MECHANISMS UNDERLYING GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: Heterogeneity of response to stromal activation in Erythrocytes.[1]

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Rimon and co-workers in Israel (1960) recently reported that the incubation of hemolysates of erythrocytes from subjects with deficiency of glucose-6-phosphate dehydrogenase (G6PD) with stroma of normal erythrocytes markedly enhanced the activity of G6PD. The enzyme activity in "deficient" hemolysates attained normal values following incubation. These results were in contradiction to the findings of Carson et al. (1959) that stroma depressed G6PD activity when the latter was measured by the reaction coupling G6PD and glutathione reductase.

In studies on the Melanesian population of New Guinea, in which G6PD deficiency has been reported previously (Kidson 1961), we have been able to confirm the results of the workers in Israel showing activation of G6PD from "deficient" subjects by incubation with stroma from erythrocytes of normal subjects. These experiments, however, have revealed heterogeneity in this response to stromal activation within the same population group, demonstrating that deficiency of an activator is not the only mechanism underlying defective activity of G6PD.

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METHODS.

Erythrocyte stroma was prepared according to the method of Ramot et al. (1961). The stroma was washed 12 times with isotonic saline to remove all traces of G6PD activity, which was assayed on all stroma washings. Hemolysates were prepared according to the procedure of Rimon et al. (1960). Activation was carried out by incubation of 1.0 ml. of hemolysate with 0.05 ml. of stroma for 1 hour at 37°C; the supernatant was then removed by centrifugation at 1500 g for 10 minutes at 4°C. G6PD activity was measured in this supernatant fraction by the method of Marks (1958) and has been expressed in units, 1 unit = $\dot{\Delta}$ OD/minute/gm.Hb.

RESULTS.

The effects of normal stroma on G6PD activity in hemolysates from "deficient" subjects are shown in Table 1. In 25 "deficient" subjects (group a) activation by normal stroma of G6PD occurred, resulting in near normal enzyme activities. This effect was observed using stroma from either normal Melanesian or normal European subjects. In 7 "deficient" subjects (group b) however, no activation occurred, using the same stromal preparations in parallel experiments; repeated incubation for longer periods of time and with higher concentrations of stroma failed to result in increased G6PD activity. Stroma from "deficient" subjects had no effect on normal or "deficient" hemolysates. We were able to confirm in the case of group (a) the findings of Ramot et al. (1961) of time, temperature and pH dependence of the activation reaction. It is evident then, that in this Melanesian population two groups of "deficient" subjects may be delineated by their response to stromal activation.

DISCUSSION.

It has been possible to confirm the presence of an activator of G6PD in normal erythrocyte stroma, thus supporting the hypothesis of Rimon et al. that this factor, enzymatic in nature, may be absent in "deficient" subjects. However, from the results it is apparent that this activator cannot

TABLE 1

Effects of normal stroma on G6PD activity in hemolysates from "deficient subjects.

Hemolysate (number tested)	Normal stroma	Activity in Units (± S.D.) pre-incubation	post- incubation
Normal (25)	-	20.0 ± 3.1	20.1 ± 3.3
	+	20.4 ± 3.2	19.7 ± 3.1
"Deficient" group (a) (25)	-	0.17 ± 0.14	0.17 ± 0.14
	+	0.17 ± 0.14	13.9 ± 3.8
group (b) (7)	-	0.32 ± 0.10	0.30 ± 0.10
	+	0.30 ± 0.10	0.31 ± 0.10

restore the enzyme of erythrocytes in all instances of deficiency. Definite heterogeneity exists among G6PD deficient subjects in New Guinea in the response to this stromal factor, about 20 per cent of the Melanesian group in the present study giving a negative reaction.

Two explanations of these data appear to be feasible: (1) The activation system could be complex with more than a single enzymatic step involved, thereby making possible single (group a) or multiple (group b) mutational effects.

(2) More probably group (a) represents simply the absence of an activator enzyme, while group (b) represents also the loss of G6PD itself or the presence of an altered G6PD protein which will not respond to the activator.

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