CHAPTER I

INTRODUCTION

1.1 Statement and significance of the problem

During exercise, increased oxygen delivery to muscle cells is required to provide continuous energy metabolism to sustain ATP. The ATP can be derived from either aerobic or anaerobic metabolism, but the latter yields less energy and results in lactic acid accumulation. If the delivery of oxygen to the working muscle is inadequate, there is an increase in lactate formation. This condition can be caused by hypoxemia. Lack of oxygen supply results in increased NADH levels, and larger amount of pyruvate is converted to lactate, producing lactic acidosis as a result of a decrease in blood pH. In addition, an elevation of blood temperature due to an increase in energy metabolism also causes a decrease in oxygen tension in muscle. Thus, the body must have an adaptive mechanism to supply adequate oxygen for working muscle. The response is an increase in 2,3-bisphosphoglycerate (2,3-BPG) levels in the red blood cells. Elevated 2,3-BPG levels have been observed in people with impaired oxygen delivery. Lung disease, anemia and hypoxia at altitude are all stimuli that increase 2,3-BPG concentrations in the red blood cells. This is a basic well known adaptive mechanism in the regulation of oxygen delivery to the peripheral tissues.

The compound 2,3-BPG is formed within red blood cells and its principal role is to facilitate oxygen release from oxyhemoglobin so that oxygen can diffuse into working muscle cells. Oxygen has a very strong attraction to hemoglobin and is quickly joined, forming oxyhemoglobin, when they combine in the lungs. This high oxygen affinity makes it more difficult for the oxygen to be freed and released to tissues where it is needed. Fortunately, 2,3-BPG is available within the red blood cell to allow for dissociation, otherwise, the oxygen would continue to circulate in the blood. Thus, the factors that occur in circulation during exercise and lead to an increase in 2,3-BPG level in red blood cells are investigated. A number of research groups have shown that exercises at altitude causes increase in red blood cell 2,3- BPG levels (Lenfant *et al.*, 1968 and Mairbanurl *et al.*, 1986). The role of 2,3-BPG in adaptation to exercise

is controversial. The results of studies at sea level on the effects of exercise on red cell 2,3-BPG levels are equivocal. Various theories have been advanced to explain the conflicting reports concerning the response of 2,3-BPG to exercise. The time of blood sampling after exercise, intensity of exercise, and the training states of the subjects are all believed to influence 2,3-BPG response (Lijnen *et al.*, 1988). Furthermore, Beulter *et al* (1987) had shown that the *in vitro* study of addition of potassium oxalate to blood stored in citrate phosphate dextrose (CPD) produces a marked improvement 2,3-BPG preservation. Determination of the level of intermediate compounds in red cells incubated with oxalate suggests the presence of inhibition at pyruvate kinase step, indicating that this is the site of oxalate action. In the similarly response, sodium fluoride, an inhibitor of enolase in glycolysis pathway may also improve 2,3-BPG preservation (Chan *et al.,* 1992). Lowney and Resina (Lowney *et al.*, 1990 and Resina *et al.*, 1994) have shown the inverse relationship between magnesium ion (Mg^{2+}) concentration and 2,3-BPG levels in exercised rat. In 1990, Stewart and coworkers (Stewart *et al.*, 1990) have shown that phosphate loading in man can increase the levels of 2,3-BPG after exercise.

Studies from a number of research groups have shown the results of the isolation, purification and properties of Bisphosphoglycerate mutase in chick and human erythrocytes that it is thermostable (reach up 45^{O}C) (Chiba *et al.*, 1975, Kappel *et al.*, 1976, Harkness *et al.*, 1977). It is possible that elevation of temperature in the vicinity of active muscles can cause inhibition of the enzyme certainly in some steps of glycolysis and ultimately results in an increase of 2,3-BPG in red blood cells.

The aim of the present study was to investigate the effects of different exercise programs on erythrocytes 2,3-BPG concentration. The influence of factors such as elevated temperature, acidosis, pO_2 and calcium ion on the regulation of 2,3-BPG synthesis was investigated.

1.2 The red blood cell

Evolution from unicellular organisms to complex metazoa requires the development of systems for the distribution of nutrients and oxygen to thel cells of the whole body. The need to supply oxygen molecules reliably, consistently, and in large amounts has been met by different animals in different ways, but the most efficient system is one which has been adopted by all vertebrates. The interior of the cell consists of highly concentrated aqueous solution of hemoglobin containing most of the glycolytic enzymes, nucleotides, 2,3-bisphosphoglycerate and other phosphorylated metabolic intermediates, glutathione and electrolytes (Beutler, 1978). Thus, one of the important properties of the red blood cell is the ability to transport oxygen from the lungs to the tissues (Figure1-1). Hemoglobin is responsible for this function, and it carries it out by combining reversibly with oxygen, loading up in the lungs and unloading in the tissues. This is a key function in the physiology of the living organism (Karger, 1971).

