fact that mice in this group received more total radiation than those in the other groups. This situation obtained because irradiation was continued as the experiment progressed, hence the mice which survived were exposed to a greater total dose of x-irradiation than those which succumbed earlier in the period. No significant differences in survival were observed among non-irradiated mice in the various groups with at least 80% of the animals in all dietary groups surviving the experimental period of 160 (42 + 118) days.

No data are available as to the cause of the diverse results obtained with the different carbohydrates. It is well established that the intestinal epithelium is highly sensitive to x-irradiation (1-6), and it is possible that multiple sublethal doses of total body x-irradiation induced changes in respect to digestion and absorption of carbohydrates that resulted in a simple sugar such as glucose being utilized more efficiently and with less toxic effects than was the case when more complex carbohydrates were fed.

*Summary.* Experiments were conducted to determine the effects of source of dietary carbohydrate on survival time of mice exposed to multiple sublethal doses of total body x-irradiation. Findings indicate that average survival time of x-irradiated mice was significantly longer on a glucose-containing diet than on rations containing sucrose, dextrin or cornstarch as the source of dietary carbohydrate.

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## A Colorimetric Method for Assay of Erythrocytic Glucose-6-phosphate Dehydrogenase.* (26418)

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A disorder known as primaquine-sensitive hemolytic anemia(1,2) in humans is proving to be increasingly useful as a tool for study of genetic homeostasis(3) and biochemical genetics(4,5,6). This disorder was first reported by Carson(7) to be associated with a deficiency in activity of glucose-6-phosphate dehydrogenase in red blood cells. More recently, 2 different enzymic expressions of the deficiency have been uncovered(8). Investigations of these disorders have been handicapped by the necessity, when the enzyme is to be measured accurately in hemolysates, for a spectrophotometer capable of operation in the ultraviolet range and equipped with a photomultiplier to compensate for the very high optical density of the hemolysate-blank. Motulsky and Campbell(9) have adapted the technic of Dickens and Glock (10) to permit useful visual estimation of the activity of erythrocytic glucose-6-phosphate dehydrogenase in genetic field work. There would seem to be a need for an objective assay requiring only relatively commonplace equipment.

A method of assaying human erythrocytic

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glucose-6-phosphate dehydrogenase has been found which preserves all of the accuracy of the previous assay as well as much of the simplicity of the method of Motulsky and Campbell. The only instrument required is a simple spectrophotometer or photoelectric colorimeter such as can be found in many clinical or chemical laboratories. In this method, which is adapted from a general assay for dehydrogenases(11), phenazine methosulfate (PMS) is used as an electron carrier between reduced triphosphopyridine nucleotide (TPNH) formed in the reaction and dichloroindophenol (DCIP). Rate of reduction of DCIP is followed at 620 mμ. At this wave length absorption of DCIP is nearly maximal, while the erythrocyte hemolysate has an absorption about 2% of that of the DCIP at the concentration used in the assay.

*Method.* The reaction mixture contains 1.0 ml of 0.5 M tris buffer, pH 7.5, 0.1 ml of 1 M $MgCl_2$, 0.4 ml of 0.01% Na 2,6-dichloroindophenol (these reactants may be mixed and stored as a stock solution in the refrigerator, provided the solution is allowed to warm to room temperature before use), 0.1 ml of 0.01 M triphosphopyridine nucleotide (TPN), 0.05-0.1 ml of a 1/20 stroma-free erythrocyte hemolysate(12), 0.5 ml of 0.05 mg/ml phenazine methosulfate, 0.5 ml of 0.02 M Na glucose-6-phosphate, pH 7.5 (because of the relatively low Michaelis constant for glucose-6-phosphate(13), the use of 0.2 ml is permissible) and water to make a total volume of 6.0 ml. TPN and glucose-6-phosphate solutions are stored frozen. The PMS solution must be protected from light and is preferably stored in the cold. All reactants except glucose-6-phosphate are added to the cuvette, which is then left at room temperature for 10 minutes. Omission of this preincubation period, during which easily oxidizable substances in the hemolysate react with DCIP, results in non-linearity of the measurement. This is an additive effect of the enzymic reaction and a non-linear, non-enzymic reduction of DCIP. After the preincubation period, glucose-6-phosphate is added to the cuvette and reduction of DCIP is followed at 620 mμ, using a water blank to zero the colorimeter. Readings are taken at 30 or 60 second intervals. The rate remains linear for 10 minutes and is proportional to the amount of hemolysate added within the limits 0.02 to 0.2 ml. A battery-operated Coleman, Jr. spectrophotometer was employed in this study. Periodic checks showed that this instrument was free enough from drift so that the initial zeroing sufficed for the entire measurement period.

