The First Case of a Complete Deficiency of Diphosphoglycerate Mutase in Human Erythrocytes

RAYMONDE ROSA, MARIE-ODETTE PREHU, YVES BEUZARD, and JEAN ROSA, *Unité de Recherches sur les Anémies, Institut National de la Santé et de la Recherche Médicale U. 91, Hôpital Henri Mondor, 94010 Creteil, France*

ABSTRACT An inherited and complete deficiency of diphosphoglycerate mutase was discovered in the erythrocytes of a 42-yr-old man of French origin whose blood hemoglobin concentration was 19.0 g/dl. Upon physical examination he was normal with the exception of a ruddy cyanosis. The morphology of his erythrocytes was also normal and there was no evidence of hemolysis. The erythrocyte 2,3-diphosphoglycerate level was below 3% of normal values and, as a consequence, the affinity of the cells for oxygen was increased. Diphosphoglycerate mutase activity was undetectable in erythrocytes as was that of diphosphoglycerate phosphatase. The activities of all the other erythrocyte enzymes that were tested were normal except for monophosphoglycerate mutase which was diminished to 50% of the normal value. The levels of reduced glutathione, ATP, fructose 1,6-diphosphate, and of triose phosphates were elevated, whereas those of glucose 6-phosphate and fructose 6-phosphate were decreased. This report sheds new light on the role of diphosphoglycerate mutase in the metabolism of erythrocytes.

INTRODUCTION

In human erythrocytes diphosphoglycerate mutase (DPGM)[1] (EC 2.7.5.4.) catalyses the formation of 2,3-diphosphoglycerate (2,3-DPG), an important effector of the dissociation of oxygen from hemoglobin; erythrocytes also contain, 2,3-diphosphoglycerate phosphatase (DPGP) (EC 3.1.3.13). These two enzyme activities have been separately studied by several authors (1–5). In previous investigations we have provided evidence that DPGM and DPGP activities are carried by a single molecule (6) which in addition contains a monophosphoglycerate mutase activity (MPGM) (EC 2.7.5.3.) (7, 8). The trifunctional enzyme has been obtained in a purified form by the use of chromatographic techniques (9, 10). This enzyme presents a single electrophoretic band (6) and represents one of the three molecular forms of erythrocyte MPGM (8). Species differences of erythrocyte DPGM have been described in the erythrocytes of mammals, such as the cat, and also in ruminants whose cells contain very low levels of 2,3-DPG (11). Thus the activity of this enzyme in vivo is probably an important determinant of 2,3-DPG levels within the erythrocytes.

Until now, only partial deficiencies of erythrocyte DPGM have been described in three unrelated families. In one of them (12) the defect was tolerated without any clinical manifestation. In contrast, the two other cases displayed a chronic hemolysis (13, 14). In the present report we describe the first case of a complete deficiency of erythrocyte DPGM. Clinical and biochemical studies have been performed on the propositus, who is homozygous for the defect, and on his two heterozygous children. These studies have thus allowed us the unique opportunity of evaluating the role of DPGM in the complex metabolic interrelationships within erythrocytes.

METHODS

Materials

With the exception of some reagents, whose source is specified in the text, all substrates and commercial enzymes used

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[1] *Abbreviations used in this paper*: ACD, acid-citrate-dextrose; 1,3-DPG, 1,3-diphosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; DPGM, diphosphoglycerate mutase; DPGP, diphosphoglycerate phosphatase; FDP, fructose 1,6-diphosphate; F6P, fructose 6-phosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; G6P, glucose 6-phosphate; MPGM, monophosphoglycerate mutase; ODC, oxygen dissociation curve; PE, phospho-enzyme; P_{50}, partial pressure of oxygen at which hemoglobin is half saturated with O_2; TP, triose phosphates.

were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Buffer salts were obtained from Merck Chemical Div., Merck & Co., Inc., Rahway, N. J. Cellogel strips were purchased from Medical Products, Chemetron Corp., Milan, Italy, whereas 2-phosphoglycolic acid, α-cellulose, and Sigma Cell type 50 were from Sigma Chemical Co., St. Louis, Mo.

