# TRANSKETOLASE AND TRANSALDOLASE REACTIONS IN THE ERYTHROCYTES OF HUMAN SUBJECTS WITH FAVISM HISTORY [1]

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Red cell hemolysates are known to possess the enzymes for the oxidation of glucose-6-phosphate to pentose phosphate (Warburg and Christian, 1932; Horecker and Smyrniotis, 1951). In these preparations the biosynthesis of pentose phosphate can also occur through an enzymatic sequence different from the oxidative shunt (Bonsignore et al, 1957; Dische, 1957). In vitro, pentose biosynthesis appears to occur mainly by the oxidation mechanism, since the acid soluble nucleotide ribose formed from glucose-2-$C^{14}$ is labeled primarily in the one position (Hiatt and Lareau, 1960).

This result raises the question of how pentose-P is formed in sufficient quantities for the cell requirements in conditions where an impairment of the oxidative mechanism exists. This occur in certain congenital diseases such as the drug or fava anemias in which a genetic deficiency in G-6-PD [2] has been demonstrated (Carson et al, 1956; Sansone and Segni, 1958; and Gross et al, 1958).

The results reported here indicate that in favism red blood cells the levels of TK and TA are significantly elevated, which may represent a compensating mechanism for pentose phosphate synthesis.

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(2) The following abbreviations are used: R-5-P, Ribose-5-phosphate; Xu-5-P, Xylulose-5-phosphate; G-6-P, Glucose-6-phosphate; F-6-P, Fructose-6-phosphate; S-7-P, Sedoheptulose-7-phosphate; GSH, Reduced glutathione; DPNH, Reduced diphosphopyridine nucleotide; Pentose-P, Pentose-phosphate; G-6-PD, Glucose-6-phosphate dehydrogenase; TK, Transketolase; TA, Transaldolase.

## Materials and Methods

Pentose phosphate isomerase and phosphoketopentose epimerase were prepared as described by Ashwell and Hickman (1957). All other substrates and enzymes used were commercial preparations.

The human subjects studied were eight Sardinians, members of a single family. One of them has experienced several acute hemolytic episodes following the ingestion of fava beans. All the subjects showed almost complete absence of G-6-PD in red blood cell hemolysates, instability of GSH in the Beutler test (1956) and the normal number of reticulocytes. Controls were normal subjects of about the same age.

Heparinized venous blood was collected, centrifuged and the plasma and leukocytes removed by suction. Erythrocytes completely free of leukocytes could not be obtained. The erythrocytes were washed 3 times with isotonic KCl buffered at pH 7.4 and hemolyzed by adding 2 volumes of cold $H_2O$. The stroma was then removed by centrifugation (25.000 x g) for 30 minutes. All these steps were carried out at +2° C.

Prior to hemolysis aliquots of the cells were removed for counting of erythrocytes and reticulocytes and determination of hemoglobin and hematocrit.

Transketolase was measured by the following reaction:

$$\text{R-5-P} + \text{Xu-5-P} \rightleftharpoons \text{S-7-P} + \text{D-glyceraldehyde-3-P}$$

The reaction mixtures contained, per ml: 40 μmoles of glycyl-glycine buffer pH 7.6; 5 μmoles of R-5-P; 2 μmoles of $MgCl_2$; 0.1 μmole of thiamine pyrophosphate; 0.3 μmoles of DPNH; 0.005 ml. of glycerophosphate dehydrogenase containing triose phosphate isomerase; 0.05 ml. of a mixture of pentose phosphate isomerase and phosphoketopentose epimerase; 0.05 ml. of hemolysate (derived from a cell suspension of hematocrit about 50).

Transaldolase was measured by the following reaction:

$$\text{F-6-P} + \text{D-glyceraldehyde} \rightleftharpoons \text{D-fructose} + \text{D-glyceraldehyde-3-P}$$

The reaction mixture contained, per ml: 40 μmoles of glycyl-glycine buffer pH 7.6; 2 μmoles of F-6-P; 20 μmoles of D-glyceraldehyde; 0.3 μmoles of DPNH; 0.005 ml. of glycerophosphate dehydrogenase containing triosephosphate isomerase; 0.05 ml. of hemolysate

(derived from a cell suspension of hematocrit about 50).

The presence of hemoglobin does not permit the direct spetrophotometric determination of DPNH oxidation, therefore the reaction mixtures were incubated at 32° C. and 1.0 ml. samples were taken every 5 minutes. The proteins were precipitated by adding 3 volumes of cold acetone and the precipitate was removed by centrifugation. Acetone was then removed by evaporation at 37° and DPNH evaluated enzymatically with the lactic dehydrogenase (Kornberg, 1955).

In the table the activities of TK and TA expressed in units per ml. of packed erythrocytes, or per $10^9$ erythrocytes, or per gr. of hemoglobin as indicated. 1 unit of enzyme is defined as that amount which causes, under sampling conditions, the oxidation of 1 micromole of DPNH per hour. The controls contained no R-5-P or no F-6-P.

TABLE I

Transketolase and Transaldolase Activities
(Mean ± Standard Error)

| Calculated per | TRANSKETOLASE | | TRANSALDOLASE | |
|---|---|---|---|---|
| | Normal Subjects | Subjects with Favism history | Normal Subjects | Subjects with Favism history |
| ml. Erythrocytes | 4.6 ± 0.4 | 7.3 ± 0.8 | 2.8 ± 0.2 | 4.5 ± 0.3 |
| $10^9$ Erythrocytes | 0.5 ± 0.04 | 0.8 ± 0.09 | 0.3 ± 0.02 | 0.5 ± 0.03 |
| gr. Hemoglobin | 15.2 ± 1.3 | 26.3 ± 3.0 | 9.5 ± 0.8 | 16.3 ± 1.1 |

## Results and Discussion

Previous results from this laboratory (Segni, 1959) have established the presence of TK and TA in hemolysates of human favism red blood cells. These enzymes have now been formed to be approxi-

mately 60 per cent more active in the erythrocytes of these subjects. This increase in the level of TK and TA may reflect a compensatory mechanism operating in these cells to provide adequate amounts of pentose phosphate by the auxiliary pathway which is normally less active than the oxidative mechanism (Hiatt and Lareau, 1960). In favism red blood cells the latter is almost completely inactive owing to the lack of G-6-P-dehydrogenase.

The ability of favism red blood cells to synthesize pyridine nucleotides has not yet been studied. Such studies are now in progress in this laboratory and preliminary results indicate that in favism erythrocytes the synthesis occurs at normal levels.

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