GLUCOSE-6-PHOSPHATE DEHYDROGENASE THERMOSTABILITY IN LEUKOCYTES OF NEGROES AND CAUCASIANS WITH ERYTHROCYTE DEFICIENCY OF THIS ENZYME*

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Erythrocyte glucose-6-phosphate dehydrogenase (G-6-P. D.) deficiency has been recognized, in varying frequency, among Negro and Caucasian populations in several parts of the world (Carson, et al., 1956; Childs, et al., 1958; Szeinberg, et al., 1958; Beutler, 1959; and Marks and Gross, 1959). This enzyme deficiency is associated with increased susceptibility to hemolysis following ingestion of certain agents. The trait appears to be due to a sex-linked gene of intermediate dominance.

The present study indicates that affected** Negroes and affected Caucasians differ with respect to the thermostability, as well as the level of activity, of G-6-P. D. in leukocytes. It is suggested that these findings reflect a difference in the genetic mechanisms which result in red cell G-6-P. D. deficiency in Negroes and in Caucasians.

Recently, it was reported that Negroes, with a marked deficiency in red cell G-6-P. D., had normal levels of this enzyme in leukocytes (Marks, et al., 1959). Subsequent observations (Marks and Gross, In Press) have indicated that among affected Caucasians the level of this enzyme in leukocytes is markedly lower than that of normal subjects (Table I). Affected Caucasians compared with affected Negroes also had significantly lower G-6-P. D. activities in their whole erythrocyte population and in young red cells.

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** The term affected is employed to denote subjects whose erythrocyte G-6-P. D. activity was below 4.5 standard deviations of the control mean value.

TABLE I

Glucose-6-Phosphate Dehydrogenase Activity in Leukocytes of Subjects with Normal and Low Levels of this Enzyme in Their Erythrocytes.

Subjects	No.	Leukocytes*	Erythrocytes*
Control	90	46.7 ± 18.9	15.1 ± 1.9
Affected			
Negro	43	42.1 ± 13.4	2.0 ± 1.5
Caucasian	20	15.5 ± 7.3	0.3 ± 0.3

* For leukocytes, a unit of enzyme activity is defined as the change in optical density units/min./10^9 leukocytes. For erythrocytes, a unit of enzyme activity is defined as the change in optical density units/min./gm.hemoglobin. The values indicated are mean values ± one standard deviation. G-6-P. D. activity in leukocytes and erythrocytes were determined by previously described methods (Marks, et al., 1959).

Studies in several laboratories have suggested that G-6-P. D. of affected Negroes and of affected Caucasians compared to that of normal subjects was less stable in crude hemolysates incubated at 38°C. (Carson, et al., 1956; Szeinberg, et al., 1958; Motulsky, et al., 1959; and Marks and Gross, 1959). In this investigation the thermostability of G-6-P. D. has been studied in lysates of white cells prepared from affected and normal subjects.

Erythrocytes and leukocytes were prepared from heparinized blood and lysed as previously described (Marks, et al., 1959). Hemolysates were diluted with glycylglycine buffer, pH 7.4, to a hemoglobin concentration of 0.1 gm./ml. Lysates of leukocytes were prepared from suspensions of 1 x 10^7 white cells/ml. of glycylglycine buffer, pH 7.4. In experiments designed to determine the effect of triphosphopyridine nucleotide (TPN) on the thermostability of G-6-P. D., samples of cell lysates were prepared in buffer containing concentrations of TPN varying between 1 x 10^{-4} and 1 x 10^{-8} molar. The whole lysates of red cells and of white cells were incubated at 38°C. for 3 hours. Aliquots taken

at hourly intervals were assayed for G-6-P. D. and 6-phosphogluconic dehydrogenase (6-P.G. D.) activities (Glock and McLean, 1953). Studies were performed on blood from 10 normal subjects, 8 affected Negro males and 5 affected Caucasian males.

Fifty to 80 percent of the initial G-6-P. D. activity remained in hemolysates of normal subjects, while none was detectable in hemolysates of affected Negroes after incubation for two hours (Figure 1). In 2 of 5 studies with affected Caucasians, no G-6-P. D. activity was measurable in the unincubated hemolysate. In the other 3, no enzyme activity was detected after incubation for one hour.

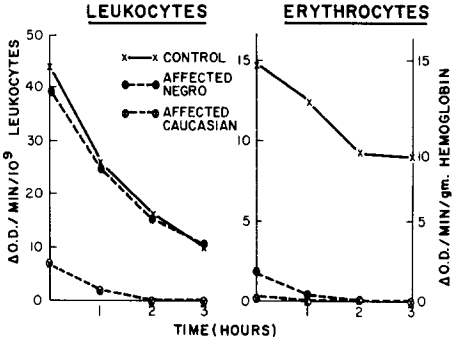

Fig. 1. Thermostability of G-6-P. D. in lysates of erythrocytes and of leukocytes incubated at 38°C.

In contrast to these findings with hemolysates, no difference was observed between affected Negroes and normal subjects with respect to G-6-P. D. stability in lysates of leukocytes (Figure 1). On the other hand, in lysates of leukocytes from affected Caucasians, G-6-P. D. activity decreased to undetectable levels after incubation for two hours. No differences were observed between normal and affected subjects with respect to 6-P.G. D. stability in either hemolysates or leukocyte lysates.

The thermostability of G-6-P. D. in hemolysates and in leukocyte lysates was enhanced by addition of TPN in a final concentration as low as 1×10^{-7} molar. In the presence of 1×10^{-5} molar TPN, G-6-P. D. activity showed no decrease in red or white cell lysates, of either normal or affected subjects, during a 3 hour incubation.

The increased heat lability of G-6-P. D. in erythrocytes and leukocytes of affected Caucasians and in erythrocytes of affected Negroes may reflect the initial low concentrations of active enzyme and not an altered thermostability of the protein. Indeed, the findings that leukocyte G-6-P. D. of normal subjects and of affected Negroes do not differ in thermostability make it unlikely that erythrocyte G-6-P. D. deficiency is a consequence of the formation of a mutant enzyme which is intrinsically more heat labile. The possibility exists that G-6-P. D. deficiency reflects an alteration in the relationship between the mutant enzyme and a factor, such as TPN, necessary to its normal stability.

The present findings suggest that there is a difference in the genetic factors determining G-6-P. D. activity in Negroes and Caucasians whose red cells are deficient in this enzyme. In addition, the expression of this enzyme deficiency is modified by cellular environment.

References

Beutler, E., Blood 44:103, 1959.

Carson, P., Flanagan, C., Ickes, C., and Alving, A., Science 124:484, 1956.

Carson, P., Schrier, S., and Alving, A., J. Lab. and Clin. Med. 48:794, 1956.

Childs, B., Zinkham, W., Browne, E., Kimbro, E., and Torbert, J., Bull. Johns Hopkins Hosp. 102:21, 1958.

Glock, G.E. and McLean, P., Biochem. J. 55:400, 1953.

Marks, P.A. and Gross, R.T., Bull. of N.Y. Acad. of Med. 35:433, 1959.

Marks, P.A. and Gross, R.T., J. Clin.Invest. 38:1023, 1959.

Marks, P.A. and Gross, R.T., J. Clin.Invest. In Press.

Marks, P.A., Gross, R.T., and Hurwitz, R.E., Nature 183:1266, 1959.

Motulsky,A., Kraut, J., Thieme, W., and Musto, D. Clinical Research 7:89, 1959.

Szeinberg, A., Sheba, Ch., and Adam, A., Nature 181:1256, 1958.