

A STUDY OF SUBJECTS WITH ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: INVESTIGATION OF PLATELET ENZYMES *

By BRACHA RAMOT, ARYEH SZEINBERG, AVINOAM ADAM, CHAIM SHEBA
AND DORA GAFNI

(From the Tel-Hashomer Hospital, Israel)

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A hereditary abnormality of the erythrocytes was described in Negroes sensitive to primaquine (1). A similar or identical defect has been detected in the erythrocytes of a considerable proportion of non-Ashkenazic Jews susceptible to favism and sensitive to various drugs (2-4). The primary defect of these erythrocytes is probably the markedly decreased activity of glucose-6-phosphate dehydrogenase (5). The cells, however, demonstrate a multitude of secondary abnormalities, namely: a low glutathione (GSH) level; glutathione instability; a decreased glycine incorporation rate into GSH *in vitro*; and an increase in glutathione reductase, aldolase and triphosphopyridine nucleotide (6-9). This hereditary erythrocyte defect is transmitted probably as a sex-linked incompletely dominant trait with various degrees of expressivity of the abnormal gene in affected females (3, 10).

In view of the importance of the hexose monophosphate shunt in glucose metabolism in various tissues and the key role of glucose-6-phosphate dehydrogenase in this metabolic pathway, it is of great interest to determine whether this genetic defect of the erythrocytes is demonstrable in other tissues of the affected subjects. In the present communication we describe the results of an investigation of glucose-6-phosphate dehydrogenase activity in platelets of normal and affected individuals.

MATERIAL AND METHODS

Blood was obtained from: a) individuals whose erythrocytes demonstrated a marked glutathione instability by the method of Beutler (11), and no glucose-6-phosphate dehydrogenase activity (most of the subjects were males); b) a control group.

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Blood was obtained and platelets were isolated by differential centrifugation as described by Gurevitch and Nelken (12). After the third washing the platelets were suspended in 1 ml. saline and were counted by a direct counting technique in duplicate (13). The platelets were then spun down at 1,300 G in an International centrifuge and 0.1 cm.³ glass powder was added to the platelet button. The platelets were then triturated for exactly one minute with a glass rod. After one minute 0.5 ml. water was added and grinding was pursued for an additional 10 seconds. Following the trituration the tubes were centrifuged for five minutes at 3,000 G. The supernatant was used for the enzyme activity determinations. All these procedures were performed at 4° C.

Glucose-6-phosphate and 6-phosphogluconic dehydrogenases were assayed by a slight modification of the Kornberg, Horecker and Smyrniotis methods, respectively (14, 15). One-tenth ml. platelet supernatant was added to 2.6 ml. Tris buffer, 0.144 M, pH 7.4, followed by 0.1 ml. triphosphopyridine nucleotide (TPN), 0.001 M, and 0.1 ml. MgCl₂, 0.3 M. The mixture was placed in a Beckmann cuvette (light path, 1.0 cm.). One-tenth ml. glucose-6-phosphate, 0.02 M, or 6-phosphogluconate, 0.02 M, respectively, was added to the cuvette. The enzymatic activity was followed by recording the increment in optical density at 340μ at 30 second intervals during three minutes or more. One unit of enzyme activity was defined as an increment in optical density of 0.001 per minute. The results were calculated per 10⁹ platelets.

RESULTS

The results are summarized in Table I. The glucose-6-phosphate dehydrogenase level of the platelets in the control group was 127.2 ± 38.5 units per 10⁹ platelets, while in the sensitive group the respective mean value was 24.0 ± 17.4 units ($p < 0.001$). On the other hand, no significant difference in the 6-phosphogluconic dehydrogenase activity was detected between the two groups of subjects ($p > 0.7$).

DISCUSSION

Genetically determined enzymatic defects raise a main question of whether the same gene con-

TABLE I
*Glucose-6-phosphate and 6-phosphogluconic dehydrogenase activity
 in platelets of normal and "sensitive" subjects*

Source of platelets	Mean G6PD* activity†	No. of samples	Mean 6PGD‡ activity†	No. of samples
Normal subjects	127.2 ± 38.5	56	75.6 ± 23.7	19
"Sensitive" subjects	24.0 ± 17.4	34	68.5 ± 28.3	15

* Glucose-6-phosphate dehydrogenase.

