JCI The Journal of Clinical Investigation

Effect of Increased Blood Oxygen Affinity on Work Performance of Rats

Robert D. Woodson, … , Bengt Wranne, James C. Detter

J Clin Invest. 1973;52(11):2717-2724. https://doi.org/10.1172/JCI107466.

Research Article

Influence of altered blood oxygen affinity on maximal performance ability was evaluated in trained rats exercising to exhaustion in a graded treadmill test. Modification of blood oxygen affinity was achieved both by 2,3-diphosphoglycerate depletion, accomplished by exposure of animals to CO_2 and by exchange transfusion with blood exposed to bisulfite or stored in acid citrate dextrose, and by carbamylation of hemoglobin, produced by exchange transfusion of blood incubated with potassium cyanate. A decrease in oxygen tension at half-saturation of hemoglobin (P_{50}) from 36 to 23 mm Hg produced a decrease in resting central venous oxygen pressure of about 12 mm Hg. During exercise it caused an average decrease in work performance of about 10%, which was equivalent to that performance decrement caused by a decrease in hemoglobin concentration of approximately 10%. When superimposed on anemia, this change in blood oxygen affinity again caused a similar decrease in performance over and above that due to anemia alone. A marked rightward shift of the in vivo oxygen dissociation curve during severe exercise may have compensated for the reduced in vitro P_{50}.

Effect of Increased Blood Oxygen Affinity on Work Performance of Rats

ROBERT D. WOODSON, BENGT WRANNE, and JAMES C. DETTER

From the Departments of Medicine and Laboratory Medicine, University of Washington School of Medicine, Seattle, Washington 98195

ABSTRACT Influence of altered blood oxygen affinity on maximal performance ability was evaluated in trained rats exercising to exhaustion in a graded treadmill test. Modification of blood oxygen affinity was achieved both by 2,3-diphosphoglycerate depletion, accomplished by exposure of animals to CO_2 and by exchange transfusion with blood exposed to bisulfite or stored in acid citrate dextrose, and by carbamylation of hemoglobin, produced by exchange transfusion of blood incubated with potassium cyanate. A decrease in oxygen tension at half-saturation of hemoglobin (P_{50}) from 36 to 23 mm Hg produced a decrease in resting central venous oxygen pressure of about 12 mm Hg. During exercise it caused an average decrease in work performance of about 10%, which was equivalent to that performance decrement caused by a decrease in hemoglobin concentration of approximately 10%. When superimposed on anemia, this change in blood oxygen affinity again caused a similar decrease in performance over and above that due to anemia alone. A marked rightward shift of the in vivo oxygen dissociation curve during severe exercise may have compensated for the reduced in vitro P_{50}.

INTRODUCTION

A variety of physiological and pathophysiological states results in a change in affinity of oxygen for hemoglobin. In some of these states, such as anemia, hypoxemia, and cardiac failure, there is a decrease in blood oxygen affinity (1–3). Under other circumstances, such as occur after transfusion of stored blood, oxygen affinity is increased (4–7). However, the importance of such changes on oxygen availability to cells is unknown. We have therefore sought to define the effect of such changes in an animal model.

METHODS

Male rats of the Sprague-Dawley strain were obtained at a weight of 150–175 g. They were trained to run on a treadmill, usually for 5 days, until they could readily run for 20 min at a velocity of 26.8 m/min and inclination of 12.5°. A catheter was then inserted via the jugular vein into the superior vena cava and allowed to remain in place throughout the experiment. 5–7 days later, after recovery of prior exercise performance, they were subjected to a test of maximal performance (W_{max} test).[1] In this test, animals were initially allowed to run at the above velocity and inclination. The angle of inclination was then increased by 2.5° every 3 min through the 18th min and the treadmill velocity by 2.7 m/min every 3 min thereafter. The endpoint was taken as that time when a rat spent 20% of a 30-s period resting on the electrically charged grid; results were expressed as running time to exhaustion. This test, described in detail elsewhere (8), was performed before and after alteration of blood oxygen affinity. In animals exposed to CO_2 (see below), it was performed 1 h before and 1 h after CO_2 exposure; in animals exchange transfused it was performed 1 day before, 0.5–2 h after, and 18–24 h after exchange. Results were compared with those in normal animals with and without catheters and in animals after acute induction of normovolemic anemia, reported elsewhere (8). Changes in performance were assessed statistically by the paired *t* test.

2,3-diphosphoglycerate (DPG) depletion was achieved in vivo in one group of trained but otherwise unmanipulated animals by exposure of animals to an atmosphere containing 15% CO_2, 21% O_2, and 64% N_2 for 22 h. Weight loss during CO_2 exposure averaged 17±1% of body wt. Six other groups of trained rats were exchange-transfused with blood obtained from normal animals of the same strain by cardiac puncture under ether anesthesia. They were transfused with:

a. Fresh heparinized blood (10 IU/ml).

Dr. Wranne's present address is: Department of Clinical Physiology, University of Linköping, S-58185 Linköping, Sweden.

Received for publication 3 November 1972 and in revised form 16 July 1973.