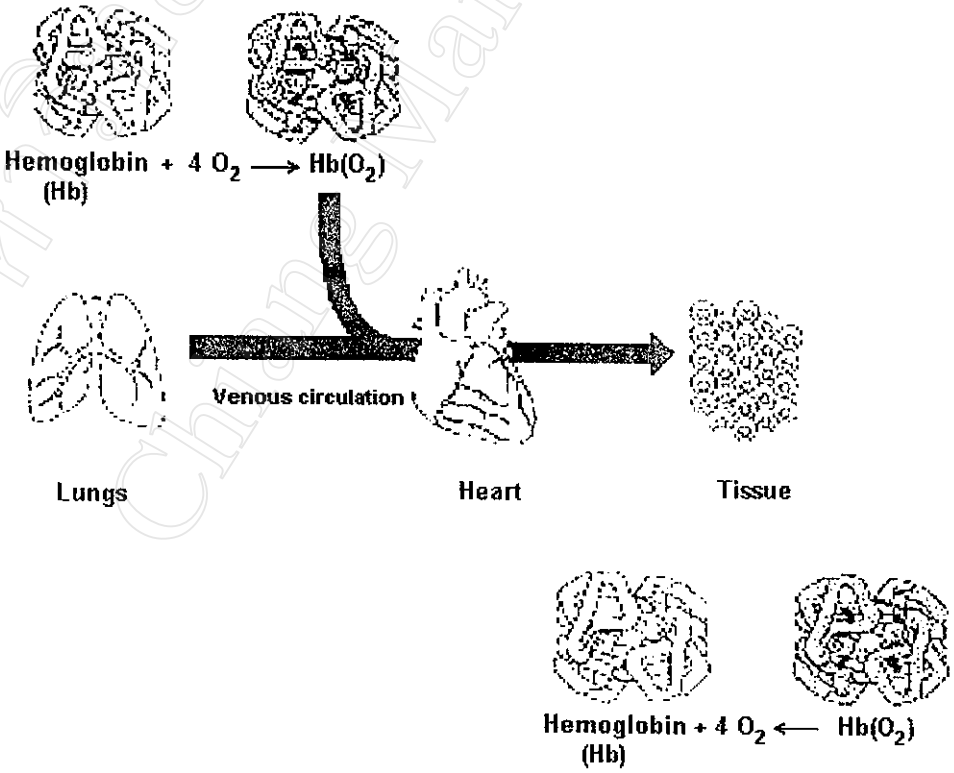


Figure 1-1: Model of the role of hemoglobin in oxygen transport (Grims, 1980).

1.3 Hemoglobin

1.3.1 Hemoglobin structure

Human hemoglobin is an approximately 64,000 daltons compact globular protein, and an elliptical shape with molecular dimension of approximately 64 x 55 x 50 Å. Normal hemoglobin molecule is composed of four subunits, each containing a protein globin chain and one heme prosthetic group. The heme prosthetic group is composed of protoporphyrin IX which is combined with an iron atom. The function of iron atom in the heme group is the oxygen binding and can be in the ferrous (+2) or the ferric (+3) oxidation state, only the +2 oxidation state can bind oxygen. Accordingly, each hemoglobin molecule is capable of combining with four molecules of oxygen (Bunn *et al.*, 1977).

Normal hemoglobin molecule contains two different type of protein globin chains, designated two α- or α-like globins and two β- or β-like globin chains. The globin chain has 141 (α) or 146 (β) amino acids in length, arranges in a series of straight joined by short non helical regions. The helical regions are designated by the letter A through H from the amino-terminal end of the polypeptide. The predominant hemoglobin in adults is Hemoglobin A (HbA), possesses 2 identical polypeptide designated α chains plus a second alike pair of polypeptides designated β chains, forming a tetramer molecule, $\alpha_2\beta_2$. They are held together by non covalent attractions to form a quaternary structure. The external surfaces of the hemoglobin tetramer are composed of amino acids with the polar side chains and the interior spaces contain only nonpolar amino acids, preserving a nonaqueous (hydrophobic) internal environment. This nonaqueous environment is essential to preserve the heme in its biologically active Fe (2+) form.

The allosteric properties of hemoglobin arise from interaction between subunits. The functional unit of hemoglobin is tetramer consisting of two kinds of polypeptide chains. In the transition from oxy-to deoxyhemoglobin, large structural changes take place at two of the four contact regions (the $\alpha_1\beta_2$ contact and the identical $\alpha_2\beta_1$ contact) but not at the others (the $\alpha_1\beta_1$ contact and the identical $\alpha_2\beta_2$ contact). The $\alpha_1\beta_1$ contact contributes principally to the structural stability of the tetramers. There is little or no movement at this contact during oxygenation. In fact, the $\alpha_1\beta_2$ contact region is designeted to act as a switch between two

alternative structures. The two forms of this dovetailed interface are stabilized by different sets of hydrogen bonds. This interface is closely connected to the heme groups, and so structural changes in it affect the hemes. Reciprocally, structural changes at the heme affect this interface. Most residues in it are the same in all species because of its key role in mediating allosteric interactions. Nearly all mutations in this interface diminish cooperative oxygen binding, whereas changes in the $\alpha_1\beta_1$ interface do not.

In oxyhemoglobin, carboxyl-terminal residues of all four chains have almost complete freedom of rotation. In deoxyhemoglobin, by contrast, these terminal groups are anchored. The terminal carboxylates and the side chains of the C-terminal residues participate in salt links (electrostatic interactions) that tie the tetramer. Deoxyhemoglobin is a tauter, more constrained molecule than oxyhemoglobin because of the presence of eight additional salt links. The quaternary structure of deoxyhemoglobin is termed the T (tense or taut) form; that of oxyhemoglobin, the R (relaxed) form (Stryler, 1995).

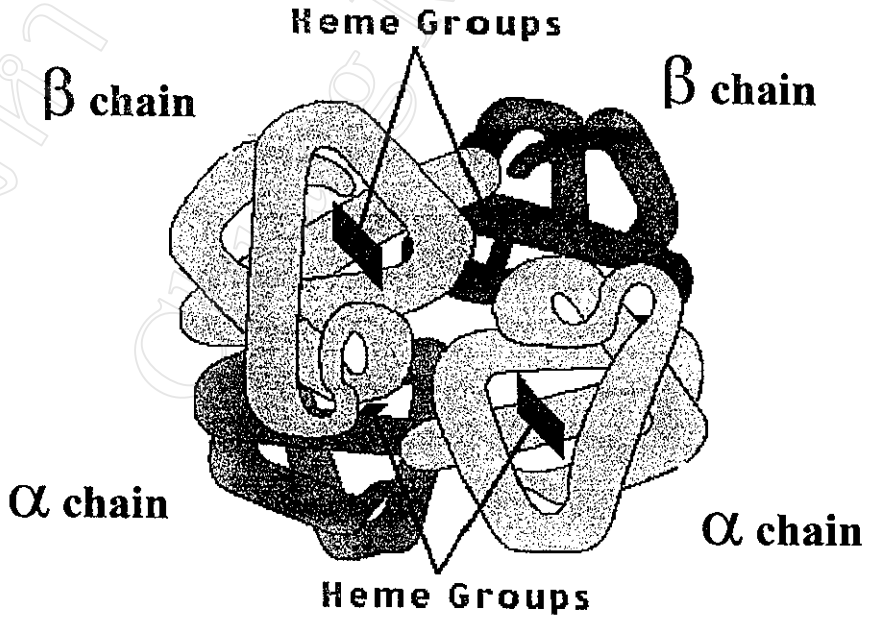


Figure1-2: The quaternary structure of hemoglobin molecule (Bunn *et al.*, 1977).

