INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

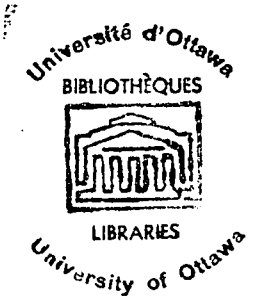
Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

A Study Of The Relationship Between Whole Blood Lactate And 2,3-Diphosphoglycerate During Submaximal Exercise Through The Anaerobic Threshold

By
Art Salmon, B.P.E.
University Of Ottawa

Thesis
Submitted To The School Of
Graduate Studies In Partial Fulfillment
Of The Requirements Of The Degree
Of Master Of Science In
Kinanthropology



School Of Human Kinetics And Leisure Studies
University Of Ottawa
1977

Acknowledgements

Sincere thanks are extended to Dr. Al Reed, Dr. Karel Rakusan and to Dr. Jim Thoden for their guidance and assistance throughout the study. Appreciation is also extended to Don Noble for his encouragement and understanding during the preparation of the study and to my sister Elaine for the countless hours at the typewriter over the past two years.

Table Of Contents

List of Tables

List of Figures

Chapter I

The Problem

Introduction

Extensive research has been devoted to the study of oxygen transport during exercise. The majority of the work has been centered around oxygen uptake at the cellular level. However, more recently the role of oxygen unloading in the erythrocyte has come to the attention of exercise physiologists. Research has found that the erythrocyte is not simply an inert mass shuttling oxygen from the lungs to the rest of the body, but forms a highly integral part of the oxygen transport mechanism.

Erythrocytes, or red blood cells, are biconcave discs formed in bone marrow. The major function of the red cell is to transport oxygen and carbon dioxide. By virtue of its biconcave shape, it is ideally suited to unloading oxygen at the tissue and taking up carbon dioxide. The transportation of oxygen and carbon dioxide in sufficient quantities to service the body's tissues is made possible by the protein hemoglobin located in the red cell. However, hemoglobin is not alone in the red cell. Within the erythocyte also lie the enzymes of its glycolytic pathway. Unique to this pathway in

red cells is the organic phosphate 2,3-diphosphoglycerate. Its presence has been shown to shift the oxygen dissociation curve to the right, thereby increasing the oxygen available to tissue. High levels of 2,3-diphosphoglycerate have been reported in patients where the amount of oxygen being transported to tissue is limited as in anemia, congestive heart failure and pulmonary vascular disease.

To what extent 2,3-diphosphoglycerate alters the oxygen affinity of hemoglobin during exercise is uncertain. It has only been in the last eight years that the role of the organic phosphate in exercise has been examined and the results are inconclusive.

Rationale for the Study

Intensity and duration have always been key variables in the examination of the effects of exercise on the human body. Research has indicated that in response to acute exercise the level of 2,3-diphosphoglycerate occasionally goes up, sometimes goes down and more often than not does not change. However, the majority of the studies have stopped short of measuring the levels of 2,3-diphosphoglycerate through various levels of graded exercise that cover both low lactate and high lactate conditions.

It should be noted that as exercise is progressively increased, blood lactate does not rise until the work rate

reaches a certain value known as the anaerobic threshold. Wasserman and Whipp (1975) reported this critical point to be approximately 50 to 60 percent of the maximal work rate. As exercise continually increases beyond this anaerobic threshold, blood lactate increases progressively with increasing work rate.

Many of the studies examining the effect of exercise on 2,3-DPG have not controlled precisely the level of work being performed or monitored closely the lactate levels reached as a result of the work performed. Therefore, the relationship between exercise lactate and 2,3-DPG has remained obscure.

Statement of the Problem

The principle aim is to study the relationship of whole blood lactate and 2,3-diphosphoglycerate during submaximal exercise at intensities which are above and below the subjects' anaerobic threshold.

Limitations of the Study

The ability to apply the findings of this study to the general population will be limited by the small number of subjects taking part and the fact that only male subjects were used. This study in no way has attempted to examine all exercise intensities and as such the findings will only be

applicable to the specific exercise levels as stated. The subjects taking part in the study were not controlled in terms of modification in their daily routine. No attempt was made to modify regular diet or activity patterns although each subject was repeatedly tested at the same time of day prior to engaging in any physical activity.

Definition of Terminology

2,3-DPG	2,3-diphosphoglycerate
ATP	adensosine-5-triphosphate
Hb	hemoglobin (reduced)
HbO	oxyhemoglobin
Po_2	partial pressure of oxygen
P_{50}	oxygen tension at 50 per cent hemoglobin saturation
NAD	Nicatinamide adenine dinucleotide
NADH	Nicatinamide adenine dinucleotide, reduced
PFK	phosphofructokinase
3-PG	3-phosphoglycerate
P	phosphorus
1,3-DPG	1,3-diphosphoglycerate
KPM	kilopond meter
VO_2max	maximum oxygen uptake
A.T.	anaerobic threshold

Chapter II

Review of the Literature

In examining the effect of exercise on whole blood 2,3-DPG levels, it is first necessary to look at the oxygen transport system as a whole. The relationship between 2,3-DPG and the role it plays in oxygen transport has been shrouded in controversy and as such requires careful examination. To this end the review of the literature will take three parts:

1) an overview of the oxygen transport system
2) an examination of 2,3-DPG in the erythrocyte
3) a review of the role of 2,3-DPG during exercise

Introduction - Oxygen Transport

Human tissue metabolism is critically dependent on an adequate supply of oxygen. The functional unit of the oxygen transport system in man is the erythrocyte. It serves this role because it contains the iron-protein conjugate, hemoglobin. The primary function of red blood cells is to bring oxygen to the tissues in adequate quantities, at a sufficient partial pressure, to permit its rapid diffusion from the blood. The ultimate supply of oxygen to the cell is determined by a number of factors which include: the content of the oxygen in the inspired air; the partial pressure of oxygen in the inspired air; the pulmonary and alveolar

ventilation; the diffusion of oxygen from the lungs and its final release to the tissues is determined in large part by the affinity of hemoglobin for oxygen (Oski and Gottlieb, 1971).

Until recently the major focus of interest in oxygen transport has centered on factors governing oxygen uptake, and little attention has been paid to the factors governing oxygen release; that is, the unloading of oxygen in the tissue capillaries. It has long been assumed that the affinity of hemoglobin for oxygen, as reflected by the hemoglobin-oxygen dissociation curve, was mainly influenced by changes in pH, Pco_2 and temperature. Within the past ten years, however, new evidence has been reported indicating that the affinity of hemoglobin for oxygen is altered in a variety of situations and that the red cell regulates these changes in oxygen affinity (Oski and Delivoria-Papadopoulus, 1970).

The oxygen-hemoglobin dissociation curve reflects the affinity of hemoglobin for oxygen (Figure 1). When oxygen diffuses into the plasma from the alveolus, almost all of it finds its way into the red cell, where it combines with hemoglobin. Only a small part of the oxygen remains in the plasma and is carried to the tissue in simple solution. Since the solubility of oxygen in aqueous solutions is very low,

(solubility coefficient 2.44 ml O_2 per 100 ml plasma per atm O_2 pressure) only 0.3 ml of oxygen will be dissolved in 100 ml of plasma with the usual partial pressure of oxygen in arterial blood (Lloyd, 1976). The red blood cell carries almost all the oxygen (98.5%) in the bloodstream by virtue of the capacity of hemoglobin to carry oxygen. When fully saturated, 1 gm of hemoglobin will hold 1.34 ml of oxygen and since the normal hemoglobin content of the blood is 15 gm per 100 ml the oxygen-carrying capacity is approximately 20 ml per 100 ml of blood (Lloyd, 1976).

Unlike the linear relationship between partial pressure and content of gases in true solution, the relationship of the partial pressure of oxygen to oxygen content is not a linear one, a fact which has important physiological implications. This relationship is exemplified by the oxygen-hemoglobin dissociation curve. The part of the oxygen-hemoglobin dissociation curve of greatest physiological importance is the part between 40mmHg and 100mmHg. At the top, large changes in the partial pressure of oxygen have a small effect on the amount of oxygen carried; below that level, small changes in partial pressure of oxygen have a large effect on the oxygen content. As blood circulates in the lung, the arterial oxygen tension rises from 40mmHg to 100-110mmHg, sufficient to ensure at least 95% saturation of

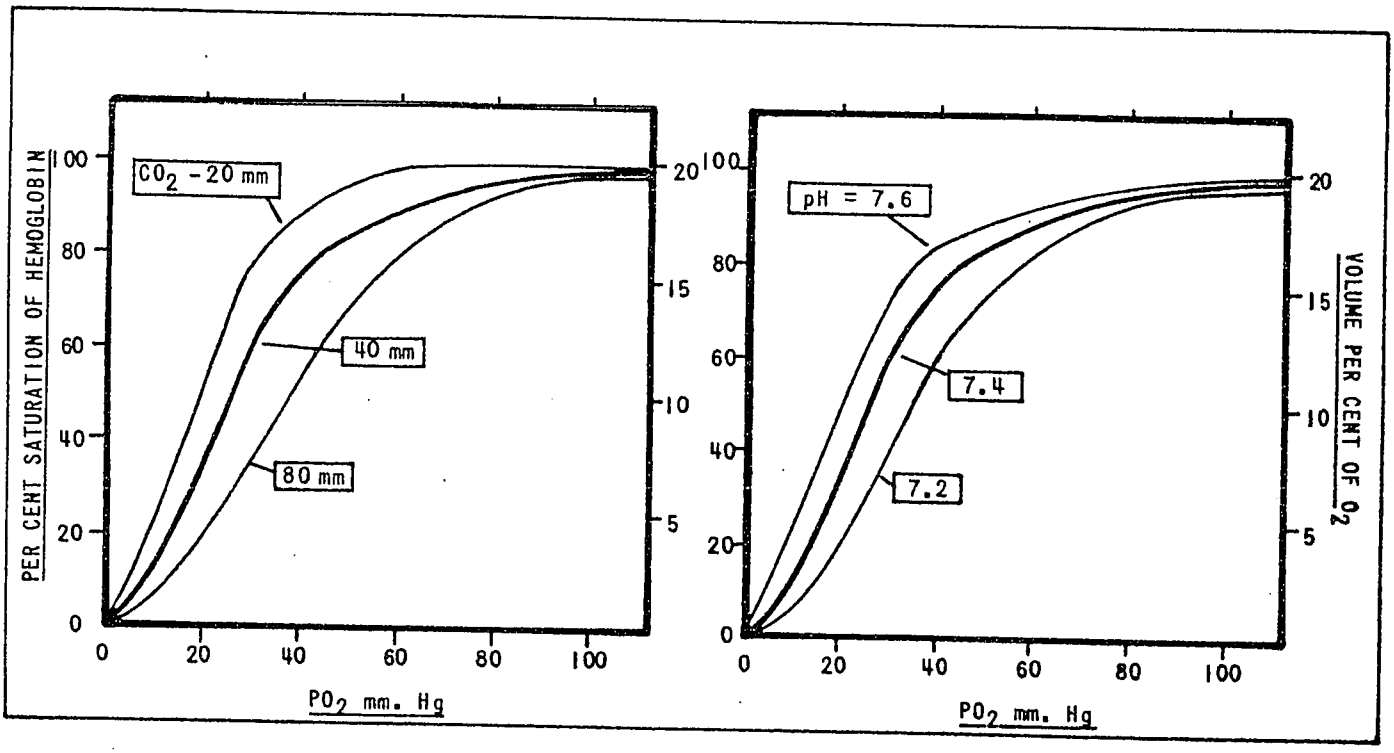


Figure 1

Normal Oxygen-Hemoglobin Dissociation Curve.

Influence of CO_2 and pH on the Curve. (Lloyd, 1976)

the arterial blood. As blood travels from the lung, the oxygen tension falls, as it is released to the tissues. When the oxygen tension has fallen to approximately 27mmHg (7.4, 37^{oc}) 50% of the oxygen bound to hemoglobin has been released. When the affinity of hemoglobin for oxygen is reduced, more oxygen is released to the tissue at a given oxygen tension.

In such situations the oxygen-hemoglobin dissociation curve is shifted to the right of its normal position. It has long been recognized that increases in blood acidity, carbon dioxide content or temperature are capable of decreasing the affinity of hemoglobin for oxygen and thus shift the curve to the right (Astrand, 1970).