Experiments

General. Routine hematologic examinations, determined by standard methods (15) and oxygen dissociation curves (ODC), were performed on freshly drawn, heparinized, venous blood specimens. Resting cardiac output was determined with technetium 99 (16). ODC of whole blood cells (2-μl samples) were recorded at 37°C, pH 7.40, and at a P_{CO_2} of 40 mm Hg with the Hem-O-Scan oxygen analyzer (American Instrument Co., Inc., Silver Spring, Md.). The ODC of hemolysates were determined on samples stripped of organic phosphates on a Dintzis column (17); ODC were measured according to the discontinuous spectrophotometric method of Benesch et al. (18). Measurements of O_2, P_{CO_2}, and pH were made at 37°C with a BSM3 Radiometer gas analyzer (Radiometer Co., Copenhagen, Denmark).

Enzyme assays

Blood collected in the presence of heparin was passed through a mixture of α-cellulose and Sigma Cell type 50. The washed erythrocytes were treated as recommended by the International Committee for Standardization in Haematology (19) and enzyme assays were performed on the hemolysate.

Assay of DPGM activity. The assay was performed according to Schröter and Kalinowski (20). The assay mixture (1 ml) contained 40 μmol triethanolamine-HCl buffer (pH 7.5), 1 μmol NAD, 7 μmol fructose 1,6-diphosphate, 7 μmol KH_2PO_4, 2 μmol 3-phosphoglycerate, 1 U aldolase, 1 U triose phosphate isomerase, and 2.5 U of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). After ≅5 min preincubation at 37°C, 0.05 ml of a 1/15 hemolysate was added. Incubation was subsequently carried out for 10 min and, when necessary, for periods up to 30 min. DPGM activity was usually evaluated by determination of the NADH formed in the course of the GAPDH reaction and in a few instances by measurement of the 2,3-DPG synthesized in the DPGM reaction. For this purpose, at the start and after 30 min incubation, 1 ml of the mixture was removed from the cuvette and deproteinized in a boiling bath for 10 min. After centrifugation, 0.5 ml of the supernate was taken for determination of 2,3-DPG as described below.

DPGP assay. The assay was performed according to the following principle which has previously been used for its electrophoretic revelation (21), i.e., in the presence of 2-phosphoglycolic acid, a strong activator of DPGP, the 3-phosphoglycerate liberated from 2,3-DPG is measured by a reaction coupled to phosphoglycerate kinase and GAPDH. The assay mixture, in a volume of 1 ml, contained 50 μmol triethanolamine-HCl buffer (pH 7.5), 10 μmol $MgCl_2$, 3 μmol Na_2ATP, 0.2 μmol NADH, 0.8 μmol 2,3-DPG, 3.3 U GAPDH, 2 U phosphoglycerate kinase and 1 μmol 2-phosphoglycolic acid as the activator of DPGP. To the incubation medium, 0.05 ml of a 1/15 hemolysate was added and incubation carried out at 37°C for 10 min and, when necessary, up to 30 min. The transformation of NADH to NAD at 334 nm was proportional to DPGP activity.

MPGM assay. The assay was performed according to a similar principle to that employed for DPGP, i.e., the 3-phosphoglycerate formed from 2-phosphoglycerate was measured by reactions coupled to phosphoglycerate kinase and GAPDH. The incubation medium was the same as that used for determination of DPGP activity except for the following: (*a*) the 0.8 μmol of 2,3-DPG was replaced by 0.08 μmol 2,3-DPG together with 0.8 μmol 2-phosphoglycerate; (*b*) 2-phosphoglycolic acid was omitted; (*c*) 10 μl of a 1/15 hemolysate was used and the incubation was carried out at 25°C.

All other glycolytic enzymes, glutathione reductase, adenylate kinase, adenosine deaminase, and acetylcholinesterase, in addition to reduced glutathione, were assayed according to Beutler et al. (19, 22). Pyrimidine 5′-nucleotidase activity was measured according to Valentine et al. (23) and inorganic phosphate according to Fiske and Subbarow (24).

Extracts for measurement of 2,3-DPG, ATP, ADP, AMP, and glycolytic intermediates

Fresh blood was collected in heparin and deproteinized extracts were prepared according to Oelshlegel et al. (25) by adding 1 vol of 1 N perchloric acid to 1 vol of blood. The extracts were then centrifuged, the supernates neutralized to a pH ≅7.5, (with a solution of 0.5 M triethanolamine and 2 M K_2CO_3), and the precipitated perchlorate removed.