1.3.2 Hemoglobin and oxygen transport

The primary function of hemoglobin is to transport oxygen. Since oxygen is not very soluble in water (the major constitutent of blood), an oxygen transport protein must be used to allow oxygen to be "soluble". Hemoglobin, the transport protein in the blood of vertebrates, which constitutes 90% of the dry weight of red cells, can carry 1.34 ml of O_2/g. An essential feature of hemoglobin is that it must bind oxygen firmly enough to remove it from pulmonary alveoli at high oxygen tensions and yet be able to release or dissociate oxygen at the low oxygen tension of tissues. Hemoglobin must bind oxygen with an appropriate degree of affinity (Jandl, 1987).

The equilibriums between oxygen and hemoglobin in whole blood at various oxygen tension or partial pressure of oxygen (pO_2) are displayed in Figure 1-3. This so-called oxygen dissociation curve. At the oxygen tension found in the air sacs of the lung, the hemoglobin in red cells becomes 97% saturated with oxygen. Thus, normal blood, having a hemoglobin concentration of 15g/100ml, would carry 1.34 x 15 or 20 ml O_2 per 100 ml blood (20 volumes/ml). Following circulation through the capillary bed, the mixed venous oxygen tension is normally about 40 mmHg. As shown by the oxygen dissociation curve for whole blood of normal individuals (Figure 1-3), at a pO_2 of 40 mmHg, hemoglobin is about 75% saturated with oxygen. Thus, blood normally gives up an average of 1.34 x 15 x (0.97-0.75) or 4.5 volumes of oxygen per 100 ml, during its circulation through the tissues. Oxygen is released at high enough pO_2 to maintain an adequate supply for intracellular utilization. Such an efficient mechanism for oxygen transport would not be possible if the oxygen dissociation curve were not sigmoidal. This sigmoidal curve reflects the fact that oxygenation of each heme increases the oxygen affinity of the others, so-called heme-heme interaction (or subunit cooperativity). If each of the four heme groups of the hemoglobin molecule bound oxygen dependently of the other, the oxygen dissociation curve would have a hyperbolic shape, like that of myoglobin. As show in Figure 1-3, such a curve would be most unsuitable for oxygen transport. In going from arterial pO_2 of 95 mmHg to mixed the venous pO_2 of 40 mmHg only 0.8 volume/100 ml O_2 would be unloaded. Further release of oxygen would be possible only at considerably lower oxygen tension (Bunn., 1977; Benesch and Benesch, 1974).Thus, the loading and unloading of oxygen at physiologic

oxygen tensions, hemoglobin must have an appropriate affinity for oxygen and heme-heme interaction.

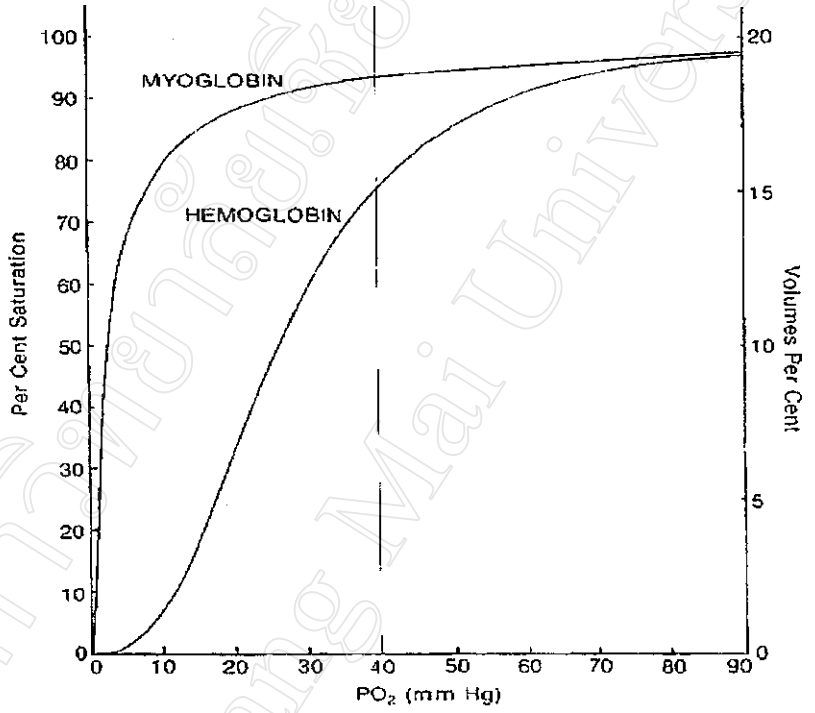


Figure 1-3: Oxygen dissociation curve of hemoglobin (whole blood) and myoglobin at 37^{O}C and pH 7.40 (Bunn *et al.*, 1977)