A certain partial pressure gradient is needed for the transfer of oxygen from capillary to tissue. Below this critical level, diffusion is impaired and tissue hypoxia results. Kety (1957) and Landis and Pappenheimer (1963) have noted that the end-capillary oxygen tension necessary for adequate tissue oxygenation is at least 20mmHg. Similarly, Berne and associates (1957) found that coronary vasodilitation occurred when the arterial oxygen pressure was 22 to 24mmHg and that loss of myocardial function was severe at an oxygen tension of 10 to 12mmHg.

A critical capillary oxygen tension cannot be defined precisely for all tissues under all metabolic conditions because of the inherent variability in requirements from tissue to tissue. Under conditions of increased oxygen need, however, the body may improve oxygen delivery to the tissues either by improving oxygen transport or by decreasing the affinity of hemoglobin for oxygen to increase oxygen unloading from blood. Improved oxygen transport is achieved by

increasing the cardiac output or by increasing the oxygen carrying capacity of the blood by increasing the hemoglobin concentration (Oski 1975, Astrand 1970). An increase in cardiac output represents rapid compensation, but is limited in its magnitude; it requires an increase in oxygen consumption that tends to offset its compensatory advantage. The second mechanism of improving oxygen transport, that of reducing hemoglobin affinity for oxygen, allows more oxygen to be delivered to the tissues at an equivalent or even higher partial pressure of oxygen. As a result, the end-capillary tissue oxygen gradient is maintained above the critical range despite a decrease in the degree of oxygen saturation of the blood.

It is this latter mechanism that results from the interaction of red blood cell organic phosphate compounds with hemoglobin that has been recognized recently as a common, rapid, and efficient means of increasing tissue oxygen delivery (Benesch and Benesch, 1967; Chanutin, 1967).

The Erythrocyte and 2,3-Diphosphoglycerate

The mammalian hemoglobin molecule within the red blood cell is composed of two sets of chemically identical chains, each containing a heme prosthetic group that can combine reversibly with oxygen (Lehninger, 1970). The polymeric structure of hemoglobin is of particular importance in

maintaining the sigmoid nature of the oxygen-hemoglobin dissociation curve. The curve represents the sequential addition of each of four molecules of oxygen to each of the four heme groups. With the saturation of each heme group, decreasingly smaller increments in oxygen tension are required for the saturation of each remaining heme. Thus, this "heme-heme interaction" results in oxygen saturations of 92% to 100% with oxygen tensions of 60mmHg to 100mmHg. Similarly, due to the slope of the curve over the range in which the majority of oxygen is either loaded or unloaded, oxygen is readily released to the tissues and hemoglobin is readily reoxygenated to near normal saturation by small changes in tissue or alveolar oxygen tension. X-ray crystalographic studies have confirmed the fact that oxygenated and deoxygenated hemoglobins differ in their confirmation (Muirhead, 1967). These confirmational changes are crucial for the interactions of hemoglobin with the organic phosphates.

The oxygen affinity of hemoglobin within the red cell is lower than that of hemoglobin in free solutions (Benesch, 1969). In commenting on this difference, Barcroft (1921) wondered in the early 1920's "Is there some third substance present . . . which forms an integral part of the oxygen-hemoglobin complex?" A few years later, Greenwald (1925) reported finding large quantities of 2,3-DPG in the

erythrocytes of pigs and in 1941 Rapoport confirmed these findings and found high levels of 2,3-DPG were also present in the red cells of man and other species. It was not until the late 1950's, however, that a physiological role for 2,3-DPG began to be uncovered.

A dramatic decrease in the affinity of hemoglobin for oxygen consequent to the binding of organic phosphates was observed virtually simultaneously in the laboratories of Benesch and Benesch (1967) and Chanutin (1967). Removal of 2,3-DPG from hemolysates by exhaustive dialysis was accompanied by marked increase in the affinity of hemoglobin for oxygen. The addition of 2,3-DPG, ATP or a number of other organic phosphates to such stripped hemoglobin solutions reversed this effect (Benesch, 1968). The magnitude of this effect was found to be sufficient to shift the P_{50} of a hemoglobin solution from about 3mmHg in the absences of 2,3-DPG, to about 13mmHg in the presence of 2mM 2,3-DPG. With oxygenation, bound 2,3-DPG was promptly released. "The results reported here strongly suggest that 2,3-DPG can act as a powerful regulator of the allosteric properties of hemoglobin in the human red cell, thus enabling it to act as an oxygen donor under physiological conditions" (Benesch, 1967).

Benesch and co-workers first suggested in 1967, and later confirmed by direct experimentation, that the displacement

of oxygen from hemoglobin by 2,3-DPG indicates a higher binding affinity for 2,3-DPG to deoxyhemoglobin than to oxygen bound hemoglobin (Benesch, 1968). Thus, the deoxyhemoglobin conformation of the molecule would be stabilized by 2,3-DPG binding, and a decreased affinity for oxygen would result. As shown in Figure 2, binding of 2,3-DPG to the hemoglobin tetramer approaches mole-for-mole binding (Benesch, 1968).

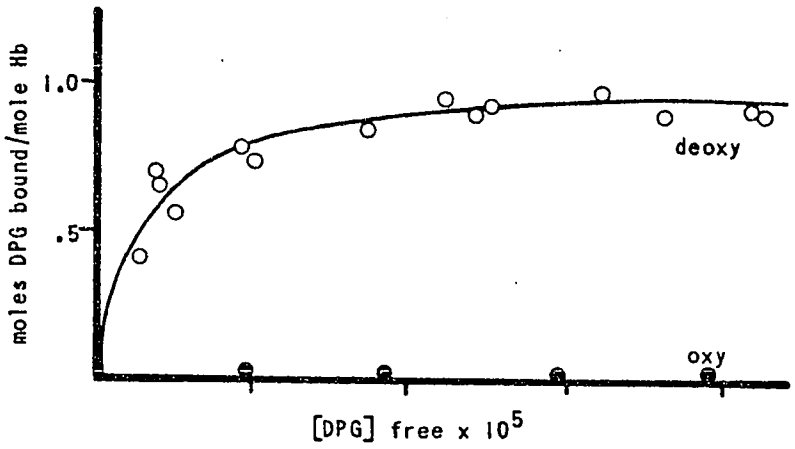


Figure 2

Binding of 2,3-DPG by Human Hemoglobin in 0.1 M NaCl, pH 7.3, 22°C (Benesch 1968)

No binding of 2,3-DPG was observed to oxyhemoglobin. Oxygen and 2,3-DPG are thus competitive for a similar confirmation, although not necessarily for the same sites on the hemoglobin molecule (Oski, 1970). Brewer and Eaton (1971) report that the exact sites of binding of 2,3-DPG and ATP to hemoglobin are not yet

known with certainty although fairly strong evidence points to a site or sites on the β-chain. Benesch and Benesch (1969) pointed out that the binding might be to the β-chain, since isolated α-chains do not bind 2,3-DPG at all and isolated β-chains were found to bind ¼ mole of 2,3-DPG per mole of β-chain. It was suggested that the binding might occur between the two β-chains within the central cavity of the hemoglobin molecule. The entrance to the central cavity of oxyhemoglobin is probably too small to admit the 2,3-DPG molecule, but, as a result of the 6-angstrom increase in the size of the cavity entrance which occurs in full deoxygenation (Muirhead, 1967), the 9 angstrom 2,3-DPG molecule could easily be accommodated by deoxyhemoglobin.

DeVerdier and Garby (1969) supported the concept that the binding site was most likely a positively charged area somewhere in the central cavity along the diad axis of symmetry. They suggested the beta-143 histidine as a likely binding site. This positively charged amino acid residue is located at the entrance to the central cavity and could form electrostatic bonds with anions such as 2,3-DPG and ATP. Bunn and Briehl (1970) have presented evidence that beta-143 histidine cannot be the sole site of binding because fetal hemoglobin, which has a greater oxygen affinity than adult hemoglobin does show some interaction with 2,3-DPG even though

this position on the gamma chain is occupied by the neutral amino acid serine which could not bind negatively charged compounds such as 2,3-DPG. Greer and Perutz (1970) as cited by Bunn (1970) were able to fit a model of 2,3-DPG into an atomic model of human deoxyhemoglobin. Bunn (1970) states that Greer and Perutz were able to place one molecule of 2,3-DPG in the internal cavity in such a way that the phosphates were within hydrogen-binding distances of the two N-terminal amino groups of the beta chains. Furthermore, they found that upon oxygenation of the hemoglobin, the N-terminals of the beta chains moved farther apart, making it impossible for both of them to bind to the same molecule of 2,3-DPG. They concluded that such a difference between the binding of 2,3-DPG to deoxy- and oxyhemoglobin would account for the effect of 2,3-DPG on oxygen affinity (Bunn, 1970).

The work of the Beneschs (1967) and Chanutin and Curnish (1967) led to the discovery that the oxygenation of hemoglobin is decisively influenced by certain organic phosphates in the red cell. Up until that time the position of the oxygen-hemoglobin dissociation curve had always been regarded as being influenced only by temperature, pH and Pco_2. Benesch and Benesch (1969, 1970) note, however, that the physiological variation of these factors is too limited to provide an efficient control mechanism, but the level of

effective organic polyphosphates, on the other hand, is a metabolically controlled environmental factor which should be capable of regulating oxygen unloading in different physiological conditions.

Eaton and Brewer (1969) reported at the 1st International Conference on Red Cell Metabolism and Function that their research indicated a negative correlation between whole blood hemoglobin and red cell 2,3-DPG in normal healthy individuals. This relationship indicated that 2,3-DPG may be of some use as a "fine tuning" mechanism for the respiratory system, allowing a more perfect integration of oxygen transport with levels of oxygen use. Eaton has been able to show increases in 2,3-DPG among residents of high altitude with a concomitant right shift in the oxygen hemoglobin dissociation curve. These increases have been reported as early as six hours after exposure to high altitude (Lenfant, 1968). Eaton (1969) also reported elevated levels of red cell 2,3-DPG seemed to be involved in adaption to the stress of prolonged exercise.

The mechanism usually advanced to explain the 2,3-DPG rise during hypoxia has been that of the increasingly desaturated hemoglobin binding more 2,3-DPG, and the enzymes of glycolysis replacing the newly bound 2,3-DPG with enough free 2,3-DPG to restore the previous free levels (Eaton and Brewer 1968, Benesch and Benesch 1969). This, then, would produce an overall increase

in the level of red cell 2,3-DPG and implies that the state of oxygenation of the red cell is at least one of the factors controlling the amount of 2,3-DPG within the circulating red cell. In this way, the oxygen delivery capacity of the red cell may be governed by a relatively simple feedback system, in which the functioning of the red cell is maintained at an adequate level merely by factors in the cell "sensing" the amount of oxygen which the cell is called upon to transport.

Astrup (1970) reported changes in the oxygen affinity of hemoglobin due to pH dependent 2,3-DPG changes. His investigations indicate that the erythrocyte 2,3-DPG level is controlled by the red cell pH, reporting changes as small as 0.01 in pH will alter the 2,3-DPG concentration as much as 4%. The changes in 2,3-DPG reported by Lenfant (1968), according to Astrup, are caused by the small changes in pH which occur as a result of the high altitude. Astrup concludes that the relationship between reduced and oxygenated hemoglobin in the vascular system determines the 2,3-DPG concentration by a primary influence on mean red cell pH.

The factors regulating red blood cell 2,3-DPG are gradually becoming clear. The basic pathway of glucose metabolism within the human red blood is shown in Figure 3.