2,3-DPG assay. 2,3-DPG was assayed enzymatically according to Rose and Liebowitz (26), i.e., by determination of the 3-phosphoglycerate liberated by the DPGP activity of rabbit muscle phosphoglycerate mutase after its stimulation by 2-phosphoglycolic acid. The 3-phosphoglycerate produced was assayed by coupling the reaction to enolase, pyruvate kinase, and lactate dehydrogenase, and measuring the oxidation of NADH in the latter reaction. The incubation mixture (1 ml) contained Tris-HCl buffer, pH 7.5 (16 μmol), KCl (3 μmol), $MgCl_2$ (4 μmol), ADP (1 μmol), NADH (0.1 μmol), 2-phosphoglycolic acid (1 μmol), enolase (4 U), pyruvate kinase (4 U), lactate dehydrogenase (5.5 U), and 20 μl of each neutralized and deproteinized extract. The reaction was initiated by the addition of phosphoglycerate mutase (12.5 U). The blank contained the complete assay mixture with the exception of 2-phosphoglycolic acid which was omitted.

ATP, ADP, AMP, and glycolytic intermediates from erythrocytes were measured according to Oelshlegel (25) in the same extracts as those employed for assay of 2,3-DPG.

Incubation of erythrocytes with inosine, inorganic phosphate, and pyruvate

Blood stored in sterile flasks containing acid-citrate-dextrose (ACD) was supplemented with 10 mM inosine, 4 mM disodium phosphate, and 4 mM pyruvate according to Oski et al. (27) and incubated at 37°C for 3 h. At the start of incubation and after 2 h, 2-ml portions of the blood were removed and deproteinized. The perchloric acid-treated samples were then processed, neutralized as described above, and subsequently assayed for 2,3-DPG, ATP, fructose 1,6-diphosphate (FDP), triose phosphates (TP), glucose 6-phosphate (G6P), and fructose 6-phosphate (F6P).

Concentrated preparation of the enzymes

Because of the undetectable activity of DPGM and DPGP in the hemolysate from the propositus, a more concentrated solution was prepared to detect any trace amounts of these enzymes that might be present. For this purpose hemolysates were passed through a carboxy-methyl Sephadex column (Pharmacia Fine Chemicals, Piscataway, N. J.) equilibrated with a 10-mM, sodium-phosphate buffer at pH 6.5. The first protein fractions eluted free of hemoglobin usually con-

TABLE I
Hematological Data of the Family

Individuals	Age	Hb	Erythrocytes	Packed cell volume	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Reticulocytes
	yr	*g/dl*	*×10¹²/liter*	*%*	*fl*	*pg*	*g/dl*	*×10⁹/liter*
C. M. (propositus)	42	17.4	5.32	54	94	32	33.5	53.10
V. M. (son)	5	14.6	4.91	43.6	89	29.5	33.3	69.10
L. M. (daughter)	4	14.3	5.14	43	83	27.0	32.9	95.10

tained the entire DPGM and DPGP activities. These fractions were pooled, concentrated by ultrafiltration, and then submitted to electrophoresis.

Electrophoretic study

Electrophoresis of DPGM, DPGP, and MPGM were carried out according to methods previously described (8) on Cellogel strips in a 0.075-M Tris-EDTA-citric acid buffer (pH 8.0) at 220 V for 3 h. The samples applied on the strips were 5 μl of 1/15 hemolysate in the case of MPGM, and 5-10 μl of the concentrated preparation of DPGM and DPGP. After electrophoresis, each enzyme activity was revealed by pouring 4 ml of a mixture containing 1% agarose, in the same medium as that used for the enzyme assay, over the respective strip. The fluorescent band of DPGM activity and the dark bands of DPGP and MPGM activities were detected with ultraviolet light (≅340 nm) after 5–30 min incubation at 25°C.

