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The Utilization of Glucose by Normal Glucose-6-phosphate Dehydrogenase and by Glucose-6-phosphate Dehydrogenase Mediterranean* (33075)

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Deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) is a widespread disorder. Efforts to explain its high prevalence in some populations have centered about possible benefits arising as a consequence of absence of this enzymatic activity. The "malaria hypothesis" (1) has received the greatest attention, and although there is a body of evidence suggesting that deficiency of the enzyme may confer some degree of immunity to malaria, this is by no means generally accepted to be the only important factor in maintaining high gene frequencies in all populations. A quite different possibility therefore deserves consideration. It may be that the loss of catalytic activity with regards to glucose-6-phosphate is merely an incidental side effect of a mutation which gives the enzyme molecule some new, beneficial substrate specificity. Indeed, the abnormal glucose-6-phosphate dehydrogenase produced in some common mutations such as G-6-PD Mediterranean uses 2-deoxyglucose-6-phosphate and galactose-6-phosphate more rapidly than does the normal enzyme (2).

Normal glucose-6-phosphate dehydrogenase has been shown to utilize unphosphorylated glucose as a substrate, although high glucose concentrations were required, and the rate of the reaction was extremely slow (3). In the present study, the capacity of one type of mutant G-6-PD, G-6-PD Mediterranean, to utilize this substrate has been investigated. At the same time, some of the kinetic characteristics of glucose utilization by the normal enzyme are described for the first time.

Materials and Methods. Partial purification of glucose-6-phosphate dehydrogenase was carried out as described previously (4). Enzyme preparations purified in this manner are free of 6-phosphogluconic dehydrogenase activity. All enzymatic assays were performed as described previously (4) except that all measurements were carried out at 37°C. Solutions of nonphosphorylated sugars were prepared at least 24 hours before use to assure complete mutorotation.

Results. A. The utilization of unphosphorylated sugars by normal and Mediterranean G-6-PD. Table I presents the utiliza-

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TABLE I. Relative Activity of Partially Purified Red Cell G-6-PD with Nonphosphorylated Sugars.

Sugar	Conc.	Activity in 0.05 *M* $NaHCO_3$ (% relative to G-6-P)	Activity without added $NaHCO_3$ (% relative to G-6-P)
Normal enzyme			
Glucose-6-P	0.6 m*M*	100	100
Glucose	1 *M*	2.8	0.24
Galactose	1 *M*	0	0
2-Deoxyglucose	1 *M*	0	0
Fructose	1 *M*	0	0
Mannose	1 *M*	0	0
Ribose	1 *M*	0	0
Gal-6-P	0.6 m*M*	1.3	3.0
2-Deoxy-G-6-P	0.6 m*M*	2.3	5.0
Mediterranean enzyme			
Glucose-6-P	0.6 m*M*	100	100
Glucose	1 *M*	10	0.8
Galactose	1 *M*	0	0
2-Deoxyglucose	1 *M*	0	0
Fructose	1 *M*	0	0
Mannose	1 *M*	0	0
Ribose	1 *M*	0	0
Gal-6-P	0.6 m*M*	9.3	22
2-Deoxy-G-6-P	0.6 m*M*	1.5	26

tion of various sugars by normal G-6-PD and G-6-PD Mediterranean. Studies were carried out with and without the addition of bicarbonate with TPN as pyridine nucleotide coenzyme. DPN was also used with added bicarbonate using galactose and glucose as substrates. No activity was observed. These studies demonstrated that of the nonphosphorylated sugars studied, only glucose served as the substrate for the enzyme. As previously reported (3), glucose utilization was strongly stimulated by the presence of bicarbonate. The utilization of glucose by the Mediterranean enzyme was more efficient when compared with the utilization of glucose-6-phosphate than with the normal enzyme.

B. The effect of various concentrations of bicarbonate. The relationship between bicarbonate concentration and enzyme velocity is shown in Fig. 1A and B. As shown there is apparently a linear relationship between the bicarbonate concentration and the ratio of the bicarbonate concentration to the velocity of the reaction, suggesting that an intermediate complex may be formed between the enzyme molecule and the bicarbonate ion. The characteristics of normal enzyme and G-6-PD Mediterranean appeared to be identical in this respect.