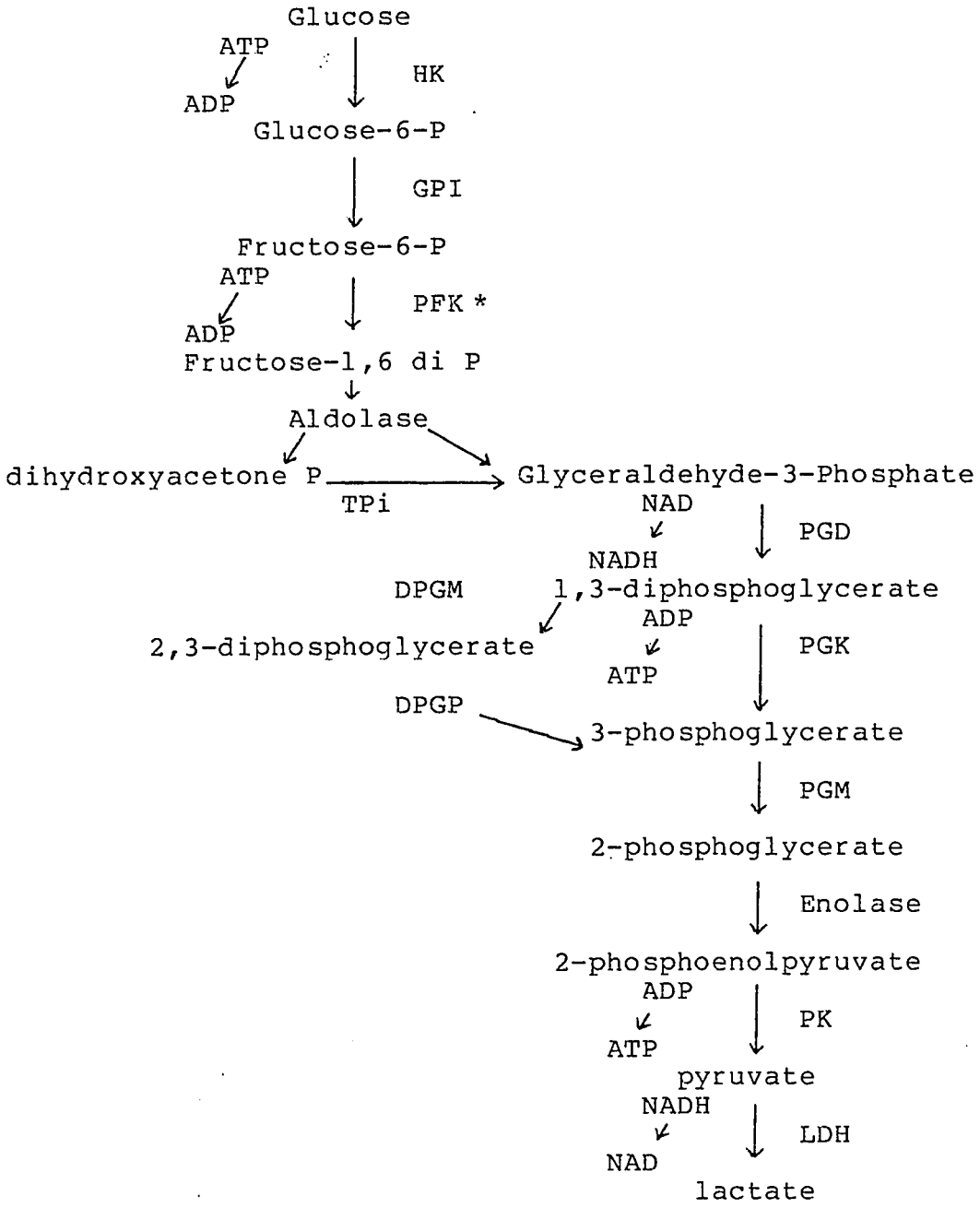


Figure 3

The Metabolism of the Human Erythrocyte

HK, Hexokinase; GPI, phosphoglucose isomerase; PFK, phosphofructokinase; TPI, triosephosphate isomerase; PGD, glyceraldehyde phosphate dehydrogenase; PGK, phosphoglyceric acid kinase; PGM, phosphoglyceromutase; DPGM, diphosphoglycerate mutase; DPGP, diphosphoglycerate phosphatase; PK, pyruvate kinase; LDH, lactic dehydrogenase. (Oski and Gottlieb, 1971)

*Rate limiting enzyme

Approximately 90% of the glucose consumed by the red cell is metabolized from glucose-6-phosphate to 1,3-diphosphoglycerate via the anaerobic pathway. At normal plasma pH of 7.4 the primary control of the red cells' glycolytic pathway is at the phosphofructokinase (PFK) step (Rose, 1966). PFK is strongly inhibited by ATP; even as low as 0.1mM. The activity of PFK is also strongly pH dependent. As pH rises, so does PFK activity, the red cell glucose consumption increases and large quantities of glucose-6-phosphate are converted to glyceraldehyde-3- phosphate. With increased pH and increasing inorganic phosphate concentration, the control of red cell glycolysis and eventual 2,3-DPG synthesis appears to shift to the steps catalyzed by glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase (Oski and Gottlieb 1971). Formation of 1,3-DPG is favoured by an increase in organic phosphate, an alkaline pH, a higher proportion of NAD and an increase in the level of glyceraldehyde-3-phosphate. These conditions are produced by a release of PFK inhibition. A rise in pH and inorganic phosphate thus serve to stimulate both phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase activity.

Despite the increase in 1,3-DPG synthesis by these conditions, the regulations of 2,3-DPG concentration is still primarily a consequence of the balance of two enzyme

reactions: 2,3-diphosphoglycerate mutase and 2,3-diphosphoglycerate phosphatase. The enzyme 2,3-DPG mutase catalyzes the irreversible reaction: 1,3-DPG + 3-PG → 2,3-DPG + 3-PG. The enzyme has a high affinity for 1,3-DPG and 3-PG and is strongly inhibited by its product 2,3-DPG (Rose, 1966). The inhibition would appear to be nearly complete at the usual intracellular concentrations of 2,3-DPG. In the presence of deoxyhemoglobin, the free 2,3-DPG is bound, the product inhibition is relieved and further 2,3-DPG synthesis is facilitated. With deoxygenation of the intact cell, red cell glycolysis also increases and 2,3-DPG levels increase.

The 2,3-DPG degradative enzyme 2,3-diphosphoglycerate phosphatase catalyzes the reaction: 2,3-DPG + H_2O → 3-PG + Pi. This enzyme appears far less active than DPG mutase (Rose, 1966).

Intracellular pH also profoundly influences 2,3-DPG metabolism either by directly regulating red cell glycolysis or possibly by direct effects on the activities of 2,3-DPG mutase and phosphatase. Increases in pH stimulate both red cell glycolysis and 2,3-DPG production while acidosis has the opposite effect (Oski, 1970).

The literature has numerous examples of environmental factors influencing the levels of erythrocyte 2,3-DPG (Eaton 1970, Brewer 1970, Oski 1970). Hypoxia, altitude, anemia,

pulmonary disease, severe exercise all cause an increase in levels of 2,3-DPG. Brewer has been able to show in humans that the level of red cell ATP is under partial genetic control (Brewer, 1970). Therefore, it would not be surprising if a similar situation pertains to another organic phosphate, 2,3-DPG. If such a situation were true, it could possibly be important in determining the range of environmentally induced change in a given individual's 2,3-DPG level. In rats, Brewer's (1970) data indicates that the offspring of the parents with high levels of 2,3-DPG have higher levels of the phosphate than the offspring of parents with lower levels of 2,3-DPG. These data imply that ATP and 2,3-DPG levels are positively correlated in rats; however, in humans Brewer concludes that the relationship is much lower, if it exists at all.

It is interesting to speculate at this time on the possible role of a right-shifted oxygen-hemoglobin dissociation curve as a consequence of being at high altitude. Impaired oxygen loading may be caused by high altitude hypoxia accompanied by alkalosis due to increased ventilation (Finch and Lenfant, 1972). 2,3-DPG levels are increased in such a case when arterial saturation is decreased, but the extent to which the associated decrease in hemoglobin affinity improves oxygen extraction depends on the amount of desaturation (Oski,

1970). When the PaO_2 is displaced to the steep portion of the oxygen hemoglobin dissociation curve, oxygen loading may be slightly impaired. A decrease in arterial PO_2 would result from a decrease in hemoglobin saturation and only to a small extent an increase in P_{50} . It is possible to calculate, using Hill's equation, that an increase in P_{50} from 27 to 30mmHg would lower the hemoglobin saturation in the pulmonary capillaries from 96.8 to 96.1%, which is a negligible drop in hemoglobin saturation. This phenomena is purely hypothetical and has not been well documented in the literature to date.

The Role of 2,3-Diphosphoglycerate in Exercise

The human red cell is capable of adaptive response to hypoxia and evidence reported earlier emphasized the important role of 2,3-DPG. The increase in the concentration of 2,3-DPG in the red cell which occurs in response to hypoxia shifts the oxygen dissociation curve to the right (Benesch and Benesch 1969, Lenfant 1968, Brewer 1970) and facilitates the release of oxygen to the tissues (Oski, 1970). Surprisingly, little has appeared in the research journals concerning the role of 2,3-DPG in the transport of oxygen during exercise. Faulkner (1970) and Eaton (1969) were among the first to report that extended physical exertion resulted in an increase in the concentration of red cell 2,3-DPG. Both Eaton and Faulkner reported separately on the same investigation in which 10 adult males were sampled for 2,3-DPG before and after engaging

in 60 minutes of basketball. They reported increases in red cell 2,3-DPG in all 10 subjects of about 18% (11.14 μ moles/gm ±1.53 at rest, 13.12 μ moles/gm ± 1.9 post exercise) and whole blood lactate levels were elevated by 60% (16.9 mg% at rest, 27.0 mg% post exercise). In a second study, to obtain a more controlled physiological stress, Faulkner and Eaton had 10 men pedal bicycle ergometers. The load was adjusted so the 5 trained subjects pedalled at 1200 KPM while 5 untrained subjects pedalled at 600 KPM. These work loads represented approximately 70% of their max. 0_2 uptake. After 30 minutes of exercise, the 2,3-DPG concentrations did not vary from resting levels. However, after 50 minutes of exercise, increases in red cell 2,3-DPG had occurred in 7 of 10 subjects. Mean 2,3-DPG levels increased from 12.84 μ moles/gm of Hb to 13.85 μmoles/gm Hb and lactate increased from 9.3 mg% to 24.4 mg%. Both authors reported that the subjects who evidenced the greatest strain had the largest increases in red cell 2,3-DPG. The three subjects on the bike who did not show an increase in 2,3-DPG displayed slight or negative changes in blood lactate concentrations during exercise. Although blood lactate does not reveal the actual muscle lactate concentration (Astrand, 1963), the blood lactate has been used on many occasions as a measure of physiological strain (Astrand 1963, Astrand and Saltin 1961). Both authors agreed that because of insufficient

data, making conclusions regarding the effect of training on red blood cell metabolism and function was very tenuous.

Shappell (1970) reported in The New England Journal of Medicine acute changes in hemoglobin affinity for oxygen among patients with angina pectoris during exercise induced by a cardiac pacer. Pacing was started at a rate that was approximately 75% of the heart rate at maximal exercise and increased 5 to 10 beats every two minutes until the patient experienced the kind of pain which would normally make him stop walking. Blood was drawn simultaneously from the radial recurrent artery and coronary sinus and measured for oxygen tension at 50% saturation (P_{50}), 2,3-DPG, ATP, and pH before, during and after angina pectoris produced by the atrial pacing. At rest, Shappell found no differences in P_{50}, however, the longer the duration of angina the more coronary-sinus P_{50} exceeded arterial P_{50}. Shappell also found that although there was a decrease in hemoglobin affinity for oxygen as displayed by the increased P_{50}, it was not accompanied by changes in the levels of erythrocyte 2,3-DPG, ATP or pH. Since there was no change in 2,3-DPG, Shappell commented on other mechanisms which might explain the increased P_{50}. Lactate did not appear to be the cause because one patient, who was free of pain, exhibited lactate production but did not have a significant P_{50} change. Similarly, another patient

demonstrated marked lactate production at rest, without a significant elevation in cardiac sinus P_{50}. Clearly this data would conflict with Faulkner (1970) and Eaton (1969) who reported a positive relationship between increased 2,3-DPG levels and increased lactate production.

In contrast to Eaton's (1969) findings of increases in 2,3-DPG with exercise, and Shappell's (1970) report of no change in the enzyme with exercise, Dempsey (1971) reported lower 2,3-DPG (μ moles/gm) levels with prolonged aerobic exercise. In examining 4 highly trained subjects who exercised to exhaustion at 65% of their maximal aerobic capacity on a treadmill, he found there was a decline below resting levels in red cell 2,3-DPG which occurred at the mid-point of each work test. He reported that neither the magnitude nor direction of change in 2,3-DPG was related to the physiologic intensity of the exercise. The subjects were on the treadmill from 50 to 120 minutes with lactate increasing from resting levels to approximately 40mg%. There was no evidence, (as reported by Eaton (1969)) that the intensity of the work correlates with the rise in 2,3-DPG. Dempsey contends that time is a critical determinant of any contribution the generation of 2,3-DPG may make to oxy-hemoglobin affinity, regardless of the apparent intensity of demand for increased oxygen delivery.

