

SEATO Medical Research Hematological Studies

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Period of Report : Annual, 1 Apr. 1965 - 31 Mar 1966

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Study Reports :

1. Title : The Normal Range of Glucose - 6 - Phosphate Dehydrogenase in Red Cells of Thai Adult Males and Females

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Objective: Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency in red blood cells, an inherited disorder, is prevalent among Thai population. This enzymatic deficiency is associated with hemolytic anemia upon exposure to a variety of drugs. Estimation of enzyme activity of red cells is necessary in the clinical diagnosis of hemolytic anemia as well as studying possible factors that influence enzyme activity.

The purpose of this study was to establish the normal range of G-6-PD activity for the Thai population, values which are necessary for subsequent work.

Methods: Quantitative analysis of G-6-PD activity of blood samples from University students and blood donors has been done according to the method of Kornberg and Horecker. The reagents used were purchased in kit form from Calbiochemical, Los Angeles, California, U.S.A. The assay was performed at 26°C. The unit of activity was defined as the changes in absorbance at 340 mμ per minute per gm. hemoglobin per 100 ml hemolysate.

In addition to the activity measurements, the following were studied with respect to their effect on activity;

Hydrogen ion concentration (PH); Dilution of hemolysate; Temperature dependence; Stability of enzyme at room temperature, refrigeration, low temperature storage; Hemoglobin level in blood sample.

Results: Mean and S.D. of enzymatic activity and hemoglobin level of Thai adult males and females are presented in table 1.

Hydrogen ion concentration: Between pH 7 and pH 8.5 (Triethanolamine buffer) the enzyme activity appeared to vary slightly, however, the optimum was found to be 7.5 (Fig. 1). At pH 9, all the samples showed decrease in enzymic activity.

Dilution of hemolysate: Dilution of hemolysate in Digitonin indicated that a concentration between 2 to 3.5 gm hemoglobin/100 ml hemolysate yielded the highest activity.

Temperature of determination: The glucose-6-phosphate dehydrogenase activity was determined 26°C, 37°C, 45°C, 55°C and 65°C. The highest activity has been found to be at 55°C (Fig. 2).

TABLE 1.- Mean values and S.D. of glucose-6-phosphate activity and hemoglobin level of Thai male and female

Blood	No.	G-6-PD activity unit		Hemoglobin level	
		Mean	S.D.	Mean	S.D.
Male donors	66	6.15	1.68	13.53	1.30
Male University students	58	8.30	2.32	14.50	.28
Female	30	9.21	3.47	10.68	1.49
Female University students	28	7.59	2.33	12.83	.75

The University students ages ranged from 20 to 25 years. Donor males and females are adults with all ages represented

Stability of enzyme: Enzyme activity was stable for 6 hours at room temperature (31°C).

Washed red cells showed significant loss of activity after storage in a refrigerator at 4°C after 14 days. Whole blood in A.C-D-I or heparin was stable for a week at 4°C.

Low temperature storage of washed red cells at -90°F showed significant losses of activity after 5 months of storage (Fig. 3).

No correlation between hemoglobin level and glucose-6-phosphate dehydrogenase activity was found.

Conclusion: Normal glucose-6-phosphate dehydrogenase activity of Thai male has been found to fall between 3 and 13 units while the activity in the normal Thai female falls between 2 and 16 units.

References:

1. Flatz, G., Nelson, E.R. (1960). Med. Arts Sci. 14 131
2. Kornberg, A. and Horecker, B.L. (1955): Methods in Enzymology I, Academic Press, N.Y.

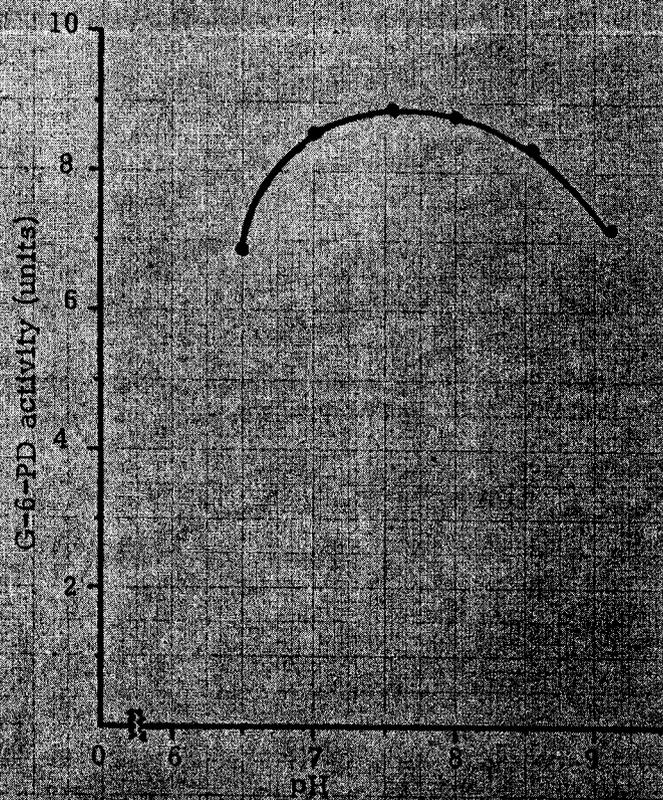


Figure 1. The effect of pH on G-6-PD activity.

