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Research Article

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Oxidant Injury of Caucasian Glucose-6-Phosphate Dehydrogenase–Deficient Red Blood Cells by Phagocytosing Leukocytes during Infection

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ABSTRACT Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency of red blood cells (RBC) may develop sudden hemolytic anemia during infection. Since phagocytizing polymorphonuclear leukocytes (PMN) are known to generate hydrogen peroxide, we explored the influence of this oxidant product of PMN on juxtaposed G6PD-deficient and normal RBC. The oxidant stress induced by phagocytosis depleted G6PD-deficient RBC of reduced glutathione (GSH) and this was associated with rapid removal of these cells from the circulation by the liver and spleen. No such effect was observed on normal RBC. Phagocytizing chronic granulomatous disease (CGD) PMN which lack hydrogen peroxide generation, failed to diminish GSH level in G6PD-deficient RBC. Thus, PMN can pose as a source of oxidant damage to G6PD-deficient RBC due to hydrogen peroxide generated during phagocytosis.

INTRODUCTION

Patients with glucose-6-phosphate dehydrogenase deficiency of red blood cells (G6PD RBC)[1] may develop hemolytic anemia during acute infection (1–4). Certain therapeutic agents, such as antipyretics (5) and antibiotics (6, 7), have oxidant properties and may induce hemolysis in some of these patients. Another less appreciated oxidant threat to G6PD RBC during infection might be the generation of hydrogen peroxide by phagocytizing polymorphonuclear leukocytes (PMN) (8). This potent oxidant is elaborated as the product of the PMN respiratory burst after the ingestion of bacteria or inert particles (9).

To explore the influence of phagocytizing PMN on juxtaposed normal and G6PD RBC, red cell glutathione (GSH) levels, methemoglobin concentration, and Heinz body formation were assessed. The oxidant stress induced by phagocytosis depleted G6PD RBC of GSH but did not significantly increase methemoglobin production and Heinz bodies were not produced. This effect was enhanced by increased numbers of phagocytizing PMN or by cyanide inhibition of a peroxide-destroying catalase. The rate of H_2O_2 production by normal PMN could be calculated from the rate of GSH depletion in G6PD RBC. Chronic granulomatous disease (CGD) PMN lack peroxide production and failed to deplete G6PD RBC of GSH. The effect of moderate H_2O_2-induced GSH depletion of G6PD RBC in vitro (without methemoglobin and Heinz body formation) upon their survival in vivo was studied in two patients with Caucasian type G6PD deficiency. When reinfused, 30–35% of these ^{51}Cr RBC were rapidly removed from the circulation and sequestered principally in the spleen and to a lesser extent in the liver. These findings support the earlier in vivo studies of Jacob and Jandl (10) who found that GSH-inhibited normal RBCs were entrapped in the spleen within hours after being reinfused in normal humans.

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[1] *Abbreviations used in this paper:* CGD, chronic granulomatous disease; G6PD, glucose-6-phosphate dehydrogenase; GSH, reduced glutathione; HMPS, hexose monophosphate shunt; KRP, Krebs-Ringer phosphate buffer; PMN, polymorphonuclear leukocytes; RBC, red blood cells.

METHODS

Cell preparation

Polymorphonuclear leukocytes (PMN). Human PMN were isolated from heparinized peripheral blood of patients with acute infections and from one patient with chronic granulomatous disease by sedimentation of red cells and differential centrifugation of PMN-rich plasma as previously described (9). White cell count was performed with a Coulter counter (Coulter Electronics, Hialeah, Fla.) and Wright's-stained smears were made. PMN were suspended in Krebs-Ringer phosphate buffer (KRP), pH 7.4 enriched with 200 mg/100 ml glucose.

Red blood cells. The heparinized peripheral blood of normals and Caucasians with G6PD deficiency was collected and then centrifuged at 3000 rpm at 4°C for 10 min. The buffy coat and plasma were removed and the blood washed thrice in cold buffered saline. The residual buffy coat was removed each time. For incubation studies, the RBC were suspended in glucose-enriched phosphate buffered saline pH 7.4 with a hematocrit of 20% to which PMN were added. PMN and RBC were enumerated and hemoglobin and hematocrit concentrations were determined on a Coulter model S cell counter. For in vivo studies, 6 cc of G6PD-deficient blood was first incubated with 50 μCi sodium ^{51}chromate for 30 min at 37°C; then the RBC were prepared as above.