Remes' (1975) study of 2,3-DPG concentration in long distance runners substantiates the earlier findings of Dempsey et al (1971). For Remes' study, 8 men and 8 women competed in a cross country running championship. The men ran 15 km while the women ran 5 km. Blood samples were taken before the race and as soon as possible after the race and measured for 2,3-DPG and lactate. His results showed that red cell 2,3-DPG (μ mole/ml) concentration decreased during strenuous physical exercise. In a similar study done on men competing in a marathon, a distance of 42 km requiring between 2.5 and 3.5 hours to complete, Remes found even greater decreases in 2,3-DPG. The decrease in red cell 2,3-DPG (μ mole/ml) concentration occurred in the athletes studied within 30 minutes to 3 hours of initiation of strenuous exercise. Remes reports no significant correlation between the change in blood lactate and 2,3-DPG concentrations.

The majority of the literature would indicate very little change in red cell 2,3-DPG with aerobic work. Eaton (1969), for example, reported no change in 2,3-DPG after 30 minutes of exercise on the bicycle ergometer. Thompson (1974), after looking at prolonged exercise consisting of walking on a treadmill at 3.5 mph at 30% of the subject's maximal oxygen consumption for 9 minutes, 40% MVO_2 for the next 9 minutes and continuing at 66% MVO_2 until exhausted,

noted that the prolonged work caused a rightward shift in the dissociation curve due primarily to increased femoral venuous temperature, but caused no consistent change in 2,3-DPG concentration. He concluded that the additive effects of pH and temperature changes alone are responsible for the shifting of the oxygen-hemoglobin dissociation curve with prolonged exhaustive work and 2,3-DPG has little or no role to play in oxygenation of working tissue.

Although Bonner (1975) found a 9.8% increase in the level of post exercise 2,3-DPG in 15 untrained women who walked to exhaustion (approximately 9 minutes) on a treadmill, he also found an increase of 9.4% in the hemoglobin level. He, therefore, concluded that when 2,3-DPG is expressed as a ratio to hemoglobin (2,3-DPG µmole/ml/Hb gm/%) there was no significant change as a result of exercise stress. It was suggested that 3 additive factors produced during strenuous exercise: decreased pH; increased hemoglobin concentration; and increased CO_2 production, result in by-product inhibition of 2,3-DPG synthesis. These data dealing with short-term maximal exercise are compatible with the consistent reports showing long term exercise to have little influence in the red cell 2,3-DPG levels (Dempsey 1971, Shappell 1970, Remes 1975).

Several investigators have examined the effect of training programs on erythrocyte 2,3-DPG levels and the results seem to be fairly inconsistent. Shappell (1971) found that although acute exercise does result in an elevated 2,3-DPG level, it does not result in an increased P_{50}, either before or after training. He did find that after an eight week training program, the resting 2,3-DPG level was increased by 5%. Rand (1975) found in highly trained world class athletes that although the resting 2,3-DPG was not elevated, the P_{50} was when compared to sedentary control groups. Leusink (1973) investigated the effects of a period of training on four female athletes and found just the opposite to what Shappell et al (1971) reported. Leusink reported no change in the 2,3-DPG concentration under the influence of training. Taunton (1972, 1974), on the other hand, found that when compared to a sedentary control group, the resting levels of 2,3-DPG of highly trained athletes was significantly higher. The resting P_{50} value for endurance athletes was also significantly elevated over a control group which is in opposition to Shappell (1971).

Taunton and Banister studied changes in 2,3-DPG in top-class middle distance athletes at rest, during maximal short-term exercise and also in sedentary subjects at rest. They found that, in addition to the elevated resting 2,3-DPG (μ mole/gm) levels in the athletes, the resting and

post-maximal exercise red cells 2,3-DPG levels in the athletic group were also significantly different. P_{50} values among this group were also significantly higher at rest than the sedentary group and higher after the exercise than at rest. The mean change in lactate above resting levels was 82.3mg% (range 35-131mg%). In a similar study in 1974, Taunton compared the effects of short-term maximal exercise (average time 5 min., 35 sec.) on levels of 2,3-DPG and P_{50} to those after a 10 mile road race. His results were not unlike his earlier study. The resting levels of 2,3-DPG of both groups of athletes were significantly higher than the mean of a sedentary control group. The resting P_{50} value of the endurance group athletes was also significantly elevated. After short-term maximal exercise, a mean 15% increase in 2,3-DPG with an increase in P_{50} from 27.2 ± .5 to 30.0 ± 9mmHg was observed. On the other hand, after prolonged endurance exercise, a small but significant decrease in 2,3-DPG and a decrease in elevated P_{50} at rest to normally determined sedentary values after exercise resulted.

Boning (1974) compared the effects of acute and chronic exercise on the oxygen dissociation curve of trained athletes and a relatively untrained group. The six trained athletes were highly competitive endurance athletes competing in rowing, cycling and long distance running. In order to measure the

acute effects of exercise, Boning had his subjects pedal a bicycle ergometer for 20 minutes as a warm-up and then increased the load every 10-15 minutes for about one hour. At rest and at each work rate, femoral venuous blood was collected by means of an indwelling catheter. He reported that physical training leads to a right shift in the oxygen-hemoglobin dissociation curve. The 2,3-DPG concentration in the trained group was also about 20% higher at rest than in the untrained group. During acute exercise, 2,3-DPG generally remains constant, as reported by Shappell and Dempsey. It is interesting to speculate on Boning's paper as to the level of exercise at which the subjects worked. He reports a maximum work rate of 24Kpm/sec (1440 Kpm) which was completed by only one of the highly trained subjects. The other subjects finished exercising at 18Kpm/sec (1080 Kpm) which is a relatively low workload. It would appear from these data that, with the exception of the final workload of 1440 Kpm performed by one subject, all work was aerobic.

Summary

Since the early days of Greenwald and the classical studies of the Beneschs and Chanutin, the role of 2,3-diphosphoglycerate as a glycolytic intermediate in the red cell capable of altering the affinity of hemoglobin for oxygen, has been well investigated. Various conditions of low

hemoglobin levels and hypoxic states have been associated with increased levels of 2,3-DPG. Increased $[H^+]$ concentration and increased temperature cause a similar effect as a rise in 2,3-DPG; a rightward shift in the oxygen-hemoglobin dissociation curve.

The effect of exercise on whole blood 2,3-DPG levels is still a matter of speculation. Eaton et al (1969) were among the first to report increases in red cell 2,3-DPG concentrations with exercise but since that time controversy has arisen. During exercise, various stimuli are at work to alter the level of 2,3-DPG and it appears from the conflicting reports that the length of the exercise and its intensity are of critical importance. In the acute aerobic studies there does not appear to be any significant elevation of 2,3-DPG. On the other hand, the high lactate or acute anaerobic studies would appear to indicate that there may indeed be changes in 2,3-DPG. Although the aerobic training studies were inconclusive, there would appear to be mounting evidence to indicate higher resting levels of 2,3-DPG among highly conditioned athletes.

Chapter III

Methodology

Subjects

All subjects in this study were male volunteers who were non-smokers. In keeping with the American College of Sports Medicine's guidelines for graded exercise testing (1975), all subjects taking part were asymptomatic with regard to heart and blood vessel disease and were under the age of 36.

Exercise Procedures

Each subject was required to visit the biokinetics laboratory on four different occasions. Each subject was tested at the same time of day (±90 minutes) to minimize any diurnal effect.

a) Day I: Upon arrival at the lab, the subjects were weighed and then asked to perform a multi-stage discontinuous bicycle ergometer test to exhaustion. The graded exercise test was performed to determine the subject's maximum oxygen consumption. Each subject pedalled for 5 minutes at 50 revolutions per minute at 300 KPM as a warm-up. Upon completion of the warm-up, a minimum 2 minute rest was taken and the subjects then pedalled at a higher workload. The workloads became more difficult every 5 minutes after the minimum 2 minute rest.

For example: 5 min @ 300 KPM - minimum 2 minute rest

5 min @ 600 KPM - 2 minute rest

5 min @ 900 KPM - 3 minute rest

Rest intervals were as long as the subject desired. Maximum oxygen consumption was considered attained when, with further increases in work rate, no further increase in oxygen consumption occurred.

b) Day 2: On their second visit to the lab the subjects performed an anaerobic threshold test to determine at what workload they crossed the anaerobic threshold. Each subject pedalled at 300 KPM for 5 minutes as a warm-up. After the warm-up, the subject continued to pedal and after each minute the workload was increased by 100 KPM until a sharp rise in carbon dioxide production and/or $\dot{V}_E$ was obtained. At that point the subject was considered to have crossed his anaerobic threshold.

c) Days 3 and 4: The testing procedure on days 3 and 4 was the same. The identical amount of work was performed each day although the duration and intensity of the workload varied. The workload was determined by the point at which each subject crossed the anaerobic threshold. For example: If the anaerobic threshold was 1200 KPM, then the total amount of work done in 5 minutes at that intensity would be 6000 KPM (50 RPM x 6 metres x 4KP x 5 minutes). Each subject first pedalled below and then above his anaerobic threshold on each

test day. If the intensity was above 4KP (our example), then the time was less than 5 minutes in order to keep the total work the same.

Unpublished observations in this laboratory indicated that a thirty minute break between work loads was adequate to allow elevated lactate levels to return to their normal resting levels if work intensity was below the anaerobic threshold. (Appendix B)

Blood Analysis Procedures

Upon arrival at the lab on test days 3 and 4, each subject had a 10cc blood sample taken from the cephalic vein and analyzed for resting levels of 2,3-DPG, lactate, Po_2, pH, Pco_2, hemoglobin, hematocrit and % oxygen saturation. 2,3-DPG was measured according to the ultraviolet method of Sigma Chemical Corporation (Sigma, 1974). Using Sigma's metabolic control sample containing 2.02 µmol/ml for 12 analyses, the mean value obtained was 2.03 µmol/ml with a range of 1.94 to 2.12 µmol/ml, with a standard deviation of ±.06 µmol/ml. Lactate was measured ultravioletly using the BMC Biochemicals Ltd Kit #15972. Hemoglobin was measured by the cyanmethemoglobin method (Stanbio Chemical Company).

The calculation of in vivo P_{50} based on specific percent saturation and venous Po_2 was performed using Hill's equation:

$$y = \frac{(P/P_{50})^n}{1 + (P/P_{50})^n}$$

in which y is the fractional saturation with oxygen, P is the partial pressure of oxygen, P_{50} is the oxygen pressure required for 50% oxygenation, and n the Hill's constant taken as 2.65. (Altman and Dittmer, 1971)

Equipment

All exercise tests were performed on a Monarch Bicycle Ergometer. Oxygen consumption and carbon dioxide production were measured on a Godart gas analyzer. Blood pH, Po_2 and Pco_2 were measured on a Corning pH/Blood Analyzer model 165. Ultra violet determination of 2,3-DPG and lactate was performed on a Bausch and Lomb Precision Spectrophotometer. The percent oxygen saturation was determined on a Radiometer type OSM 1 oxygen saturation meter.

Statistical Analyses

A one-way analysis of variance with repeated measures was used to analyze the data with subjects and intensity as the independent variables and 2,3-DPG, lactate, pH, Po_2, and P_{50} as the dependent variables. A Pearson Product Moment Correlation was used to determine if a relationship existed between 2,3-DPG and lactate for each subject over his four exercise intensities and between pH and P_{50}.

Means for all resting values were based on data obtained on two separate days while all exercise values were based on one post-exercise sample.

Chapter IV

Results

The primary purpose of the study was to determine if a relationship exists between 2,3-diphosphoglycerate and lactic acid at various exercise intensities above and below the anaerobic threshold. Table I describes the physical characteristics of the six male subjects taking part in the study.

TABLE I

Physical Characteristics of Subjects

Subject	Age (Yrs)	Weight (kg)	VO_2MAX (ml/kg min)	Anaerobic Threshold (KPM)
DN	35	91.3	35.2	1050
LM	21	85.4	53.5	1350
DM	23	75.9	52.1	1350
JF	23	78.2	57.3	1350
JW	34	77.7	52.7	1350
BB	25	65.9	66.5	1350

Mean values obtained at each workload for 2,3-diphosphoglycerate, Hemoglobin, P_{50} and lactate are expressed in Table II and Figure 4. A one way analysis of variance with repeated measures was employed to determine if there was any statistically significant change in the levels of 2,3-diphosphoglycerate with exercise. No significant difference was found in the levels of 2,3-diphosphoglycerate when expressed in terms of μmole/ml (Table III) or μmole/gm (Table IV).