1.3.3 Hemoglobin and 2,3-Bisphosphoglycerate

The oxygen affinity of hemoglobin within red cells is lower than that of hemoglobin in free solution. As early as 1921, Josept Barcroft wondered, " Is there some third substance present ..... which forms an integral part of the oxygen-hemoglobin complex ?" It is therefore surprising that the discovery by Greenwald in 1925 (Greenwald *et al.*, 1925) that 2,3-bisphosphoglycerate is present in a high concentration in the red cells of many species. Although it is the most abundant organic phosphate of the red cell, it is present in only trace amounts in other tissues. Its concentration within the red cell is normally about 5 mmoles per liter of packed cells, roughly equivalent to the concentration of hemoglobin tetramer and about four fold that of ATP. In 1967 Chanutin and Curnish (Chanutin and Curnish, 1967) and Benesch and Benesch (Benesch and Benesch, 1967) independently demonstrated that 2,3-bisphosphoglycerate (2,3-BPG or known as 2,3-diphosphoglycerate, 2,3-DPG) binds to hemoglobin and has a large effect on its affinity for oxygen. 2,3-BPG lowers the oxygen binding affinity by a factor of 26. 2,3-BPG is physiologically important by facilitating oxygen release from hemoglobin while red cells are passing through tissue capillaries. Without 2,3-BPG would remain fully oxygenated in peripheral capillaries. Furthermore, this compound only change the over oxygen affinity and has no influence on the other allosteric properties of hemoglobin such as the "heme-heme interaction" and the Bohr effect. As shown in figure 1-4, an addition of low concentrations of 2,3-BPG (0.2 to 2.0 mM) results in a progressive decrease in oxygen affinity. ATP is the second most abundant organic phosphate in human red cells. Normally, its concentration is about one-fourth that of 2,3-BPG. ATP is almost as effective as 2,3-BPG. However, because ATP has a much higher affinity for divalent cations than has 2,3-BPG, the bulk of red cell ATP is bound to magnesium ion. The MgATP complex has no significant effect on the oxygenation of hemoglobin. Thus, red cell ATP probably has no effect on the oxygen affinity of whole blood. In contrast to human red cells, ATP is prime mediator of oxygen affinity of fishes and most amphibians (Bunn *et al.*, 1977).

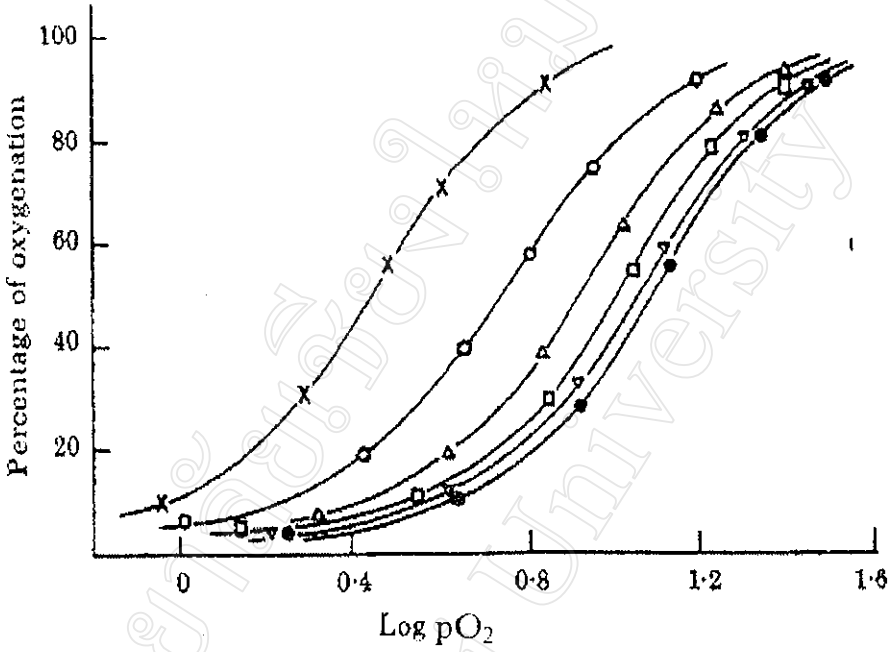


Figure 1-4: The effect of 2,3-BPG on the oxygenation of phosphate-free hemoglobin.

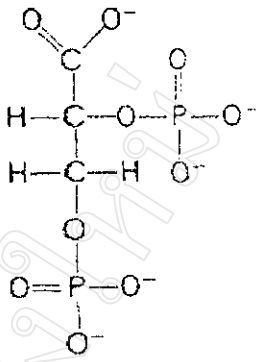
x = no 2,3-BPG; o = 0.2 mM 2,3-BPG; Δ = 0.4 mM 2,3-BPG; □ = 0.6 mM 2,3-BPG; ∇ = 0.8 mM 2,3-BPG; ● = 1.0 mM 2,3-BPG (Benesch and Benesch, 1969).

The binding between 2,3-BPG and hemoglobin

The structure of 2,3-BPG is shown in Figure 1-5. It has tritratable acid groups. At physiologic pH, 2,3-BPG has about 3.5 negative charges. binds to hemoglobin in the ratio of one molecule of 2,3-BPG per tetramer. This is an unusual stoichiometry---an $\alpha_2\beta_2$ tetramer would be expected to possess at least two binding sites for a small molecule. The presence of just one binding site immediately suggests that 2,3-BPG binds on the asymmetry axis of the hemoglobin molecule in the central cavity. This cavity is of sufficient size for 2,3-BPG only when the space between the H helices of the β chains is wide enough. The x-ray analysis has showed that the binding site for 2,3-BPG is constituted by multiple positively charged residues on each β chain: the α-amino group, His 2, Lys 82, and His 143. These groups interact with the strongly negative charged 2,3-BPG, which carries nearly four negative charges at physiologic pH. 2,3-BPG is stereochemically complementary to this constellation of positively charged groups facing the central cavity of the hemoglobin molecule (Figure 1-5)

On oxygenation, 2,3-BPG is extruded because the central cavity becomes too small. Specifically, the gap between the H helices of the β chains becomes narrowed. Also, the distance between the α-amino groups increases from 16 to 20°A, which prevents them from simultaneously binding the phosphates of a 2,3-BPG molecule. Thus, 2,3-BPG stabilizes the deoxyhemoglobin quaternary structure by cross-linking the β chains. In other words, 2,3-BPG shifts the equilibrium toward the T form. As mentioned previously, the carboxyl-terminal residues of deoxyhemoglobin form eight salt links that must be broken for oxygenation to occur. The binding of 2,3-BPG contributes additional cross-links that must be broken, and so the oxygen affinity of hemoglobin is diminished (Stryler, 1995).