[1] *Abbreviations used in this paper:* ACD, acid citrate dextrose; BE, base excess; DPG, 2,3-diphosphoglycerate; P_{50}, oxygen tension at half-saturation of hemoglobin; W_{max} test, test of maximal performance.

b. Fresh heparinized blood to which 0.15 ml acid citrate dextrose (ACD) solution (NIH Formula A)/ml of blood was added, after which the blood was refrigerated for 0.5–1.5 h before transfusion.

c. Blood depleted of DPG by storage in ACD for 1 wk at 4°C.

d. Blood depleted of DPG by incubation with sodium metabisulfite. Washed red cells were incubated for 4 h at 37°C in 20 vol of a solution containing 130 mM NaCl, 2.7 mM KCl, 10 mM glucose, and 10 mM sodium metabisulfite (9) and buffered with 20 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] (10). Cells were then washed three times in a solution lacking bisulfite, but otherwise similar, and resuspended in their own plasma.

e. Blood with decreased DPG and decreased total hemoglobin concentration. Blood was depleted of DPG as in (*d*) and resuspended in excess plasma so as to produce various reductions of hemoglobin concentration.

f. Blood with increased hemoglobin oxygen affinity induced by carbamylation of hemoglobin (11). Packed unwashed red cells were suspended at an approximate hematocrit of 75% in glucose and potassium cyanate (J. T. Baker Chemical Co., Phillipsburg, N. J.) such that the final concentrations were 33 mM for glucose and either 16 or 32 mM for cyanate. The suspension was then incubated with slow shaking for 2 h at 37°C, after which the cells were washed three times in the washing buffer described for subgroup (*d*) and resuspended in plasma.

Exchange transfusion was performed over a period of approximately 30 min with the animal unanesthetized and unrestrained. Blood was stored at 37°C for 5–15 min before infusion and exchanged in portions of about 4 ml with care taken to infuse exactly the volume of blood withdrawn. Usually two times the predicted blood volume of the animal (6% of body weight) (12) was exchanged. Exchange efficiency, estimated from the volume of blood exchanged, averaged 89±5% (SD) and agreed closely with the value of 92±8% calculated from the change in DPG level of circulating cells. Samples for blood gas measurements were obtained anaerobically during transfusion in glass syringes, stored on ice, and analyzed with Radiometer electrodes connected to a Model 27 Radiometer pH meter (Radiometer A/S, Copenhagen, Denmark). Base excess (BE) was calculated using the Severinghaus nomogram for man (13).

We have previously shown that performance of animals in the W_{max} test is linearly related to hemoglobin concentration. Because hemoglobin concentration of animals exchanged with whole blood sometimes differed from that of animals before transfusion (maximal difference = 7%), running times in animals exchanged were corrected according to the relationships:

$$corr.\ run_{day\ 2} = obs.\ run_{day\ 2} + (obs.\ run_{day\ 1} - exp.\ run_{day\ 2})$$

where

$$exp.\ run_{day\ 2} = \left(a + b\frac{Hb_{day\ 2}}{Hb_{day\ 1}}\right) obs.\ run_{day\ 1}$$

The symbols *corr. run, obs. run,* and *exp. run* refer respectively to the corrected, observed, and expected running time in minutes, Hb refers to hemoglobin concentration in grams per 100 milliliters, the subscripts day 1 and 2 refer to measurements before and after transfusion, and *a* and *b* refer to the y-intercept and slope of the regression of W_{max} on hemoglobin concentration described elsewhere (8).

Hemoglobin concentration was determined as cyanmethemoglobin and hematocrit by the microhematocrit technique. Red cell organic phosphates were separated by column chromatography and measured spectrophotometrically as described previously (1). The position of the oxygen dissociation curve and the Bohr effect were measured by the mixing technique at 37°C (14). Oxygen affinity is expressed as P_{50}, which is the oxygen tension at half-saturation of hemoglobin with oxygen at pH 7.4 and 37°C. P_{50} was further corrected to BE = 0, which is equivalent to P_{CO_2} = 40 mm Hg at pH 7.4. Correction to zero BE was made according to the relationship for man: $\Delta \log P_{50} = \Delta BE(f)$, where $f = 0.0022$ for DPG values above 0.89 mol/mol Hb and 0.0052 for DPG values at and below 0.15 mol/mol Hb (15). Interpolation was used to obtain f values for intermediate DPG levels.

RESULTS

Levels of DPG, ATP, P_{50}, and BE of fresh heparinized rat blood, of fresh blood to which ACD was added, of blood stored for 1 wk in ACD, and of blood after incubation with sodium metabisulfite or potassium cyanate are shown in Table I. Fig. 1 shows that there is a highly significant linear relationship between DPG and P_{50} ($r = 0.88$, $P < 0.001$). P_{50} of carbamylated blood is reduced independently of the DPG level.

Levels of DPG, ATP, P_{50}, and BE of circulating cells after CO_2 exposure and after exchange transfusion with the various bloods are also given in Table I. Exposure to CO_2 for 22 h reduced mean DPG and P_{50} from control values by 41% and 6 mm Hg, respectively. After exchange transfusion with bisulfite-incubated or ACD-stored blood, reductions in DPG and P_{50} averaged 71% and 13 mm Hg, respectively. Exchange with carbamylated blood reduced P_{50} by 15 mm Hg.