RESULTS

Case report. C. M., born in Paris in 1936 of French extraction, was found on routine testing in 1974 to have a hemoglobin level of 19.0 g/dl blood. He was then treated by phlebotomy every 2 mo for 2 yr and was referred in April 1977 to the Henri Mondor Hospital. Except for ruddy cyanosis, he was normal upon physical examination. His spleen was not enlarged and no renal mass was apparent. Extensive cardiac and pulmonary investigations revealed no abnormality and his resting cardiac output was normal. Arterial blood gas values were normal (P_{O_2}, 92 mm Hg; P_{CO_2}, 45 mm Hg; oxygen saturation, 96.5%). The absence of hemolysis was shown by the normal levels of haptoglobin and bilirubin. The hematological findings are detailed in Table I. In brief, they show an hematocrit of 54%, normal erythrocyte morphology, and normal leukocyte and platelet counts; the reticulocyte count was 1.2% (uncorrected). Total blood volume, erythrocyte volume, and plasma volume were 88.0 ml, 44.0 ml, and 41.0 ml/kg body weight, respectively, thus revealing an absolute erythrocytosis. Analysis of the propositus' hemoglobin by the sensitive isofocusing method (28) did not show the presence of an abnormal hemoglobin. The level of hemoglobin A_2 (2.3%) was normal. The oxygen affinity curve of his stripped hemolysate was normal. In contrast, the oxygen affinity of his whole erythrocytes, as determined on the Hem-O-Scan, was increased (Fig. 1), the partial pressure of oxygen at which hemoglobin is half saturated with O_2 (P_{50}) being 17.3 mm Hg at pH 7.4 and P_{CO_2} of 5% (normal values: 26.5±1 mm Hg). The contrast between the normal oxygen affinity of his stripped hemolysate and the high oxygen affinity of his intact erythrocytes was explained by the level of erythrocyte 2,3-DPG which was ≅3% of the normal mean (Table II).

Family study. The two parents of the propositus could not be studied. His 5-yr-old son and his 4-yr-old daughter were examined. In the absence of clinical

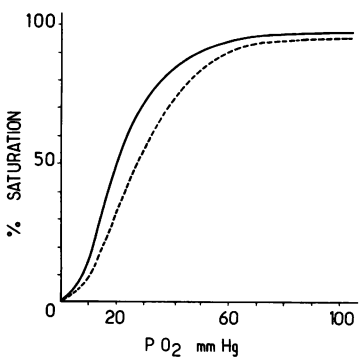

FIGURE 1 Oxygen dissociation curves of 2 μl of whole blood freshly drawn from propositus (——) and control (– – –). Measurements were performed with a Hem-O-Scan; 2 μl of samples; temperature 37°C; CO_2, 5%; pH 7.4.

TABLE II
Family Study of the Correlation between 2,3-DPG and P_{50} Values

	2,3-DPG	P_{50}
	μmol/g/Hb	*mm Hg*
Propositus	0.4	17.3
Son	9.2	19.5
Daughter	10.6	22.5
Normal adult values	15±2	26.5±1

2,3-DPG concentrations are expressed as micromoles per gram of hemoglobin. The P_{50} is expressed in millimeters of mercury at which hemoglobin is half saturated with O_2.

abnormalities, their hemoglobin and hematocrit (Table I) were in the upper range of normal for this age group. This can be related to their erythrocyte 2,3-DPG levels, which were found to be decreased, relative to the normal values. The erythrocyte P_{50} values were in accordance with the intracellular 2,3-DPG levels (Table II).

Erythrocyte enzymatic study. The very low level of 2,3-DPG in the propositus erythrocytes led us to study the three enzymes which involve this compound in their reactions, i.e., DPGM, DPGP, and MPGM. The results are summarized in Table III.

The DPGM activity of the propositus erythrocytes was undetectable under the usual conditions even after recording NADH formation for 30 min. To detect a very low DPGM activity, a more sensitive technique was employed which involved measurement of the 2,3-DPG synthesized. We did not, however, detect any additional formation of 2,3-DPG even after 30 min of incubation. It could be concluded that, at least under these conditions, DPGM activity was undetectable. In the propositus' two children, erythrocyte DPGM activity was half the normal value.

DPGP activity was also undetectable in the propositus' erythrocytes and was half of the normal value in the two children.

MPGM activity was also about half of normal in the propositus' erythrocytes and close to the normal in his two children.

Activities of other enzymes. Enzymes of the Embden-Meyerhof pathway, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, adenosine deaminase, adenylate kinase, pyrimidine 5′-nucleotidase, and acetylcholinesterase were within normal limits in both the propositus' and in his children's erythrocytes.

Study of some other erythrocyte compounds. The levels of the glycolytic intermediates, ATP, ADP, and AMP in the propositus' erythrocytes are shown in Fig. 2. The very high levels of TP and FDP contrast with the small amounts of G6P and F6P. The amount of

TABLE III
Family Study of DPGM, DPGP, and MPGM

	DPGM	DPGP	MPGM
		U/g Hb/min	
Propositus	0	0	27
Son	2.53	1.17	36
Daughter	2.45	1.15	39
Normal adult values	5.72±0.68	2.64±0.45	46±8

The activities of DPGM are expressed as micromoles per gram of hemoglobin per minute of NADH formed in the reaction, whereas those of DPGP and MPGM are micromoles of NADH oxidized per gram of hemoglobin per minute during the reactions.