Red blood cell ghosts. For certain in vitro incubations, RBC ghosts resealed with GSH were prepared from normal red cells washed thrice in 0.15 M sodium phosphate buffer, pH 7.4 and then resuspended with a hematocrit of 50%. They were then lysed with 15 vol. of 20 mM Tris buffer pH 8.0, and the ghosts were washed with 20 mM Tris and then washed again with 10 mM Tris containing 10 mM reduced glutathione at pH 7.4. All centrifugations were at 10,000 *g* for 20 min at 4°C. The white ghosts were then incubated at 50% v/v for 5 min at 37°C in 5 mM tris buffer with 5 mM GSH which also contained at final concentrations, 0.5 mM ATP and 1 g/100 ml albumin. After the incubation, a 5 ml suspension of 50% ghosts was added to 5 ml KRP pH 7.4, centrifuged, and resuspended in KRP.

Incubations

All incubations were performed in siliconized flasks at 37°C in a Dubnoff metabolic shaker at 100 oscillations per min. Most were for 120 min. Portions were removed at intervals throughout the incubations for measurement of reduced glutathione levels, methemoglobin, and for inspection of Heinz body preparations. A typical incubation contained 0.5 ml PMN at 1.0×10^8 per ml, 1.0 ml red blood cells at 1.0×10^9 per ml, 0.15 ml 10 mM KCN titrated to pH 7.4, with 1 normal HCL or phosphate- buffered saline pH 7.4, and either 0.05 ml undiluted latex spherules or 0.05 ml water. Control flasks contained either PMN or RBC. In certain experiments PMN were separated from RBC and incubated in dialysis bags immersed in the RBC suspension; usually PMN and RBC were together in suspension. In some incubations, including those preparations for the in vivo ^{51}Cr RBC studies, an artificial hydrogen peroxide-generating system of glucose and glucose oxidase was used instead of PMN. These preliminary incubations were performed using sterile techniques involving glucose oxidase sterilized by a millipore filtration technique. In exploratory experiments, it was found that the hydrogen peroxide-generating glucose oxidase did not elute the ^{51}Cr-label from the red cells and that the enzyme could be removed from the RBC after incubation by washing the cells thrice with KRP. In other experiments, red cells ghosts filled with GSH were used instead of RBC to determine the rate of GSH oxidation with peroxide-generating glucose oxidase.

Chemical methods

GSH was determined by the method of Beutler, Duron, and Kelly (11). RBC GSH was calculated from the total GSH minus the PMN GSH and expressed as μg GSH per 10^{10} RBC. PMN GSH remained constant throughout the incubations. A standard curve was prepared for each assay using known concentrations of commercial GSH. The rate of GSH oxidation in G6PD RBC (which cannot regenerate GSSG to GSH [12]) was determined and the rate of H_2O_2 generation by normal PMN during phagocytosis was calculated from this rate utilizing the stoichiometric reaction PMN $H_2O_2 + 2$ GSH RBC $\rightarrow$ GSSG RBC $+ 2$ H_2O. H_2O_2 generation from glucose and oxygen by glucose oxidase was calculated from the rate of GSH oxidation in red cell ghosts filled with GSH and in G6PD RBC. Such ghosts do not exhibit hexose monophosphate shunt (HMPS) activity and cannot then regenerate GSH from GSSG. The rate of oxygen consumption by the glucose and glucose oxidase system was determined using a Clark membrane-oxygen electrode equipped with a scale expansion recorder. Sine 1 mole of oxygen generates 1 mole H_2O_2, peroxide production was calculated from the rate of oxygen consumption (O_2 + glucose $\rightarrow$ H_2O_2 + gluconic acid).

The rate of oxygen consumption by human PMN during phagocytosis has been previously determined (9), and this value was compared with the rate of H_2O_2 production by PMN based upon the rate of GSH oxidation in the G6PD-deficient RBCs. An additional experiment was performed using PMN from the same sample to determine both of the above rates i.e., oxygen consumption and H_2O_2 production.