The time-course of DPG restoration after exchange transfusion with bisulfite-incubated cells is given in Fig. 2. There was only a slight increase in DPG values 0.5–2 h after transfusion, which was the time of the W_{max} test. Half-restoration time for DPG averaged 8.9 h. 18–24 h after transfusion, DPG, ATP, and P_{50} were essentially normal. In the case of animals exchange-transfused with carbamylated blood, the P_{50} was unchanged at 24 h from the value obtained immediately after transfusion.

The effect of exchange transfusion on central venous oxygen pressure at rest is shown in Fig. 3. As exchange with fresh heparinized and fresh ACD blood yielded similar findings, resulting data were pooled. Findings with bisulfite-incubated and ACD-stored blood were similarly pooled. After transfusion of blood with normal oxygen affinity, central venous oxygen pressure remained unchanged at 37 mm Hg. Transfusion with low DPG blood such that P_{50} of circulating cells decreased by 13 mm Hg produced a decrease of venous

TABLE I

Characteristics of Blood Used for Exchange Transfusion and of Animals after Exchange and CO_2 Exposure

	DPG	ATP	P_{50}(7.4, BE = 0)	BE
	mol/mol Hb	*mol/mol Hb*	*mm Hg*	*meq/liter*
Fresh heparinized blood				
Blood transfused	1.70±0.18 (64)	0.21±0.05 (64)	36.1±1.9 (20)	−4±3 (22)
Animals post-transfusion	1.63±0.09 (16)	0.25±0.03 (16)	35.3±2.5 (7)	−2±4 (7)
Fresh ACD blood				
Blood transfused	1.53±0.10 (6)	0.15±0.02 (6)	—	−32±4 (4)
Animals post-transfusion	1.69±0.12 (10)	0.24±0.03 (10)	—	1±2 (10)
Bisulfite-incubated blood				
Blood transfused	0.15±0.06 (20)	0.12±0.05 (20)	22.9±0.9 (4)	−19±6 (4)
Animals post-transfusion	0.47±0.13 (26)	0.12±0.03 (26)	23.1±2.3 (15)	−8±5 (15)
Stored ACD blood				
Blood transfused	0.17±0.10 (7)	0.10±0.04 (7)	21.2±4.0 (5)	−27±12 (5)
Animals post-transfusion	0.53±0.13 (12)	0.16±0.03 (12)	—	−2±6 (13)
Cyanate-incubated blood				
Blood transfused	1.56±0.07 (4)	0.11±0.02 (4)	—	—
Animals post-transfusion	1.66±0.08 (8)	0.17±0.02 (8)	21.3±2.4 (6)	−6±3 (6)
Animals after CO_2 exposure	1.01±0.11 (9)	0.11±0.04 (9)	30.3±2.3 (9)	0±5 (9)

Values are ±1 SD. The numbers in parentheses are the number of observations.

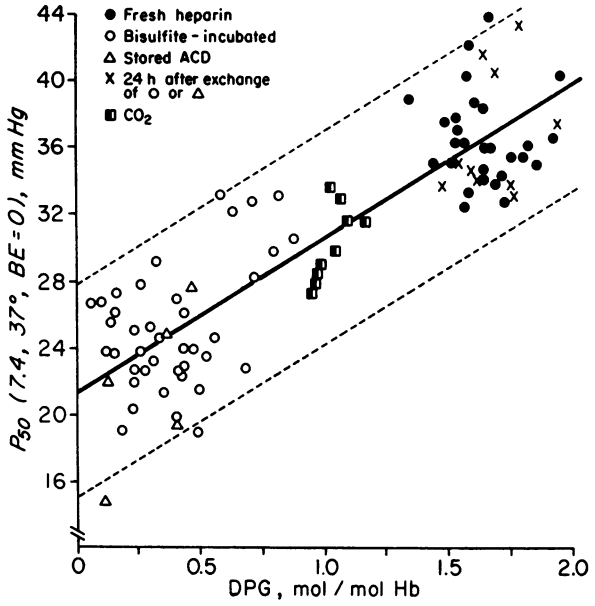

FIGURE 1 Relationship of P_{50}, measured at 37°C and corrected to pH, 7.4 and BE, 0, to DPG. Symbols in each category refer to samples taken either from blood prepared for transfusion or from rats immediately after transfusion with that blood. The crosses refers to samples taken from animals 24 h after transfusion with ACD-stored or bisulfite-incubated blood. The regression line (fitted by least squares)±2 SE of the estimate is shown. Fresh ACD and cyanate-incubated samples were excluded from the regression. The equation of the line is $P_{50} = 9.3\ DPG + 21.4$ ($r = 0.88$, $P < 0.001$).

oxygen tension averaging 12 mm Hg. Arterial oxygen pressure was normal throughout the transfusion, averaging 85±15 mm Hg (SD, $n = 36$). Arterial CO_2 pressure averaged 34±8 mm Hg, and pH averaged 7.41±0.08.

