, an element which is a necessary cofactor for many of the enzymatic conversions which take place in glycolysis (including the reaction which hexokinase catalyzes) (Rose, 1968). Variations in levels of DPG, and consequent changes in the amounts of free magnesium, may thus indirectly modify the activity of many of the enzymes of glycolysis.

Our understanding of this complex set of interactions is not yet complete enough to allow mathematic representation of this feedback system. It is evident, however, that DPG level is a vital control of both the level of metabolism and the efficiency of operation of the erythrocyte.

The control of respiratory efficiency through DPG and DPG-like compounds may be a widespread phenomenon, at least among the vertebrates.

Benesch, et al. (1968) have suggested that a substance called inosine hexophosphate (IHP) serves the same purpose in avian blood as DPG does in mammalian blood. Their data indicate that there is a definite relationship between the IHP content of chicken red cells and the oxygen affinity of the blood as the chick develops. In addition, work now in progress (Grover and Brewer) has shown that the DPG content of the red cells of llamas and probably goats increases when the animals are taken to high altitude.

In the course of evolution, organisms have developed a great variety of mechanisms with which to buffer environmental stresses. There seem to be some relatively ubiquitous stresses imposed by life which have only one genre of solution. One such solution appears to be the control of red cell function through modification of cellular metabolism.

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