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Role of Elevated Glucose Concentrations in the Hemolysis of Glucose-6-Phosphate Dehydrogenase Deficient Erythrocytes[1] (38474)

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Hemolytic episodes in patients with erythrocyte glucose-6-phosphate dehydrogenase (G6PD) deficiency are known to occur during diabetic acidosis (1). The mechanism for this hemolysis is not understood. Whether hyperglycemia has a role in diabetic acidosis-induced hemolysis of G6PD deficient red blood cells is not known. Travis and coworkers have reported that elevated glucose concentrations stimulated the hexose monophosphate shunt (HMP) of normal erythrocytes, presumably due to activation of the sorbitol pathway and the associated oxidation of dihydronicotinamide adenine dinucleotide diphosphate (NADPH) (2). The experiments described in this report were designed to answer whether glucose-induced NADPH-oxidation increased the vulnerability of G6PD deficient erythrocytes to oxidant injury. Specifically, the effects of normal and elevated glucose concentrations on NADPH-dependent reactions integrally involved with hemoglobin protection were measured.

Methods. Red blood cells (RBC's) from normal and Black G6PD deficient males were collected in EDTA and washed thrice in a pH 7.4 glycylglycine-buffered salt solution (GBS) with the following composition: 140 m*M* NaCl, 5 m*M* KCl, 1 m*M* Na_2HPO_4, 1 m*M* $MgCl_2$ and 20 m*M* glycylglycine. Following the last wash, erythrocytes were resuspended in GBS to a hematocrit of 25–30%. Glucose was added to the incubation medium such that the final concentration was 5 or 50 m*M*. As the glucose concentration was varied, equiosmolal amounts of sucrose were added to maintain a constant osmolality in all experimenta suspensions. The HMP shunt was assessed by measurement of glucose-1-^{14}C oxidation to $^{14}CO_2$ under resting conditions and in the presence of 10 m*M* sodium ascorbate (3). Glutathione (GSH) stability and hemoglobin oxidation were determined in normal and G6PD deficient RBC's incubated with 5 m*M* NaCN, 10 m*M* ascorbate and 5 or 50 m*M* glucose. Glutathione was measured by the method of Beutler (4). Hemoglobin oxidation was assessed by comparing the absorbance of a given sample at 620 nm with the absorbance at 540 nm (5). Methemoglobin formation was determined in normal and G6PD deficient RBC's incubated with 0.2 m*M* hydroxylamine-dapsone in the presence of 5 or 50 m*M* glucose (6).

Results. The resting HMP shunt was stimulated when the concentration of glucose in the incubation medium was increased (Table I). The rate of $^{14}CO_2$ production increased significantly when ascorbate was present in the incubation medium, and this ascorbate-induced stimulation of glucose-1-^{14}C oxidation was augmented further when the medium glucose concentration was elevated.

In the presence of cyanide and ascorbate, normal RBC's were able to maintain GSH levels, while the concentration of GSH predictably fell in G6PD deficient erythrocytes (Table II). No difference in the decrease of GSH was noted, however, when G6PD deficient RBC's were incubated with ascorbate and 5 or 50 m*M* glucose. Hemoglobin oxidation, as manifested by the ratio of absorption at 620 nm to the absorbance at 540 nm, was greater in cyanide and ascorbate-treated G6PD deficient cells than in similarly treated normal erythrocytes. No difference in the magnitude of hemoglobin oxidation was noted, however, when the

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additional effects of 5 and 50 m*M* glucose were compared.

The degree of methemoglobin formation in normal and G6PD deficient erythrocytes incubated with 0.2 m*M* hydroxylamine-dapsone is recorded in Table III. More methemoglobin was formed in normal than in G6PD deficient RBC's, but no difference was noted in either normal or enzyme deficient cells when the effects of 5 and 50 m*M* glucose were compared.

Discussion. The HMP shunt of normal erythrocytes is regulated by the rate of NADPH oxidation to NADP since the latter is a required cofactor of the initial reaction of this pathway (7). Ascorbate stimulates the HMP shunt because the intracellular products of this oxidant (H_2O_2 and dehydroascorbate) directly lead to an increased oxidation ot NADPH. The observation that elevated glucose concentrations stimulated the resting pentose pathway confirmed the previous work of Travis *et al.* (2). In addition, elevated glucose concentrations further enhanced the ascorbate-stimulated HMP shunt. This glucose-induced stimulation of the HMP shunt most probably is due to NADPH oxidation associated with the conversion of glucose to sorbitol. In many tissues this reaction is catalyzed by aldose reductase, an enzyme which requires NADPH as a cofactor, but human erythrocytes apparently lack this enzyme (8). Hexonate dehydrogenase is present in red blood cells, however, and is capable of converting glucose to sorbitol with the simultaneous oxidation of NADPH to NADP (9). This enzyme has a very high Km for glucose (approximately 400 m*M*) and thereby could be responsive to the changes in glucose concentrations used in these experiments. Thus, increased glucose concentrations probably activate hexonate dehydrogenase, increase NADPH oxidation, and thereby stimulate the HMP shunt pathway.