The assays reported here were run at room temperature. The amount of PMS specified, which is lower than that used previously(11), gives a maximum reaction rate with glucose-6-phosphate dehydrogenase. Higher concentrations are inhibitory. The pH employed in the present assay is below the pH optimum of this enzyme as measured by other methods(8). However, with PMS as electron carrier, the reaction rate is greater at pH values close to 7.5 than at higher pH values. Activity of the enzyme may be expressed as mOD units change in absorbancy per minute per ml of hemolysate or as mμmoles of DCIP reduced per minute per ml of hemolysate.

*Results and discussion.* To test the method, activities of erythrocyte hemolysates from a normal and from a glucose-6-phosphate dehydrogenase deficient individual as well as mixtures of the 2 hemolysates were determined by the present method (PMS method) and by the adaptation of Zinkham *et al.*(12) of the method of Kornberg and Horecker(14) (UV method). Assays by the latter method were carried out at pH 8.0. The cuvette temperature on the day these assays were performed was about 33°C in the UV method and 25-27° in the PMS method. The results are shown in Fig. 1. Rates determined by each method are proportional to amount of enzyme present. The lower activity by the PMS method is due in part to the lower pH at which the PMS assay is run and, in this experiment in which temperature equilization was not attempted, to the temperature difference. When measurements were made at the same temperature by the two methods, and corrected for the increased activity at pH 8.0(8), turnover rates by the 2 methods were approximately equal. In the

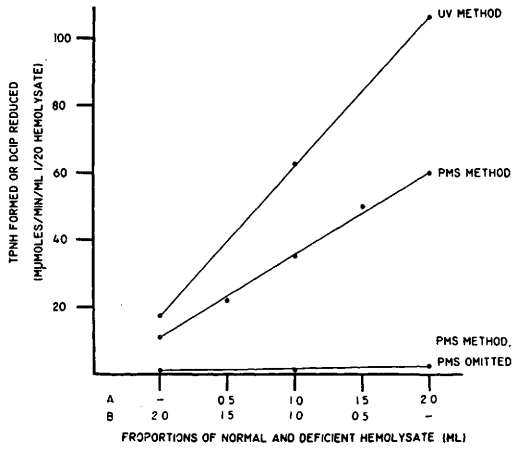


FIG. 1. Measurement of glucose-6-phosphate dehydrogenase activity of normal and deficient hemolysates and mixtures of the two by PMS method and UV method. Values for DCIP reduction are calculated from an observed molar extinction coefficient of $17.6 \times 10^3$ for DCIP in 0.083M tris, pH 7.5.

A. Hemolysate from normal subject.
B. " " deficient subject.

PMS assay there is essentially no activity when PMS is omitted, indicating that in these stroma-free hemolysates diaphorase activity capable of coupling TPNH and DCIP is absent.

In addition to the advantage of requiring only readily available equipment, the present method makes it possible, because of the higher extinction coefficient of DCIP at 620 mμ than of TPNH at 340 mμ and because of the lower absorbancy of hemoglobin at the former wave length, to measure lower activities of the dehydrogenase in hemolysates than has hitherto been possible. The ratio

$$\frac{\Delta \text{O.D.}_{\text{PMS METHOD}}/\text{O.D. of hemolysate at 620 m}\mu}{\Delta \text{O.D.}_{\text{UV METHOD}}/\text{O.D. of hemolysate at 340 m}\mu}$$

is equal to approximately 110. The question of the necessity of a diaphorase, which arises in a colorimetric determination of dehydrogenase activity, is obviated since the present method is independent of a diaphorase activity. A few experiments have been done which suggest that this method should be readily adaptable to visual estimation of erythrocytic glucose-6-phosphate dehydrogenase.

*Summary.* A colorimetric method for estimation of glucose-6-phosphate dehydrogenase activity of erythrocytic hemolysates is described. The method reported employs phenazine methosulfate as an electron carrier between TPNH formed in the reaction and dichloroindophenol. Rate of reduction of the latter compound is followed spectrophotometrically. The method gives turnover rates comparable to those obtained with the ultraviolet spectrophotometric method and is more sensitive than the latter. The assay utilizes a standard spectrophotometer or photoelectric colorimeter and is independent of diaphorase activity. The method should be readily adaptable to visual estimation of glucose-6-phosphate dehydrogenase of erythrocytes.

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