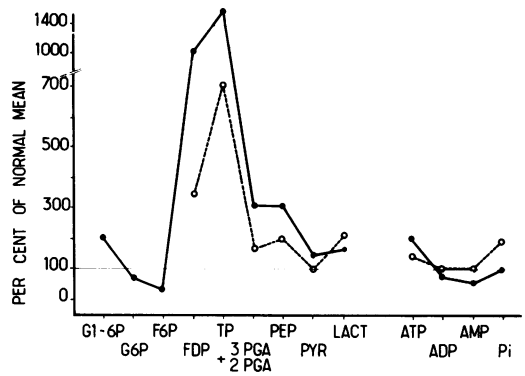

FIGURE 2 Crossover plot of the concentration of glycolytic intermediates in the erythrocytes from the propositus (— ● —) and from his son (– – ○ – –). The glycolytic compounds were determined after deproteinization in neutralized acid extracts on fresh heparinized blood. Results are expressed for each intermediate as a percentage of the normal mean calculated from 10 controls. PEP denotes phospho-enol-pyruvate; Pi denotes inorganic phosphates; PYR represents pyruvate; LACT denotes lactate.

ATP is higher than normal, whereas those of ADP and of AMP are lower. The inorganic phosphate level was normal but the concentration of reduced glutathione was 70 mg per 100 ml of erythrocytes, a value higher than normal (50±11 mg).

Effect of storage. Storage in ACD of normal erythrocytes induces a rapid decrease in 2,3-DPG content and significant alteration in the levels of several phosphorous compounds (Fig. 3). In the erythrocytes of the propositus after 2 wk storage, the concentrations of these compounds were strikingly modified and had become very close to that of the control. More precisely, the values of FDP and TP which were markedly in-

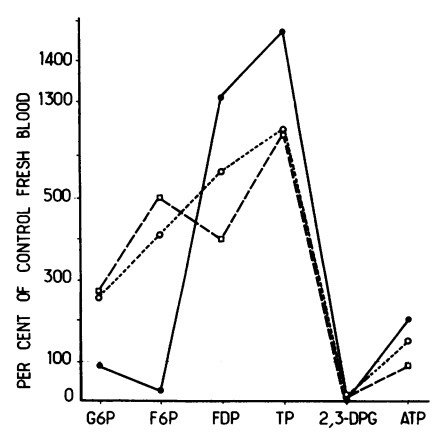

FIGURE 3 The concentrations of glycolytic intermediates in heparinized fresh blood (— ● —) and in ACD-collected blood from the propositus (– – ○ – –), after 2 wk storage, are compared to those in ACD collected blood from a control (– – □ – –), after the same period of storage. Results are expressed as a percentage of the concentrations in normal fresh blood.

creased in his fresh blood fell close to those of the control, whereas the concentration of G6P rose in a similar manner to that in the control.

Incubation with inosine, inorganic phosphate, and pyruvate. The results of incubation of the propositus' erythrocytes with inosine, inorganic phosphate, and pyruvate are compared to those of a control in Table IV and Fig. 4. A slight increase in 2,3-DPG concentration was detected in the propositus' erythrocytes, while its level was markedly elevated in the control, to as much as three times the normal value. In addition, all the glycolytic intermediates were increased in concentration in both the propositus' and in the control's erythrocytes, but their relative levels differed. In the control's erythrocytes, G6P showed the greatest elevation of all the ester phosphates, whereas in the propositus' cells, FDP was the most increased.

Electrophoretic study. Although the activities of erythrocytes DPGM and DPGP were undetectable in the propositus and low in his children, we attempted to study the electrophoretic pattern of these enzymes. Despite the use of highly concentrated preparations, we could not detect any band of DPGM activity in the sample from the propositus. On the contrary, one band of enzymatic activity appeared in his children's erythrocytes and this band migrated as did the control sample. Similarly DPGP activity did not appear in the sample from the propositus but was revealed in his children's erythrocytes as one band that moved as the control moved and to the same position as the band of DPGM activity. Electrophoresis of MPGM activity did not necessitate the use of a concentrated preparation as did DPGM because MPGM activity was sufficiently high in the propositus' erythrocytes to permit its identification in crude hemolysates. The pattern obtained from the propositus was different from that of his children and from the control (Fig. 5). Three bands of MPGM activity appeared in the children's hemolysates, showing a pattern identical to that of the control, whereas only two bands were detected in the hemolysate from the propositus. These bands did not show any modification of charge when compared with the

TABLE IV
*2,3-DPG Level in Erythrocytes of Control and Propositus Incubated with Inosine, Pyruvate, and Inorganic Phosphate**

	Erythrocytes	
	0 Time	2 h
	μmol/ml	
Propositus	0.12	0.55
Control	1.88	6.91

* See conditions in legend of Fig. 4.

