FAMILIAL DIPHOSPHOGLYCERATEMUTASE DEFICIENCY. INFLUENCE ON THE OXYGEN AFFINITY CURVES OF HEMOGLOBIN

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1. Introduction

It is now well established that the accumulation of 2,3-diphosphoglycerate (DPG) observed in pyruvate kinase deficiency gives rise to a decrease of the oxygen affinity of hemoglobin when studied in intact red cells [1–3]. The opposite phenomenon would be expected in conditions creating a low level of intraerythrocytic DPG, such as diphosphoglycerate mutase (DPGM) deficiency. However no such case has yet been reported.

The present report concerns two related subjects with partial DPGM deficiency. As a consequence of this defect their red cells exhibit a low level of DPG. Since DPG has been shown to exert a regulatory action both on energetic metabolism [4–6] and on the oxygen affinity of hemoglobin [7, 8], the metabolic state of the red cells and the oxygen equilibrium of hemoglobin were studied.

In the two subjects investigated, the following consequences of DPGM deficiency were observed: an apparent activation of phosphofructokinase (PFK), as revealed by the crossover plot of glycolytic intermediates; a shift to the left of the oxygen equilibrium, curves in intact red cells, which disappears after hemolysis.

2. Material and methods

The enzymes and intermediates of Embden-Meyerhof pathway, and the overall rate of glycolysis, were measured as described elsewhere [9–12]. DPGM was assayed according to Schröter [3].

Oxygen affinity was determined spectrophotometrically according to Benesch et al. [14] in whole cells and lysed cells, at three different pH values using standard buffers of identical ionic strength.

3. Results and discussion

3.1. *Metabolic consequences of the enzyme defect (table 1, fig. 1)*

DPGM is decreased by 50%, DPG by 70%, these values are close to those reported by Schröter in his heterozygous subjects [15].

The measurement of metabolic intermediates (fig. 1) discloses an apparent positive crossover point at the level of PFK, whose *in vitro* value in optimal conditions is nevertheless normal. This result does not agree with an inhibition of hexokinase by DPG, observed by some authors [4, 5], but questioned by others [16]: the finding of a low level of hexosemonophosphates does not support this hypothesis.

The high levels of triosephosphates and fructosedi-

phosphate probably result from the decreased activity of DPGM, which further shows the metabolic importance of this enzyme in normal erythrocytes. The reduced level of hexosemonophosphates may arise from an activation of PFK due either to the accumulation of fructosediphosphate, which is a known activator of the enzyme; or to the depletion of red blood cells in DPG, if we assume an inhibition of PFK by DPG which was reported in muscle and brain [17], but has not yet been described in erythrocytes, as far as we know. Among other known effectors of PFK, adenine nucleotides and glucose-1,6-diphosphate are present at normal levels. The apparent activation of PFK does not significantly affect the overall rate of glycolysis (table 1).

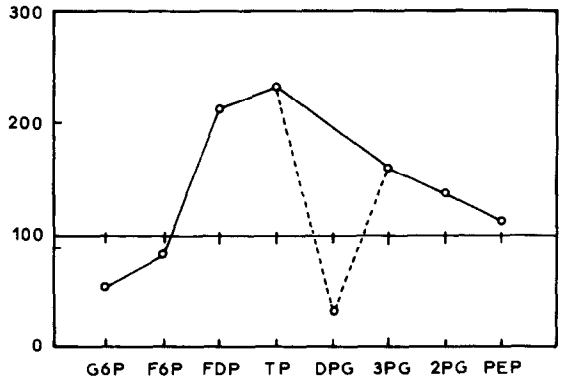

Fig. 1. Crossover plot of glycolytic intermediates in the propositus, per cent normal values (published elsewhere [10, 11]). Same results were observed in the father (not shown).
Abbreviations:
G6P : glucose-6-phosphate
F6P : fructose-6-phosphate
FDP: fructosediphosphate
TP : triosephosphates
DPG: 2,3-diphosphoglycerate
PG : phosphoglycerate
PEP : phosphoenolpyruvate.

3.2. *Effect on oxygen binding*

When studied in whole cells, the oxygen equilibrium curves were shifted towards the left with a decreased P_{50} at all the pH values (fig. 2). When studied on lysed cells, the curves were identical to those of a normal subject (fig. 3). The allosteric properties of the molecule: cooperativity for oxygen binding as measured by the coefficient of Hill, $\bar{n}$, and the Bohr effect were normal (table 2).

The abnormal oxygen dissociation curve present

Table 1

	Normal		Propositus	Father
	Mean	± 1 S.D.		
Diphosphoglycerate mutase (1)	0.31	± 0.05	0.17	0.17
Phosphofructokinase	1.30	± 0.20	0.92	
Hexokinase	0.14	± 0.03	0.21	0.12
2,3-Diphosphoglycerate (2)	4580	± 610	1430	1980
Glucose-1,6-diphosphate	97	± 17	112	114
ATP	1527	± 180	1630	1870
ADP	170	± 21	183	180
AMP	30		24	11
Glycolysis (3)				
Glucose consumed	0.022	± 0.004	0.027	
Lactate formed	0.035	± 0.007	0.050	

(1) Enzymes in international units/ml red blood cells;
(2) intermediates in nmoles/ml red blood cells;
(3) overall rate of glycolysis in μmoles/min/ml red blood cells at 37°, pH 7.4.