TABLE II

Mean Values (±S.D.) for 2,3-DPG, Lactate, Hemoglobin and P_{50} at Each Workload Above and Below the Anaerobic Threshold (A.T.)

	Rest	450 KPM Below AT	150 KPM Below AT	150 KPM Above AT	450 KPM Above AT
2,3-DPG (μmole/ml)	5.04	4.86	4.90	4.84	4.70
	±.46	±.34	±.46	±.48	±.41
2,3-DPG (μmole/gm)	12.28	11.84	12.48	11.38	11.91
	±.91	±.98	±1.24	±.92	±1.38
Hemoglobin (gm%)	17.84	18.85	18.20	20.01	19.3
	±.82	±1.01	±1.20	±.99	±1.1
Lactate (mg%)	9.94	26.48	35.91	55.66	82.33
	±3.92	±13.19	±10.31	±18.01	±11.09
P_{50} (mmHg)	25.9	27.0	27.2	27.6	27.5
	±1.2	±1.0	±1.0	±1.0	±1.0

Figure 4

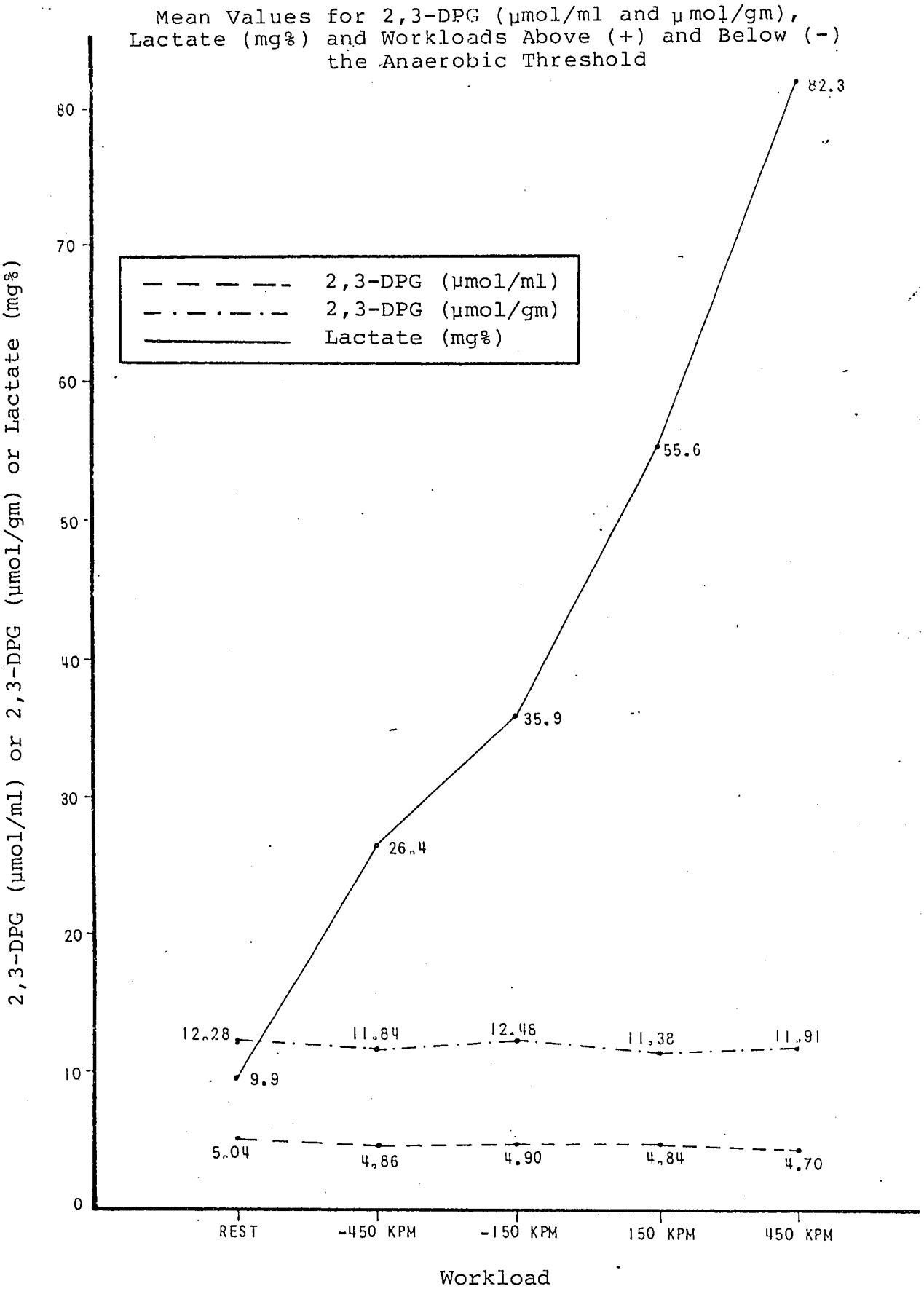

Mean Values for 2,3-DPG (µmol/ml and µmol/gm), Lactate (mg%) and Workloads Above (+) and Below (-) the Anaerobic Threshold
2,3-DPG (µmol/ml)
2,3-DPG (µmol/gm)
Lactate (mg%)
2,3-DPG (µmol/ml) or 2,3-DPG (µmol/gm) or Lactate (mg%)
0
10
20
30
40
50
60
70
80
82.3
55.6
35.9
26.4
12.28
11.84
12.48
11.38
11.91
9.9
5.04
4.86
4.90
4.84
4.70
REST
-450 KPM
-150 KPM
150 KPM
450 KPM
Workload

TABLE III

ANOVA Table for 2,3-DPG (μmole/ml) as a Function of Increasing Exercise Intensity

Source	SS	dF	MS	F
Among Subjects	5.2805	5	1.0561	
Within Subjects	.4964	5	0.0992	1.436* (ns)
Error	1.7284	25	0.0691	
TOTAL	7.5053	35		

*critical value of F = 2.60

TABLE IV

ANOVA Table for 2,3-DPG (μmole/gm) as a Function of Increasing Exercise Intensity

Source	SS	dF	MS	F
Among Subjects	28.8841	5	5.7768	
Within Subjects	4.8699	5	0.9739	1.698* (ns)
Error	14.3334	25	0.5733	
TOTAL	48.0874	35		

*critical value of F = 2.60

To confirm a significant increase in the levels of lactate as the workloads increased above the anaerobic threshold, a repeated measures one way analysis of variance was performed, as illustrated in Table V, and proved to be statistically significant at the .05 level.

In order to determine if a relationship existed between the rise in lactate and the values obtained for 2,3-DPG (μmole/gm), a Pearson Product Moment Correlation was determined for each subject. Table VI reflects the correlations obtained. Only one subject demonstrated a statistically significant correlation of -.965 indicating an inverse relationship between lactate and 2,3-diphosphoglycerate.

TABLE V

ANOVA Table of Lactate (mg%) as a Function of Increasing Exercise Intensity

Source	SS	dF	MS	F
Among Subjects	2513.511	5	502.702	
Within Subjects	23880.338	5	4776.0676	58.66*
Error	2035.119	25	81.407	
TOTAL	28428.968	35		

*critical value of F = 2.60

TABLE VI

Pearson Product Moment Correlation Between 2,3-DPG (μmole/gm) and Lactate (mg%)

Subject	Correlation
JF	-.706
JW	-.379
BB	-.324
LM	-.437
DN	-.965*
DM	-.025

*Critical value of the correlation coefficient = ± .754 @ .05 level

Table VII and Figure 5 represent the results of the blood gas analysis at each workload. Percent hemoglobin saturation, pH, Po_2 and Pco_2 are included.

TABLE VII

Blood Gas Analysis

(Mean Values ± S.D.)

	REST	450 Below AT	150 Below AT	150 Above AT	450 Above AT
Po_2 (mmHg)	36.55	56.06	46.60	40.20	42.01
	±3.12	±14.70	±11.79	±8.33	±7.64
Pco_2 (mmHg)	40.80	31.70	33.33	34.48	35.71
	±5.02	±5.02	±2.57	±7.20	±5.11
% SAT	71.12	84.00	78.16	70.33	73.16
	±4.48	±9.52	±8.35	±11.85	±11.09
pH	7.41	7.40	7.40	7.34	7.29
	±.03	±.02	±.03	±.04	±.03

An analysis of data obtained from Hill's equation indicated a significant increase in P_{50} with exercise over resting values. Table VIII displays the repeated measures analysis of variance.

Figure 5

Blood Gas Analysis of Po_2, Pco_2 and % Hb Saturation at Workloads Above (+) and Below (-) the Anaerobic Threshold

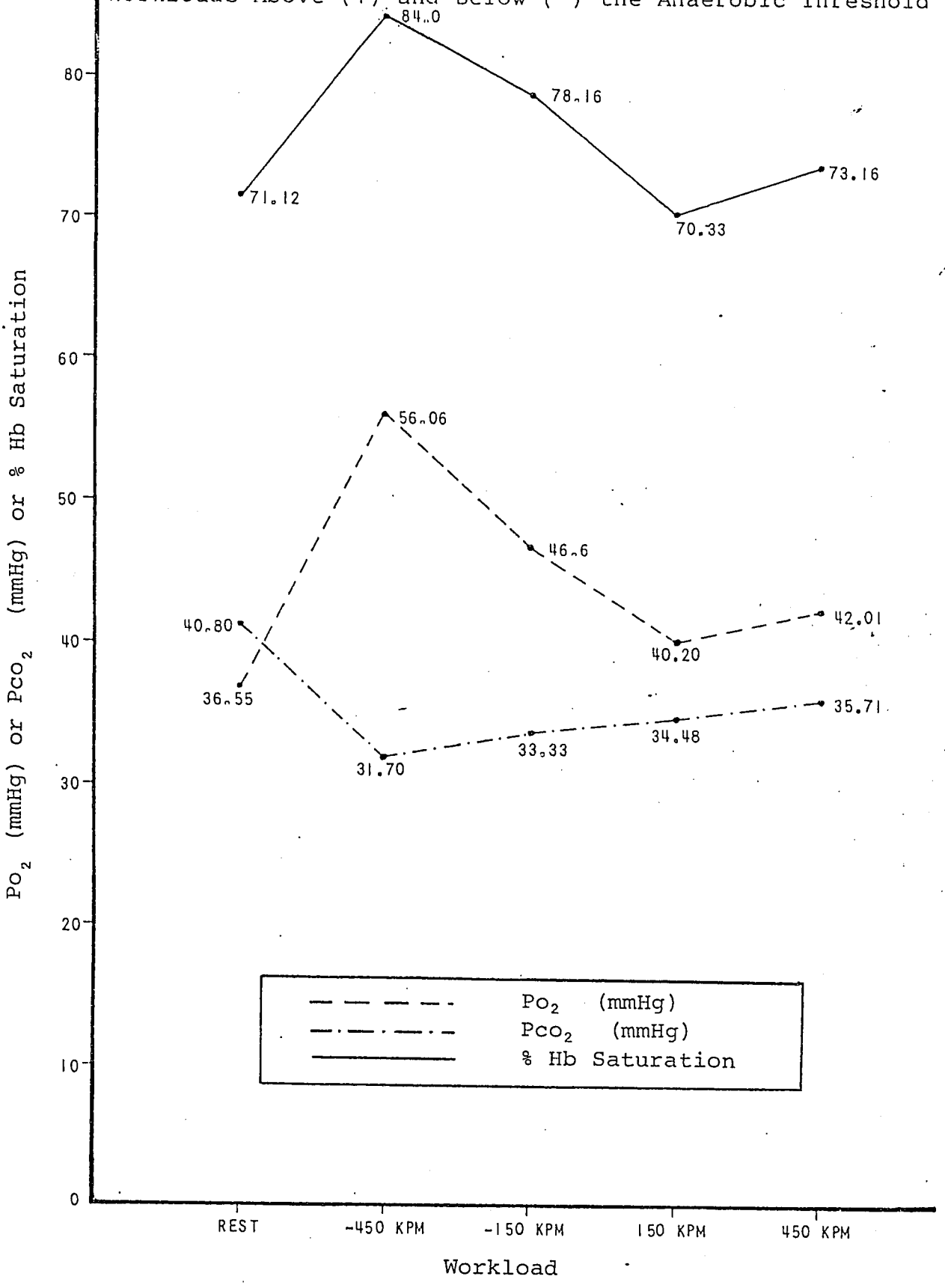


TABLE VIII

ANOVA Table for P_{50} as a Function of Increasing Exercise Intensity

Source	SS	dF	MS	F
Among Subjects	33.731	5	6.7462	
Within Subjects	20.004	5	4.0094	10.895*
Error	9.180	25	0.3672	
TOTAL	62.915	35		

*critical value of F = 2.60

Table IX represents the data obtained from an analysis of variance with repeated measures to determine if there was any significant change in the pH as a result of the graded exercise. As confirmed by the significant F value of 10.21 the $[H^+]$ concentration did increase as the workload increased.

