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FAVISM-PRODUCING FAST VARIANT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN POLAND

PRELIMINARY REPORT[1]

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In all, 24 variants (alleles) of X-linked erythrocyte glucose-6-phosphate dehydrogenase have been described in man (Betke *et al.* 1967). Nine of them apparently do not change red blood cell life span. The other nine do not affect the red blood cell life span in normal conditions, yet cause an acceleration of hemolysis in homo- and hemizygotes after the use of oxidant drugs, aniline derivatives ("primaquine sensitivity"). Six rare variants of glucose-6-phosphate dehydrogenase cause in hemizygotes congenital nonspherocytic hemolytic anemia. Some variants in the group of "primaquine sensitivity" predispose to the hemolytic disease known as favism, which may occur after ingestion of fava beans, or even after the inhalation of fava bean pollens. The latter reaction has been described up to now only in populations with high incedence of the alleles: $Gd^{Mediterranean}$ in South Europe and Middle East (Betke *et al.* 1967; Maj, 1965), Gd^{Athens} in Greece (Harris, 1969; Stamatoyannopoulos *et al.*, 1966), and Gd^{Canton} in South-East Asia (Harris 1969; Ochocka, Chmielewska 1968).

In Poland, frequency of the mutant gene for glucose-6-phosphate dehydrogenase in erythrocytes seems to be very low: a few families with congenital nonspherocytic hemolytic anemia and three families with favism have been described so far (Franczak and Sławińska 1967; Jabłońska *et al.*, 1964; Maj, 1965; Ochocka and Chmielewska, 1968; Pawlak and Szydłowski, 1966; Rokicka, Michałowska *et al.*, 1968; Rożynkowa, 1969, Urasiński *et al.*, 1966).

In the present paper preliminary characterization of favism-producing variant in the population of Poland is being reported.

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MATERIAL AND METHODS

The glucose-6-phosphate dehydrogenase deficient test subject J. M., male, 57, was in good condition during the study. Ahemolytic crisis after ingestion of fava beans in both his grandsons has been reported (Franczak and Sławińska, 1967). Blood of a normal male, 39 years old served as a control. The blood samples were collected on ACD (acid citrate dextrose; citric acid 0.8, glucose 2.45, natrium citrate 2.2, H_2O ad 100,0) solution and kept at 4°C.

Assays of glucose-6-phosphate dehydrogenase activity were carried out according to the method of Zinkham *et al.* (1958). Hemolysates for measuring the enzyme activity were prepared by adding 9 volumes of hemolysing solution (NADP 10 μM, beta mercaptoethanol 7mM, EDTA sodium, pH 7.0 - 2.7 mM) to 1 volume of washed, packed red blood cells (Betke *et al.*, 1967). Readings were taken with a "Spectromom 202" spectrophotometer at 60 sec intervals, 340 mμ and 25±2°C.

Partial purification of the erythrocyte glucose-6-phosphate dehydrogenase on DEAE-cellulose, according to the procedure of Kirkman (1962), was started two hours after collecting the blood. Twenty-ml samples of ACD-blood from both subjects were used as the source of the enzyme. The enzyme-containing precipitates were suspended in 1 ml of ammonium sulphate solution and dialysed for 4 hrs. All operations were carried out at 4°C. The dialysed mutant enzyme was obtained in a final volume of 1.5 ml, its activity was 0.002 EU/ml. The normal enzyme was diluted to the concentration of 0.006 EU/ml.

Starch-gel electrophoresis was carried out on a vertical system, as described by Smithies (1959), using Tris-chloride buffer, pH 8.4, according to Kirkman and Hendrickson (1963). 20 - 30 μl samples of the dialysed enzyme solution were placed in one slot. Electrophoresis was performed at 4°C and 12 mA current. The duration of the run was 16 hrs. Following electrophoresis, the gel was stained while protected from light, for about 90 min. at 37°C with MTT [3(4.5-Dimethyl Thiazolyl-2)-2.5-diphenyl Tetrazolium Bromide, Sigma] stain according to Matthai *et al.*, (1966).

For K_m G-6-P determination the rate measurements were carried out on purified enzyme preparations in the standard reaction mixture (Zinkham *et al.*, 1958), with several different glucose-6-phosphate (natrium salt, Boehringer) concentrations, varying from 0.2 mM to 2 mM.

RESULTS

Trace amounts only of glucose-6-phosphate dehydrogenase activity (0,1 - 0,4% of normal) heve been found in the hemolysate of J.M. Mobility of the partially purified mutant enzyme from J.M., following electropho-