Methemoglobin (13), Heinz bodies (14), glucose-6-phosphate dehydrogenase (15), and red cell transaminase (16) were determined by standard techniques. Red cell survival and organ sequestration of ^{51}Cr RBC was carried out on two patients with mild hemolysis due to Caucasian type G6PD deficiency. Patient's cells were preincubated in the H_2O_2-generating system and the reaction was stopped when GSH was diminished but only small amounts of methemoglobin had formed and no Heinz bodies were visible. As a control for the specific effect of H_2O_2 generation, the in vitro procedure was repeated in the absence of glucose oxidase and the blood reinfused into one patient. In addition, normal RBCs were chromated, then preincubated in the buffer system for 90 min, and infused into a normal subject to determine the effect of glucose-glucose oxidase system on normal RBCs. Informed consent by the subjects was obtained for each of these studies.

RESULTS

Effect of normal PMN on GSH of normal and G6PD-deficient red cells. As noted in Fig. 1, RBC GSH levels did not fall when normal RBC were incubated with PMN either at rest or during phagocytosis of latex spherules throughout a 2 hr incubation at 37°C in the presence of 1 mM KCN to inhibit catalase. This was true for normal RBC over the range of RBC to PMN ratios used to

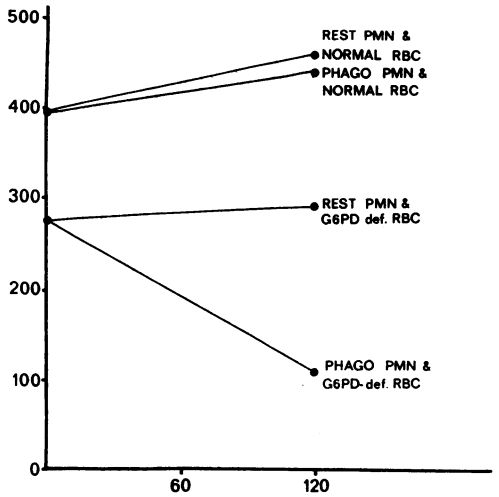

FIGURE 1 Effect of normal resting and phagocytizing PMN on GSH levels of normal and G6PD-deficient red blood cells. The ratio of RBC to PMN was 50:1. 1 mM KCN was added to all incubates at 37°C. Results expressed as μg GSH per 10^{10} RBC on the ordinate and time in minutes on the abscissa.

study G6PD RBCs. In contrast, GSH of G6PD-deficient RBC was initially lower and was further depressed when incubated with phagocytizing PMN. In order to determine the effect of concentration of PMN on GSH stability of G6PD-deficient RBC, these RBC were incubated with varying numbers of phagocytizing PMN. As noted in Fig. 2, GSH depletion was directly proportional to PMN concentration. However, if the ratio of G6PD RBC to PMN was increased to 1000:1 similar to the blood, no GSH was depleted (data not shown). If the ratio of G6PD RBC to PMN was reduced to 10:1, GSH was depleted during incubation with resting as well as phagocytizing PMN even in the absence of KCN, an inhibitor of catalase (Fig. 3). Phagocytosis and KCN which both increase H_2O_2 from PMN, further enhanced the depletion of GSH in G6PD RBC. Neither methemoglobin nor Heinz body formation was observed in the GSH-depleted RBCs.

The effect of cell to cell interaction of GSH depletion of G6PD-deficient RBC by PMN was determined by incubating PMN either in dialysis bags or directly in contact with RBC. As noted in Fig. 4, GSH was not diminished in G6PD-deficient RBC that were separated from PMN in dialysis bags. Phagocytosis did not increase the extent of GSH depletion in this case. In contrast, marked GSH depletion without phagocytosis and enhanced depletion with phagocytosis was observed when RBC and PMN were juxtaposed.