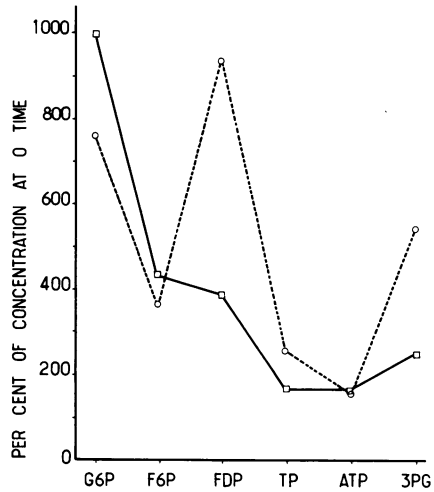

FIGURE 4 The levels of glycolytic intermediates, in ACD blood stored for 2 wk, after incubation in 10 mM inosine, 4 mM Na_2 PO_4, and 4 mM pyruvate at 37°C for 2 h. Deproteinized extracts were made both before and after 2 h incubation. The values of the intermediates after 2 h incubation are expressed as percentages of their respective concentrations at 0 time. — □ — Denotes control erythrocytes and - - ○ - - represents propositus erythrocytes.

corresponding bands from the control hemolysate. It should be observed that the band that was absent in the propositus corresponded to that which has been demonstrated to carry DPGM and DPGP activities (8).

DISCUSSION

This paper reports the first case of a homozygous deficiency of erythrocyte DPGM and the associated low level of 2,3-DPG. This diminished 2,3-DPG value resulted in an increased erythrocyte oxygen affinity and a moderate compensatory erythrocytosis. The defect

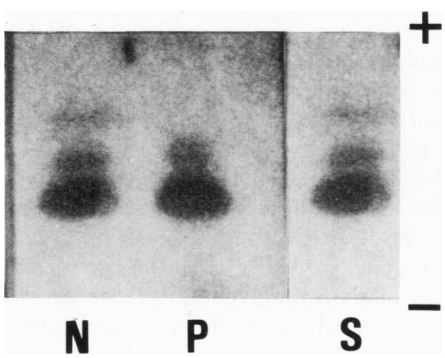

FIGURE 5 Electrophoretic patterns of MPGM activity from the hemolysates of a control (N), of the propositus (P), and of his son (S). Electrophoresis was performed on cellulose acetate strips with a Tris-EDTA citric buffer at pH 8.0. MPGM specific staining and detection with ultraviolet light at ≅340 nm revealed the MPGM activity as dark bands on a fluorescent background.

was well tolerated by the propositus who exhibited no sign of hypoxemia, displayed normal activity, and spent several holidays at high altitude without difficulty. Such a situation closely resembles that presented by subjects heterozygous for a hemoglobin with high oxygen affinity in whom the disorder is mostly benign. There was no evidence for hemolysis in our propositus nor in his children in contrast with the cases reported by Schröter (13) and Travis et al. (14) who have described two unrelated families having low DPGM activities associated with hemolysis. In the case of Travis et al., an associated disorder responsible for the described microspherocytosis could cause the hemolysis.

The crossover plot of the levels of glycolytic substrates in the propositus' erythrocytes (Fig. 2) documents the effect of the absence of 2,3-DPG on erythrocyte glycolysis. This plot is characterized by high levels of TP, FDP, and phosphoglycerates which contrast with low levels of both G6P and F6P. Similar modifications, though less pronounced, have been found in the children's erythrocytes, and also in an unrelated heterozygous propositus (12). Again these results contrast with those obtained by Travis et al. (14) who did not find any modification in the glycolytic substrates of their patient's erythrocytes. In our propositus, the markedly increased amounts of TP and FDP are probably the consequence of an accumulation of 1,3-phosphoglycerate (1,3-DPG). Unfortunately a direct determination of this phosphorus compound could not be performed on account of its lability in the extracts. Such an elevation is, however, highly probable because under normal conditions more than half of the 1,3-DPG passes through the Rapoport shunt (29), which is blocked in the propositus' erythrocytes. An additional mechanism could also explain the high levels of FDP in the propositus' erythrocytes and the low levels of G6P and F6P. Thus, these modifications could arise from a stimulation of phosphofructokinase which could be repressed in normal erythrocytes by 2,3-DPG (30). Such a stimulation of phosphofructokinase would also contribute to the high levels of TP and of 1,3-DPG.