As blood with increased hemoglobin-oxygen affinity was thus shown to decrease central venous P_{O_2} and presumably tissue P_{O_2} without apparent adverse se-

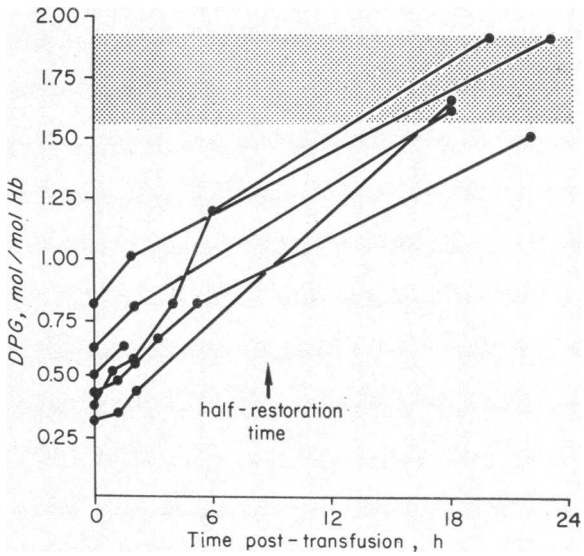

FIGURE 2 Recovery of DPG after transfusion of depleted cells. Each line indicates changes in one animal.

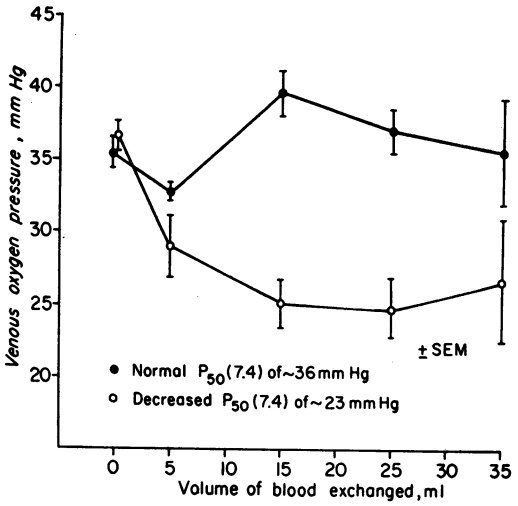

FIGURE 3 Central venous oxygen pressure during transfusion with normal blood and with blood having reduced P_{50} due to DPG depletion.

quellae, effect of increased blood oxygen affinity on work capacity was assessed in animals whose oxygen consumption was increased by exercise. Results are shown in Fig. 4 and Table II. When DPG and P_{50} were reduced by CO_2 exposure (mean decrease of P_{50} = 6 mm Hg), performance was unchanged from that preceding CO_2 exposure ($P < 0.10$). When P_{50} was decreased by exchange of blood depleted of DPG by bisulfite incubation (mean P_{50} decrease = 13 mm Hg), there was a mean decrease in performance 0.5–2 h later of 9% ($P < 0.025$). By 24 h, DPG and P_{50} were again normal and performance was normal. Upon transfusion of ACD-stored blood (mean decrease of DPG = 69%), there was no change in performance. Exchange transfusion using carbamylated blood (mean P_{50} decrease = 15 mm Hg) caused an acute decrease in performance of 9% ($P < 0.10$); 1 day later the decrease in P_{50} averaged 16 mm Hg, and average performance decreased by 18% from the basal value ($P < 0.025$). These results may be compared to findings in normovolemic anemia, in which a decrease in hemoglobin concentration of about 10% produces a similar decrease in performance (8).

In further studies, the effect of increased blood oxygen affinity combined with anemia was investigated. As shown in Fig. 5, the decrement in performance of this group after exchange is greater than that observed in anemia alone ($P < 0.005$), and again corresponds to a decrease in hemoglobin concentration of 10–15%.

TABLE II
Performance of Animals after Various Manipulations

		Running time			Day-to-day change in running time of individual rats		
		Day 1	Day 2	Day 3	Day 2–Day 1	Day 3–Day 2	Day 3–Day 1
			min			*min*	
Exchange transfusion with fresh heparinized blood	mean	19.1	19.5	19.2	0.4	−0.1	0.7
	SD	2.2	2.8	2.8	2.2	1.0	3.1
	number	10	10	7			
Exchange transfusion with fresh ACD blood	mean	19.3	20.2	20.5	0.8	0.7	1.2
	SD	1.8	3.9	3.2	2.9	1.7	2.8
	number	9	8	9			
Post-respiratory acidosis	uean	23.0	22.2	—	−0.8	—	—
	SD	2.4	3.3	—	2.2	—	—
	number	9	9	—			
Exchange transfusion with bisulfite-incubated blood	mean	19.2	17.4	20.5	−1.8*	3.1‡	1.3
	SD	2.7	3.6	3.4	2.6	2.4	2.7
	number	12	12	12			
Exchange transfusion with stored ACD blood	mean	17.7	18.2	19.7	0.5	1.5	2.0
	SD	2.1	4.1	4.7	3.0	3.1	3.3
	number	7	7	7			
Exchange transfusion with cyanate-incubated blood	mean	20.2	18.4	16.6	−1.8	−1.8	−3.6*
	SD	3.1	4.9	5.4	2.7	3.8	3.7
	number	8	8	8			

* $P < 0.025$.
‡ $P < 0.005$.

