TECHNICAL / SCIENTIFIC REPORT

22-72

CHLORAMPHENICOL-INDUCED HEMOLYSIS IN CAUCASIAN
GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

By

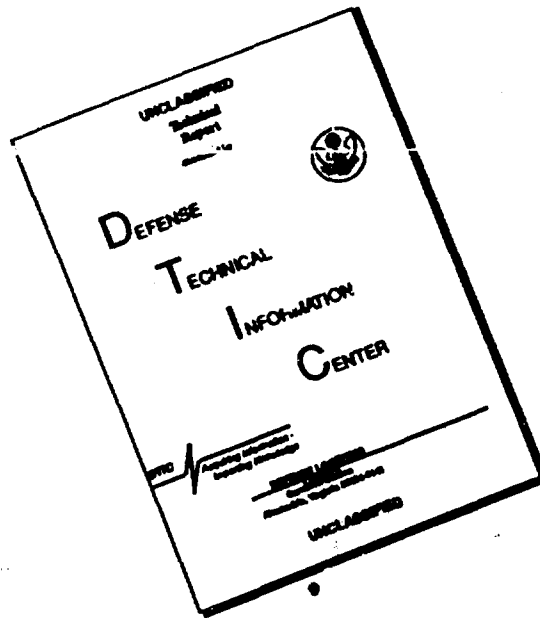Ronald P. McCaffrey, Charles H. Halsted
Mohammed Fathy Abdel Wahab, R. Paul Robertson

U. S. NAVAL MEDICAL RESEARCH UNIT No. 3

(CAIRO, THE ARAB REPUBLIC OF EGYPT)

FPO NEW YORK 09527

DISCLAIMER NOTICE

THIS DOCUMENT IS BEST
QUALITY AVAILABLE. THE COPY
FURNISHED TO DTIC CONTAINED
A SIGNIFICANT NUMBER OF
PAGES WHICH DO NOT
REPRODUCE LEGIBLY.

DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
U.S. Naval Medical Research Unit No. 3 FPO New York 09527	UNCLASSIFIED
	2b. GROUP

3. REPORT TITLE

CHLORAMPHENICOL-INDUCED HEMOLYSIS IN CAUCASIAN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final

5. AUTHOR(S) (First name, middle initial, last name)

Ronald P. McCaffrey, Charles H. Halsted, Mohammed Fathy Abdel Wahab and R. Paul Robertson

6. REPORT DATE **10 March 1970**	7a. TOTAL NO. OF PAGES **5**	7b. NO. OF REFS **22**
8a. CONTRACT OR GRANT NO c. PROJECT NO **MR005.20.01-0058**	9a. ORIGINATOR'S REPORT NUMBER(S) **NAMRU-3-TR.22-72**	
	9b. OTHER REPORT NO (S) (Any other numbers that may be assigned this report)	

10. DISTRIBUTION STATEMENT
Distribution of this report is unlimited

11. SUPPLEMENTARY NOTES **Published in** **Ann. Int. Med. 74(5):722-726. 1971**	12. SPONSORING MILITARY ACTIVITY Bureau of Medicine and Surgery Department of the Navy Washington, D.C. 20390

13. ABSTRACT

Three white G6PD-deficient patients suffered severe hemolytic reactions during treatment for typhoid fever with chloramphenicol. Two of these patients were studied when free of infection to determine the hemolytic potential of chloramphenicol in the noninfected G6PD-deficient white person. It was found to be mildly hemolytic under these conditions, suggesting that a drug-disease synergism was primarily responsible for the clinical hemolytic reactions. The febrile state itself, or changes in plasma amino acids accompanying infection, may be responsible for disease-related hemolysis.

i

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Glucose-6-Phosphate Dehydrogenase Deficient patients Typhoid fever Chloramphenicol hemolytic reactions Clinical observations						

Chloramphenicol-Induced Hemolysis in Caucasian Glucose-6-Phosphate Dehydrogenase Deficiency

Lt. RONALD P. MCCAFFREY, MC, USNR, CHARLES H. HALSTED, M.D., Surg. USPHS,

MOHAMMED FATHY ABDEL WAHAB, M.B., Ch.B., DTM & H., D.M.,

Lt. R. PAUL ROBERTSON, MC, USN, Cairo, United Arabic Republic

Three white G6PD-deficient patients suffered severe hemolytic reactions during treatment for typhoid fever with chloramphenicol. Two of these patients were studied when free of infection to determine the hemolytic potential of chloramphenicol in the noninfected G6PD-deficient white person. It was found to be mildly hemolytic under these conditions, suggesting that a drug-disease synergism was primarily responsible for the clinical hemolytic reactions. The febrile state itself, or changes in plasma amino acids accompanying infection, may be responsible for disease-related hemolysis.

