

## BODY OF REPORT

SEATO CRC Study No. 7                      Stability of Glucose-6-Phosphate Dehydrogenase in Blood Samples

Project No. 3A 025601 A 811                Military Medical Research Program  
S. E. Asia

Task 01:                                        Military Medical Research Program  
S. E. Asia

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SEASIA (Thailand)

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Division of Clinical Research

Department of Clinical Pathology

Nutritional Biochemistry Section

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**Objective:** To study the stability of glucose-6-phosphate dehydrogenase in blood samples collected with various anticoagulants.

**Methods:** Four different methods of collection and preparation of samples were used in this study:

1. a. Seven venous blood samples were collected in heparin, the plasma was discarded, the red cells were washed three times with 0.9% saline and kept at 4°C.

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b. Fifteen samples were collected using heparin and treated the same as in a, but stored at  $-90^{\circ}\text{C}$ .

2. Seven samples were collected using ACDI and were treated the same as in the heparin series.

3. Seven venous blood samples were collected in heparin and kept as whole blood at  $4^{\circ}\text{C}$ .

Samples from 1a, 1b, and 2 were used as such to determine G6PD activity. Samples 3 and 4 were washed three times with 0.9% saline before the determinations. The quantitative test of G6PD activity was conducted by measuring the increase in absorbance at 340  $\mu$  caused by conversion of TPN to TPNH.

Results: Heparinized and acid-citrate-inosine red cells showed a loss of enzymic activity at the beginning of the third week. (Figure 1). The statistical analysis of variance showed significant loss at 5% level at the end of the third week.

Whole blood kept in heparin and ACDI shows a loss of enzymic activity after ten days (Figure 1). The samples of higher range activity tend to lose faster than the normal range activity. The possibility of having different forms of G6PD enzyme or the contribution of 6-phosphogluconic dehydrogenase could be possible explanations. After ten days the loss is statistically significant at 5% level.

Hemolysis of heparinized blood kept at  $4^{\circ}\text{C}$  started at the end of third week of storage.

There is no difference in the use of heparin and ACDI as anticoagulants in the determination of G6PD activity. This study also showed that keeping the whole blood in ACDI at  $4^{\circ}\text{C}$  has an advantage in preventing hemolysis while enzymatic activity is unaffected.

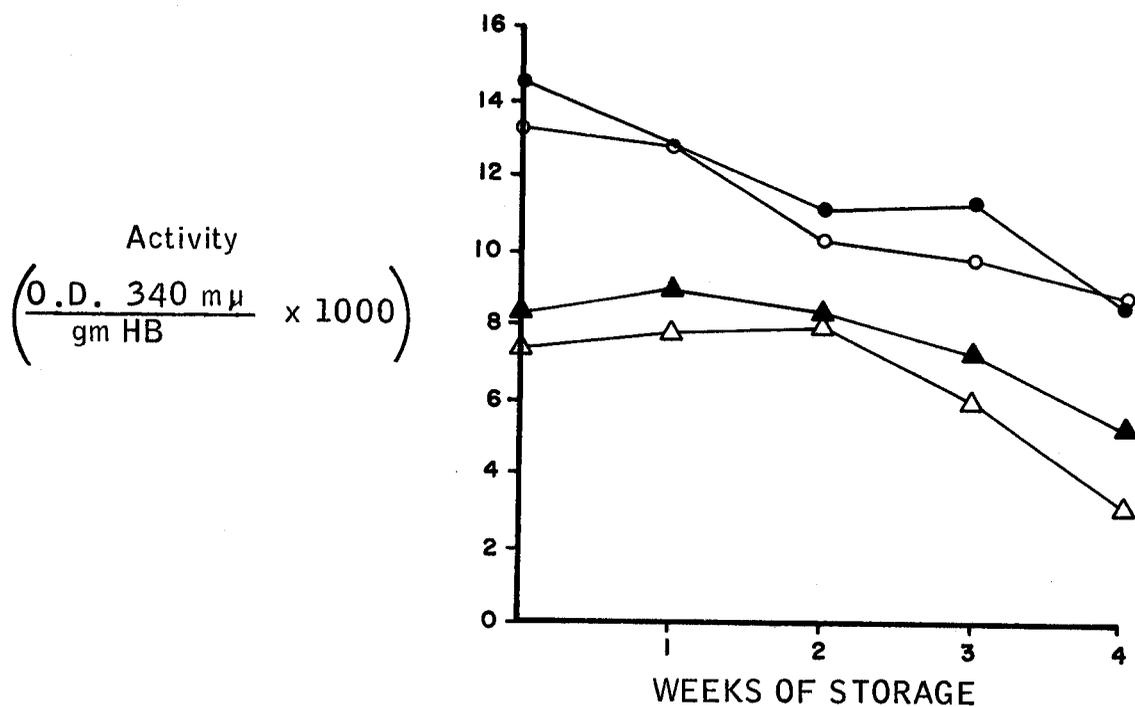
Low temperature storage studies have been inconclusive and additional work is underway in this area.

#### Conclusion:

1. Washed red cells should not be kept in refrigerator at  $4^{\circ}\text{C}$  longer than 14 days.
2. Whole blood should not be kept longer than a week.
3. There is no difference in the use of heparin and ACDI as anticoagulants.

FIGURE 1

EFFECT OF STORAGE ON THE VARIOUS PREPARATIONS USED IN THE DETERMINATION OF G-6-PD



- = Heparin whole blood
- = ACDI whole blood
- ▲ = Inosine washed cells
- △ = Heparin washed cells

CRC Pilot Study

Hemolysis in G-6-PD Deficient Lepers Due to Drug Therapy

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Prapradaeng Leprosarium

Objective: The objective of this study was to ascertain if the chemotherapy of Leprosy caused significant red blood cell hemolysis in individuals deficient in RBC glucose-6-phosphate dehydrogenase.

Description: One hundred and fifty male subjects from the Prapradaeng Leprosarium was evaluated for anemia and/or reticulocytosis. From this group 51 subjects were selected who had hematocrits below 35% and/or reticulocyte counts greater than 1%. Blood was obtained by venipuncture from 50 of these subjects for the determination of hemoglobin, hematocrit, bilirubin, reticulocyte count, G-6-PD activity, and blood drug level (DDS, Diamino-diphenyl-sulfone). In addition records were reviewed to determine dosage schedule and duration of present dose levels.

Results: Forty-seven subjects had hemoglobin levels below 14gm/100 ml. Nine subjects had reticulocyte counts greater than 1.5% bilirubin levels were normal. However, there was no correlation between any of these measurements and dosage of drug, drug blood level, or enzyme activity.

Conclusion: Red blood cell hemolysis as measured by reticulocyte counts or bilirubinemia did not appear to be related to DDS therapy even in subjects deficient in RBC G-6-PD.