The rate of GSH depletion in G6PD RBC by resting and phagocytizing PMN was determined by repeated sampling over a 120 min incubation period and, as noted in Fig. 5, the rate was exponential and was approximately 270 μg GSH $\rightarrow$ GSSG/10^{10} RBC per hr (or 0.880 μmoles) during phagocytosis. Since the ratio of RBC: WBC was 20:1, then 0.880 μmoles GSH was oxidized by 5.0×10^8 PMN/hr or 17 mμmoles H_2O_2 were produced by 10^7 PMN/hr. In another similar study with a RBC: WBC ratio of 10:1, phagocytozing PMN produced 9 mμmoles $H_2O_2/10^7$ PMN per hr. Prior studies in this laboratory using manometric techniques (9) have shown that human PMN consume approximately

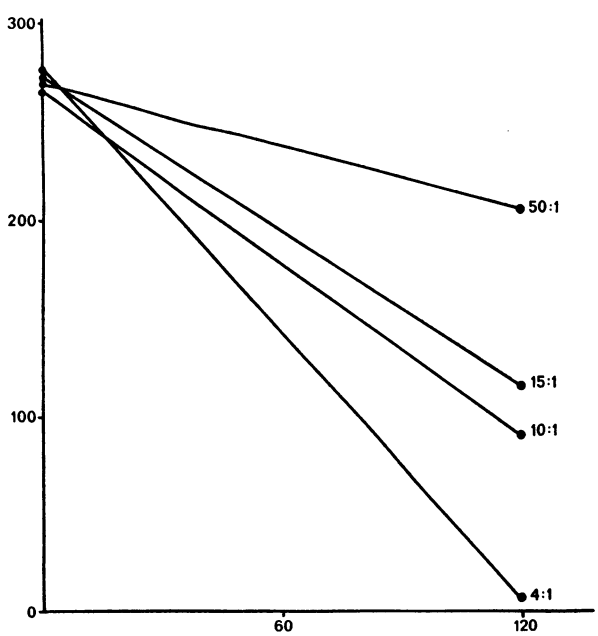

FIGURE 2 Effect of varying RBC to PMN ratio on GSH levels in G6PD RBC. All incubates contained latex and 1 mM KCN. Ratios indicate RBC to PMN for each incubate. Results expressed as μg GSH per 10^{10} RBC on the ordinate and time in minutes on the abscissa.

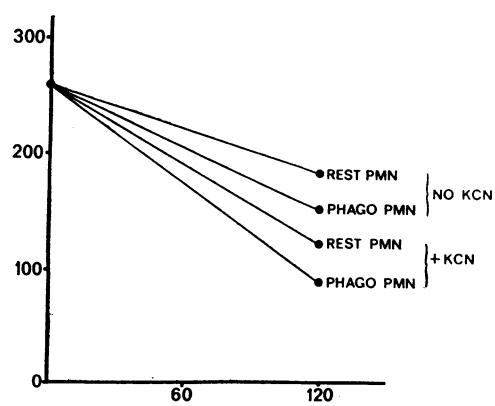

FIGURE 3 Effect of enhanced H_2O_2 production by phagocytosis and by 1 mM KCN on GSH levels in G6PD RBC. Ratio of RBC to PMN was 10:1. Results expressed as μg GSH per 10^{10} RBC on the ordinate and time in minutes on the abscissa.

2.5 μl (112 mμmoles) $O_2/10^7$ PMN per hr during phagocytosis. Therefore, the theoretical rate of H_2O_2 generated during phagocytosis would be 112 mμmoles $H_2O_2/10^7$ PMN per hr. As noted in Table I, experiment 5, we found that the respiratory burst by PMN using the oxygen electrode technique was 93 mμmoles/10^7 PMN per hr and that 28 mμmoles GSH was oxidized/10^7 PMN per hr for the same sample of PMN. This variability in the rate of GSH oxidation can be noted throughout the experiments and is most likely due to the heterogenous sources of PMN donors. It seems likely from the above calculations and experiments that between 10 and 15% of the H_2O_2 produced by PMN during phagocytosis, leaks out of the PMN and becomes available for GSH oxidation of juxtaposed G6PD RBC.

Effect of chronic granulomatous disease (CGD) PMN on GSH of G6PD RBC. CGD PMN which are deficient in H_2O_2 production, were incubated with G6PD-deficient RBC. As noted in Fig. 6, CGD PMN, both at rest and during phagocytosis, as well as in the presence and absence of KCN, produced only minimal depletion of GSH from G6PD-deficient RBC compared with the effect obtained with normal PMN incubated at the same time under identical experimental conditions.