TABLE I[a]

Normal RBC's	HMP Shunt activity (nmoles $^{14}CO_2$/ml RBC/hr)
5 m*M* glucose	0.223 ± 0.015
50 m*M* glucose	0.323 ± 0.026
5 m*M* glucose + 10 m*M* ascorbate	0.728 ± 0.035
50 m*M* glucose + 10 m*M* ascorbate	0.812 ± 0.047

[a] The rate of glucose-1-^{14}C oxidation to $^{14}CO_2$ in normal RBC's incubated in 5 or 50 m*M* glucose, with or without 10 m*M* ascorbate added to the incubation medium. Results expressed as mean and standard deviation.

The experiments reported here were designed to assess the functional significance of the extra NADPH oxidation which occurs in the presence of elevated glucose concentrations. The slightly increased NADPH oxidation predictably would not alter other NADPH-dependent reactions in metabolically replete normal RBC's. In G6PD deficient red cells which have a limited capability for maintaining an NADPH reductive potential, however, a theft of NADPH by

TABLE II[a]

	GSH (nmoles/ml RBC)		Hemoglobin oxidation (OD at 620/OD at 540)
	(T-0)	(T-2)	(T-2)
Normal RBC's			
Ascorbate + 5 m*M* glucose	2.30 ± 0.19	2.18 ± 0.17	0.114 ± 0.018
Ascorbate + 50 m*M* glucose	—	2.20 ± 0.21	0.082 ± 0.015
G6PD deficient RBC's			
Ascorbate + 5 m*M* glucose	1.96 ± 0.18	0.41 ± 0.20	0.217 ± 0.022
Ascorbate + 50 m*M* glucose	—	0.38 ± 0.16	0.229 ± 0.019

[a] Glutathione and hemoglobin oxidation measured in normal and G6PD deficient RBC's after 2-hr incubation with 5 m*M* NaCN, 10 m*M* ascorbate and 5 or 50 m*M* glucose. Results expressed as mean and standard deviation.

TABLE III[a]

	Methemoglobin (% of total hemoglobin)
Normal RBC's	
5 m*M* glucose	37.8 ± 5.8
50 m*M* glucose	41.4 ± 7.3
G6PD deficient RBC's	
5 m*M* glucose	26.9 ± 4.1
50 m*M* glucose	29.4 ± 5.0

[a] Methemoglobin formation in normal and G6PD deficient RBC's incubated for 1 hr with 0.2 m*M* hydroxylamine dapsone and 5 or 50 m*M* glucose. Results expressed as mean and standard deviation.

glucose conversion to sorbitol might deleteriously limit the ability of these cells to protect hemoglobin from an oxidative assault. As seen in Table II, GSH was stable and there was no significant hemoglobin oxidation by ascorbate in normal RBC's. On the other hand, GSH decreased and a substantial amount of hemoglobin was oxidized in G6PD deficient RBC's incubated with ascorbate. Of paramount interest, however, the rate of GSH and hemoglobin oxidation in these cells was not significantly different in 5 or 50 m*M* glucose. These studies suggest that the reductive potential required for glutathione reductase and protection of hemoglobin in normal and G6PD deficient erythrocytes was not significantly altered by elevated glucose concentrations.

Methemoglobin production by hydroxylamine dapsone is another reaction which requires NADPH for maximal activity (6). As previously reported, normal RBC's incubated with this agent produced more methemoglobin than similarly treated G6PD deficient erythrocytes, presumably because more NADPH was available in normal RBC's to activate and recycle the drug (Table III). In the present experiments with normal and G6PD deficient RBC's, it was anticipated that hydroxylamine dapsone would produce less methemoglobin at elevated glucose concentrations if the glucose-induced theft of NADPH was significant. As seen in Table III, however, no difference in methemoglobin formation was noted when the effects of 5 and 50 m*M* glucose were compared. These observations again indicate the relative insignificance of the NADPH diversion in the presence of elevated glucose concentrations.

Infection is the most common cause of hemolysis associated with erythrocyte G6PD deficiency, and most probably is the common denominator inducing oxidant injury and triggering diabetic acidosis. During infection, bacteria and macrophages produce peroxides which are capable of entering and producing oxidant injury in G6PD deficient RBC's (10). In view of the observed increase in HMP shunt activity when glucose concentrations are elevated, hyperglycemia conceivably could add an extra burden to G6PD deficient cells in the presence of an oxidant stress. In the experiments described here, however, no evidence of increased oxidant sensitivity was documented under these conditions *in vitro*. This partially may reflect the relative insensitivity of the parameters available to evaluate oxidant injury. In any event, elevated glucose concentrations during diabetic acidosis do not appear to pose any major problem to the integrity of G6PD deficient erythrocytes. It should be emphasized that NADPH oxidation per se does not cause injury to G6PD deficient RBC's. It is only when an exogenous oxidant is present that competitive loss of NADPH could be detrimental to G6PD deficient erythrocytes. Whether other metabolites produced during diabetic acidosis have either oxidant activity or are capable of greater NADPH oxidation than glucose remains to be determined.

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