THE SUSCEPTIBILITY of individuals deficient in glucose-6-phosphate dehydrogenase (G6PD) to hemolysis on exposure to a wide variety of drugs has become well known since the initial description of hemolysis in American negroes given the antimalarial agent, primaquine (1). More recently (2-4), many disease states, primarily bacterial and viral infections, have been associated with hemolysis in enzyme-deficient subjects, in the absence of drug exposure of any type. In situations where a disease and a drug are associated with a hemolytic reaction it has often been impossible to state whether the illness, the drug, or the combination was responsible for the hemolysis. Moreover, in a few instances some of the drugs commonly

listed as being "hemolytic" in G6PD deficient subjects have been thus categorized on the basis of clinical observations in disease states that are now known to be hemolytic in themselves (5). These drugs include chloramphenicol, nitrofurantoin (Furadantin®)-related compounds, quinidine, and acetylsalicylic acid. Their role in precipitating hemolysis should be considered no more than "circumstantial" until a clear association with hemolysis is observed in deficient subjects in the absence of a disease state.

We recently had occasion to observe serious hemolytic reactions in three white G6PD-deficient subjects during chloramphenicol treatment of 58 patients for typhoid fever. These hemolytic crises were the most serious side effects encountered with chloramphenicol; on the basis of this circumstantial evidence it was suggested that ampicillin be used in enzyme-deficient subjects in place of chloramphenicol in the treatment of typhoid fever (6). About the same time another group reported hemolysis in five similar patients during treatment of typhoid fever with chloramphenicol (7). In these cases, however, it was thought that the disease process, not the chloramphenicol, was the prime hemolytic stimulus. Thus, in order to define further the role of chloramphenicol in these situations, two of our patients were studied in detail under controlled conditions, when free of infection. The results are reported here.

Patients and Methods

CASE 1

Patient A.L.S., a 9-year-old Egyptian boy, was ad-

mitted to the Abbasiah Fever Hospital, Cairo, after a 4-day period of fever, headache, cough, abdominal discomfort, nausea, vomiting, and diarrhea. He received no treatment before admission. He appeared acutely ill; his temperature was 40 C and pulse 86. There was generalized abdominal tenderness and moderate hepatosplenomegaly. Scattered rhonchi were audible in both lungs. The clinical impression on admission was typhoid fever and hepatosplenic schistosomiasis. Appropriate laboratory data were obtained; Salmonella typhi were subsequently isolated from blood, stool, urine, and bone marrow cultures. Schistosoma mansoni ova were found in stool specimens. The hematocrit on admission was 28. Treatment was begun on the first hospital day with oral chloramphenicol, 50 mg/kg body weight daily. On the fifth hospital day jaundice and hemoglobinuria were noted. The temperature had remained between 38.5 C and 40 C since admission. The hematocrit at this time was 13. Stools were repeatedly guaiac negative. A diagnosis of red cell G6PD deficiency was made at this time (using the glutathione stability test (8)), and, based on this, the patient was thought to be suffering from severe hemolysis. The chloramphenicol therapy was discontinued. He was treated with blood replacement, intravenous fluids, and steroids. Oral ampicillin, 100 mg/kg body weight daily, was started. He defervesced 3 days later. On the fifth day of ampicillin treatment he was remarkably improved; his hematocrit was 30 and reticulocyte count, 25%.

Approximately a year later he was readmitted to the hospital for further study.