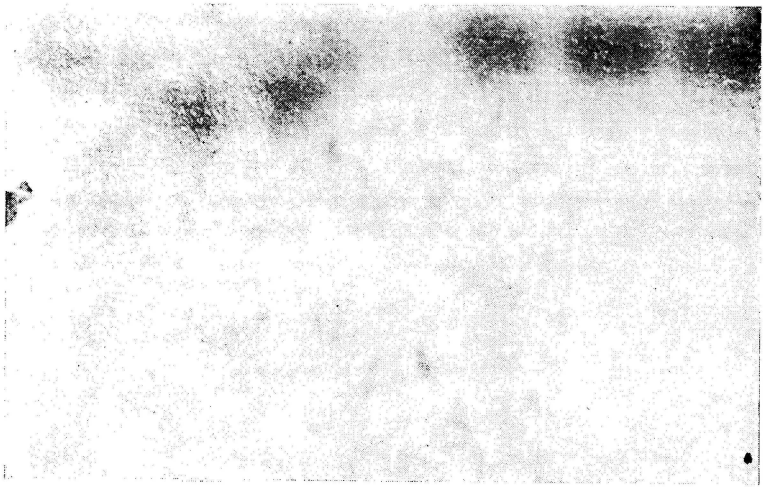

Fig. 1. Electrophoretic mobility of glucose-6-phosphate dehydrogenase. Slots from right to left: 1, 5, 6, 7 — mutant enzyme (J. M.); 2, 3 — normal enzyme; 4 — no sample

resis at pH 8.4, was estimated to be about 105 - 110% of the normal (Fig. 1). K_m G-6-P of the mutant glucose-6-phosphate dehydrogenase has been estimated to be 26 μM (Fig. 2).

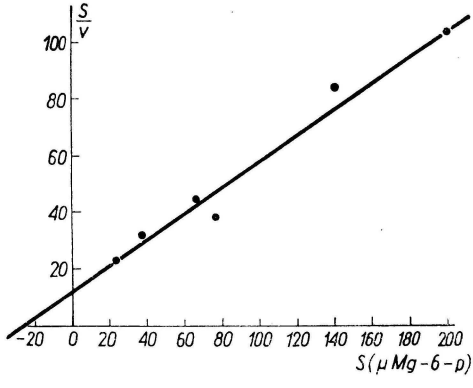

Fig. 2. K_m G-6-P of mutant (J. M.) erythrocyte glucose-6-phosphate dehydrogenase

DISCUSSION

In Table 1, the results characterizing the enzyme under study are compared with the corresponding values of the normal Gd^B and of the three glucose-6-phosphate dehydrogenase variants known to produce favism.

The enzyme activity in hemolysates of the studied subject (J.M.) is

similar to that in homo- and hemizygotes of $Gd^{Mediterranean}$. On the other hand, only Gd^{Canton} resembles the described one in respect of its electrophoretic mobility. The K_m for G-6-P of the enzyme under study is found to be both in the range described for $Gd^{Mediterranean}$ and in that for

Table 1. Partial Characterization of the Studied Enzyme (J.M.) and of Some Other Variants of Human Erythrocyte Glucose-6-phosphate Dehydrogenase

Reference	Variant	Activity in hemolysates (% of normal)	Electrophoretic mobility (% of normal)	K_m G-6-P (μM)
1	Gd^B	100%	100%	50 - 78
—	J.M. (studied)	0.1 - 0.5	105 - 110	26
1	$Gd^{Mediterranean}$	0 - 5	100	19 - 26
17	Gd^{Athens}	12 - 45	98	16 - 19
1	Gd^{Canton}	4 - 24	105	20 - 36

Gd^{Canton}. The mild Greek type of deficiency with the glucose-6-phosphate dehydrogenase activity in hemolysates between 12 and 45% of normal is heterogenous (B e t k e *et. al.*, 1967). The fast moving, but still uncharacterized variant, different from well characterized Gd^{Athens}, was found in a male with this type of deficiency (S t a m a t o y a n n o p o u l u s *et. al.*, 1967). Nevertheless activity of the studied variant in hemolysates does not fall into the range described for the mild Greek type of deficiency.

These results may suggest the presence of a new defective allele at the glucose-6-phosphate dehydrogenase locus in J.M. Further characterization is required for definite differentiation of this enzyme from the known variants.

SUMMARY

A favism-producing fast-moving variant of erythrocyte glucose-6-phosphate dehydrogenase has been described in a male from the Polish population. Electrophoretic mobility of the variant at pH 8.4 has been found to be 105 - 110% of normal, K_m for G-6-P was 26 μM. Enzyme activity in hemolysates has been found 0.1 - 0.4% of normal. A possibility that the enzyme is distinct from the known favism-producing variants of erythrocyte glucose-6-phosphate dehydrogenase has been subject to discussion.

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Streszczenie

ODMIANA DEHYDROGENAZY GLUKOZO-6-FOSFORANOWEJ O ZWIĘKSZONEJ RUCHLIWOŚCI ELEKTROFORETYCZNEJ WYWOŁUJĄCA OBJAWY FAWIZMU

Podaje się wstępną charakterystykę odmiany dehydrogenazy glukozo-6-fosforanowej erytrocytów o zwiększonej ruchliwości elektroforetycznej (105 - 110% normy w pH 8.4), stwierdzonej u mężczyzny z polskiej populacji. W rodzinie badanego opisano występowanie objawów fawizmu. Aktywność enzymu w hemolizacie wynosiła 0.1 - 0.4% normy; K_m dla glukozo-6-fosforanu — 26 μM. Omawia się różnice pomiędzy właściwościami badanego enzymu a właściwościami znanych odmian dehydrogenezy glukozo-6-fosforanowej powodujących występowanie objawów fawizmu.