The trigger for the R to T transition of hemoglobin is the movement of the iron in the out of the plan of the porphyrin ring. Both steric and electrostatic factors mediate this trigger. Thus, a terminal change in the position of Fe(+2) relative to the porphyrin ring induces significant switching of the conformations of hemoglobin and crucially affects its biologic function in response to an environmental signal (Murray *et al.*, 1993).



2,3-bisphosphoglycerate (2,3-BPG)

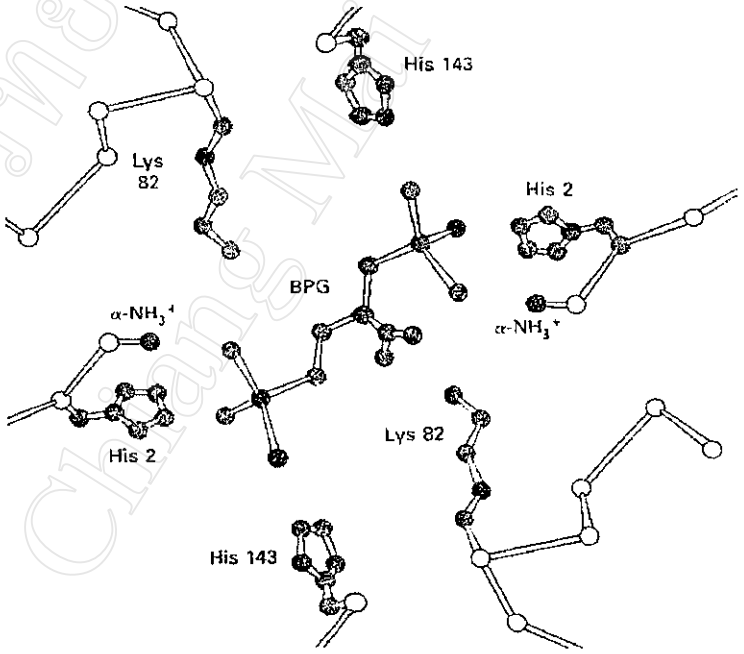


Figure 1-5: Mode of binding of 2,3-BPG to human deoxyhemoglobin. 2,3-BPG interacts with three positively charged groups on each β chain (Arnone, 1972).

1.4 Red cell 2,3-Bisphosphoglycerate

In mammal, the functional properties of hemoglobin are regulated through 2,3-bisphosphoglycerate, the glycolytic intermediate present in high concentration inside red blood cell where it binds to the deoxy form of hemoglobin and decrease its oxygen affinity. Consequently, 2,3-BPG plays a key role in facilitating the supply of oxygen to the tissues.

1.4.1 Metabolism

The primary function of red blood cell is the efficient transport of oxygen. As emphasized in the preceding section, the red cell is especially well engineered for this purpose. It has differentiated to the extent that only essential metabolic pathways are in operation. Thus, the adult erythrocyte has a relatively simple organization: a metabolically active membrane enclosing a homogeneous cytoplasm that contains by weight 34% hemoglobin and about 2% nonheme proteins, primarily carbonic anhydrase and glycolytic enzymes. The red cell has very limited metabolisms in (a) maintenance of the sodium-potassium pump in order to prevent colloid osmotic lysis; (b) maintenance of hemoglobin in the reduce (ferrous) state, since the oxidized form, methemoglobin (ferrihemoglobin), is unstable to bind oxygen; (c) repair of the membrane by acylation of phospholipids; and (d) protection of the cell membrane and interior against oxidant stress. These functions can be accomplished with a relatively low expenditure of ATP (the metabolic currency of the cell) and reducing equivalent, NADH and NADPH.

During its maturation in the bone marrow, the red cell loses its organelles and, with them, the capability for mitochondrial respiration and fatty acid and protein synthesis. Only two metabolic pathways remain intact in the mature erythrocyte: anaerobic-glycolysis (the Embden-Meyerhof pathway) and the pentose phosphate pathway (hexose monophosphate shunt). These metabolic processes are diagrammed in Figure 1-6. Under physiologic conditions, glucose is the only important substrate for red cell metabolism. It enters the cell by facilitated diffusion. Once phosphorylated, the glycolytic intermediates are locked within the cell until they are metabolized to pyruvate and lactate. These two nonphosphorylated products are free to diffuse out of the red cell and constitute the prime source of plasma pyruvate and lactate under resting conditions.

In the red cell, 2,3-BPG is by far the most abundant organic phosphate. In this respect, the red cell differs dramatically from other tissues in which 2,3-BPG is present only in the minute concentration which is required for it to serve as a cofactor in the phosphoglycerate mutase reaction (see Figure1-7).