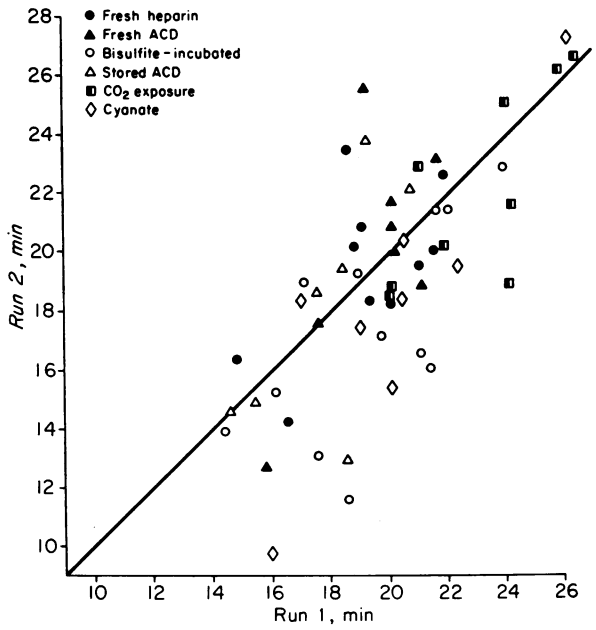

FIGURE 4 Running time of animals before (run 1), and after (run 2) various manipulations. Filled symbols refer to animals with normal blood oxygen affinity, open symbols to animals with substantially decreased P_{50}, and half-filled symbols to animals with intermediate reductions of P_{50}. Note that open symbols tend to lie below the line of identity.

DISCUSSION

In these studies blood with increased oxygen affinity was shown to produce a decrease in central venous oxygen tension in resting animals without apparent adverse effect. The magnitude of the decrease is approximately that expected from the shift in the oxygen dissociation curve alone, the small residual difference being explainable by the difference in BE (4 meq/liter). If we assume an unchanged oxygen uptake, this finding suggests that no compensatory increase in cardiac output occurred. While the measurements made cannot exclude a compensatory redistribution of blood flow, extraction of oxygen by the body as a whole would have still occurred at a lower oxygen pressure.

Use of an exercise test as a means of detecting impaired oxygen delivery depends on its sensitivity, reproducibility, and ability to elicit maximal work (or some fixed proportion thereof). We have previously presented data supporting the consistency of this test on day-to-day testing and its maximal nature; that it does measure decreased oxygen transport is shown by the proportional decrease in work capacity with anemia (8), a finding consistent with results in man (16, 17). While performance of individual groups of animals could have been influenced by factors coincident with the increase in blood oxygen affinity, such as acid load, citrate, residual bisulfite, or cyanate, the comparability of findings when blood oxygen affinity was increased in a variety of different ways emphasizes the validity of our results.

Steady-state work requires an adequate oxygen supply to exercising skeletal muscle groups and to cardiac muscle, as well as to other tissues for metabolic functions related to exercise. Results in this study show no change in performance when P_{50} was decreased by about 6 mm Hg. There was, however, a definite decrease in working ability of animals, when, by different means, P_{50} was decreased from about 36 mm to 23 mm Hg. This decrease in performance is equivalent to that seen with a decrease in hemoglobin concentration of 10–15%. Additional data on the influence of oxygen affinity on work performance are provided by the studies in normal men of Vogel, Gleser, Wheeler, and Whitten (18) and of Pirnay, Dujardin, Deroanne, and Petit (19). They found that the decrease in maximal oxygen consumption was the same during carbon monoxide poisoning as during an equivalent decrease in oxygen content due to hypoxemia, implying that the approximately 6 mm Hg decrease in P_{50} of the remaining normal hemoglobin due to CO-liganded heme groups did not produce an additional constraint on oxygen delivery.

During exercise certain effects occur that may facilitate oxygen release in muscle and thereby account for the lack of a greater difference in W_{max} between animals with normal and with increased blood oxygen affinity. First, compensation may result from the Bohr effect, in which local acidosis appreciably reduces in vivo blood oxygen affinity in working muscle. Similarly, there is a marked local increase in P_{CO_2} (17), which

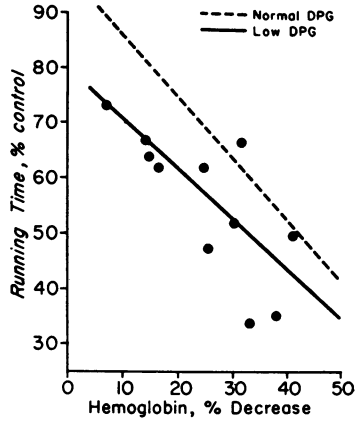

FIGURE 5 Relation between change in hemoglobin concentration and change in performance of animals with anemia alone and with anemia plus increased blood oxygen affinity. The dashed line is from data in reference 8; the solid line is the least squares regression of the points indicated.

would also decrease oxygen affinity. Additionally, muscle temperature is increased during exercise, which would shift the oxygen dissociation curve further to the right. While these effects would be expected in animals with normal and with increased in vitro blood oxygen affinity, they may be of more benefit in the latter instance by maintaining capillary oxygen pressure above a critical minimum. Furthermore, the combined influence of pH and P_{CO_2} on oxygen affinity is considerably greater in DPG-depleted human blood than in normal blood (15); preliminary experiments (unpublished) have disclosed a similar effect in rat blood. This should provide an additional advantage to animals with DPG-depleted blood.