TABLE IX

Changes in $[H^+]$ Concentration as a Function of Increased Workloads

Source	SS	dF	MS	F
Among Subjects	0.00692	5	0.001392	
Within Subjects	0.07507	5	0.015017	10.21*
Error	0.03674	25	0.001469	
TOTAL	0.11873	35		

*critical value of F = 2.60

Table X and Figure 6 represent the relationship between pH and P_{50}. A Pearson Product Moment Correlation was determined for each subject and the results displayed in Table X.

TABLE X

Pearson Product Moment Correlation Between pH and P_{50}

J.W.	-0.736
B.B.	-0.657
D.N.	-0.471
D.M.	-0.389
J.F.	-0.669
L.M.	-0.129

Critical value of the correlation coefficient = ± .754 @ .05 level

Table XI represents the changes in P_{50} as a function of pH. A one-way analysis of co-variance with one concomitant variable resulted in a non-significant F value of 2.48.

TABLE XI

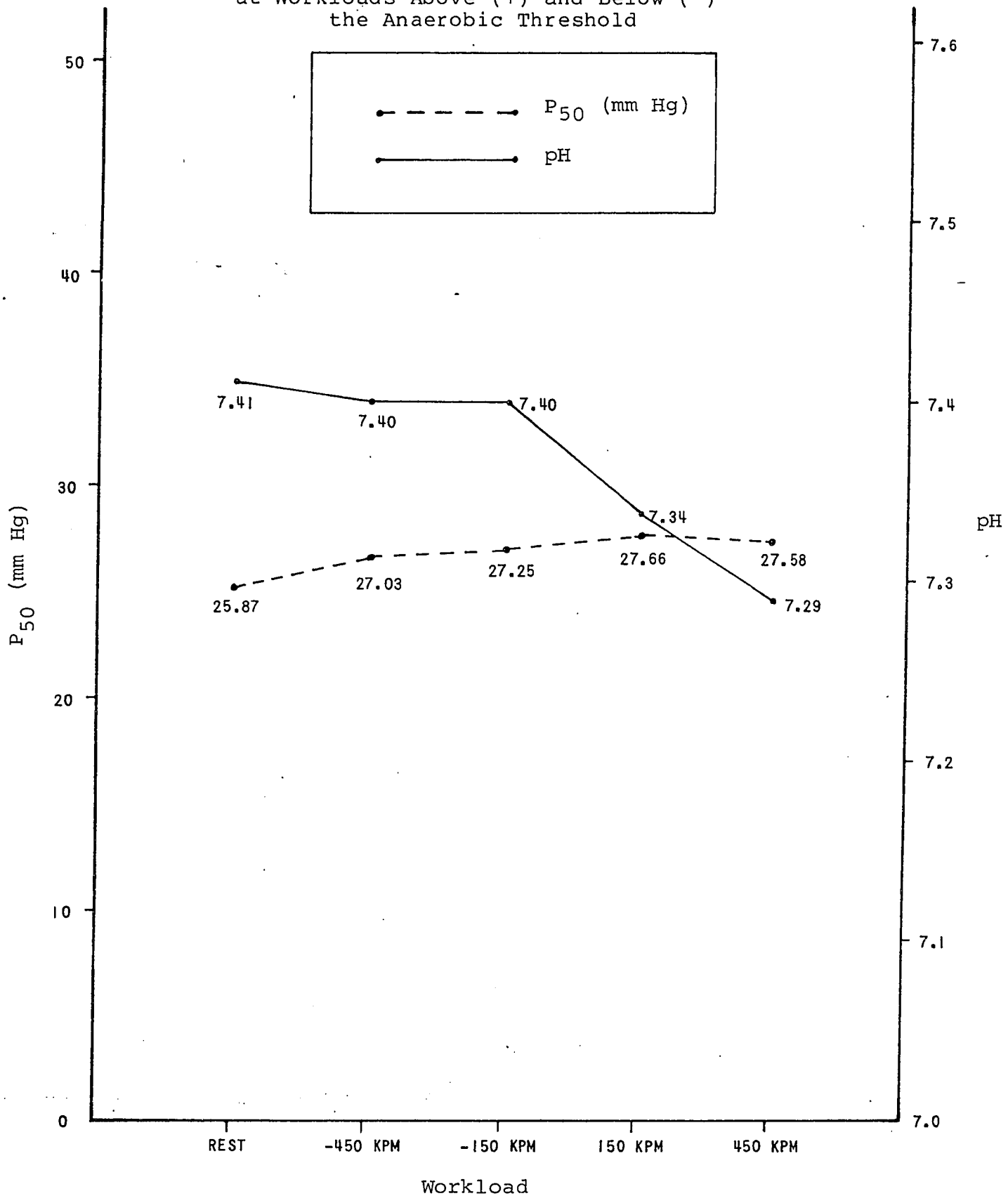
ANOVA Table for Adjusted Statistics for P_{50} as a Function of pH

Source	SS	dF	MS	F
Among Groups	14.065	4	3.516	
Within Groups	39.632	28	1.415	2.48*
TOTAL	53.697	32		

*critical value of F = 2.71

Figure 6

Relationship Between pH and P_{50} at Workloads Above (+) and Below (-) the Anaerobic Threshold



Chapter V

Discussion

In recent years the investigations into the effects of exercise on hemoglobin affinity for oxygen have produced controversial results. Eaton et al (1969) reported an increase in 2,3-DPG (μmol/ml) after exercise lasting one hour in duration and noted the increase was positively correlated with an increase in blood lactate levels. Dempsey et al (1971) described an opposite trend in which the level of 2,3-DPG (μmol/ml) declined below resting levels during exercise. Numerous other studies have fallen along this continuum adding to the apparent conflict in the literature.

The results of this study would indicate that there is neither an increase nor a decrease in the level of 2,3-DPG μmol/ml RBC or 2,3-DPG μmol/g Hb as a result of strenuous exercise. Although there was no significant change in the levels of 2,3-DPG, significant increases were noted in the P_{50} following exercise. These data, suggesting little or no change in 2,3-DPG during graded exercise above and below the anaerobic threshold, are in agreement with Bonner (1975) who found little or no change during a maximal exercise test of approximately 9 minutes duration. This type of exercise resulted in a nonsignificant 1.5% increase in the 2,3-DPG level. Wranne et al (1974) found similar results when he

noted no change in maximal work performance in human subjects whose 2,3-DPG levels were depleted by 35.7% of their normal levels. Similarly, Woodson et al (1972) reported maximal treadmill performance to be unaffected in rats depleted of 25% of their normal 2,3-DPG content.

Recent studies have shown a rightward shift in the P upon ascent to altitude which occurs within hours of ascent (Lenfant, 1968, 1970). Furthermore, such a change has uniformly been associated with increased erythrocytic 2,3-DPG levels. In the present study, a similar increase in the estimated P_{50} has been demonstrated to occur within minutes, but no change in erythrocytic 2,3-DPG was demonstrated. This phenomena has also been demonstrated by Shappell et al (1970) who showed a decrease in hemoglobin affinity for oxygen occured across the heart during myocardial ischemia associated with angina pectoris. This increased P_{50} occured without change in 2,3-DPG, ATP or pH.

The present study has demonstrated a decrease in the affinity of hemoglobin for oxygen which cannot be explained on the basis of changes in intraerythrocyte 2,3-DPG concentration. In the inital reports relating 2,3-DPG to the oxygen transport function of hemoglobin, both Chanutin and Curnish (1967) and the Benesches (1967) observed that other red cell organic phosphates, particularly ATP and ADP, had an effect on P_{50}

nearly as great as 2,3-DPG, when compared on an equimolar basis. Although these organic phosphates were not measured in this study, it is unlikely that increments in erythrocytic ATP concentrations sufficient to cause the observed P_{50} changes would have occured in the subjects, considering the observations of Shappell et al (1971) that ATP did not rise significantly in three subjects during exercise.

Based on data from the literature, it appears that several factors caused by the intense exercise: decreased pH, increased hemoglobin concentration and increased carbon dioxide production, would result in little or no change in the level of 2,3-DPG. The data reported here would substantiate the work of Thompson et al (1974) which indicated that the rightward shift of the oxygen dissociation curve during heavy exercise is primarily resultant from the Bohr effect (decreased pH) and essentially unaffected by 2,3-DPG concentrations. In the present investigation, the decrease in pH between rest and the final exercise load averaged 7.43 to 7.29. This decrease in pH alone would account for about 70% of the reported shift in the estimated P_{50} (Lloyd, 1976). The decreased pH during intense exercise caused by increased lactic acid concentrations could prevent a change in the level of 2,3-DPG in a dual manner. It may act directly upon the hemoglobin molecule blocking the binding site of 2,3-DPG resulting in by-product inhibition or it may act generally within the erythrocyte causing an inhibition of glycolysis resulting in a reduction in all

glycolytic intermediates including 2,3-DPG. Bonner (1975) notes that the hyperventilation of exercise is insufficient to counteract the inhibition of 2,3-DPG synthesis resulting from the increased lactic acidosis.

The hemoglobin concentrations reported in this study (mean = 17.8 gm/100 ml) are slightly higher than normal (13-16 gm/100 ml) (Anthony & Kolthoff, 1971). This could have resulted from a shift in plasma volume due to extremely high temperature and humidity in the laboratory as a consequence of a failure in the building's cooling system. Although it is reported in the literature that increased hemoglobin concentration inhibits 2,3-DPG synthesis, in this study an examination of the hematocrit values revealed that they increased to the same extent as the hemoglobin concentration and, therefore, the intracellular hemoglobin concentration remained constant. It is therefore unlikely that hemoglobin concentration was a significant factor in this case.

The increase in CO_2 production during strenuous exercise may have also contributed to the by-product inhibition of 2,3-DPG since both molecules are bound at the same site on the hemoglobin complex. Tomita and Riggs (1971) observed that CO_2 reacts with the uncharged $R\text{-}NH_2$ group. At a constant pH, an increased Pco_2 shifts the equilibrium $R\text{-}NH_3^+ \rightleftharpoons R\text{-}NH_2 + H^+$ to the right resulting in less $R\text{-}NH_3^+$ being available to combine with 2,3-DPG. Therefore, there is an increase in unbound

2,3-DPG producing by-product inhibitions.

Bunn and Jandl (1970) described a series of interacting factors that contribute to the control of resting levels of red cell 2,3-DPG (Figure 7). As can be seen from Figure 7, an increase in deoxyhemoglobin would result in less free 2,3-DPG because deoxyhemoglobin would bind free 2,3-DPG. As a result, by-product inhibition would be removed and the 2,3-DPG mutase reaction with 1,3-DPG would result in an increase in 2,3-DPG.

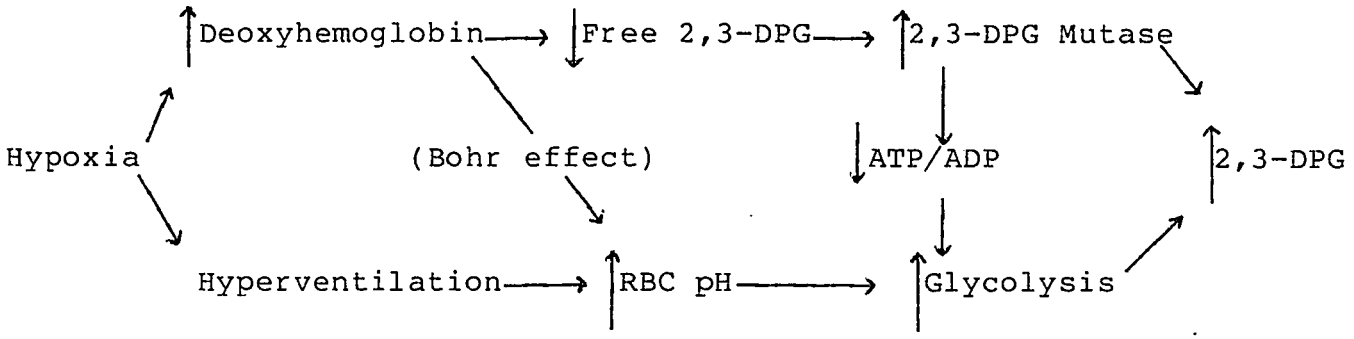


Figure 7

Interacting Factors Regulating 2,3-DPG Level During Hypoxia

(Bunn & Jandl)

Bunn and Jandl also point out that hyperventilation associated with hypoxia (due to high altitude for example) could result in small increases in pH. As a result, glycolysis would be stimulated by activating phosphofructokinase (PFK) and an increase in 2,3-DPG results. Bonner (1975), however, found that the hyperventilation of exercise was inadequate to

counteract the inhibitory effect of a decrease in pH which stemmed from an increase in lactic acid production. Therefore, rather than stimulating PFK, this rate limiting enzyme of the glycolytic pathway would be inhibited resulting in no change in the level of 2,3-DPG. It is very possible then that a combination of factors resulted in little or no change in the level of 2,3-DPG in this study.