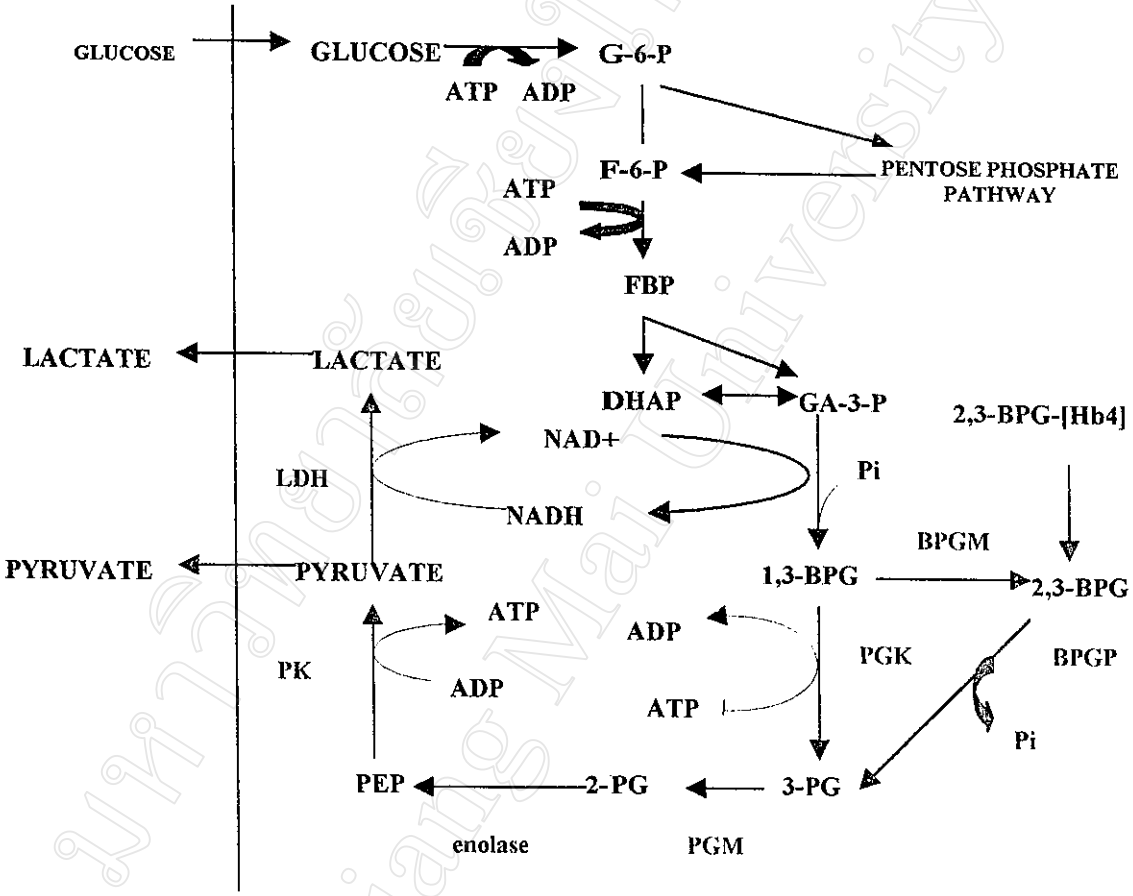


Figure 1-6: Glycolytic pathway within red cell. Glucose enters red cell and is metabolized to pyruvate and lactate, which escape from the cell (Bunn *et al.*, 1977).

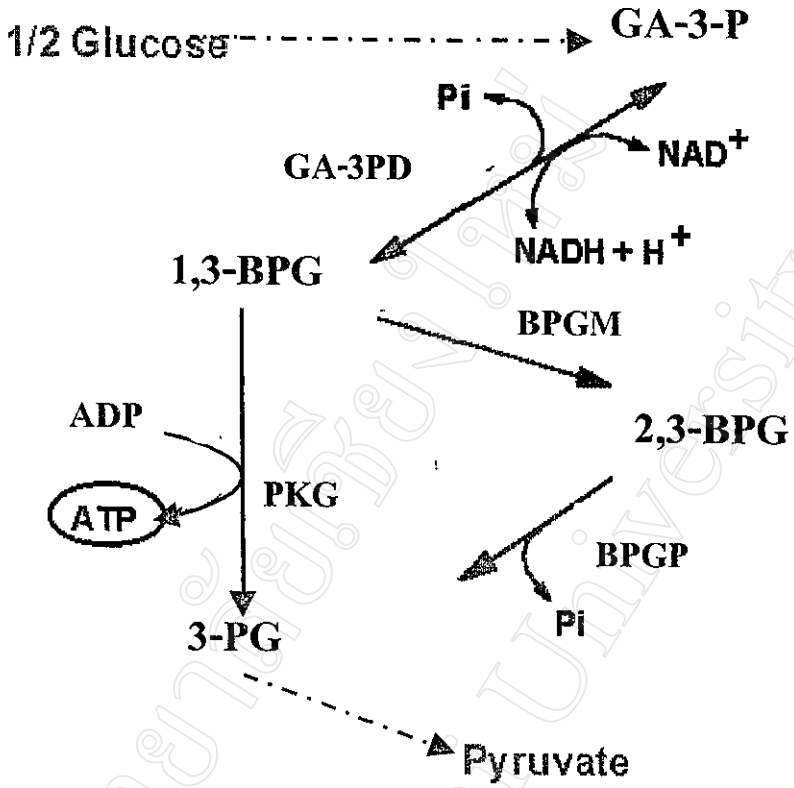


Figure1-7: The Rapoport-Luebering shunt. 1,3-Bisphosphoglycerate (1,3-BPG) can be isomerized to 2,3-BPG or, alternatively, can be converted directly to 3-phosphoglycerate with the formation of one equivalent of ATP (Russel, 1996).

1.4.2 Regulation

During red cell glycolysis, 2,3-BPG is produced, along with 3-phosphoglycerate (3-PG), from the common substrate 1,3-bisphosphoglycerate (Figure1-7). It undergoes conversion to 3-PG and therefore acts as a bypass to the phosphoglycerate kinase (PGK) step. The two pathways compete for their substrate and form either ATP or 2,3-BPG. This action of the Emden-Meyerhof pathway is the Papoport-Luebering shunt and the regulation between the two branches is complex. It seems very likely that the two steps in the 2,3-BPG branch are catalysed by two different enzymes, namely 2,3-BPG mutase (or Bisphosphoglycerate mutase, BPGM, EC 5.4.2.4) and 2,3-BPG phosphatase (or Bisphosphoglycerate phosphatase, EC 3.1.3.13) (Rose and Liebowitz, 1970). The concentration of 2,3-BPG depends on the balance between its rate of formation from 1,3-BPG by BPGM and its degradation by BPGP. Although the level of 2,3-BPG in red cells is some 100 times that of 3-PG, only around 20% of 1,3-BPG is metabolized via 2,3-BPG (Gerlach and Dunhm, 1972). Like ATP, 2,3-BPG is an inhibitor of its own synthesis because of its action on the mutase; in addition 3-PG is a cofactor in the mutase reaction while PGK is stimulated by an increase in the ADP/ATP ratio. Thus, this shunt may be envisaged as a self-regulating system designed to maintain levels both of ATP and 2,3-BPG, the one at the expense of the other (Grimes, 1973).

In essence, the level of red cell 2,3-BPG is determined by three factors: (a) the rate of formation of substrate 1,3-BPG; (b) the relative amount of 1,3-BPG going into the Rapoport-Luebering shunt versus that undergoing conventional glycolysis; and (c) the rate at which 2,3-BPG is hydrolysed. It is apparent that the regulation of the concentration of 2,3-BPG is the resultant of a complex array of independent and interdependent factors, a number of which are altered by environmental stimuli. We shall consider these in order.

