THE HYDROGEN ION CONCENTRATIONS AND ERYTHROCYTE GLYCOLYSIS

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Recently several reports appeared on the control of glycolysis in living cells, notably tumor cells (Wu, 1964) and cerebral tissues (Lowry et al. 1964). In these studies, the glycolytic intermediates of the cells are assayed enzymatically and the controlling points are analyzed by the "crossover theorem" of Chance et al.(1958).

Erythrocytes seemed to be one of the most suitable cells for this kind of analysis, as glycolysis is predominant compared to other metabolic activities. The pattern of glycolytic enzymes are systematically studied by Chapman et al. (1962) and some of the intermediates were assayed chromatographically by Bartlett (1959) and Yoshikawa et al.(1960). Hexokinase has been considered as the rate-limiting step in the glycolytic chain of the cells by several investigators (Rapoport et al.,1961, Chapman et al., 1962, Rose and O'Connell, 1964), as the hexokinase activity is the lowest in the glycolytic enzymes and glycolytic rates of the red cells are found to be parallel to those of hexokinase in several conditions, e.g. aging, storage and in different species of animals. The optimum pH for the glycolysis of the cells lies at pH 8.1, which coincides with those of hexokinase.

In this paper, the contents of glycolytic intermediates of human erythrocytes except 1,3-diphosphoglycerate are reported, especially in relation to the

Abbreviations: ACD: acid-citrate-dextrose (the media for the preservation of blood). G6P: glucose 6-phosphate. F6P: fructose 6-phosphate. FDP: fructose diphosphate. GAP: glyceraldehyde 3-phosphate. DHAP: dihydroxyacetone phosphate. 2,3DPG: 2,3-diphosphoglycerate. 2PGA: 2-phosphoglycerate. 3PGA: 3-phosphoglycerate. PEP: phosphoenolpyruvate.

TABLE I

The levels of glycolytic intermediates in erythrocytes (mμmole/ml red cells or blood*)

	Heparinated (1 hr. incubation)	ACD blood (stored for 48 hrs.)
G6P	58	46
F6P	10	7
FDP	22	<1
DHAP	97	<1
GAP	13	<1
3PGA	83	<1
2PGA	20	<1
PEP	16	<1
2,3DPG	4800	3900
ATP	1300	1200
ADP	97	130
AMP	60	55
NAD	23	21
Pyruvate*	51	200
Lactate*	2900	3000

pH of the suspending medium. The hydrogen ion concentration is found to affect mainly the step of phosphofructokinase.

EXPERIMENTAL

The blood was obtained from local blood bank and leucocytes were separated by centrifugation. Blood or red cell suspension was deproteinized by adding the same volume of ice cold 0.6 N perchloric acid using glass homogenizer. The supernatant obtained by refrigerated centrifuge was neutralized to pH 4 - 5 with 5 N K_2CO_3. The contents of water in red cells and plasma were calculated as 70% and 93% respectively. The measurements of the intermediates were performed spectrophotometrically at 340 mμ using Gilford Recording Attachment (Full scale: 0.1, 0.4 or 1.0 O.D.) except 2,3-diphosphoglycerate which was measured chemically according to Bartlett (1959). Methods of enzymatic assays are according to Bergmeyer (1962). The enzymes used were from C.F.Boehring u. Sohne.

RESULTS AND DISCUSSIONS

Table I shows the levels of glycolytic intermediates in heparinated blood incubated for 1 hr. at 37° and ACD blood stored for 48 hrs. at 4°. The existence of dihydroxyacetone phosphate, glyceraldehyde phosphate and phosphoenolpyruvate

was ascertained besides the intermediates already reported by Bartlett (1959). The ratios, [F6P]/[G6P], [DHAP][GAP]/[FDP], [DHAP]/[GAP], [2PGA]/[3PGA] and [PEP]/[2PGA] in heparinated blood were found to be 0.17, 5.7×10^{-5}, 7.5, 0.24 and 0.8 respectively, and are close to the equilibrium constants, 0.47, 6.8×10^{-5}, 22, 0.43 and 3, respectively (the data from Hess (1962) at pH 7.0 and 20°). This shows that glucosephosphate isomerase, aldolase, triosephosphate isomerase, phosphoglycerate mutase and enolase are maintained close to equilibrium and might not be the rate-limiting steps in the glycolysis. The potentially rate-limiting steps in the cells will be the following four, namely, hexokinase, phosphofructokinase, phosphoglycerate kinase and pyruvate kinase.

The big differences were observed in the glycolytic intermediates between heparinated blood and ACD blood. Although, the levels of sugar monophosphates, 2,3-diphosphoglycerate and nucleotides were maintained in ACD blood compared to fresh blood, the intermediates between fructose diphosphate and phosphoenolpyruvate could not be detected in ACD blood. This is in agreement with the observation of Bartlett and Shafer (1960) who failed to detect fructose diphosphate in ACD blood.

This observation led authors to investigate the effect of the pH of the incubating medium on the glycolytic intermediates, as the disappearance of fructose diphosphate might be induced by the decrease of the pH. Fig. 1 shows the levels of the intermediates in relation to the pH of the suspending medium. As pH increases, the formation of lactate and the concentration of fructose diphosphate in the cells increase, while those of glucose 6-phosphate and fructose 6-phosphate (not shown in the figure) decrease intensively. This observation shows that "crossover" point lies between sugar monophosphate and diphosphate: phosphofructokinase may be regarded as controlling the glycolytic rate in this conditions of the cells, rather than hexokinase which has been suggested by several investigators.

This is one of the demonstrations that phosphofructokinase is the rate-limiting step in the glycolysis of living cells. Phosphofructokinase is known to be responsible for the "Pasteur-effect" (Lynen et al. 1959. Lowry et al. 1964.

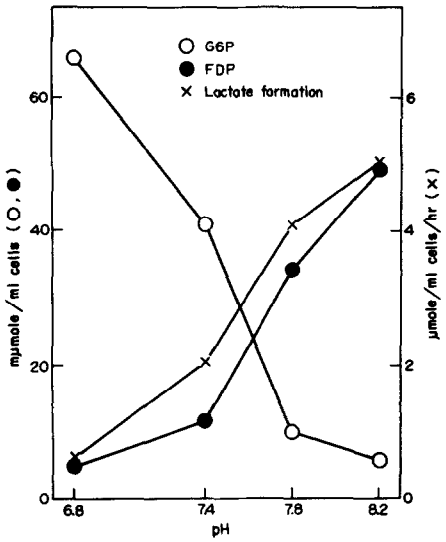

Fig. 1. The change of the glycolytic intermediate levels by pH. The red cells were washed 4 times in phosphate-free media of different pH (100 mM NaCl, 10 mM KCl, 50 mM triethanolamine HCl, 10 mM glucose). The cells were incubated for 1 hr. at 37°.

Wu, 1964). The decrease of the glycolysis in red cells by inhibiting the active cation transport has been demonstrated and phosphofructokinase is suggested to be responsible for this effect (Minakami et al. 1964).

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