† In units per 10⁸ platelets ± standard deviation.

‡ 6-Phosphogluconic dehydrogenase.

trols a certain enzyme in different tissues. In congenital galactosemia the galactose-1-P uridyl transferase is absent in the red cells and liver (16). Therefore, it is of great interest to determine whether the glucose-6-phosphate dehydrogenase is absent in tissues other than red cells. The present investigation demonstrated that the hereditary deficiency of glucose-6-phosphate dehydrogenase in certain population groups is not restricted to the erythrocytes and that a similar defect is demonstrable in the platelets as well. To exclude the possibility of erroneous results due to differences in platelet fragility, Dr. Nelken was kind enough to perform a platelet fragility test on enzyme deficient platelets and found it to be normal (17). In a number of experiments pyrophosphatase activity was determined and the activity of this enzyme was found to be similar to that found in normal platelets (18). Finally, the finding of similar levels of 6-phosphogluconic dehydrogenase in normal and deficient platelets would strengthen our results. On the other hand, Marks, Gross and Hurwitz have found glucose-6-phosphate dehydrogenase activity to be normal in the leukocytes and slightly decreased in the livers of affected individuals (19). There is no explanation at present for the similarity of the findings in erythrocytes and platelets and their difference from the findings in livers and leukocytes.

Glucose-6-phosphate dehydrogenase activity declines in the erythrocyte as a function of cell age (20). Even in deficient erythrocytes the younger red cell population has a higher enzyme level than the older cells (21). The glucose-6-phosphate dehydrogenase in erythrocytes affected by the hereditary defect is more heat labile than in normal cells (4, 22). Therefore, there is a possibility that the enzymatic defect in the

erythrocytes is not due to a lack of this enzyme in the cells, but to an enzyme that is qualitatively abnormal and is degraded more rapidly. If this assumption is correct it is possible that erythrocytes and platelets that are non-nucleated cells and presumably unable to synthesize proteins could not compensate for an increased enzyme turnover, while the liver and leukocytes do have such a synthetic potential and therefore could do so. This is our working hypothesis at present. It is also possible that only cells exhibiting a decline in glucose-6-phosphate dehydrogenase as a function of their age will demonstrate this enzymatic defect.

The hereditary defective erythrocytes respond with a hemolysis *in vivo* to the administration of various drugs, such as primaquine, furofantine, para-aminosalicylic acid, sulfanilamide and sulfapyridine (1, 2). No thrombocytopenia, however, was observed by us during a hemolytic event, nor has it been described in the literature. Beutler, Robson and Bittenwieser have shown that there is a relation between the drop in GSH and the appearance of hemolysis *in vivo* (6). They have also shown that GSH of sensitive erythrocytes is destroyed when oxygenated cells are incubated with acetylphenylhydrazine. Furthermore, hemoglobin solutions prepared from red cells which have been incubated with acetylphenylhydrazine were shown to destroy GSH. The recent finding of an enzyme, GSH peroxidase, in erythrocytes that could mediate the oxidative destruction of GSH by a hemoglobin peroxide compound is of interest (23). All these data demonstrate a possible relation between hemoglobin, GSH destruction and hemolysis.

The lack of hemoglobin in platelets might be related to their immunity to the damaging effect of drugs. On the other hand, this could be re-

lated to the short survival time of platelets in general and the great capacity of the marrow to compensate for the peripheral destruction (24). The possible presence of other TPNH generating systems in platelets should also be considered.

SUMMARY

Glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activity were determined in platelets of individuals whose erythrocytes showed a deficiency of the former enzyme. A significantly lower level of glucose-6-phosphate dehydrogenase was found in these platelets, whereas the 6-phosphogluconic dehydrogenase activity was normal.