CASE 2

Patient M.S.A., a 16-year old Egyptian youth, was admitted to the Abbasiah Fever Hospital, Cairo, after a 6-day period of fever, headache, cough, abdominal distention, nausea, vomiting, diarrhea, and, later, constipation. He had received a small but unknown quantity of aspirin before admission. He appeared acutely ill; his temperature was 40 C and pulse 80. There were generalized abdominal tenderness, moderate hepatosplenomegaly, and scattered rhonchi. Clinical diagnoses of typhoid fever and hepatosplenic schistosomiasis were made on admission. Appropriate base-line laboratory studies were done. Blood cultures grew out Salmonella typhi. Microscopic examination of stool showed Schistosoma mansoni ova. The hematocrit on admission was 29. Treatment was begun on the first hospital day with oral chloramphenicol, 50 mg/kg body weight daily. On the fourth hospital day jaundice and hemoglobinuria developed. The temperature up to this time had remained in the range of 39 to 40 C. The hematocrit was now 17. Stools were repeatedly negative on testing for blood. At this time a diagnosis of red cell G6PD deficiency (using the screening test of Jacob and Jandl (9)) was made, and the patient was considered hemolytic on this basis. The chloramphenicol therapy was discontinued, and oral ampicillin, 100 mg/kg body weight daily, was substituted. Blood replacement, intravenous fluids, and steroids were added. He did well thereafter; by discharge 14 days later his hematocrit was 35.

Approximately a year later he returned to the hospital for further study.

Both patients entered into the study with informed,

voluntary consent*. They had been well in the interval. Both were found to be free of typhoid infection on repeated culture of blood, urine, and stool. Hepatosplenomegaly, and S. mansoni ova in stool were still present. Their hematocrits were 37 and 40, respectively, at this time; reticulocyte counts were 1.3% and 0.9%. By assay (10) and starch gel electrophoresis (11), their residual red cell G6PD was similar to Caucasian-type G6PD (Gd, Mediterranean).

Base-line serial hematocrits, reticulocyte counts, and bilirubin were measured over several days. Stools were repeatedly tested for occult blood throughout the study, using guaiac. An autologous red cell survival study using ⁵¹Cr random labeling was begun (12). After establishment of the ⁵¹Cr disappearance slope over the first several days, a 7-day course of oral chloramphenicol, 35 mg/kg body weight daily, was given to both subjects, and plots of the ⁵¹Cr elimination rate were continued. Concurrent measures of hematocrits, reticulocyte counts, and bilirubin values were made during these periods.

Results

The red cell survival curves for each patient are charted in Figures 1 and 2. The course of the first patient was complicated by the development of a 2-day period of fever (temperature, 40 C), from day 7 to day 9 of the ⁵¹Cr survival study. Blood cultures taken during this time subsequently grew out Escherichia coli, sensitive to chloramphenicol. During the period of fever and bacteremia there was no change
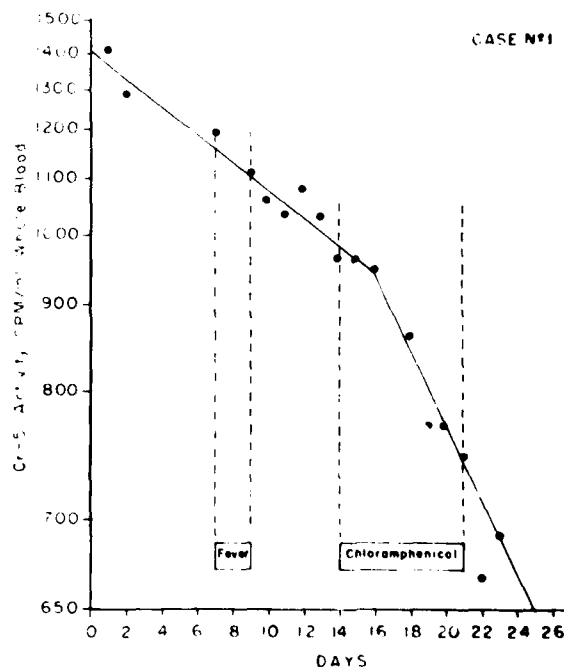
Figure 1. Red cell survival curves for Patient A.L.S.