These data dealing with short-term high intensity exercise are compatible with the consistent reports showing long-term exercise to have little influence on the erythrocyte 2,3-DPG levels (Dempsey 1971, Thompson 1974). Studies of long distance runners have shown either no change or a slight decline from resting levels. Under these long-term, steady state conditions, Thompson et al (1974) suggested that an adequate oxygen supply to the tissue is maintained by the shift in the oxygen dissociation curve caused by the additive influences of pH and temperature. Taunton (1974) suggested that long-term exercise is predominantly aerobic, therefore, the amount of deoxygenated hemoglobin is relatively low and, via by-product inhibition, further 2,3-DPG synthesis is inhibited.

Eaton et al (1969) was the first to illustrate a strong positive correlation between the change in blood lactate and in red cell 2,3-DPG after 50 minutes of exercise. In his study the subjects who showed the greatest exercise induced stress

(i.e., the largest increase in lactate) also demonstrated the largest increase in red cell 2,3-DPG. It is worth noting that the mean increases in lactate in Eaton's (1969) study were 15mg%. In the present study, the mean lactate was much higher since the task was relatively short-term, high intensity exercise compared to Eaton's work. Contrary to Eaton's (1969) work, this study showed no evidence of any positive correlation between 2,3-DPG and lactate. In fact, there was more of a trend towards a negative correlation with one subject exhibiting a correlation of -.965. Shappell (1970) in his study of acute changes in hemoglobin affinity for oxygen during angina pectoris demonstrated a rightward shift in the oxyhemoglobin dissociation curve without any change in 2,3-DPG. He also noted that lactate did not seem to be the agent producing this change because one patient who was free of pain exhibited lactate production but did not have a significant P_{50} change. In another patient, marked lactate production occured at rest but without a significant elevation in the cardiac sinus P_{50}.

The literature dealing with exercise and 2,3-DPG has generally dealt with young, highly trained subjects. Although five of the six subjects in this study had oxygen consumptions over 50 ml/kg/min, one subject, DN, had only a VO_2 of 35.2 ml/kg/min. It is interesting to note that it was this subject who had the highest negative correlation between lactate and 2,3-DPG (-.965). The shift to the right of the oxygen-

hemoglobin dissociation curve was also far less dramatic in this subject than in the other more highly trained subjects. These observations of one subject are not meant in any way to indicate a trend but simply pointed out as an interesting observation and worthy of further investigation.

Throughout the literature, there would seem to be one question which has remained unanswered, i.e., What causes the rightward shift in the oxygen dissociation curve during exercise? This paper has demonstrated a decrease in the affinity of hemoglobin for oxygen in the blood of six subjects which cannot be explained by an increased 2,3-DPG level. Other researchers (Rand 1975, Shappell 1971, Oski 1971) have suggested that some unknown substance which rapidly decays in vitro shifts the oxygen dissociation curve. It appears that pH alone is not a statistically significant factor in the rightward shift of the estimated P_{50} . Although statistically significant at the .10 level, the changes in P_{50} as a function of pH could not be conclusively attributed to pH at the .05 level. It also appears that temperature, Pco_2 and the mean corpuscular hemoglobin concentration cannot totally explain some of the large rightward shifts reported in the literature.

Generally, these data support the contention that graded exercise through the anaerobic threshold has little influence on the resting levels of erythrocyte 2,3-DPG. Other factors, such as decreased pH, increased Pco_2 and increased

temperature, are presumably the dominant factors producing an oxygen gradient between the capillary and the tissue mitochondria during physical exertion. It appears unlikely that the metabolite 2,3-DPG is in any way affected by lactate levels or provides any tangible physiologic benefit in the adaptation of the oxygen transport system to exercise stress. These observations of one subject are not meant in any way to indicate a trend but simply pointed out as an interesting observation and worthy of further investigation.

Throughout the literature, there would seem to be one question which has remained unanswered, i.e., What causes the rightward shift in the oxygen dissociation curve during exercise? This paper has demonstrated a decrease in the affinity of hemoglobin for oxygen in the blood of six subjects which cannot be explained by an increased 2,3-DPG level. Other researchers (Rand 1975, Shappell 1971, Oski 1971) have suggested that some unknown substance which rapidly decays in vitro shifts the oxygen dissociation curve. It appears that pH alone is not a statistically significant factor in the rightward shift of the estimated P_{50}. Although statistically significant at the .10 level, the changes in P_{50} as a function of pH could not be conclusively attributed to pH at the .05 level. It also appears that temperature, Pco_2 and the mean corpuscular hemoglobin concentration cannot totally explain some of the large rightward shifts reported in the literature.

Generally, these data support the contention that graded exercise through the anaerobic threshold has little influence on the resting levels of erythrocyte 2,3-DPG. Other factors, such as decreased pH, increased Pco_2 and increased temperature, are presumably the dominant factors producing an oxygen gradient between the capillary and the tissue mitochondria during physical exertion. It appears unlikely that the metabolite 2,3-DPG is in any way affected by lactate levels or provides any tangible physiologic benefit in the adaptation of the oxygen transport system to exercise stress.

Chapter VI

Summary, Conclusions and Recommendations

Six male volunteers performed graded submaximal exercise below and above their anaerobic threshold in order to determine if any relationship existed between whole blood lactate and 2,3-diphosphoglycerate. It was found that although the oxygen affinity for hemoglobin was reduced as a result of the exercise, no change occured in the levels of 2,3-DPG. There was no substantial correlation between lactate and 2,3-DPG exhibited, although a trend towards a negative relationship was observed.

It was concluded from the study that:

1) During graded exercise through the anaerobic threshold, the concentration of 2,3-DPG in the venuous blood erythrocytes remains virtually unchanged;

2) There is no significant relationship between the rise in blood lactate levels as a result of strenuous exercise and the level of post-exercise 2,3-DPG;

3) 2,3-DPG provides little or no physiological benefit as measured by P_{50} in the transport of oxygen during short-term submaximal exercise.

In the light of the present results and the findings reported by other investigators, it may be supposed that the divergent opinions concerning the effect of exercise on the

erythrocyte concentration of 2,3-DPG are due to non-homogenity of the subjects examined with respect to their level of fitness as well as the type and intensity of work performed. In order to conclusively determine the role of 2,3-DPG during exercise induced stress, further research with strict control on work intensity and duration is necessary. Such an investigation should include an analysis of 2,3-DPG responses during long-term aerobic exercise through short-term high intensity work. An examination of the effect of exercise on 2,3-DPG of unfit subjects would also be prudent.

WHOLE BLOOD LACTATE AND 2,3-DIPHOSPHOGLYCERATE DURING SUBMAXIMAL EXERCISE THROUGH THE ANAEROBIC THRESHOLD

Art Salmon, B.P.E.

Graduate Student, School of Human Kinetics and Leisure Studies,
University of Ottawa, Ottawa, Ontario. K1N 6N5

Abstract--To what extent exercise alters the level of 2,3-DPG is, at present, controversial. The present study examined the relationship between whole blood lactate and 2,3-DPG during submaximal exercise above and below the anaerobic threshold. Six male subjects (<36 years old) had blood samples taken and examined for 2,3-DPG, lactate, Po_2, Pco_2, pH, $\%O_2$ saturation, hemoglobin and hematocrit before and after exercising. On each of two test days the total amount of work done remained constant, only the intensity and duration of the work done was varied. Statistical analysis revealed no significant change in post exercise 2,3-DPG levels and no significant correlation with blood lactate levels. The significant shift in the P_{50} could not be explained on the basis of changes in 2,3-DPG and as such it was concluded that 2,3-DPG provided little or no physiological benefit in the transport of oxygen during short-term submaximal exercise.

Bibliography

Adair, G. and J. Barcroft. The Identity of Hemoglobin in Human Beings. J. Physiology 55:332, 1921.

Altman, P.L. and D.S. Dittmer. In Respiration and Circulation. Federation of American Society for Experimental Biology, Bethesda, Maryland, 1971.

Anthony, C.A. and N.J. Kolthoff. Textbook of Anatomy and Physiology. C.V. Mosby, St. Louis, 1971.

Astrand, P.O., J. Hollbock, R. Hedman and B. Saltin. Blood Lactates after Prolonged Severe Exercise. J. App. Phys. 18:619, 1963.

Astrand, P. and K. Rodall. Textbook of Work Physiology. McGraw-Hill Co., New York, 1970.

Astrand, P.O. and B. Saltin. O_2 Uptake During the First Minutes of Heavy Muscular Exercise. J. App. Phy. 16:971, 1961.

Astrup, P. Dependence of Oxyhemoglobin Dissociation and Introerythocytic 2,3-DPG on Acid-Base Status of Blood. Advances in Experimental Medicine and Biology. Vol. 6. Plenum Press, New York, 1970.

Astrup, P. and M. Rorth. Dependency on Acid-Base Status of Oxyhemoglobin Dissociation and 2,3-DPG Level in Human Erythrocytes. Scand. J. Clin. Lab Invest. 26:47-52, 1970.

Benesch, R. and R. Benesch. Intracellular Organic Phosphates as Regulators of Oxygen Release by Hemoglobin. Nature. 221:618, 1969.

Benesch, R. and R. Benesch. The Effect of Organic Phosphates from the Human Erythrocytes on the Allosteric Properties of Hemoglobin. Biochem. Biophys. Res. Commun. 26:162, 1967.

Benesch, R. and R. Benesch. The Reaction Between Diphosphoglycerate and Hemoglobin. Federation Proceedings. Vol. 29, #3, 1970.

Benesch, R. and R. Benesch. The Oxygenation of Hemoglobin in the Presence of 2,3-DPG: Effect of Temperature, pH, Ionic Strength and Hemoglobin Concentration. Biochemistry. 8:2567, 1969.

Benesch, R., R. Benesch and Y. Enoki. The Interaction of Hemoglobin and Its Subunits with 2,3-DPG. Proc. Nat. Acid. Sci. U.S.A. 61:1102-1106, 1968.

Benesch, R. and R. Benesch. The Interaction of Hemoglobin and Its Subunits with 2,3-DPG. Nature. 221:618, 1969.

Berne, R.M., J.R. Blockman and T.H. Gardner. Hypoxemia and Coronary Blood Flow. J. Clin. Invest. 36:1011, 1957.

Bonner, H.W. and C.A. Tate. Changes in Erythrocyte 2,3-DPG in Women Following Short Term Maximal Exercise. Europ. J. Appl. Physiol. 34:227, 1975.

Brewer, G. Studies of Red Cell Glycolysis and Interactions with Carbon Monoxide, Smoking and Altitude. Experimental Medicine and Biology. Vol. 6. Plenum Press, New York, 1970.

Bunn, H.F. and R.W. Briehl. The Interaction of 2,3-DPG with Various Human Hemoglobins. J. Clin. Invest. 49:1088, 1970.

Bunn, H.F. and Jandl. Control of Hemoglobin Function within the Red Cell. New England Journal of Medicine. 282: 1414, 1970.

Chanutin, A. and R. Curnish. Effect of Organic and Inorganic Phosphates on the Oxygen Equilibrium of Human Erythrocytes. Arch. Biochem. and Biophy. 121:96, 1967.

Corning Biomedical Instruments 165-pH Blood Gas Analyzer Instruction Manual. Corning Scientific Inst. Medfield, Mass., 1972.

Dempsey, J.A. et al. Muscular Exercise, 2,3-DPG and Oxyhemoglobin Affinity. Int. Z. Anger Phy. 30:34, 1971.

Duhm, J. Effects of 2,3-DPG and Other Organic Phosphate Compounds on Oxygen Affinity and Intracellular pH of Human Erythrocytes. Pflugers Arch. 326:341-356, 1971.