C. Reaction products. It has been shown previously that in alkaline media high concentrations of monosaccharides may reduce pyridine nucleotides to a form which resembles enzymatically active pyridine nucleotides both on the basis of absorption and fluorescent spectra, but which do not react with enzymes which normally oxidize the pyridine nucleotides (5). The capacity of the reduced pyridine nucleotide formed when red cell G-6-PD oxidizes glucose to react with enzymes has therefore been investigated using the glutathione reductase–oxidized glutathione system as a hydrogen receptor. Glutathione reductase and GSSG were added to a cuvette in which red cell G-6-PD, glucose, and TPN had been permitted to react for 20–30 min. The optical density of the system at 340 mμ rapidly fell and returned to base-line levels. This suggests that the TPNH which is formed in the reaction represents a physiologically active form of the coenzyme.

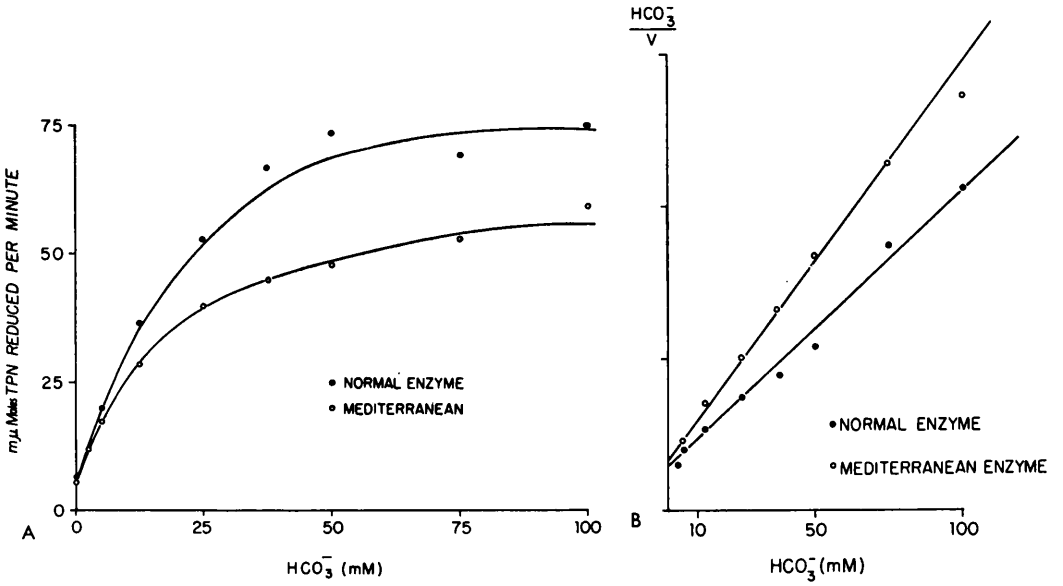

FIG. 1A and B. The effect of bicarbonate concentration on the rate of oxidation of glucose by partially purified human glucose-6-phosphate dehydrogenase. The glucose concentration was 1 *M*; other conditions as described in the text.

D. pH optimum. The relationship between enzyme activity with glucose and hydrogen ion concentration was determined using the triple buffer system described by Kirkman (6), with and without the additional incorporation of 0.05 *M* bicarbonate. The pH optimum curves of the two types of enzymes were very similar in the presence of bicarbonate (Fig. 2). Bicarbonate did not produce an alteration of the pH curve of normal enzyme, and efforts to determine the pH optimum of the Mediterranean enzyme in the absence of bicarbonate were unsuccessful because of the low activities achieved.

E. Michaelis constants. The Michaelis constant of normal and Mediterranean G-6-PD for glucose has been determined in the presence of bicarbonate. In the case of the normal enzyme, K_m determinations could also be carried out in the absence of added bicarbonate. The K_m of the enzymes for TPN was determined spectrofluorometrically. The results of these studies are given in Table II.