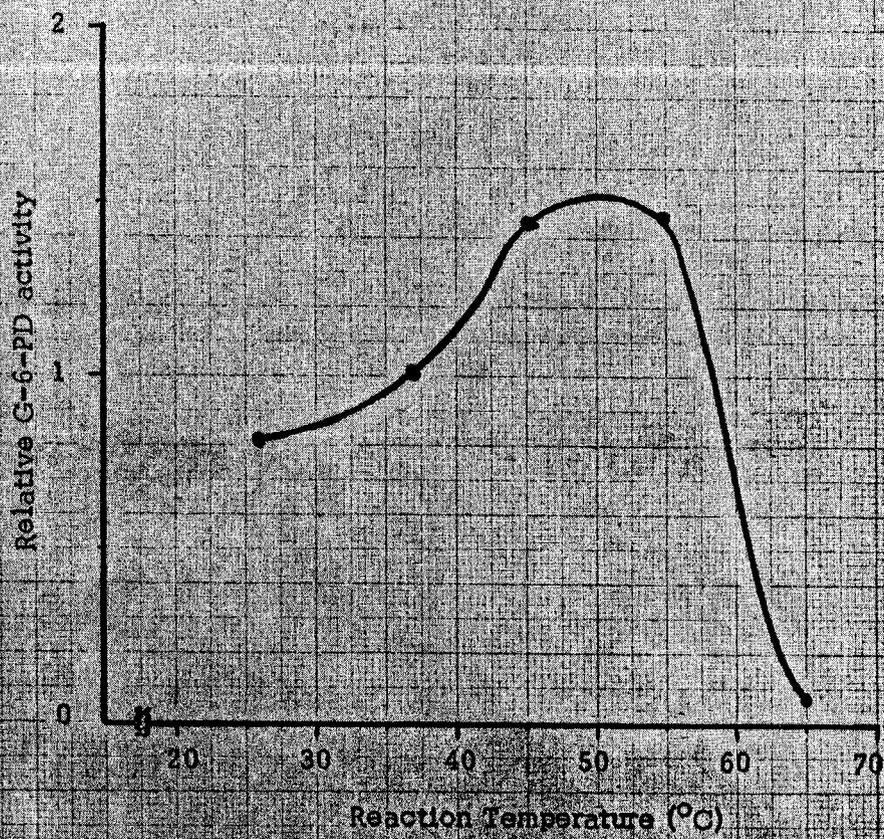


Figure 2. The effect of reaction temperature on G-6-PD activity.

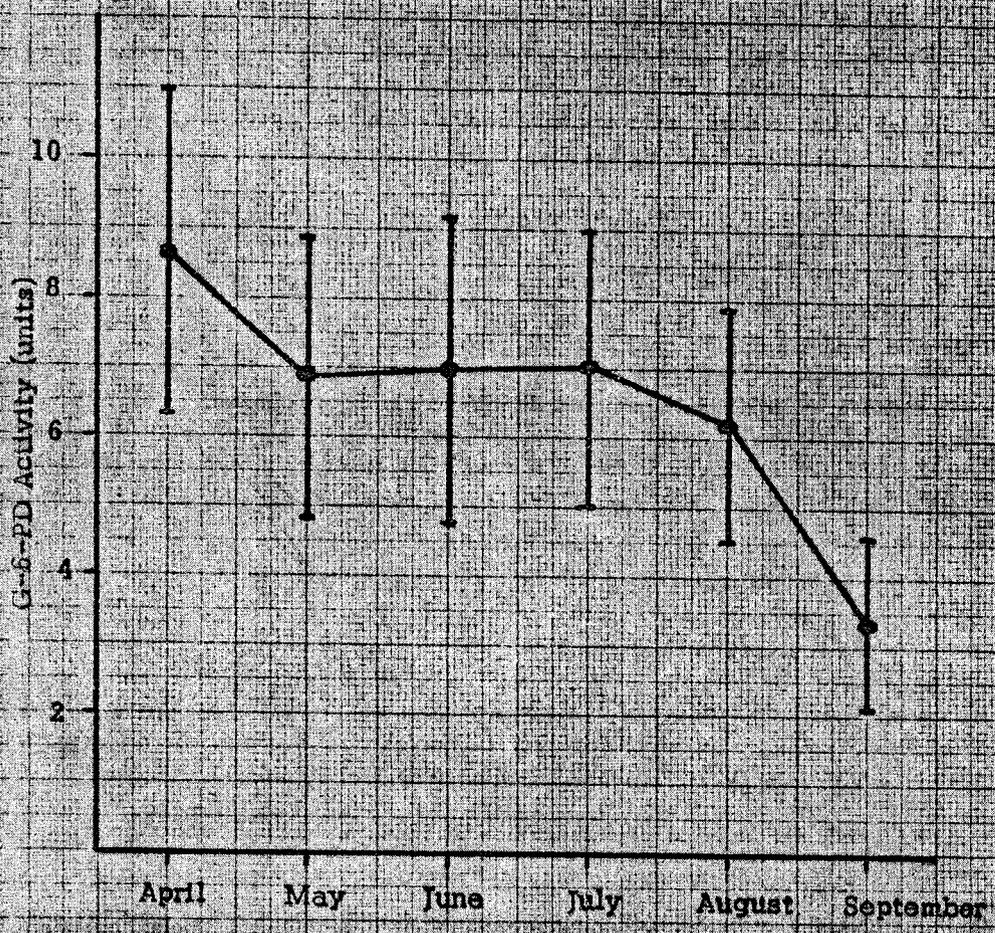


Figure 3. The effect of long-term, low-temperature (-90°C) storage on G-6-PD activity.