Quantitative implications of these effects on oxygen delivery may be examined using the Fick relationship

$$\dot{V}_{O_2} = \dot{Q} \cdot Hb \cdot 1.39(Sa_{O_2} - Sv_{O_2}),$$

where $\dot{V}_{O_2}$ is oxygen consumption in ml/min, $\dot{Q}$ is cardiac output in liter/min, Hb is the hemoglobin concentration in g/liter, 1.39 is the oxygen-binding capacity of hemoglobin in ml/g, and Sa_{O_2} and Sv_{O_2} are the arterial and venous fractional oxygen saturations, respectively. (The small quantity of physically dissolved oxygen is disregarded.) As cardiac output at maximal exercise reaches a maximal value despite appreciable reduction in oxygen content due to anemia (16) or hypoxemia (20), it seems reasonable to assume that this would also occur when P_{50} is changed. If we assume hemoglobin concentration is also constant, oxygen delivery to exercising skeletal muscle will depend entirely upon $Sa_{O_2} - Sv_{O_2}$, which in turn depends upon the position of the in vivo oxygen dissociation curve and the P_{O_2} in arterial and venous blood, respectively. Assuming conditions likely to occur at maximal exercise,[2] P_{50} in DPG-depleted blood would increase from the in vitro value of 23 mm Hg to an in vivo value of 37 mm Hg in the artery and 53 mm Hg in the vein, while P_{50} of normal blood would increase from the in vitro value of 36 mm Hg to 57 and 77 mm Hg, respectively.

Fig. 6 shows the relationship between P_{50} and maximal oxygen delivery, expressed as $Sa_{O_2} - Sv_{O_2}$, (*A*) at rest and (*B*) with exercise. The upper and lower abscissas have been drawn so that the differing in vivo arterial and venous P_{50} values due to exercise are in vertical alignment. The data were calculated assuming an arterial pressure of 100 mm Hg and venous oxygen pressures of 10 and 20 mm Hg.[3] As shown, an increase in in vivo P_{50} from 22 to 36 mm Hg, assuming a critical P_{O_2} of 20 mm Hg, should increase maximal oxygen extraction at rest (left panel) from 56 to 74% (equivalent to a 32% increase in oxygen delivery). A somewhat smaller effect would result if the critical P_{O_2} were 10 mm Hg. Under conditions of exercise, the difference between in vivo arterial and venous P_{50} should result in a somewhat greater extraction at maximal exercise at a given P_{50} (right panel). Furthermore, the relationship of P_{50} and maximal oxygen extraction is relatively flat with increasing P_{50} due to exercise.[4] As indicated above, conditions at maximal exercise may shift the venous P_{50} of DPG-depleted and normal blood to 53 and 77 mm Hg, respectively. It thus appears that little effect on maximal oxygen extraction would result from blood with low in vitro P_{50}, a conclusion in agreement with the experimental results.

While the above calculations were based on blood with increased oxygen affinity due to DPG depletion, a similar decrease in work performance was found in animals when P_{50} was decreased by carbamylation of hemoglobin; in this case a slightly reduced Bohr effect would be expected (32). While detailed measurements of the Bohr effect in carbamylated whole blood

[2] The following conditions were assumed to occur in venous blood from exercising muscle: temperature 43°C, pH 7.0, and BE −10 meq/liter. These values are based on the increased core (8, 21) and muscle temperature (22) in the working rat, a pH of 7.1 at 37°C in femoral venous blood of man at maximal exercise (23–26) that becomes 7.02 at 43°C (13), and the rise in lactic acid with work (8, 24, 25). In arterial blood the following conditions were assumed: temperature, 42°C; pH 7.2; and BE, −10 meq/liter; temperature was selected 1° below muscle temperature (27) and pH as reported by Saltin et al. (25) (pH of 7.26 at 37°C = pH of 7.19 at 42°C). In vivo P_{50} was then calculated from measured P_{50} with the Bohr effect and the BE correction described in Methods; the standard temperature correction (13) was used, as preliminary experiments in whole blood have shown no effect of DPG on this factor.

[3] An arterial oxygen pressure of 100 mm Hg during exercise was assumed from resting values in rats and the minimal effect of exercise on Pa_{O_2} in normal man. Most studies have shown values for Pv_{O_2} at maximal exercise between 10 and 20 mm Hg. Saltin et al. reported a value for femoral venous oxygen pressure of 12 mm Hg (25), which equals 16.7 mm Hg at 43°C. Shappell et al. reported a value for P_{O_2} in femoral venous blood in moderately trained and untrained subjects of 17 and 10 mm Hg (23), equivalent to 24 and 14 mm Hg at 43°C, respectively. Stainsby and Otis determined that the critical oxygen pressure (lowest oxygen pressure before oxygen consumption starts to fall) of venous blood in the exercising dog hind limb is 10 mm Hg (28). Oxygen saturation over a range of arterial and venous P_{50} values (Fig. 6) were calculated for these oxygen pressures using the polynomial equation fitted by Kelman (29) to the standard oxygen dissociation curve (13).

[4] At higher P_{50} values there is a paradoxical decrease in calculated maximal extraction, which is due to increasing arterial desaturation with rising P_{50}. The extent to which this occurs is probably less than shown, as the effect of DPG and CO_2 on oxygen affinity at higher levels of oxygen saturation is appreciably reduced (30, 31).

