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Polish Variant of Glucose-6-Phosphate Dehydrogenase (G-6-PD Lublin)

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Summary. A new, favism-inducing variant of glucose-6-phosphate dehydrogenase in erythrocytes is described in a Polish family. The enzyme activity has been 0—4% of normal. The enzyme displayed normal heat stability, electrophoretic mobility 105—110% of normal, K_m for NADP: 16—22 μM, K_m for G-6-P: 26 μM, and the utilization of 2-deoxy-G-6-P: 2—3%.

In Poland the frequency of the mutant genes for glucose-6-phosphate dehydrogenase (G-6-PD) in erythrocytes seems to be very low. Totally 5 families with congenital nonspherocytic hemolytic anemia due to G-6-PD deficiency and 4 families with favism have been described so far (Horst, 1970). A favism-producing variant of G-6-PD found in 1 of these families has been characterized by us.

Family Data

The presently investigated male, J. M., 58, apart from anemia and jaundice after ingestion of fava beans in 1957, has not showed signs nor complained of symptoms suggesting accelerated hemolysis. Hemolytic crises after ingestion of fava beans have been reported in two of his grandsons (Franczak and Sławińska, 1967; Rożynkowa *et al.*, 1970). The G-6-PD activity in red blood cells of these boys has been 2 and 4% of normal activity.

Materials and Methods

Partial purification of the G-6-PD from erythrocytes of J. M. as well as studies of the enzyme properties were done according to the methods recommended by Beutler *et al.* (1968). Following chemicals were used: Cellulose DEAE 11 (Whatman); NADP and G-6-P (both natrium salts, Boehringer); phenazine methosulphate, 2 mercaptoetanol (type I), 2-deoxy-G-6-P (natrium salt) and MTT (Sigma). The determinations of substrate and coenzyme concentrations were performed with G-6-PD purified in our laboratory. Hydrolysed starch was prepared according to Smithies (1955). Electrophoresis was performed in the vertical system.

Results

The activity of G-6-PD in hemolysate of J.M. has been 0—2% of normal activity. The electrophoretic mobility of the variant enzyme on starch gel in a tris chloride buffer pH 8,4 has been 105—110% of normal (Fig. 1). K_m for NADP has been 16—22 μM, K_m for G-6-P 26 μM and the utilization of 2-deoxy-G-6-P

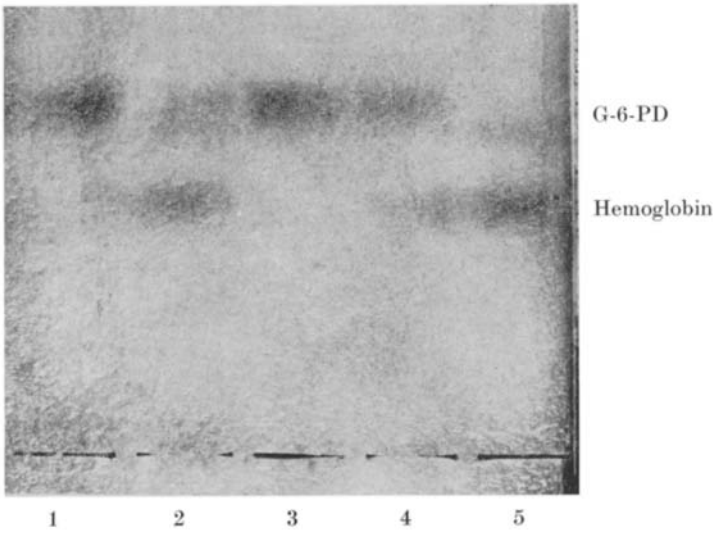

Fig. 1. Electrophoretic mobility of erythrocyte glucose-6-phosphate dehydrogenase. *1, 3, 4* purified enzyme of J.M. (G-6-PD Lublin), *5* control hemolysate, *2* purified enzyme of J.M. and control hemolysate

has been 2—3% of utilization of G-6-P as a substrate. Heat stability of the enzyme at 44°C has been as follows (in per cent of the initial activity): 100 after 20 min, 85 after 40 min and 67 after 60 min (corresponding values for control enzyme have been 90, 90 and 75).

Discussion

The table collates the properties of the studied enzyme and of established G-6-PD variants having similar electrophoretic mobilities and decreased activity. All variants discussed below appear distinct from the investigated one. The electrophoretic mobility, K_m for G-6-P, utilization of 2-deoxy-G-6-P and heat stability of the studied enzyme resemble the data for G-6-PD A— variant (Yoshida *et al.*, 1967). However, lower activity in hemolysates and higher values of K_m for NADP as well as the hemolysis after ingestion of fava beans in our case show the difference. In G-6-PD Union (Motulsky and Yoshida, 1969) and G-6-PD Markham (Kirkman *et al.*, 1968) very high utilization of 2-deoxy-G-6-P (160—220%) and low K_m for G-6-P (4—12 μM as compared to 26 μM in our studies) have been described. G-6-PD Canton seems also to be different because of the greater utilization of 2-deoxy-G-6-P (4—15%) and normal K_m for NADP (McCurdy *et al.*, 1966). In newly reported variant G-6-PD Constantine activity of the enzyme in hemolysates is about 16% of normal as compared to 0—4% in our case (Kissin and Cotte, 1970). G-6-PD Ohio shows very low heat stability and causes symptoms of chronic hemolytic anemia (Pinto *et al.*, 1966).

Discussed data support the conclusion that the studied variant of G-6-PD is distinct from variants reported in the literature. For the new allele the name Gd^{Lublin} is proposed.

Table. *Comparison of properties of the studied enzyme (G-6-PD Lublin) with properties of established G-6-PD variants having similar electrophoretic mobilities and decreased activities*

Variant	Enzyme activity in rbc (% of normal)	Electrophoretic mobility (% of normal)	K_m G-6-P (μM)	K_m NADP (μM)	2-dG-6-P utilization (%)	Heat stability	Population origin	Symptoms of favism or of C.N.H.A.[a] reported	Reference
B (normal)	100	100	50—78	2,9—4,4	< 4	normal	various	—	WHO Scientific Group, 1967
A—	8—20	110	normal	normal	< 4	normal	black race	—	Yoshida *et al.*, 1967
Canton	4—24	105	20—36	2—2,4	4—15	slightly reduced	South Chinese	—	McCurdy *et al.*, 1966
Constantine	16	110	19	1,9	4	normal	North African	—	Kissin and Cotte, 1970
Markham	1,5—10	fast	4,4—6,3	?	162—222	?	New Guinea	—	Kirkman *et al.*, 1968
Union	< 3	fast	8—12	3,6—5,6	180	?	Filipino	—	Motulsky and Yoshida, 1969
Ohio	2—16	110	slightly increased		< 4	very low	Italian	C.N.H.A.	Pinto *et al.*, 1966
Lublin	0—4	105—110	26	16—22	< 4	normal	Polish	favism	Pawlak *et al.* (this report)

[a] C.N.H.A. = congenital nospherocytic hemolytic anemia.

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