Glycolysis

The synthesis of 2,3-BPG in the red cell is directly dependent upon the rate of glycolysis—i.e., the rate at which glucose is converted to lactate. From the measurements of various glycolytic intermediates before and after a given change in incubation conditions, one can demonstrate which enzymatic steps of the glycolytic sequence remain in equilibrium and which are perturbed by the change. The latter, called crossover points, indicate those enzymes which are subject to the metabolic control. In the red cell, phosphofructokinase (PFK) is a particularly sensitive step. The positive and negative factors which help control the activity of this enzyme are shown by the following reaction:

$$\text{Fructose-6-phosphate} + Mg^{2+}\text{ATP} \xrightarrow[-:\text{ATP},\ H^{+}]{+:\text{ADP},\ Pi} \text{Fructose-1,6-bisphosphate} + Mg^{2+}\text{-ADP}$$

This reaction is unusual in that it is activated by its product (ADP) and inhibited by its substrate (ATP). In addition, PFK is activated by inorganic phosphate and inhibited by hydrogen ion. Thus, the rate of red cell glycolysis varies directly with pH and with organic phosphate (Bunn et al., 1977).

The ratio of NAD^+ to NADH

The ratio of NAD^+ to NADH is an important determinant of the rate of synthesis of 1,3-BPG (see Figure 1-6). Thus, the incubation of red cells with appropriate oxidants such as pyruvate or methylene blue will increase this ratio and, by enchancing levels of 1,3-BPG, will promote the synthesis of red cell 2,3-BPG (Bunn et al., 1977).

The ratio of ADP to ATP

Which of the two reaction pathways that 1,3-BPG takes is determined in part by the ratio of ADP to ATP. A relative increase in ADP will favor the conversion of 1,3-BPG to 3-phosphoglycerate. Conversely, if ADP is relatively decreased, the synthesis of 2,3-BPG will be favored. This probably explains why levels of red cell 2,3-BPG and ATP are often reciprocal. The determination of the ADP/ATP ratio involves a complex interaction between the glycolytic rate and the metabolic demands of the cell.

Activity of Bisphosphoglycerate mutase

Another important factor determining the metabolic fate of 1,3-BPG is the activity of the Bisphosphoglycerate mutase (BPGM):

$$\text{1,3-BPG} \xrightarrow{\text{BPGM}} \text{2,3-BPG}$$

This enzyme competes with PGK for 1,3-BPG as a substrate. It changes 1,3-BPG to 2,3-BPG, thereby dissipating the energy of the high-energy acylphosphate bond. It is inhibited by its product, 2,3-BPG and by inorganic phosphate, whereas it is activated by 2-phosphoglycolate and by increased pH levels.

The activity of the BPGM increases directly with pH up to pH 9.75. The increase in red cell 2,3-BPG induced by alkalosis is probably in part due to this pH effect on the mutase as well as the stimulation to glycolysis discussed above. It requires 3-PG for its activity. Thus, the activity of this enzyme in vivo is probably an important determinant of the concentration of 2,3-BPG within the red cell (Bunn *et al.*, 1977, William *et al.*, 1983).

Activity of Bisphosphoglycerate phosphatase

Bisphosphoglycerate phosphatase (BPGP) hydrolyzed 2,3-BPG to 3-phosphoglycerate:

$$2,3\text{-BPG} + H_2O \xrightarrow{\text{BPGP}} 3\text{-PG} + P_i$$

This enzyme has been purified and studied by Harkness and Roth (Harkness and Roth, 1969) and by Rose and Liebowitz (Rose and Liebowitz, 1970). It must be distinguished from PGM, which has some BPGP activity, particularly in the presence of activator such as 2-phosphoglycolate or pyrophosphate (Keitt, 1971). BPGP is activated by some anions, including chloride and inorganic phosphate, whereas monophosphoglycerate (2-PG and 3-PG) are competitive inhibitors. BPGP has a pH optimum at about 6.2. This is a third factor (along with the above mentioned effects of pH on bisphosphoglycerate mutase and on glycolysis) which explains the direct relationship between red cell 2,3-BPG and pH. Under physiologic conditions, the activity of BPGP is probably controlled by the levels of inorganic phosphate, the monophosphoglycerates, and the intracellular pH.

Identity of the two enzymes

After the physiologically important observation that the presence of 2,3-BPG profoundly influences the complexation between oxygen and hemoglobin, it has been demonstrated that BPGM and BPGP have identical electrophoretic (Rosa *et al.*, 1973) and chromatographic properties and they may indeed be the same protein. Sasaki and coworkers (Sasaki *et al.*, 1975) have suggested that intracellular 2,3-BPG concentration may be regulated by one protein or one enzyme. The enzyme has a molecular weight of 57,000, comprising of two subunits of similar mass (28,600/each) and thermostability property (reach up 45°C) (Kappel and Hass, 1976). The trifunctional enzyme regulates the level of 2,3-BPG in human red blood cells via a main synthase

activity (BPGM) and an additional phosphatase activity (BPGP), leading to 2,3-BPG degradation. The hydrolysis of 2,3-BPG is physiologically stimulated by 2-phosphoglycolate, a normal constituent of red blood cells. The enzyme also displays a mutase reaction similar to that of the glycolytic enzyme monophosphoglycerate mutase or phosphoglycerate mutase (PGM, EC 5.4.2.1) which reversibly converts glycerate-3-phosphate (3-PG) to glycerate-2-phosphate (Ravel *et al.*, 1997).

1.4.3 Clinical relevance of 2,3-BPG

2,3-BPG plays an important role in human erythrocytes by regulating their oxygen transport. Since the original observation in 1967 (Chanutin and Curnish, 1967, Benesch and Benesch, 1967) that 2,3-BPG binding to hemoglobin could alter its oxygen affinity, much interest has been focused on the clinical implications. The erythrocyte has an intrinsic control mechanism for manipulating tissue oxygenation under varying conditions, and clinical implication of this have now been partially evaluated.