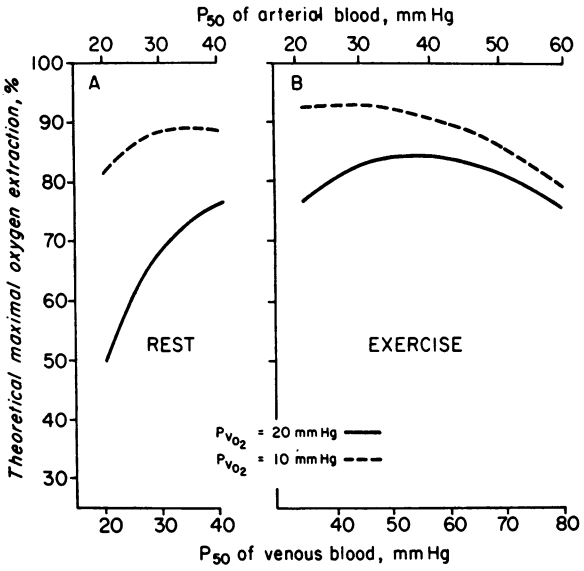

FIGURE 6 Effect of P_{50} on theoretical maximal oxygen extraction (A) at rest and (B) with exercise. The upper and lower abscissas have been drawn so that the differing in vivo arterial and venous P_{50} values due to exercise (B) are in vertical alignment.

are not available, changes in pH and other factors would still lead to an increased P_{50} with exercise.

In the above treatment it has been assumed that equilibrium measurements suffice to describe the effect of ligands and temperature on blood oxygen association and dissociation under in vivo conditions. While equilibration between cells and plasma may not be completed during the transit of blood through capillaries (33), there are insufficient data to estimate the quantitative significance of such effects at this time.

The applicability of the present findings to other situations and tissues may be limited, as the extent of adverse influence of increased in vitro blood oxygen affinity appears to have been reduced by compensatory mechanisms occurring in vivo during muscular exercise. By contrast, changes in blood oxygen affinity in human subjects usually occur in the presence of other disorders which adversely affect tissue oxygen supply, and which may not be associated with compensatory mechanisms of the type observed during exercise. Additionally, certain organs, such as the brain, which are more sensitive to oxygen lack (34), may be more adversely affected by an increase in P_{50}. Holsinger, Salhany, and Eliot have recently reported an adverse effect of DPG-depleted blood on myocardial function in a small number of dogs with severe myocardial ischemia (35). The extent of clinical consequences due to changes in blood oxygen affinity thus remains to be defined.

ACKNOWLEDGMENTS

The authors express gratitude to Clement A. Finch, M.D., for advice and criticism, to Louise Miller, Charlotte Aucutt, Rosa Linda Franada, Joanne Mukhalian, and Mary Anne Ramborger for superb technical assistance, and to Jan Thelen, Marcia Shuman, and Diane Chaddock for preparing the manuscript.

This study was supported by Public Health Service Contract NIH-71-2105 and by Research Grants HL-06242 and HL-12174 from the National Institutes of Health. Dr. Wranne acknowledges a Swedish Medical Research Council travel grant (B71-19R-3293).

REFERENCES

1. Torrance, J., P. Jacobs, A. Restrepo, J. Eschbach, C. Lenfant, and C. Finch. 1970. Intraerythrocytic adaptation to anemia. *N. Engl. J. Med.* **283**: 165.
2. Lenfant, C., J. Torrance, E. English, C. Finch, C. Reynafarje, J. Ramos, and J. Faura. 1968. Effect of altitude on oxygen binding by hemoglobin and on organic phosphate levels. *J. Clin. Invest.* **47**: 2652.
3. Woodson, R. D., J. D. Torrance, S. D. Shappell, and C. Lenfant. 1970. The effect of cardiac disease on hemoglobin-oxygen binding. *J. Clin. Invest.* **49**: 1349.
4. Valtis, D. J., and A. C. Kennedy. 1954. Defective gas-transport function of stored red blood-cells. *Lancet.* **1**: 119.
5. Gullbring, B., and G. Ström. 1956. Changes in oxygen-carrying function of human hemoglobin during storage in cold acid-citrate-dextrose solution. *Acta Med. Scand.* **155**: 413.
6. Bunn, H. F., M. H. May, W. F. Kocholaty, and C. E. Shields. 1969. Hemoglobin function in stored blood. *J. Clin. Invest.* **48**: 311.
7. Valeri, C. R., and F. B. Collins. 1971. Physiologic effects of 2,3-DPG-depleted red cells with high affinity for oxygen. *J. Appl. Physiol.* **31**: 823.
8. Wranne, B, and R. D. Woodson. 1973. A graded treadmill test for rats: maximal work performance in normal and anemic animals. *J. Appl. Physiol.* **34**: 732.
9. Parker, J. C. 1969. Influence of 2,3-diphosphoglycerate metabolism on sodium-potassium permeability in human red blood cells: studies with bisulfite and other redox agents. *J. Clin. Invest.* **48**: 117.
10. Good, N. E., G. D. Winget, W. Winter, T. N. Connolly, S. Izawa, and R. M. M Singh. 1966. Hydrogen ion buffers for biological research. *Biochemistry.* **5**: 467.
11. Cerami, A., and J. M. Manning. 1971. Potassium cyanate as an inhibitor of the sickling of erythrocytes in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 1180.
12. Wang, L. 1959. Plasma volume, cell volume, total blood volume and F_{cells} factor in the normal and splenectomized Sherman rat. *Am. J. Physiol.* **196**: 188.
13. Severinghaus, J. W. 1966. Blood gas calculator. *J. Appl. Physiol.* **21**: 1108.
14. Lenfant, C., P. Ways, C. Aucutt, and J. Cruz. 1969. Effect of chronic hypoxic hypoxia on the O_2-Hb dissociation curve and respiratory gas transport in man. *Respir. Physiol.* **7**: 7.
15. Wranne, B., R. D. Woodson, and J. C. Detter. 1972. Bohr effect: interaction between H^+, CO_2, and 2,3-DPG in fresh and stored blood. *J. Appl. Physiol.* **32**: 749.
16. Ekblom, B., A. N. Goldbarg, and B. Gullbring. 1972. Response to exercise after blood loss and reinfusion. *J. Appl. Physiol.* **33**: 175.