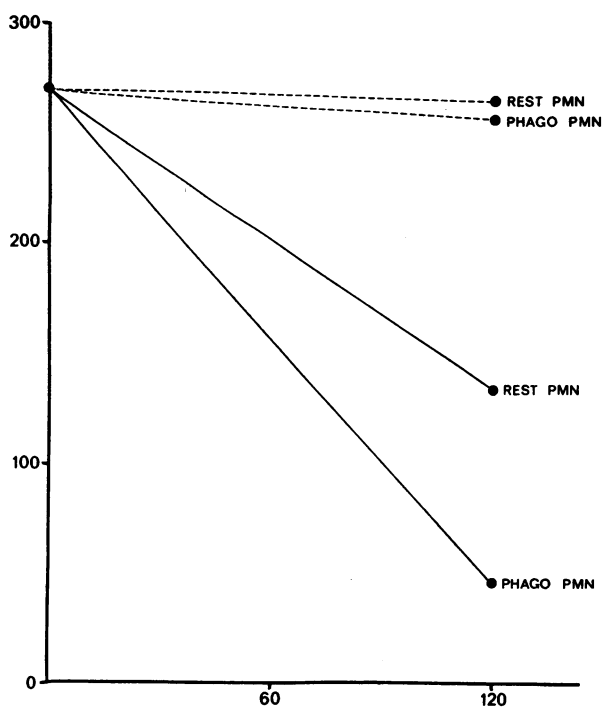

FIGURE 4 Effect of separation of PMN from RBC by dialysis tubing upon GSH levels of G6PD RBC. Results obtained with PMN in dialysis bags are indicated by the dotted lines. Results obtained with PMN in usual suspension with RBC are indicated by the solid lines. The ratio of RBC to PMN was 32:1. Results expressed as μg GSH per 10^{10} RBC on the ordinate and time in minutes on the abscissa.

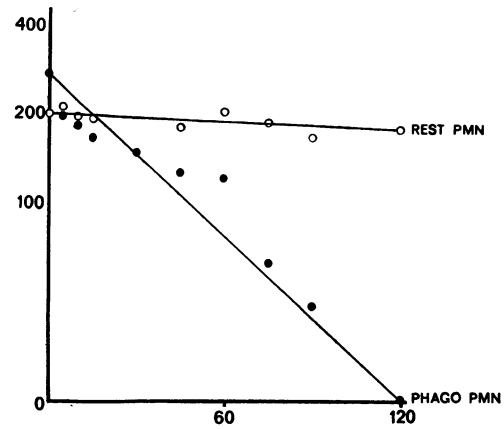

FIGURE 5 Determination of the exponential rate of GSH depletion in G6PD RBC by resting and phagocytizing PMN at a ratio of 20 RBC to 1 PMN. Results expressed as μg GSH per 10^{10} RBC on the ordinate and time in minutes on the abscissa.

Effect of a H_2O_2 generating system on RBC "ghosts" enriched with GSH. The rate of GSH depletion from GSH-enriched "ghosts" of normal RBC was determined using glucose and glucose oxidase as a source of H_2O_2 generation. The amount of glucose oxidase used in these experiments was first determined from the rate of oxy-

TABLE I

Estimation of the Percentage of Total H_2O_2 Produced by Glucose Oxidase or by Phagocytosing PMN Utilized for Oxidation of GSH of Red Cell Ghosts or of Intact G6PD RBC

Experiment	Column A	Column B	Column C
			Per cent of total H_2O_2 involved in GSH oxidation
	mμmoles H_2O_2 produced/hr	*mμmoles GSH oxidized/hr*	
1	113	6.5	2.8
2	292	3.8	0.7
3	298	3.2	0.6
4	840	19.7	1.2
5	93	28.0	15.2

Data in column A represents maximal H_2O_2 production calculated from the measured rate of oxygen consumption and based on equimolar ratios of each substance.

Experiments 1, 2, 3 were performed with ghosts of normal RBC and experiment 4 with intact G6PD RBC. Experiment 5 was with intact G6PD RBC and normal PMN at 23:1 ratio and results are expressed per 10^7 PMN. The data in column C are calculated from 2 × column A/column B × 100 since 1 mole H_2O_2 oxidizes 2 moles of GSH. See text for explanations.