REFERENCES

1. Beutler, E., Dern, R. J., Flanagan, C. L., and Alving, A. S. The hemolytic effect of primaquine. VII. Biochemical studies of drug-sensitive erythrocytes. *J. Lab. clin. Med.* 1955, **45**, 286.
2. Szeinberg, A., Sheba, C., Hirshorn, N., and Bodonyi, E. Studies on erythrocytes in cases with past history of favism and drug-induced acute hemolytic anemia. *Blood* 1957, **12**, 603.
3. Szeinberg, A., Sheba, C., and Adam, A. Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes. *Blood* 1958, **13**, 1043.
4. Szeinberg, A., Sheba, C., and Adam, A. Enzymatic abnormality in erythrocytes of a population sensitive to *vicia faba* or haemolytic anaemia induced by drugs. *Nature (Lond.)* 1958, **181**, 1256.
5. Carson, P. E., Flanagan, C. L., Ickes, C. E., and Alving, A. S. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 1956, **124**, 484.
6. Beutler, E., Robson, M., and Bittenwieser, E. The mechanism of glutathione destruction and protection in drug-sensitive and non-sensitive erythrocytes. *In vitro* studies. *J. clin. Invest.* 1957, **36**, 617.
7. Szeinberg, A., Adam, A., Ramot, B., Sheba, C., and Myers, F. The incorporation of isotopically labelled glycine into glutathione of erythrocytes with glutathione instability. Submitted for publication.
8. Schrier, S. L., and Kellermeyer, R. W. Compensatory mechanisms in primaquine-sensitive erythrocytes (abstract). *J. clin. Invest.* 1958, **37**, 928.
9. Schrier, S. L., Kellermeyer, R. W., and Alving, A. S. Coenzyme studies in primaquine-sensitive erythrocytes. *Proc. Soc. exp. Biol. (N. Y.)* 1958, **99**, 354.
10. Childs, B., Zinkham, W., Browne, E. A., Kimbro, E. L., and Torbert, J. V. A genetic study of a defect in glutathione metabolism of the erythrocyte. *Bull. Johns Hopk. Hosp.* 1958, **102**, 21.
11. Beutler, E. The glutathione instability of drug-sensitive red cells. *J. Lab. clin. Med.* 1957, **49**, 84.
12. Gurevitch, J., and Nelken, D. ABO groups in platelets. *J. Lab. clin. Med.* 1954, **44**, 562.
13. Dacie, J. V. *Practical haematology*. London, J. & A. Churchill Ltd., 1950, p. 111.
14. Kornberg, A., and Horecker, B. L. Glucose-6-phosphate dehydrogenase in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. New York, Academic Press Inc., 1955, vol. I, p. 323.
15. Horecker, B. L., and Smyrniotis, P. Z. Phosphogluconic acid dehydrogenase from yeast. *J. biol. Chem.* 1951, **193**, 371.
16. Kalckar, H. M., and Maxwell, E. S. Biosynthesis and metabolic function of uridine diphosphoglucose in mammalian organisms and its relevance to certain inborn errors. *Physiol. Rev.* 1958, **38**, 77.
17. Nelken, D., Gilboa, N., and Gurevitch, J. Studies on osmotic fragility of human blood platelets. III. Normal values. Submitted for publication.
18. Ramot, B., and Gafni, D. Unpublished data.
19. Marks, P. A., Gross, R. T., and Hurwits, R. K. Studies on the gene action in erythrocyte glucose-6-phosphate dehydrogenase deficiency: Tissue Enzyme levels. *Nature (Lond.)*. In press.
20. Marks, P. A. A relationship between human erythrocyte aging *in vivo* and the activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenases (abstract). *J. clin. Invest.* 1957, **36**, 913.
21. Johnson, A. B., and Marks, P. A. Glucose metabolism and oxygen consumption in normal and glucose-6-phosphate dehydrogenase deficient human erythrocytes. *Clin. Res.* 1958, **6**, 187.
22. Carson, P. E., Schrier, S. L., and Alving, A. S. Inactivation of glucose-6-phosphate dehydrogenase in human erythrocytes (abstract). *J. Lab. clin. Med.* 1956, **48**, 794.
23. Mills, G. C. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J. biol. Chem.* 1957, **229**, 189.
24. Craddock, C. G., Jr., Adams, W. S., Perry, S., and Lawrence, J. S. The dynamics of platelet production as studied by a depletion technique in normal and irradiated dogs. *J. Lab. clin. Med.* 1955, **45**, 906.