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The Influence of Anaerobiosis on Human Erythrocyte Metabolism (39670)

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Incubation of human erythrocytes in a nitrogen atmosphere *in vitro* increases glycolytic rate and 2,3-DPG[1] content (1, 2), changes which have been attributed to the well-known rise in pH that occurs upon deoxygenation of hemoglobin (1). It is not clear, on the other hand, to what extent the absence of O_2 itself contributes to this effect under physiological conditions. In the present study, experiments were conducted in which media buffered at different pH were used to eliminate the pH variable. The results indicate that significant metabolic changes can be ascribed to the absence of O_2 alone.

Methods. Blood was collected from young adult males in acid–citrate–dextrose medium and erythrocytes were separated by centrifugation at 800*g* for 8 min. After the plasma and buffy coat were removed, the packed cells were washed three times with cold Tris–Ringer's medium containing (m*M*): NaCl, 120; KCl, 5.6; $MgCl_2$, 2; Na_2HPO_4, 1; glucose, 10; and tris(hydroxymethyl)aminomethane (Tris, pH 7.40 at 37°), 24. Erythrocyte suspensions with a hematocrit of 25% were prepared with Tris–Ringer's media buffered at four different pH levels; at each pH, the media were titrated so as to cause the pH of anaerobic erythrocytes to match that of aerobic erythrocytes after a given period of incubation. Humidified N_2 was delivered at 8.0 ft^3/hr to deoxygenate erythrocytes and samples were incubated at 37° in a Dubnoff shaker. The N_2-incubated samples were maximally deoxygenated (< 10 mmHg) by 90 min of incubation. Intracellular pH was measured in hypotonic lysates which were prepared by adding 1 ml of packed cells to 2 ml of water. In control studies with oxygenated and deoxygenated erythrocytes, pH values obtained from hypotonic and freeze-thaw lysates were essentially the same. All pH measurements were recorded at 37° with an Instrumentation Laboratory Model 113 pH/gas analyzer.

Protein-free extracts were prepared and standard enzymatic procedures were employed for the measurement of 2,3-DPG (3), FDP, triose-P, 3-PGA, and lactate (4). G6-P, F6-P, PEP, and pyruvate concentrations were determined by a fluorometric procedure (5). Hemoglobin was evaluated by the cyanmethemoglobin method (6). The hemoglobin concentration was greater in cells incubated at alkaline pH (data not shown), signifying the well-known decrease in cell volume as pH is raised (7). Since no difference was noted between hemoglobin concentrations of erythrocytes incubated in air and in N_2 and since the difference between the hemoglobin values at the pH extremes was only 8%, the metabolic data are presented in terms of cell volume. The metabolite profiles (Figs. 1 and 2) were basically the same when the data were expressed in terms of grams of hemoglobin.

Results. Lactate production. When erythrocyte samples were suspended in Tris–Ringer's media that had been buffered to pH 7.70 and 7.40 at 37° and the suspensions were incubated in air (Expt A) and in N_2 (Expt B), respectively, the pH values in both sets of erythrocytes were the same after 90 min of incubation (Table I). After an additional 2 hr of incubation, the final pH values were also identical although they had both decreased by 0.1 unit. Lactate production was 16% higher in the cells incubated in N_2. When a third erythrocyte sample was placed in the same pH 7.70 medium as the oxygenated suspension (Expt A) but was deoxygenated, this suspension (Expt C) had initial and final intracellular pH values

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[1] Abbreviations used: 2,3-DPG, 2,3-diphosphoglycerate; G6-P, glucose 6-phosphate, F6-P, fructose 6-phosphate; FDP, fructose 1,6-diphosphate; triose-*P*, triose phosphate; 3-PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PK, pyruvate kinase.

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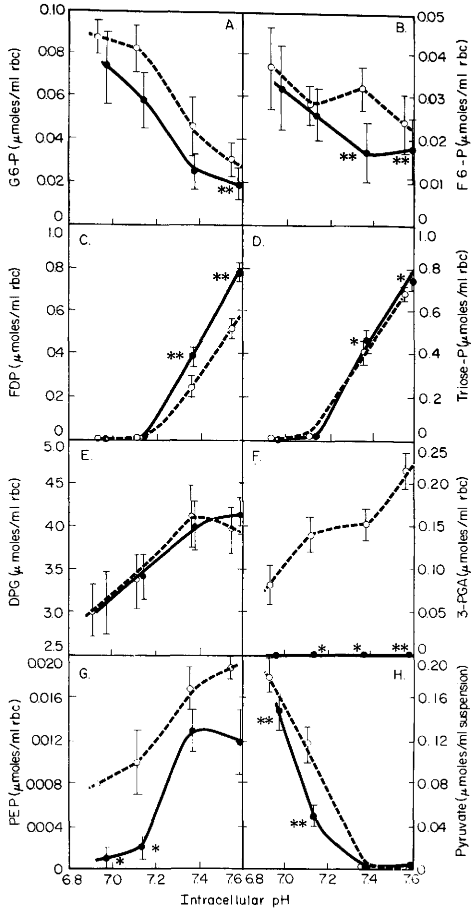