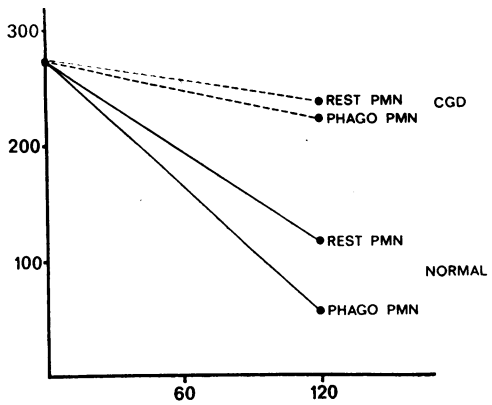

FIGURE 6 Lack of GSH oxidation of G6PD RBC by CGD PMN. Responses elicited by CGD PMN are indicated by the dotted lines. Responses elicited by normal PMN are indicated by the solid lines. In both the ratio of RBC to PMN was 10:1. Results expressed as μg GSH per 10^{10} RBC on the ordinate and time in minutes on the abscissa.

gen consumed by the enzyme as measured with the oxygen electrode. The efficiency of this system with regard to theoretical rates of H_2O_2 produced during glucose oxidase, catalyzed oxygen consumption was compared with rates of GSH oxidation in RBC ghosts as well as in G6PD-deficient RBC. Results are indicated in Table I. The artificial system was less efficient than phagocytosing PMN, i.e., only 0.6–2.8% of the theoretical rate of H_2O_2 produced by the artificial system was available for GSH oxidation in both the RBC ghosts fortified with GSH and the intact G6PD RBC compared with 15% for PMN.

Effect of in vitro H_2O_2 generation on in vivo survival of G6PD-deficient red cells. Two Caucasian patients with mild compensated hemolytic anemia due to G6PD deficiency were studied. Their salient hematologic features are given in Table II and showed that neither was anemic but both had a young population of RBC as indicated by red cell oxaloacetic transaminase levels consistent with response to mild hemolysis. Their ^{51}Cr-labeled RBC were suspended in glucose-enriched buffer with and without the hydrogen peroxide-generating enzyme, glucose oxidase. As indicated in Table III, GSH was depleted to 10% in patient E. E. and to 40% in patient S. F.; methemoglobin levels were less than 10% in E. E. and 5% in S. F. No Heinz bodies were evident. At the end of this incubation, each patients' RBC were washed five times, resuspended in buffer, and reinfused into them.

The ^{51}Cr survival curves are illustrated in Fig. 7 and show that 30–35% of the GSH-depleted cells were destroyed within 1 day. There was prompt organ accumulation of ^{51}Cr particularly in the spleen but also in the liver in both patients (Fig. 7B and 7C). These effects upon RBC survival were not observed when the G6PD RBC were incubated in the absence of the hydrogen peroxide-generating system (Fig. 7A). Incubation of normal RBCs for 90 min in the glucose buffer system was carried out at glucose oxidase concentrations which did not affect normal RBC GSH levels. A ^{51}Cr survival of 35 days was observed after reinfusion of this blood into the normal recipient.

DISCUSSION

The elaboration of hydrogen peroxide by phagocytizing PMN was first described by Quastel (8). This potent oxidant is the product of the cyanide-insensitive respiratory burst that occurs shortly after phagocytes ingest particles or bacteria (20). Paul and Sbarra have detected hydrogen peroxide in the dialysate from homogenates of phagocytosing PMN (21). The prime respiratory enzyme which catalyzes this reaction between atmospheric oxygen and intracellular reduced pyridine nucleotides is thought to be NADH oxidase (9, 22), although other oxidases have been identified in human phagocytes (23–25). CGD PMN, in contrast to normal PMN, lack a respiratory burst (26), fail to generate hydrogen peroxide (17–19), and are deficient in NADH oxidase activity (27). The availability of hydrogen peroxide produced by phagocytes for such diverse functions as bacterial killing or oxidant damage to RBC has not been accurately quantitated. It has previously been shown that only 3% of the peroxide produced during the respiratory burst is available intracellularly for the catalatic oxidation of formate-^{14}C (9). The findings presented in this report based upon the rate of GSH oxidation in G6PD-deficient RBC by juxtaposed phagocytizing PMN, suggest that approximately 10–15% of the peroxide generated during the respiratory burst is available to oxidize other cells. This effect is enhanced by cell—cell interaction since G6PD RBC separated from PMN (which were placed in small dialysis bags during the incubation) maintained