17. Sproule, B. J., J. H. Mitchell, and W. F. Miller. 1960. Cardiopulmonary physiological responses to heavy exercise in patients with anemia. *J. Clin. Invest.* **39**: 378.
18. Vogel, J. A., M. A. Gleser, R. C. Wheeler, and B. K. Whitten. 1972. Carbon monoxide and physical work capacity. *Arch. Environ. Health.* **24**: 198.
19. Pirnay, F., J. Dujardin, R. Deroanne, and J. M. Petit. 1971. Muscular exercise during intoxication by carbon monoxide. *J. Appl. Physiol.* **31**: 573.
20. Stenberg, J., B. Ekblom, and R. Messin. 1966. Hemodynamic response to work at simulated altitude, 4,000 m. *J. Appl. Physiol.* **21**: 1589.
21. Gollnick, P. D., and C. A. Ianuzzo. 1968. Colonic temperature response of rats during exercise. *J. Appl. Physiol.* **24**: 747.
22. Brooks, G. A., K. J. Hittelman, J. A. Faulkner, and R. E. Beyer. 1971. Tissue temperatures and whole-animal oxygen consumption after exercise. *Am. J. Physiol.* **221**: 427.
23. Shappell, S. D., J. A. Murray, A. J. Bellingham, R. D. Woodson, J. C. Detter, and C. Lenfant. 1971. Adaptation to exercise: role of hemoglobin affinity for oxygen and 2,3-diphosphoglycerate. *J. Appl. Physiol.* **30**: 827.
24. Pernow, B., J. Wahren, and S. Zetterquist. 1965. Studies on the peripheral circulation and metabolism in man. IV. Oxygen utilization and lactate formation in the legs of healthy young men during strenuous exercise. *Acta Physiol. Scand.* **64**: 289.
25. Saltin, B., G. Blomquist, J. H. Mitchell, R. L. Johnson, K. Wildenthal, and C. B. Chapman. 1968. Response to exercise after bed rest and after training. *Circ. Suppl.* **38**: 7.
26. Hermansen, L., and J.-B. Osnes. 1972. Blood and muscle pH after maximal exercise in man. *J. Appl. Physiol.* **32**: 304.
27. Saltin, B., and L. Hermansen. 1966. Esophageal, rectal, and muscle temperature during exercise. *J. Appl. Physiol.* **21**: 1757.
28. Stainsby, W. N., and A. B. Otis. 1964. Blood flow, blood oxygen tension, oxygen uptake and oxygen transport in skeletal muscle. *Am. J. Physiol.* **206**: 858.
29. Kelman, G. R. 1966. Digital computer subroutine for the conversion of oxygen tension into saturation. *J. Appl. Physiol.* **21**: 1375.
30. Tyuma, I., K. Imai, and K. Schimizu. 1972. Organic phosphates and the oxygen equilibrium function of some human hemoglobins. *In* Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status. (Proceedings of the Alfred Benzon Symposium IV. Copenhagen, 17–22 May, 1971). M. Rørth and P. Astrup, editors. Academic Press, Inc., New York. 131.
31. Garby, L., M. Robert, and B. Zaar. 1972. Proton- and carbamino-linked oxygen affinity of normal human blood. *Acta Physiol. Scand.* **84**: 482.
32. Kilmartin, J. V., and L. Rossi-Bernardi. 1971. The binding of carbon dioxide by horse haemoglobin. *Biochem. J.* **124**: 31.
33. Forster, R. E. 1972. Interaction of CO_2 and O_2 exchange in red blood cells. *In* Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status. (Proceedings of the Alfred Benzon Symposium IV, Copenhagen, 17–22 May, 1971). M. Rørth and P. Astrup, editors. Academic Press, Inc., New York. 494.
34. Cohen, P. J., S. C. Alexander, T. C. Smith, M. Reivich, and H. Wollman. 1967. Effects of hypoxia and normocarbia on cerebral blood flow and metabolism in conscious man. *J. Appl. Physiol.* **23**: 183.
35. Holsinger, J. W., J. M. Salhany, and R. S. Eliot. 1973. Physiologic observations on the effect of impaired blood oxygen release on the myocardium. *Adv. Cardiol.* In press.