FIG. 1. Concentrations of metabolites in erythrocytes incubated in air (O- - -O) and in N_2 (●——●) as a function of intracellular pH. Each point is the mean of three experiments, except for the pyruvate and 2,3-DPG values which are the means of six experiments. The vertical lines bracketing each point represent plus and minus one SEM. P values were calculated with the t test for paired experiments. $^*P < 0.05$; $^{**}P < 0.01$.

that were 0.1 unit greater and a 60% greater lactate production.

Concentrations of glycolytic intermediates and cofactors. In a second series of experiments, erythrocytes were suspended in media with different pH values so that similar pH values would be attained in aerobic and anaerobic erythrocytes after 150 min of incubation, *i.e.*, 60 min after deoxygenation was complete in the anaerobic erythrocytes.

When pH was raised in the air-incubated control cells, G6-P and F6-P values decreased (Fig. 1A, B) and FDP and triose-P values increased at pH values greater than 7.2 (Fig. 1C, D). These changes have been reported before and ascribed to an increase in phosphofructokinase activity (8). When erythrocytes were incubated in N_2, G6-P and F6-P concentrations were lower and FDP and triose-P concentrations were higher at relatively alkaline pH values where the latter compounds were measurable. This result indicates that phosphofructokinase activity was also accelerated by anaerobiosis at pH values greater than 7.2.

Following deoxygenation, the most marked variation occurred in the 3-PGA concentration, since this compound was undetectible (<0.006 μmole/ml rbc) in N_2 at any pH examined (Fig. 1F). PEP was also de-

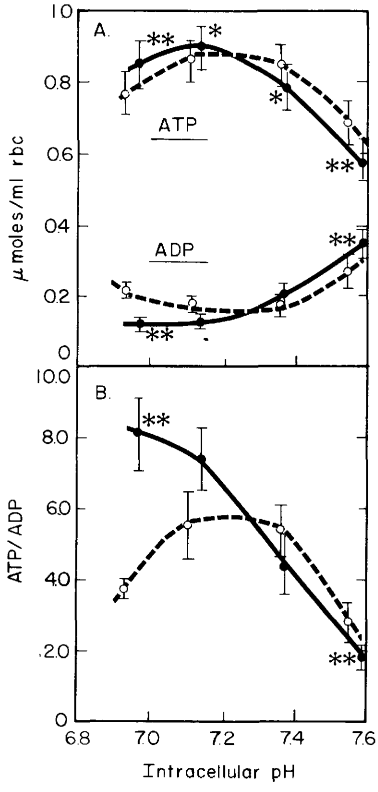

FIG. 2. (A) ATP and ADP concentrations in erythrocytes incubated in air (O- - -O) and N_2 (●——●) as a function of intracellular pH. (B) The ratio of measured ATP and ADP concentrations in erythrocytes incubated in air (O- - -O) and in N_2 (●——●) as a function of intracellular pH. Each point is the mean of six experiments. The vertical lines bracketing each point represent plus and minus one SEM. P values were calculated with the t test for paired experiments. $^*P < 0.05$ and $^{**}P < 0.01$.

TABLE I. INFLUENCE OF ANAEROBIOSIS ON LACTATE PRODUCTION BY HUMAN ERYTHROCYTES UNDER CONDITIONS OF CONTROLLED AND UNCONTROLLED pH.[a]

Expt	Gas phase	pH Initial	pH Final	Lactate production (μmol/ml rbc/hr)
A	Air	7.18 ± 0.01	7.09 ± 0.01	1.71 ± 0.07
B	N_2	7.17 ± 0.01	7.08 ± 0.01	1.99 ± 0.06[b]
C	N_2	7.26 ± 0.01	7.18 ± 0.02	2.75 ± 0.26[b]

[a] Washed erythrocytes were incubated in air (Expt A) and in N_2 (Expt B) after they were suspended in Tris-Ringer's medium buffered at pH 7.70 and 7.40, respectively. Under these conditions, initial and final intracellular pH readings were the same after 90 and 210 min of incubation, respectively. A third set of erythrocytes were incubated in N_2 (Expt C) after they were suspended in the same buffer (pH 7.70) as the oxygenated erythrocytes (Expt A). The means ± SEM from three experiments are shown.

