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

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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 favaism 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 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 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 340 m 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 107 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 107 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-
trols a certain enzyme in different tissues. In
congenital galactosemia the galactose-1-P uridylic
transferase is absent in the red cells and liver
(16). Therefore, it is of great interest to deter-
mine whether the glucose-6-phosphate dehydro-
genase is absent in tissue other than red cells.
The present investigation demonstrated that the
hereditary deficiency of glucose-6-phosphate dehydro-
genase 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 Hurwits have found glucose-
6-phosphate dehydrogenase activity to be nor-
mal 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 leuko-
cytes.

Glucose-6-phosphate dehydrogenase activity de-
clines 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 af-
fected 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
erthrocytes 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 eryth-
rocytes 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 pres-
et. 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, furadantine,
para-aminosalicylic acid, sulfanilamide and sul-
fapyridine (1, 2). No thrombocytopenia, how-
ever, was observed by us during a hemolytic
event, nor has it been described in the literature.
Beutler, Robson and Buttenwieser 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 acetyl-
phenylhydrazine were shown to destroy GSH.
The recent finding of an enzyme, GSH perox-
idase, in erythrocytes that could mediate the oxy-
dative destruction of GSH by a hemoglobin per-
oxide 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
drugs. On the other hand, this could be re-
CONCOMITANT G6P DEHYDROGENASE DEFICIENCY IN PLATELETS, RED CELLS

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

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