CASE N°2
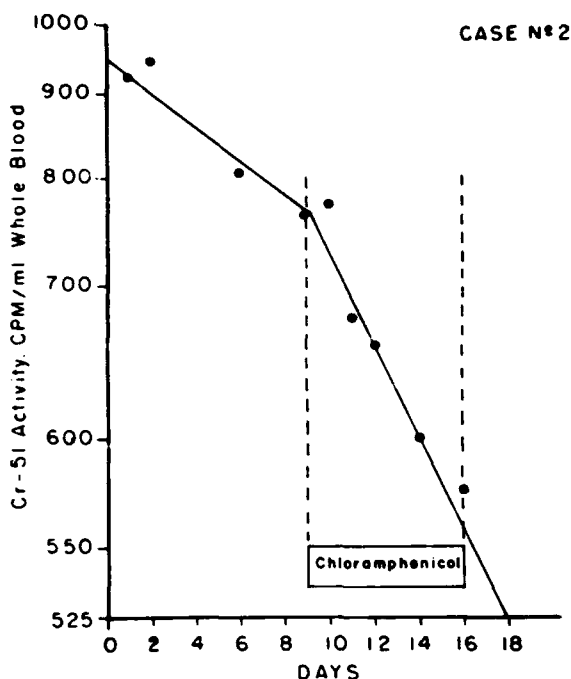
Figure 2. Red cell survival curves for Patient M.S.A.

Discussion

The role of a wide variety of drugs in initiating and perpetuating hemolytic reactions in G6PD-deficient subjects has been repeatedly documented (13). Uncontrolled clinical observations have suggested that a number of disease states, especially febrile infectious processes, can act in a similar manner, thus obscuring the role of simultaneously applied drugs in these hemolytic reactions (3). Clear evidence that chloramphenicol can induce hemolysis in G6PD-deficient subjects in the absence of such a disease state has been lacking. On the basis of the data presented here, it is evident that chloramphenicol has mild but definite hemolytic potential in G6PD-deficient white persons. Its hemolytic potential in deficient negro subjects remains to be defined.

The mild reactions observed under controlled conditions are in contrast to the fulminating hemolysis that was seen clinically during the typhoid illness. The admission hematocrits of 28 and 29, when compared with the normal recovery hematocrits, may indicate the presence of a hemolytic process before the institution of treatment. In this regard, the role of the aspirin taken by the second patient before admission is unknown. Unfortunately, reticulocyte counts from this period are not available. Assuming the presence of disease-induced hemolysis on admission, the addition of the mildly hemolytic chloramphenicol may have resulted in a drug-disease synergism, ending in fulminating hemolysis. In this regard, a drug-drug
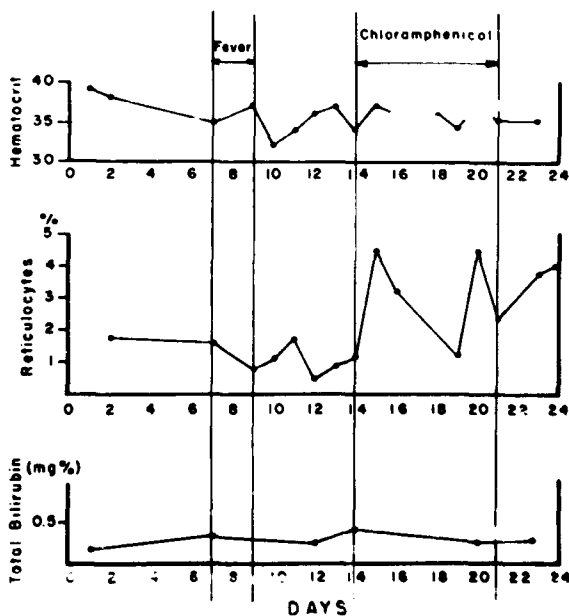
in the ⁵¹Cr slope. On the 14th day of the ⁵¹Cr study the 7-day course of chloramphenicol was started and was followed by an obvious acceleration in the ⁵¹Cr decay rate. By extrapolation from pre- and post-chloramphenicol slopes. the predrug T½ was 33 days and postdrug T½, 17 days. The second patient had an uncomplicated course. The chloramphenicol was introduced on the seventh day of the ⁵¹Cr study, followed by a similar accelerated removal of labeled red cells. Here the (extrapolated) red cell survival time fell from an expected T½ of 30 days to 16 days.

In addition to the definite changes in the ⁵¹Cr slopes, there was evidence for hemolysis with standard laboratory measures. Figures 3 and 4 tabulate concurrent hematocrits, reticulocyte counts, and bilirubin values. In both patients a definite reticulocytosis followed the introduction of the chloramphenicol. Hematocrits and bilirubin values remained essentially unchanged. A "rebound" reticulocytosis after removal of the chloramphenicol suggests erythropoietic suppression, in addition to hemolysis, during the period of chloramphenicol administration in Patient M.S.A.