TABLE II
Laboratory Results of Patients with Caucasian G6PD Deficiency

Patient	Hb	Retic. count	Red cell transaminase/ μM/min per 10^{10}		Red cell G6PD/ μM/min per 10^{10}
	g/100 ml	%			
E. E.	14.5	1.8	Top	1.20	0.13
			Bottom	1.01	0.27
S. F.	15.0	1.2	Whole blood	0.77, 0.82	0.49, 0.52
			Top	1.11	0.61
			Bottom	0.71	0.77
		Normal		0.68 ±0.08	2.83 ±0.31

GSH levels in contrast to the G6PD RBC incubated with PMN. CGD PMN, which are deficient in the ability to form peroxide, caused only minimal loss of GSH from G6PD RBC. Conditions which favor peroxide accumulation, i.e. phagocytosis and inhibition of intracellular catalase with KCN, had minimal effects on G6PD RBC incubated with CGD PMN, but these conditions enhanced peroxide production and RBC GSH depletion by normal PMN.

The hemolytic effect of the antimalarial drug, primaquine, on red cells of Negro males which were deficient in G6PD led to the discovery and elucidation of the importance of the hexose monophosphate shunt (HMPS) as the biochemical shield of cells against oxidant stress (28). The maintainance of GSH levels in RBC depends upon generation of adequate NADPH by the HMPS. Beutler showed that RBC GSH levels were diminished in vivo in G6PD deficiency due to inadequate NADPH production by the shunt (29). Allen and Jandl found that thiol groups play a key role in preventing oxidative injury to red cells and hemoglobin (30). They noted that if sufficient oxidant stress was delivered to RBC by compounds such as acetylphenylhydrazine, the following metabolic sequences occurred: (*a*) GSH was oxidized to GSSG, a portion of the oxidized GSH formed mixed disulfides with the globin sulfhydryls of hemoglobin,

TABLE III

Effect of H_2O_2 Generating System In Vitro (Glucose and Glucose Oxidase) upon GSH and Methemoglobin of G6PD-Deficient ^{51}CR Red Blood Cells Compared with Normal Red Cells before Reinfusion of RBC to Patients

	Washed RBC suspended in buffer		
Time of incubation	Hgb	Methemoglobin	GSH
min	*g/100 ml*	*g/100 ml*	*μg/10¹⁰ RBC*
Patient E. E.			
0	10.9	0	529
30		0	159
60		0.46	79
90		0.83	66
after four washings		0.91	60
Patient S. F.			
0	11.3	0.0	592
30		0.12	350
60		0.49	314
after five washings		0.66	248
Normal			
0	10.5	0.0	811
90		0.10	811
after four washings		0.12	795

* See text for details.

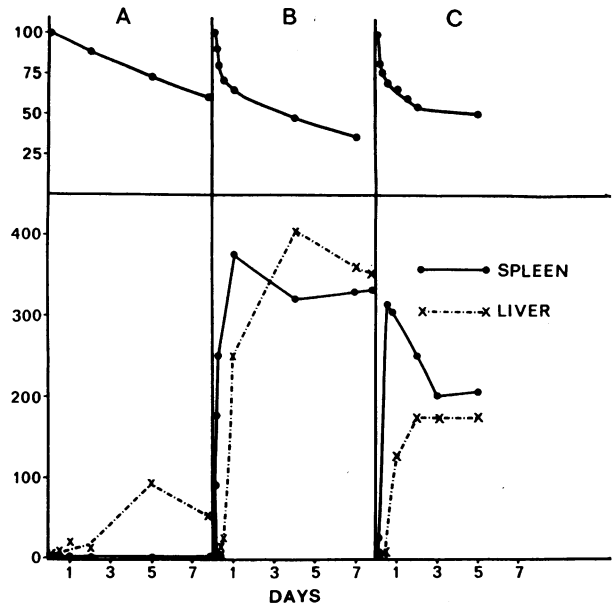

FIGURE 7 In vivo effect of prior in vitro oxidation of GSH of labeled sample of G6PD RBC. ^{51}Cr RBC survival expressed as percentage of initial value is indicated on the top panel and liver and spleen sequestration counts expressed as counts/minute per microcurie injected are indicated on the bottom panel. Panel A represents observations on patient E. E. after RBC were incubated in buffer with glucose but without glucose oxidase; panel B and C represent similar observations on patients E. E. and S. F., respectively, except that RBC GSH was depleted in vitro by peroxide generated from glucose and oxygen by glucose oxidase.