The total quantity of 1,3-DPG would then become available to phosphoglycerate kinase and be directed towards lactate formation, thereby increasing both the level of the intermediary products and the rate of ATP formation. Thus, the high level of ATP in the propositus' erythrocytes probably is the consequence of the stimulation of phosphoglycerate kinase as a result of the increase of available 1,3-DPG. In addition, the very high concentration of FDP, a well-known activator of pyruvate kinase, should contribute to this elevation of ATP.

The low concentration of G6P in the propositus' erythrocytes is not consistent with the inhibitory effect of 2,3-DPG on hexokinase found by some authors (31). This apparently contradictory finding is probably because of an increased utilization of G6P which is produced in excess by stimulated hexokinase and passes through the hexose monophosphate shunt; this pathway should itself be stimulated by the low level of 2,3-DPG (32). Such a stimulation could also contribute to the large amounts of TP, which are a convergence point of the pentose and Embden-Meyerhof pathways.

Another problem concerns the increase in glucose 1,6-diphosphate level that is related to the decrease in G6P concentration. This apparently paradoxical finding could be explained by a stimulation of phosphoglucomutase; the phosphorylated form of this enzyme reacts with G6P to give a glucose 1,6-diphosphate-enzyme complex that is subsequently transformed into glucose 1-phosphate and phospho-enzyme (PE) according to the two-step mechanism: (*a*) G6P + PE $\rightleftarrows$ glucose 1,6-di-PE, (*b*) Glucose 1,6-di-PE $\leftrightarrows$ glucose 1-P + PE. Thus, under normal conditions, glucose 1,6-diphosphate is not released during the course of the normal reaction. As demonstrated by Alpers (33), phosphoglucomutase may be phosphorylated by 1,3-DPG when the latter is present at a high concentration in the medium. Under these conditions, it competes with glucose 1,6-diphosphate which is released from the enzyme complex by the following sequence: (*c*) Glucose 1,6-di-PE + 1,3-DPG $\leftrightarrows$ glucose 1,6-di-P + PE + 3-phosphoglyceric acid. Thus, the PE can again react with G6P, the level of the latter decreasing in favor of the formation of glucose 1,6-diphosphate. Such an eventuality could be considered in the propositus' case because a high concentration of 1,3-DPG could result from the DPGM defect.

It is of some interest to compare the levels of glycolytic intermediates in the erythrocytes from the propositus with those from some animal species that exhibit a low level of 2,3-DPG in their erythrocytes (34). The concentrations of these compounds in the erythrocytes of these animal species are quite different from those found in the propositus. These data apparently indicate that the role played by 2,3-DPG in regulating the metabolite flow inside the erythrocytes is not of predominant importance. In all probability the observed differences are a result of enzymatic ratios that vary from one species to another. Some other different data can be observed in human erythrocytes that have been stored in ACD solution and that exhibit a fall in their 2,3-DPG concentration (34). FDP and TP were slightly increased in this condition. Moreover, the erythrocytes of the propositus, when stored under the same conditions, showed a fall in the level of all their glycolytic compounds which were originally elevated, i.e., FDP, TP, and 3-phosphoglyceric acid, leading to an increase in lactate. On the contrary, when the erythrocytes from the propositus and from the control were incubated in a medium containing inosine, inorganic phosphate, and pyruvate, the resulting increases in

FDP, TP, and 3-phosphoglyceric acid were more elevated in the propositus' than in the control cells, whereas the level of G6P was more increased in the latter. Thus, the erythrocytes of the propositus appear to represent unique material in which to evaluate the complex metabolic interrelationships within erythrocytes.

Small but measurable amounts of 2,3-DPG are present in the propositus' erythrocytes. These results must be correlated with the absence of any detectable DPGM activity. Perhaps this discrepancy results from the fact that our technique for estimation of DPGM is unable to detect the minute amounts of DPGM activity which are nevertheless sufficient to produce the low quantities of 2,3-DPG present in the propositus' erythrocytes. An alternative explanation arises from the observation of Laforet et al. (35), according to which the low quantities of 2,3-DPG could be synthesized by the MPGM in erythrocytes. It should be recalled that these results could not be reproduced by Sheibley and Hass (36) with purified enzyme.