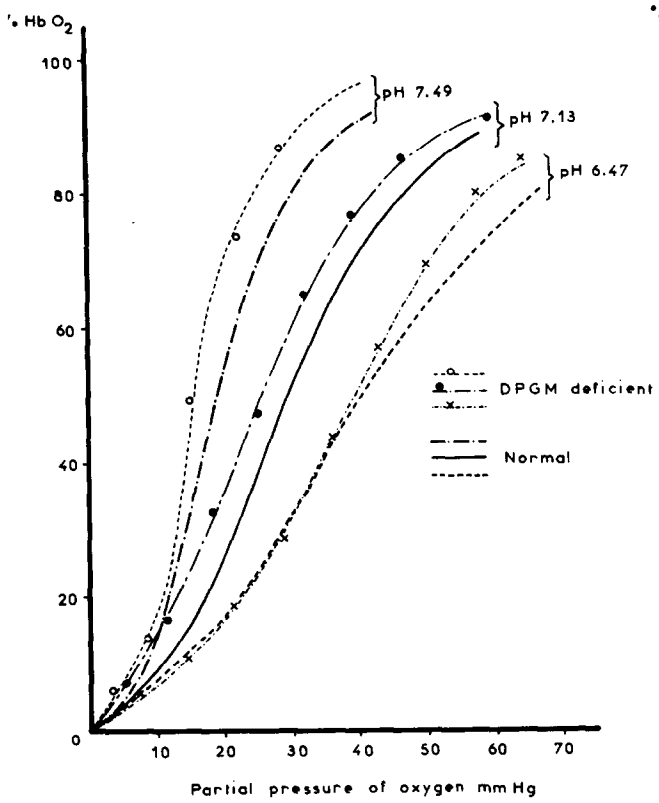

Fig. 2. Comparison of the oxygen dissociation curve of hemoglobin of a normal subject and of the propositus deficient in dpgm in intact red cells. Isotonic phosphate buffers. Three different pH values at 37°.

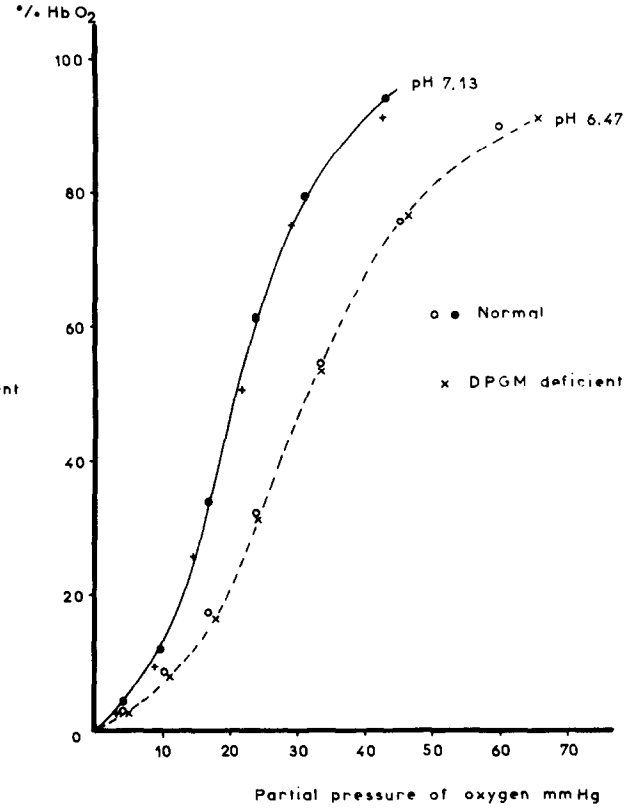

Fig. 3. Comparison of the oxygen dissociation curves of hemoglobin of a normal subject and of the propositus deficient in DPGM in lysed red cells. Same conditions as for intact cells.

Table 2
Comparison of values of the oxygen equilibrium of hemoglobin in normal subjects and in a patient with DPGM deficiency.

		Normal subject	DPGM deficient
Intact red cells	pH 7.13 P_{50}	29–30	25.5
	n	2.7–2.9	2.77
	pH 7.49 P_{50}	21–22	15.5
	n	2.2–2.7	2.77
	pH 6.47 P_{50}	38–39	39
	n	2.6	3.07
	Alkaline Bohr effect	0.4	0.42
	Acid Bohr effect	0.19–0.24	0.27 and 0.30
Lysed red cells	pH 7.13 P_{50}	21–23	20.75
	pH 6.47 P_{50}	32	31.5

in intact erythrocytes becomes normal when the cells are lysed. This phenomenon reflects the fact that the abnormality is not due to a difference of structure of the hemoglobin molecule, but rather to the abnormal level of intracellular DPG. When the hemoglobin molecule itself is abnormal, such a difference is not observed [18].

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