(*b*) methemoglobin was formed, (*c*) and denatured hemoglobin precipitated to form Heinz bodies. Jacob and Jandl further studied the effects of sulfhydryl blockade on normal RBC and noted that blockade of RBC membrane sulfhydryls produced a rapid loss of such RBC from the circulation and sequestration of these cells predominantly in the spleen (10, 31). Our studies provide further evidence of the noxious role of oxidant stress on G6PD RBC and indicate that the H_2O_2 produced by phagocytosing PMN can induce deleterious effects in G6PD RBC or on GSH levels in hemoglobin and enzyme-free ghosts of normal RBC.

The effects of H_2O_2 production on in vivo survival were very striking. We did not wish to expose human G6PD RBC to phagocytosing human PMN before reinfusion of the RBC because of the risk of contamination of the RBC by bacteria or viruses in the PMN. However, exposure of the G6PD RBC to equivalent levels of H_2O_2 produced by sterile glucose oxidase markedly decreased their survival. The glucose oxidase concentration was titered to mimic the oxidant potency of the PMN system, i.e. depletion of G6PD GSH of RBC without significant methemoglobin production and no

Heinz body formation. Although no significant cell retention was found when these RBC were passed through 3.5 μ millipore filters in vitro, reinfusion of their own GSH-depleted RBC into two G6PD-deficient Caucasians produced a rapid loss of 35–40% of RBC from their circulation. This was associated with rapid accumulation and persistence of radioactivity over the spleen and subsequently over the liver as well. The initial rapid sequestration of red cells was not observed when the ^{51}Cr RBCs were infused after the usual incubation in the *absence* of the glucose oxidase-hydrogen peroxide–generating system. The precise mechanism for RE sequestration of these GSH-depleted cells remains undefined, but could involve alteration of electrostatic charge on the surface of the RBC (32).

Depletion of GSH from ghosts of normal red cell required an amount of hydrogen peroxide generated from glucose and glucose oxidase similar to that required for depletion of GSH in intact G6PD-deficient RBC. Mills (33) has isolated and partially purified a glutathione peroxidase from human red cells which is absent in red cell ghost preparations. The similar responses of normal RBC ghosts and intact G6PD RBC suggests that GSH oxidation by H_2O_2 in RBC may be nonenzymatic.

The results of our study suggest that in areas where there is an accumulation of PMN in vivo, such as with local infection or inflammation surrounded by hyperemia or in the "septic spleen," sufficient hydrogen peroxide can be generated to deplete GSH levels in juxtaposed G6PD-deficient RBC. This membrane injury could lead to sequestration of these red cells in the spleen and liver and so accentuate the extent of the hemolytic process in affected patients. The lower G6PD activity in Caucasians than in Negroes with this RBC deficiency (6) may render the former group more susceptible to infection induced hemolysis. Three Caucasians of English, Sicilian, and Ashkenazic Jewish ancestry, respectively, displayed more profound hemolysis subsequent to a single dose of 45 mg of primaquine base than did three Negro males with G6PD RBC deficiency (34). Since only the older red cells are deficient in Negroes with G6PD RBC deficiency, the mechanism proposed in this investigation may play a role for inducing hemolysis only on that proportion of cells most susceptible to oxidant damaged. It remains to be determined if such a mechanism does or does not pertain in Negro-deficient subjects when they have infections. Although there is usually an associated deficiency of G6PD in the leukocytes of affected Caucasians (35), the levels are rarely below 20% of normal and the oxidative capacities of such phagocytes are not diminished.[2] Thus they readily provide a source of oxidant damage to RBC during infection.

[2] Baehner, R. L., and R. B. Johnston, Jr. Oxidative and bactericidal studies of G6PD-deficient PMN. Manuscript in preparation.

ACKNOWLEDGMENTS

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