Electrophoretic patterns have shown that bands of DPGM and DPGP were absent from the propositus' erythrocytes, confirming previous reports that provided evidence that DPGM contained DPGP activity (6, 9, 10). The absence of any detectable DPGP activity confirms the results of Hass and Miller (37) who concluded that only one DPGP existed in erythrocytes. These data are not in accordance with the observations of Sheibley and Hass (36) on the purified enzyme, but the techniques used by the latter to determine DPGP differed from our own and thus could explain these divergent findings. Such a DPGM deficiency should be a result either of an absence of synthesis of this molecule, or of the existence of an unstable molecule, or of the presence of an inactive enzyme. Detection of eventual cross-reactive material should be undertaken to try to solve this problem.

The data on MPGM values in the erythrocytes of our family are of interest. They involve MPGM values close to normal and a normal electrophoretic pattern in the children's erythrocytes, which contrasts with a diminution of 50% in the MPGM activity of the propositus' cells and with a disappearance of the most anodic band on the MPGM electrophoretic pattern. Its absence is consistent with our previous demonstration (8) that this band corresponded to a MPGM activity carried by the DPGM-DPGP molecules. Nevertheless, because the part of MPGM activity carried by DPGM represents only 5% of the total MPGM activity of the erythrocytes, the DPGM deficiency cannot explain the absence of 50% of MPGM activity in the propositus' cells. Also intriguing are the MPGM values close to normal found in the children's erythrocytes. To solve this problem, one can formulate the hypothesis that either a lack of synthesis or a modification of

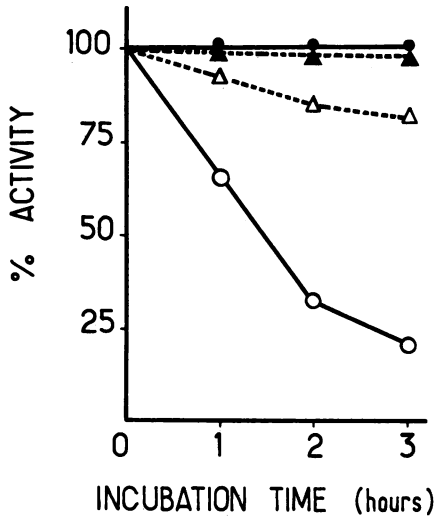

FIGURE 6 Heat stability of MPGM as a function of time in crude 1/15 hemolysates from the propositus (— ○ —) and from control (- - △ - -) and after addition of 5 mM 2,3-DPG in hemolysates from the propositus (— ● —) and from control (- - ▲ - -). Incubation was performed at 50°C. The noncentrifuged hemolysates contained 0.7 mM β-mercaptoethanol and 1 mM EDTA. Results are expressed as a percentage of the MPGM activity of each nonincubated hemolysate.

a peptide chain common to DPGM and MPGM has occurred. The structures of these two enzymes have been partially studied and their molecules have been found to be constituted of two chains of the same molecular weight (10, 36, 38). However, the molecular weights of the two chains of MPGM differed from those of the DPGM chains (39). Under these conditions, the hypothesis of a common chain seems an unlikely explanation.

A more attractive hypothesis involves a possible protective effect of 2,3-DPG on MPGM. After consideration of the results obtained by Omenn and Hermodson (40) in brain and by Borders and Wilson (41) in erythrocytes, we have studied the effect of 2,3-DPG on MPGM thermostability in hemolysates from the propositus and from a control (Fig. 6). In the absence of 2,3-DPG in the hemolysate, the MPGM of the propositus lost about 70% of its activity after 2 h at 50°C, whereas that of the control lost only 15%. In the presence of 2,3-DPG, no loss of MPGM activity could be detected either in the propositus or in control. These preliminary results lead us to suppose that the partial MPGM deficiency observed in the propositus' erythrocytes is because of the low level of 2,3-DPG. Thus, MPGM would be normally synthesized in the erythroblasts of the propositus, but as a result of the DPGM deficiency resulting in insufficient concentration of 2,3-DPG this enzyme would be unstable and would partially lose its activity during erythrocyte maturation. Because the children's erythrocytes contain apprecia-

ble amounts of 2,3-DPG, substantial denaturation of MPGM should not occur.

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