[b] $P < 0.05$, when compared to Experiment A.

pressed (Fig. 1G). These results indicate that pyruvate kinase activity was also accelerated in N_2, causing both PEP and 3-PGA to be decreased. The increase in 2,3-DPG with pH (2) was also observed in our study (Fig. 1E) but no significant change was noted with anaerobiosis.

In air-incubated control cells, pyruvate decreased with increasing pH (Fig. 1H) since NADH availability is greater with increasing glycolytic rate (9). Thus, the lower pyruvate concentrations in N_2 (Fig. 1H) (when these compounds were measurable) were also consistent with a higher glycolytic rate, especially as the latter variation was observed in a N_2 environment (Table I).

When ATP concentration was plotted as a function of pH (Fig. 2A), bell-shaped curves were obtained which were basically the same variations noted before in the case of the oxygenated cells (10). The curve for deoxygenated cells were located slightly to the left of the curve for oxygenated cells (Fig. 2A), and the graphs for ADP were essentially mirror images of the ATP graphs. The ATP/ADP ratio also displayed a bell-shaped form for oxygenated cells; in contrast, the graph for deoxygenated cells followed a downward course from a value at relatively acid pH, which was higher than that noted for oxygenated cells (Fig. 2B).

Discussion. When erythrocytes were incubated in a N_2 atmosphere after the pH increase associated with deoxygenation had been compensated for, an acceleration of glycolysis was observed which could be attributed to anaerobiosis alone (Table I). The fact that 3-PGA and PEP concentrations decreased under these conditions, instead of increasing as would be expected with a pH elevation (Fig. 1), indicates that the anaerobic effects were not due to a residual pH rise. The influence of anaerobiosis on lactate production was small since only a 16% activation was found. In contrast, the increase in glycolytic rate that occurred when pH was uncontrolled was three times greater (Table I).

Judging from the changes in concentration of the glycolytic intermediates (Fig. 1), two of the nonequilibrium reactions in glycolysis, the phosphofructokinase (PFK) and pyruvate kinase (PK) reactions (11), were activated in a N_2 environment. These effects may have been due to the reduction in free 2,3-DPG concentration which follows the increased binding of this compound to deoxyhemoglobin (12). Thus, the allosteric inhibition of PFK by free 2,3-DPG (13) may have been attenuated by the decreased concentration of this negative effector causing lactate production to increase under anaerobic conditions. PK activity is also elevated by a lowered free 2,3-DPG concentration with a consequent reduction in allosteric inhibition (10). In addition, an increase in free Mg^{2+} concentration is associated with the increased 2,3-DPG binding to deoxyhemoglobin due to the release of this cation from binding to 2,3-DPG (14, 15). Since MgADP concentration may be raised under these conditions, PK activity may be elevated for this reason as well (15).

The most notable response to anaerobiosis was the absence of a measurable 3-PGA concentration. In an earlier study by Hamasaki *et al.* (16), 3-PGA concentration was decreased by only 30% in N_2. In this case, the activation of PFK by a nonphysiological medium P_i concentration (30 m*M*), appears to have overridden the increased 3-PGA utilization by PK. These authors attributed the increases in PK as well as PFK activities to a decrease in the ATP/ADP ratio (16). Our results (Fig. 2) indicate that the latter change could contribute to the PK activation at alkaline pH but not at acid pH.

The PK effect may be significant because this enzyme may be modulating carbon flow between the principal and alternate glycolytic pathways. When free 2,3-DPG concentration declines in N_2, product inhibition of 2,3-DPG mutase decreases (18) and carbon flow can be diverted toward 2,3-DPG synthesis and binding to deoxyhemoglobin. Although this would have a salutary effect on O_2 delivery to the tissues (19), ATP synthesis could be seriously jeopardized. If PK activity were also raised, the flow of substrate through the phosphoglycerate kinase reaction may not be reduced and the rate of ATP synthesis may be essentially normal. The decrease in 3-PGA concentration in the presence of increased lactate production indicates that these conditions prevailed in our studies. The ATP concentration, moreover, was not seriously altered under anaerobic conditions.

Summary. Anaerobiosis caused a small increase in lactate production when oxygenated and deoxygenated human erythrocytes had similar initial and final pH values. This change could be attributed to PFK activation. Under physiological conditions, PK also appeared to be activated, as judged by the marked decrease in 3-PGA concentration. When the pH rise associated with deoxygenation was allowed to take place, a threefold greater increase in lactate production was observed.

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