Duhm, J. On the Mechanisms of the Hypoxia-Induced Increase of 2,3-DPG in Erythrocytes. Pflugers Arch. 326:254, 1971.

Eaton, J.W. and G. Brewer. Variation In 2,3-DPG and ATP Levels in Human Erythrocytes and Effects on Oxygen Transport. Advances in Experimental Medicine and Biology. Vol. 6. Plenum Press, New York, 1970.

Eaton, J.W. and G. Brewer. Response of the Human Red Cell to Muscular Activity. Proc. Soc. Exp. Biol. Med. 132:886-887, 1969.

Faulkner, J., G. Brewer and J. Eaton. Adaptation of the Red Cell to Muscular Exercise. Advances in Experimental Medicine and Biology. Plenum Press, New York, 1970.

Greenwald, J. A New Type of Phosphoric Acid Compound Isolated From Blood with Some Remarks on the Effect of Substitution on the Rotation of ∝-glyceric Acid. J. Biol. Chem. 63:339, 1925.

Greer, J. and M. Perutz. Cited in Bunn, H.F. and R.W. Briehl. The Interaction of 2,3-DPG with Various Human Hemoglobins. J. Clin. Invest. 49:1088, 1970.

Guidelines for Graded Exercise Testing and Exercise Prescription. American College of Sports Medicine. Lea and Tebiger, Philadelphia, 1975.

Guyton, A.C. Textbook of Medical Physiology, 4th Edition. W.B. Saunders Co., Toronto, 1971.

Kety, S. Determinants of Tissue Oxygen Tension. Fed. Proc. 16:666, 1957.

Landis, E.M. and J.R. Poppenheimer. Exchange of Substances Through the Capillary Walls. Handbook of Physiology. The American Physiology Society, 1963.

Lehninger, A.L. Biochemistry. Worth Publishing Inc., NY, 1970.

Lenfont, C. et al. Adaptations to Hypoxia. Red Cell Metabolism and Function. Edited by G. Brewer. Plenum Press, New York, 1970.

Lenfont, C. et al. Effect of Altitude on Oxygen Binding By Hemoglobin and on Organic Phosphate Levels. J. Clinc. Invest. 47, 1968.

Leusink, J.A. The Influence of an Intensive Period of Training on a Number of Hemotological Parameters in Male and Femal Topathletes. S. Belge de Physiologie et de Pharmacologie. Sept. 1973.

Lloyd, T.C. Respiratory Gas Exchange and Transport. Physiology. Little, Brown and Co., Boston, Mass., 1976.

Muirhead, H. Structure and Function of Hemoglobin. M. Mol. Biol. 28:117, 1967.

Oski, F.A. and M. Delivoria-Papodopoulos. The Red Cell, 2,3-Diphosphoglycerate and Tissue Oxygen Release. Journal of Paediatrics. Vol. 77, No. 6, pp. 941-956, 1970.

Oski, F.A. and A. Gottlieb. The Interrelationships Between Red Blood Cell Metabolites, Hemoglobin and the Oxygen-Equilibrium Curve. Progress in Hematology. Vol. 6. Edited by E. Brown and C. Moore. Grune and Stratton, New York, 1971.

Rand, P.W., J.M. Norton, N. Barker and M. Lowell. Influence of Athletic Training on Hemoglobin-Oxygen Affinity. Am. Jour. of Phys. 224:6:1334, 1973.

Raporport, S. and G.M. Grust. Distribution of Acid-Soluable Phosphorus in the Blood Cells of Various Vertebrates. J. Biol. Chem. 138:269, 1941.

Remes, K., M. Harkonen, P. Vuopio, and P. Peltokallio. The Decrease in Red Cell 2,3-DPG Concentration in Long Distance Running. J. Sport Med. 15, 1975.

Rose, I.A. and J.V.B. Warms. Control of Glycolysis in the Human Red Cell. J. Biol. Chem. 241:4848, 1966.

Shappell, S.D. et al. Adaption to Exercise: Role of Hemoglobin Affinity for Oxygen and 2,3-DPG. J. Appl. Phys. 30, No. 6, 1971.

Shappell, S.D., J.A. Murray, M.G. Nasser, R.E. Wills, J.D. Torrance and J.M. Lenfont. Acute Change in Hemoglobin Affinity for Oxygen During Angina Pectoria. New England Journal of Medicine. 282:1219, 1970.

Sigma Technical Bulletin #35-UV. Sigma Chemical Co., St. Louis, Miss., 1974.

Taunton, J. and E. Banister. The Increase of 2,3-DPG with Maximum Exercise. Proc. C.A.S.S., 1972.

Taunton, J.E., C.A. Taunton and E. Banister. Alterations in 2,3-DPG and P_{50} with Maximal and Submaximal Exercise. Med. Sc. Sport. 6:4, pp. 238-241, 1974.

Test-Combination Lactate (uv-method), B.M.C. Biochemicals Catalogue No. 124.

Thompson, J.M. and J. Dempsey. Oxygen Transport and Oxyhemoglobin Dissociation During Prolonged Muscle Work. J. Appl. Physiol. 37:5, 1974.

Tomita, S. and A. Riggs. Studies of the Interaction of 2,3-DPG from Mouse, Man and Elephant. J. Biol. Chem. 246, 547-554, 1971.

Wasserman, K. and B. Whipp. Exercise Physiology in Health and Disease. American Review of Respiratory Disease. Volume 112, 1975.

Woodson, R.D., B. Wranne and J. Better. Effect of Erythrocyte 2,3-DPG and Hemoglobin Concentration on Oxygen Delivery and Work Performance. Clinic Res. 20, 628, 1972.

Wranne, B., L. Nordgren and R.D. Woodson. Increased Blood Oxygen Affinity and Physical Work Capacity in Man. Scand. J. Clinic. Lab. Inv. 33, 347-352, 1974.

APPENDIX A

RAW DATA

BB

	REST		900 KPM	1200 KPM	1500 KPM	1800 KPM
2,3-DPG (μmole/ml) (R.B.C.)	2.07	2.09	2.31	2.32	2.37	2.34
2,3-DPG (μmole/ml) (Hemoglobin)	4.71	4.86	5.13	5.15	5.15	4.97
2,3-DPG (μmole/gm)	12.39	11.88	13.27	13.34	12.53	13.07
Hematocrit	44	43	45	45	46	47
Hemoglobin	16.7	17.6	17.4	17.4	18.9	17.9
Lactate	9.8	4.5	12.8	22.6	51.0	90.8
pH	7.44	7.41	7.43	7.41	7.33	7.25
Po_2	38.1	40.6	34.7	36.2	26.3	50.9
%Hb Saturation	72	74	65	68	46	83
Pco_2	46.1	43.0	35.1	34.9	44.7	32.6
P_{50}	26.7	27.4	27.5	27.3	28.0	27.7

JF

	REST		900 KPM	1200 KPM	1500 KPM	1800 KPM
2,3-DPG (µmole/ml)	2.46	2.42	2.39	2.36	2.27	2.30
2,3-DPG (µmole/ml) (R.B.C.)	5.85	5.40	5.03	4.91	4.54	4.60
2,3-DPG (µmole/gm) (Hemoglobin)	13.29	13.51	12.32	12.36	10.46	11.86
Hematocrit	42.0	47.0	47.5	48.0	50.0	50.0
Hemoglobin	18.5	17.9	19.4	19.1	21.7	19.4
Lactate	6.0	6.0	23.6	30.4	52.4	85.4
pH	7.41	7.40	7.38	7.37	7.36	7.32
Po_2	39.4	38.3	46.4	37.8	41.9	49.7
%Hb Saturation	79	78	81	72	75	84
Pco_2	32.0	44.0	27.4	34.6	26.5	28.3
P_{50}	24.0	24.0	26.7	26.7	27.4	26.9

DM

	REST		900 KPM	1200 KPM	1500 KPM	1800 KPM
2,3-DPG (μmole/ml)	1.86	2.11	2.07	2.08	2.11	2.21
2,3-DPG (μmole/ml) (R.B.C.)	4.42	4.39	4.60	4.37	4.69	4.33
2,3-DPG (μmole/gm) (Hemoglobin)	11.13	12.78	10.78	12.16	10.98	12.20
Hematocrit	42.0	48.0	45.0	47.5	46.5	51.0
Hemoglobin	16.7	16.5	17.7	17.1	19.2	18.1
Lactate	13.0	17.4	29.0	33.6	61.0	70.0
pH	7.40	7.42	7.42	7.40	7.31	7.27
Po_2	32.0	32.1	56.5	39.4	36.2	40.0
%Hb Saturation	66	66	88	75	70	76
Pco_2	45.8	41.1	35.7	34.7	39.3	43.8
P_{50}	25.0	25.1	26.0	26.0	25.9	25.9

LM

	REST		900 KPM	1200 KPM	1500 KPM	1800 KPM
2,3-DPG (µmole/ml)	2.27	2.32	2.36	2.45	2.44	2.54
2,3-DPG (µmole/ml) (R.B.C.)	4.93	5.16	4.72	5.16	4.97	4.79
2,3-DPG (µmole/gm) (Hemoglobin)	12.34	13.41	12.16	14.58	12.20	13.44
Hematocrit	46.0	45.0	50.0	47.5	49.0	53.0
Hemoglobin	18.4	17.3	19.4	16.8	20.0	18.9
Lactate	4.8	9.0	9.1	33.4	28.0	66.8
pH	7.42	7.31	7.42	7.47	7.36	7.35
Po_2	34.6	33.4	50.6	40.0	37.0	28.6
%Hb Saturation	69.0	67.5	85.0	75.0	69.0	51.0
Pco_2	35.9	45.4	35.8	27.8	40.3	39.0
P_{50}	25.5	25.4	26.0	26.5	27.2	28.0

DN

	REST		600 KPM	900 KPM	1200 KPM	1500 KPM
2,3-DPG (μmole/ml)	2.09	2.02	1.96	1.96	1.89	1.91
2,3-DPG (μmole/ml) (R.B.C.)	4.86	4.69	4.36	4.26	4.10	4.15
2,3-DPG (μmole/gm) (Hemoglobin)	11.17	10.68	10.37	10.53	9.74	9.14
Hematocrit	43	43	45	46	46	46
Hemoglobin	18.7	18.9	18.9	18.6	19.4	20.9
Lactate	10.0	14.8	37.4	39.4	52.6	82.4
pH	7.46	7.43	7.40	7.35	7.40	7.32
Po_2	36.7	33.6	79.7	61.0	48.1	37.7
%Hb Saturation	68	66	95	90	81	69
Pco_2	32.0	42.7	22.5	35.1	26.7	38.1
P_{50}	27.5	26.0	27.0	27.8	28.0	27.8

JW

	REST		900 KPM	1200 KPM	1500 KPM	1800 KPM
2,3-DPG (µmole/ml)	2.36	2.31	2.47	2.41	2.59	2.43
2,3-DPG (µmole/ml) (R.B.C.)	5.61	5.63	5.37	5.60	5.63	5.40
2,3-DPG (µmole/gm) (Hemoglobin)	12.96	11.88	12.16	11.93	12.39	11.79
Hematocrit	42	41	46	43	46	45
Hemoglobin	18.2	18.7	20.3	20.2	20.9	20.6
Lactate	12.0	12.0	47.0	56.1	89.0	98.6
pH	7.44	7.41	7.38	7.37	7.26	7.25
Po_2	40.7	39.2	68.3	65.2	51.7	45.2
%Hb Saturation	74	74	90	89	81	76
Pco_2	39.3	42.6	33.7	32.9	29.4	32.5
P_{50}	27.4	26.5	29.0	29.2	29.5	29.2

APPENDIX B

In order to determine the length of time required for lactate levels to return to resting levels after the first sub-anaerobic threshold bicycle ride, two non-participating subjects were given anaerobic threshold tests as described on day two in Chapter 3. On the following day they returned to the lab and a resting blood sample was analyzed for lactate. The subject then pedalled a bicycle ergometer for 5 minutes at a workload just below his anaerobic threshold. Blood samples were taken immediately after the ride, 10 minutes post exercise, 20 minutes post exercise and 30 minutes post exercise. It was noted that 30 minutes post exercise resulted in lactate levels having returned to normal.

Lactate Levels (mg%)

	Rest	Post Exercise	10 min. Post Ex.	20 min. Post Ex.	30 min. Post Ex.
Subject A	12.0	37.5	23	16	11.0
Subject B	9.0	31.0	19	13	10.5