Foetal blood

The recognition that oxygen unloading to the tissues is decisively controlled by 2,3-BPG has given rise to an explanation for the higher oxygen affinity for foetal as compared with maternal blood. The data on concentrations of 2,3-BPG in these two kinds of blood are conflicting and suggest that the difference can not be accounted for in this way. Tyuma and Shimizu (1969) have shown that, while the oxygen affinity of stripped hemoglobin F is actually lower than that adult hemoglobin, the effect of 2,3-BPG in lowering the oxygen affinity is much greater for the adult than for the foetal hemoglobin. The binding of the phosphates to deoxyhemoglobin F must therefore be significantly weaker than to deoxyhemoglobin A, provided these compounds are again only bound to the deoxy form. It will be fascinating to search for the original of this situation in terms of the well known structural differences between hemoglobin F and hemoglobin A (Benesch and Benesch, 1969).

Transfusion of stored blood

The importance of ensuring an adequate content of 2,3-BPG in blood for transfusion has also received attention, since blood stored in acid-citrate-dextrose (ACD) loses its 2,3-BPG rapidly, so that only 20% remains at the end of two weeks. Citrate-phosphate-dextrose (CPD) preserves the erythrocyte in a more physiological state, and small additions of pyruvate and inosine may maintain the 2,3-BPG level through out the period of storage. In healthy blood recipients, the 2,3-BPG content is rapidly replenished (60% by 24 hours), but it is possible that massive transfusion of old blood with depleted 2,3-BPG content may not have an immediately beneficial effect on the patient (Editor, 1972).

Congenital enzyme deficiency

Some of the congenital deficiencies of enzymes involved in anaerobic glycolysis are of special interest, particularly pyruvate kinase deficiency, are associated with striking rises in 2,3-BPG (Tanaka and Paglia, 1971), whereas defects on the glycolytic pathway before the formation of 2,3-BPG are associated with decreased levels. The clinical importance of this was shown by the difference in ability of two affected individuals with hexokinase and pyruvate kinase deficiency, respectively, to respond to exercise.

Altitude adaptation

A particularly interesting problem in respiratory physiology is the mechanism of high altitude adaptation in man. Lenfant *et al* (1968) strongly suggests that this phenomenon is also controlled by adjustments in the level of 2,3-BPG in the blood. A change in altitude from the sea level to about 15,000 feet was accompanied within 24-36 hours by a shift of the oxygenation curve to the right and a simultaneous elevation of the concentrations of 2,3-BPG by about 50%. Both changes have reversed with the return to sea level. The same increases in oxygen affinity and decrease in concentration of 2,3-BPG occurred in indigenous high lenders when they descended to areas of normal barometric pressure. This indicates that the adjustment of the concentration of 2,3-BPG takes place within existing red cells rather than by erythropoietic mechanisms.

The oxygen deficit at high altitudes will lead to an increased concentration of deoxyhemoglobin in the venous circulation. Only this form of hemoglobin binds 2,3-BPG and therefore the synthesis of this substrate will be simulated by its removal, because, as we have pointed out, it acts as an inhibitor of serveral steps in its formation.

Hypoxemia

A shift of the oxygenation curve to the right, presumably to facilitate unloading of oxygen, has now been reported in a number of clinical conditions associated with hypoxemia such as congenital heart disease, chronic obstructive pulmonary disease and various type of anemia. It will be of great interest to ascertain how far these effects can be traced to changes in the level of effective 2,3-BPG. In the case of certain anemias, the increased reticulocytosis must certainly lead to a red cell population enriched in 2,3-BPG. This will, for example, apply to anemias caused by bleeding and it is temping to speculate whether this ancient therapeutic procedure was an unwitting attempt to bring more oxygen to the tissues.

1.5 Exercise and 2,3-Bisphosphoglycerate

During exercise, requiring a continuous supply of ATP to sustain tissue function (muscle contraction, especially). The ATP can be derived from either aerobic or anaerobic metabolism, but the latter yields less energy and results in lactic acid accumulation. Thus, exercise requires an adequate supply of oxygen to maintain of aerobic metabolism. Molecular oxygen is essential for aerobic organisms. Exposure of tissue or cells to hypoxia induces a variety of adaptive or pathogenic responses. If the delivery of oxygen to the working muscle is inadequate, there is an increased in lactate formation. Lack of oxygen supply results in increased NADH levels, and more amount of pyruvate is converted to lactate, producing lactic acidosis as a result of a decrease in blood pH. Aerobic exercise is fundamentally dependent on intake, transport, and utilization of oxygen by the body. The efficiency and effectiveness of this aerobic system in many ways include improved cardiac (heart) performance, expanded plasma volume, augmented red blood cell mass, greater proliferation of capillaries, enhanced number of organelles, and increased enzyme activity, to name a few. These responses vary in adaptation time and tend to be slow in progression as the athlete nears peak attainable fitness. Thus, the body adapts in many ways to boost its tolerance to the stress of exercise. The response is probably an increase in 2,3-BPG levels in the red blood cells. Elevated 2,3-BPG levels have been observed in people with impaired oxygen delivery. As mentioned, lung disease, anemia and hypoxia at altitude are all stimuli that increase

2,3-BPG concentrations. This is a basic and well known adaptive mechanism in the regulation of oxygen delivery to peripheral tissues. But, the role of 2,3-BPG in adaptation to exercise is controversial. The results of studies at sea level on the effects of exercise on red cell 2,3-BPG levels are equivocal. Thus, the precise mechanism(s) by which 2,3-BPG concentration is regulated has continued to be the subject of debate and an understanding of the regulation of 2,3-BPG concentration is important for understanding blood oxygen transport (Editor, 1972).

1.6 Objectives of the study

1. To investigate the effect of different exercise programs on erythrocyte 2,3-bisphosphoglycerate levels in male Wistar rat.
2. To investigate the factors, such as pH, temperature, pO_2 and calcium ion on Wistar rat erythrocyte 2,3-bisphosphoglycerate levels.