Table 1 contains estimates of the severity of hemolysis based on the ⁵¹Cr data. Losses above physiologic destruction amounted to 13% to 15% of the red cell mass after the course of chloramphenicol. During the period of typhoid illness, based on calculations from hematocrit changes, an estimated 60% of the red cell mass was destroyed over approximately the same time.

Figure 3. Concurrent hematocrits, reticulocyte counts, and bilirubin values for Patient A.L.S.
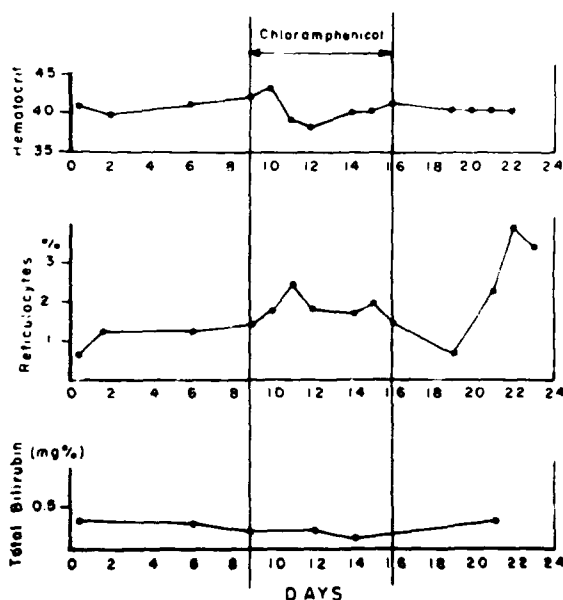
Figure 4. Concurrent hematocrits, reticulocyte counts, and bilirubin values for Patient M.S.A.

in plasma amino acids was seen 2 to 3 days after the onset of symptoms. All amino acids were affected, including precursors of the tripeptide glutathione, which is low in quantity and "unstable" in G6PD-deficient red cells and is probably the critical component involved in protecting red cells from oxidative injury (13). The importance of these changes in plasma amino acids is suggested by the work of Batalden, Swaim, and Lowman (19), who report a specific dietary requirement for glutathione precursors for normal red cell glutathione content. Other data on red cell glutathione turnover (20, 21) may be consistent with a limiting effect of plasma precursors on red cells only marginally compensated for this component. The implications of this possibility in terms of amino acid replacement during infected states are obvious.

synergism has been postulated for aspirin when added to primaquine in a febrile patient (14). No hemolytic reactions were noted in a similarly sized group of patients treated at the same time with ampicillin for typhoid fever (6), although several admission hematocrits in the ampicillin-treated group were in the range of the patients studied here. Since the frequency of G6PD deficiency would be expected to be the same in both groups (about 5% overall frequency among Egyptians (15)), the role of chloramphenicol in these reactions is definite. Thus, it would be prudent to avoid giving this drug to white G6PD-deficient subjects.

The mechanism or mechanisms of disease-induced hemolysis remain speculative. The 2 days of fever and bacteremia noted in the first patient were probably not present long enough to initiate hemolysis. Karle (16, 17) has shown that a sustained 2- to 3-C elevation of body temperature in the rabbit can result in significant degrees of hemolysis. Accelerated red cell destruction is almost exclusively confined to older cells and cells low in all enzymes, including G6PD. The patients studied here continued to be febrile during their clinical hemolytic crises. Possible parallels between hemolysis in experimental pyrexia and disease-induced hemolysis in G6PD-deficient subjects need to be explored.

Another possibility that deserves consideration is suggested by work on plasma amino acid changes in experimental typhoid infection in human volunteers (18). In all subjects who became ill a significant drop

Addendum

Since completion of our manuscript, Chan and associates (22) have shown that chloramphenicol does not intensify the hemolysis seen in G6PD-deficient Chinese subjects with typhoid fever. This lack of synergism may be caused by the qualitative differences between the G6PD Canton variant present in the Chinese patients and the G6PD Mediterranean variant present in our Egyptian patients. This suggests that chloramphenicol-typhoid synergism may be limited to white G6PD-deficient subjects.

