ENZYMATIC ABNORMALITY IN ERYTHROCYTES OF A POPULATION SENSITIVE TO *VICIA FABA* OR HÆMOLYTIC ANÆMIA INDUCED BY DRUGS

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HÆMOLYTIC reactions due to *Vicia faba* or certain drugs (sulpha drugs, *p*-aminosalicylate or naphthalene) occur in Israel only in dark-complexioned non-Ashkenazy Jews of Oriental or Mediterranean origin. Our previous investigations revealed glutathione deficiency and instability[1,2] in the erythrocytes of these cases. Carson *et al.* described a defect of glucose-6-phosphate dehydrogenase in erythrocytes of American primaquine-sensitive subjects, having similar glutathione abnormalities[3–5]. Following these reports, we investigated the activity of this dehydrogenase in the erythrocytes of our sensitive population.

Thirty control subjects and twenty-six 'sensitive subjects' whose red blood-corpuscle glutathione was sensitive to acetyl phenyl hydrazine were investigated.

The reaction system was essentially identical with that of Carson *et al.*[3]. The activities of glutathione reductase, glucose-6-phosphate (G-6-P) and 6-phosphogluconate dehydrogenases were estimated by the quantities of reduced glutathione (GSH) formed by the coupled reactions (1)–(2) or (1)–(3).

$$GSSG + TPNH + H^+ \xrightarrow[\text{reductase}]{GSSG} 2GSH + TPN^+ \quad (1)$$

$$G\text{-}6\text{-}P + TPN^+ \xrightarrow[\text{dehydrogenase}]{G\text{-}6\text{-}P} 6\text{-}P\text{-}G + TPNH + H^+ \quad (2)$$

$$6\text{-Phosphogluconate} + TPN^+ \xrightarrow[\text{dehydrogenase}]{6\text{-phosphogluconate}} \text{pentose phosphate} + CO_2 + TPNH + H^+ \quad (3)$$

In all experiments, fresh (not more than 2 hr. old) heparinized blood was used. It was centrifuged in the cold, the red corpuscles were washed thrice with cold saline (4° C.) and hæmolysed with 5 vol. of cold distilled water. The hæmolysates were usually divided into four parts, each being treated differently: (*a*) immediate dialysis for 18 hr. in the refrigerator with 0·067 *M* phosphate buffer *p*H 7·4; (*b*) incubation at 37° C. for 1 hr. prior to dialysis; (*c*) centrifugation for 1 hr. in a refrigerated centrifuge at 16,000*g* followed by dialysis of the supernatant hæmolysate; (*d*) incubation of the supernatant (obtained as in (*c*)) at 37° C. for 1 hr. before dialysis.

2 ml. of hæmolysate were mixed with constituents appropriate for each reaction and the volume was made up to 3 ml. with *tris* buffer. The concentrations of the various reagents in the final reaction mixtures were: *tris* (hydroxymethyl) aminoethane 0·048 *M*, *p*H 7·4; triphosphopyridine nucleotide 1×10^{-5} *M*; glucose-6-phosphate 5×10^{-4} *M*, 6-phosphogluconate 2×10^{-4} *M*; oxidized glutathione (GSSG) $3{\cdot}5 \times 10^{-4}$ *M* (0·64 mgm./3 ml. mixture). All the reagents were dissolved in the *tris* buffer. After incubation for 15 min. at room temperature, the reaction was stopped by the addition of 5 ml. of 3 per cent metaphosphoric acid. The reduced glutathione was determined by the method of Grunert and Philips as modified by Beutler[5].

When the incubation mixtures contained 6-phosphogluconate (coupled reactions 1 and 3) the amount of reduced glutathione formed in all the samples (normal and sensitive) was similar (range 0·20–0·30 mgm./3 c.c. mixture). It may be concluded, therefore, that no abnormality in the activities of 6-phosphogluconate dehydrogenase and glutathione reductase was demonstrated in blood of sensitive subjects.

Table 1. Reduced Glutathione found in Experiments with Glucose-6-phosphate as Hydrogen Donor (Coupled Reactions 1–2)

Subjects	No. of subjects	GSH mgm./3 ml. mixture formed Type of hæmolysate			
		a	*b*	*c*	*d*
Normal ♂ or ♀	30	0·21–0·32	0·20–0·30	0·20–0·34	0·19–0·43
Sensitive ♂	15	0	0	0–0·04*	0–0·02*
Sensitive ♀	2	0	0	not per	formed
Sensitive ♀	4	0·06–0·12	0·04–0·10	0·30–0·35	0·23–0·30
Sensitive ♀	2	0·20–0·28	0·06–0·10	0·32–0·35	0·20–0·22
Sensitive ♀	3	0·20–0·30	0·19–0·24	0·26–0·35	0·22–0·30

Hæmolysates (*a*), (*b*) contained stroma; (*c*) and (*d*) were supernatants after high-speed centrifugation in the cold. Hæmolysates (*a*), (*c*) were kept at 4° C. throughout the preparation; (*b*) and (*d*) were incubated at 37° C. for 1 hr. before dialysis.
* Only four subjects tested.

When glucose-6-phosphate was used as the hydrogen donor (coupled reactions 1 and 2), different grades of abnormalities were observed (Table 1). In the hæmolysates from all sensitive males and two females no glucose-6-phosphate dehydrogenase activity could be detected in samples which contained stroma, even if kept all the time at 4° C. After removal of stroma only trace activities could be detected in some of them. In most samples from sensitive women, normal activity was seen when stroma-less supernatants were examined, but hæmolysates containing stroma showed markedly diminished activity. Only in some cases incubation at 37° C. was required to show this effect. Finally, in three women which were sensitive to Beutler's test no abnormality in this dehydrogenase activity could be shown.

In most cases containing unstable erythrocyte glutathione, an abnormality of glucose-6-phosphate dehydrogenase activity was demonstrated. This abnormality showed variable expression, which was probably sex linked or sex conditioned.

[1] Szeinberg, A., Sheba, C., Hirshorn, N., and Bodonyi, E., *Blood*, **12**, 603 (1957).
[2] Szeinberg, A., Asher, Y., and Sheba, C., *Blood* (in the press).
[3] Carson, P. A., Flanagan, C. L., Ickes, C. E., and Alving, A. S., *Science*, **124**, 484 (1956).
[4] Carson, P. A., Schrier, S. L., and Alving, A. S., *J. Lab. and Clin. Med.*, **48**, 794 (1956).
[5] Beutler, E., *J. Lab. and Clin. Med.*, **49**, 84 (1957).