ACTIVATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE OF ENZYME DEFICIENT SUBJECTS: 1. Activation by stroma of normal erythrocytes*

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It has been recently reported that erythrocyte glucose-6-phosphate dehydrogenase (G-6-P.D.) activity was decreased when hemolysates were incubated with stroma of either normal or enzyme deficient subjects. (Carson et al. 1959). These authors used as an assay method for the enzymatic activity the coupled reaction of G-6-P.D. and glutathione reductase measuring the amount of reduced glutathione (GSH) formed.

We could confirm their results using the same technic; however when a direct assay method for G-6-P.D. was used, namely measuring the reduction of TPN, we observed no effect of stroma on normal hemolysates. Furthermore, normal erythrocyte stroma markedly enhanced the activity of G-6-P.D. of "sensitive" hemolysates**.

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** Hemolysates which exhibit a very low G-6-P.D. activity will be referred to as "sensitive".

METHODS

Hemolysates were prepared from washed erythrocytes by diluting the packed red cells with four volumes of cold distilled water and then centrifuged at 28.000g for 1 hour. Stroma was prepared by the method of Danon, Nevo and Marikovski (1956).

The activation reaction mixture included one ml. of hemolysate and 0.25 ml. of a suspension of red cell ghosts containing 7.5 mg. dry material. Various combinations of the reaction mixture were incubated at 37°C. At the end of the incubation the reaction mixture was centrifuged for five minutes at 1000g and the supernatant was assayed for G-6-P.D. activity by a slight modification of the method of Kornberg and Horecker as published in a previous communication. (Ramot, Szeinberg, Adam, Sheba and Gafni, 1959).

RESULTS

Table I represents the effect of stroma from normal and "sensitive" erythrocytes on both types of hemolysates.

Table I

The effect of stroma on G-6-P.D. activity in hemolysates

Number of experiments	Reaction mixtures		Range of activity in units*	
	Stroma	Hemolysate	0 time	1 hour
9	Normal	Normal	18.0 - 27.0	18.0-27.0
11	"Sensitive"	"Sensitive"	0 - 3.2	0 - 3.2
12	"Sensitive"	Normal	26.0 - 27.0	26.0-27.0
40	Normal	"Sensitive"	0 - 3.2	3.1-28.0

* Unit = Δ OD/min/gr Hb.

It is clear that stroma of normal erythrocytes exerts an activating effect upon the hemolysate of "sensitive" erythrocytes, increasing its activity to about normal levels.

This activation reaction is temperature dependent as can be seen from table II, being very slow below 20°C.

Table II

The effect of normal stroma on G-6-P.D. activity of sensitive hemolysates at various temperatures

Temp. of incubation	Activity* after incubation for		
	0 time	30 min.	60 min.
1°	3.3	3.3	4.0
10°	"	4.7	6.7
20°	"	5.4	7.9
30°	"	6.7	8.7
40°	"	8.7	8.7
50°	"	3.3	3.3

* in units.

At 37°C the activation proceeds rather fast and ends in about 10 minutes. This activation reaction is pH dependent its optimum being about pH 7.2 .

DISCUSSION

An activating effect of normal stroma on G-6-P.D. of "sensitive" hemolysates was demonstrated. Erythrocyte stroma of enzyme "sensitive" subjects did not exhibit this effect. This, to our knowledge, is the first demonstration of a stromal factor lacking in enzyme deficient subjects. The fact that "sensitive" stroma did not suppress

the activity in normal hemolysates suggests that we are dealing with an activator of G-6-P.D. present in the normal stroma rather than with an inhibitor of this enzyme present in the "sensitive" stroma.

From the data obtained, namely the time, pH and temperature dependence, it appears that this activation reaction is of an enzymatic nature. The possible mechanism of the activating effect is at present under investigation.

Studies on the properties of G-6-P.D. from erythrocytes of "sensitive" subjects has not demonstrated up to now any qualitative differences from that of normal subjects. (Kirkman 1959).

Thus the presence of an activator of erythrocyte G-6-P.D. in normal stroma and its absence in stroma of "sensitive" erythrocytes may explain the differences between normal and "sensitive" subjects in the activity of the G-6-P.D. system.

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