Discussion. Even though the capacity of G-6-PD Mediterranean to utilize unphosphorylated glucose is three or four times as great as the capacity of normal enzyme to utilize the nonphosphorylated sugar, the higher rate of utilization does not compensate

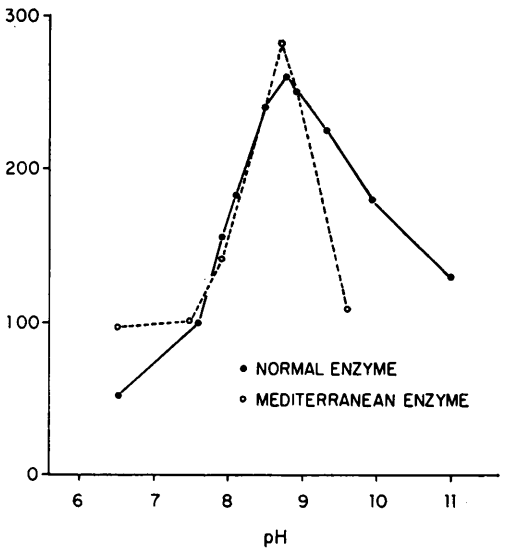

FIG. 2. The relationship between pH and activity of partially purified normal and Mediterranean enzyme with 1 *M* glucose as substrate. The pH values are expressed as the pH of the buffer at 37°C when diluted as used in the assay mixture. Activity is expressed as percentage of the activity with pH 7.5 buffer.

TABLE II. Michaelis Constants of Red Cell G-6-PD with Glucose Substrate.

Enzyme	K_m glucose (M)	K_m TPN (μM)
Normal	0.8	14
Mediterranean	0.8	10

for the 20- to 100-fold diminution of the amount of enzyme normally found in the red cells. Furthermore, the K_m of the Mediterranean enzyme for glucose seems to be as unphysiologically high as that of the normal enzyme.

It may readily be calculated that at physiologic glucose concentrations of 0.006 M either the normal or the Mediterranean enzyme would utilize glucose at only approximately 0.75% of the V_{max}. If the red cells of a normal individual have the capacity to metabolize glucose-6-phosphate at the rate of 9000 mμmoles/gm of hemoglobin per min through G-6-PD, they could only metabolize 3.4 mμmoles of glucose/gm of hemoglobin per min through this pathway, assuming that the V_{max} utilization of glucose is only 5%[2] of that of glucose-6-P. An individual with the Mediterranean type of G-6-PD deficiency, with an enzyme activity of 500 mμmoles /gm of hemoglobin per min with G-6-P as substrate could then only metabolize 0.65 mμmoles of glucose through the shunt pathway.[2] By comparison it may be noted that the capacity of hexokinase to phosphorylate glucose is of the order of 500 mμmoles/gm of hemoglobin per min.

Thus, although the relative capacity of the Mediterranean enzyme to metabolize unphosphorylated glucose is increased, it is more than offset by the overall decrease in enzyme activity. Further, it is apparent that because of the very high K_m, physiologic utilization of glucose by this pathway is probably negligible. It is recognized that other unknown *in vivo* activators might alter this interpretation, but in the absence of knowledge of activators other than bicarbonate, it is concluded that the utilization of nonphosphorylated glucose by the red cells for the reduction of TPN or DPN cannot be an important factor in genetic selection for the G-6-PD Mediterranean enzyme.

Summary. The utilization of glucose and other nonphosphorylated sugars by partially purified normal G-6-PD and G-6-PD Mediterranean was investigated. Both enzymes have the capacity to oxidize glucose with a K_m of approximately 0.8 M. The utilization of glucose by both enzymes was markedly stimulated by the presence of bicarbonate, and the activity with Mediterranean enzyme was greater than that of normal G-6-PD. The pH optimum of utilization of glucose by the two enzymes was very similar. Galactose, 2-deoxyglucose, fructose, mannose, and ribose did not serve as substrate for either type of G-6-PD.

[2] Calculated using data given in Table I, in the presence of bicarbonate, and with K_m *of 0.8 M* for glucose. Under these circumstances v at 1 M is 0.55 of V_{max}. The enzyme is considered to be saturated with glucose-6-phosphate at 0.6 mM concentration.

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