References

1. HOCKWALD RS, ARNOLD J, CLAYMAN CB, et al: Status of primaquine. IV. Toxicity of primaquine in Negroes. *JAMA* 149:1568-1574, 1952
2. MENGEL CE, METZ E, YANCEY WS: Anemia during acute infections. *Arch Intern Med* (Chicago) 119:287-290, 1967
3. BURKA ER, WEAVER Z, MARKS PA: Clinical spectrum of hemolytic anemia associated with glucose-6-phosphate dehydrogenase deficiency. *Ann Intern Med* 64:817-825, 1966

4. PHILLIPS SM, SILVERS NP: Glucose-6-phosphate dehydrogenase deficiency, infectious hepatitis, acute hemolysis, and renal failure. Ann Intern Med 70:99-104, 1969

5. WORLD HEALTH ORGANIZATION: Standardization of Procedures for the Study of Glucose-6-Phosphate Dehydrogenase. Report of a WHO Scientific Group. Technical Report Series No. 366, Geneva, World Health Organization, 1967

6. ROBERTSON RP, WHAB MEA, RAASCH FO: Evaluation of chloramphenicol and ampicillin in salmonella enteric fever. New Eng J Med 278:171-176, 1968

7. HERSKO C, VARDY PA: Hemolysis in typhoid fever in children with G6PD deficiency. Brit Med J 1:214-215, 1967

8. BEUTLER E, DERN RJ, FLANAGAN CL, et al: The hemolytic effect of primaquine VIII. Biochemical studies on drug sensitive erythrocytes. J Lab Clin Med 45:286-295, 1955

9. JACOB HS, JANDL JH: A simple visual screening test for glucose-6-phosphate dehydrogenase deficiency employing ascorbate and cyanide. New Eng J Med 274:1162-1166, 1966

10. KORNBERG A, HORECKER BL: Glucose-6-phosphate dehydrogenase, in Methods in Enzymology, vol. 1, edited by COLOWICK SP, KAPLAN NO. New York, Academic Press, 1955, p. 330

11. STAMATOYANNOPOULOS G, YOSHIDA A, BACOPOULOS C: Athens variant of glucose-6-phosphate dehydrogenase. Science 157: 831-833, 1967

12. MOLLISON PL, VEALL N: The use of the isotope Cr-51 as a label for red cells. Brit J Haemat 1:62-74, 1955

13. CARSON PE, FRISCHER H: Glucose-6-phosphate dehydrogenase deficiency and related disorders of the pentose phosphate pathway. Amer J Med 41:744-761, 1966

14. BREWER GJ, ZARAFONETIS CJD: The hemolytic effect of various regimens of primaquine with chloroquine in American Negroes with G6PD deficiency and the lack of an effect of various antimalarial suppressive agents on erythrocyte metabolism. Bull WHO 36:303-308, 1967

15. MCCAFFREY RP, AWNY AY: Glucose-6-phosphate dehydrogenase deficiency in Egypt (with a note on the Methemoglobin reduction test). Blood 36:793-796, 1970

16. KARLE H: Significance of red cell age to red cell destruction during experimental fever. Brit J Haemat 15:221-229, 1968

17. KARLE H: The site of abnormal erythrocyte destruction during experimental fever. Ibid., pp. 475-485

18. FEIGIN RD, KLAINER AS, BEISEL WR, et al: Whole blood amino acids in experimentally induced typhoid fever in man. New Eng J Med 278:293-298, 1968

19. BATALDEN P, SWAIM WR, LOWMAN JT: Diet induced red cell glutathione deficiency. J Lab Clin Med 71:312-318, 1968

20. STEINBERG A, ADAM A, RAMOT B, et al: The incorporation of isotopically labelled glycine into glutathione of erythrocytes with glucose-6-phosphate dehydrogenase deficiency. Biochim Biophys Acta 36:65-73, 1959

21. HOCHBERG A, RIGBI M, DIMANT E: The incorporation in vitro of glycine and L-glutamic acid into glutathione of human erythrocytes. Biochim Biophys Acta 90:464-471, 1964

22. CHAN TK, CHESTERMAN CN, MCFADZEAN AJS, et al: The survival of glucose-6-phosphate dehydrogenase deficient erythrocytes in patients with typhoid fever on chloramphenicol therapy. J Lab Clin Med 77:177-184, 1971