MEDICAL GRAND ROUNDS

Parkland Memorial Hospital

April 4, 1963

PRIMAQUINE SENSITIVE ANEMIA

Case History [redacted])

The patient is a 24 year old [redacted] woman who has been followed at [redacted] since 1954. In November, 1955 she was admitted for delivery of a term pregnancy. Mild pre-eclampsia was diagnosed on the basis of albuminuria, and a blood pressure of 142/40; however, she had an uneventful delivery, receiving no medication other than Fergon and Pitocin, morphine and phenobarbital. Her hemoglobin on discharge was 10.8 gm/100 ml.

She was next seen on [redacted]-57 in the 7th month of pregnancy complaining of pain on the right side of three days duration and fever beginning on the day prior to admission. Urinalysis revealed 5 to 10 pus cells. The hemoglobin was reported at 8.4. She was treated with Achromycin I.V. for 24 hours and discharged. The patient was admitted on [redacted]-57 for the delivery of her second child. Her hemoglobin on admission was 12.0. The delivery was uneventful, and the patient was discharged on [redacted]-57 having received medications consisting only of Fergon and codeine.

She was next seen 15 days later with a two day history of fever, chills, right flank pain followed by nausea, vomiting and cloudy urine. Hemoglobin on admission was 8.5, hematocrit 28%. The urine was loaded with white cells. Urine culture grew out E. Coli and she was treated with intravenous Achromycin, 1 gram, and Penicillin, 0.5 m.u. followed by achromycin 250 mg. q-6-h P.O. Five days later the urinalysis showed 10 to 15 cells, however, the patient complained of nausea and vomiting following the oral Achromycin and she was consequently shifted to intravenous Achromycin, and Furadantin was added in an oral dose of 50 mg. q.i.d.

The first dose of Furadantin was given at 2:00 PM on [redacted]57 and every four hours during the day thereafter for two days. On the morning of [redacted]-57 while being ambulated in preparation for discharge, the patient suddenly fainted. A hemoglobin obtained at that time showed a drop to 4.8 mg.%. The hematocrit was 15. The plasma was reported to be "jaundiced". By the following day, the patient's hemoglobin had further decreased to 2.6 gm%, the hematocrit to 10.3%. Reticulocyte count was 7.4% with 10 nucleated red cells, WBC 31,200. Urobilinogen was noted in the urine, and the serum bilirubin had risen 5.4 mg.%. Furadantin was discontinued after the 10:00 PM dose on [redacted]-57, and thereafter the patient received no drugs whatsoever. Reticulocyte count obtained on [redacted]57 was 6.7%. The patient was treated with 2 units of whole blood and on the following day, her hemoglobin was reported as 8.6 and hematocrit 23. Another unit of blood was given on [redacted]-57 and by the time of discharge on [redacted]-57 her hemoglobin had risen to 12.0, and the hematocrit 35%. A bone marrow obtained on [redacted]-57 showed hypercellular marrow with erythrocyte:granulocyte ratio of 1:1. The erythrocyte hyperplasia is of the normoblastic type. No marrow iron. Impression: erythrocytic hyperplasia. The sickle cell prep was negative. Serology was negative. Coombs test was likewise negative. The patient was discharged with the diagnosis of iron deficiency anemia and acute hemolytic anemia secondary to Furadantin.

The patient was next seen 3-1/2 months later ([redacted]57) when she was again admitted to [redacted] with a two day history of chills, fever, pain in the left side but no dysuria. Hemoglobin on admission was 8.8 gm.%, hematocrit 32%. The urine contained approximately 100 WBC, and E. Coli was again cultured from a catheterized specimen. The pyelonephritis was initially treated with Achromycin and Penicillin and streptomycin. However, on 11-28-57 in addition to this therapy she was again begun on Furadantin, 50 mg. q.i.d. Over the following 6 days the patient's hemoglobin dropped to 7.3 and her hematocrit decreased to 24.

Her reticulocyte increased from a premedication level of 2.5% to 4.5% and reached a maximum on [redacted]-57 of 10.2%. Furadantin was discontinued on [redacted]-57 and at the time of discharge on [redacted]-57 this patient's hemoglobin was 7.6 gr.%.

The patient was next seen in this hospital on [redacted]58 at which time she was five months pregnant. The hemoglobin at that time was 7.8, hematocrit 28.5.

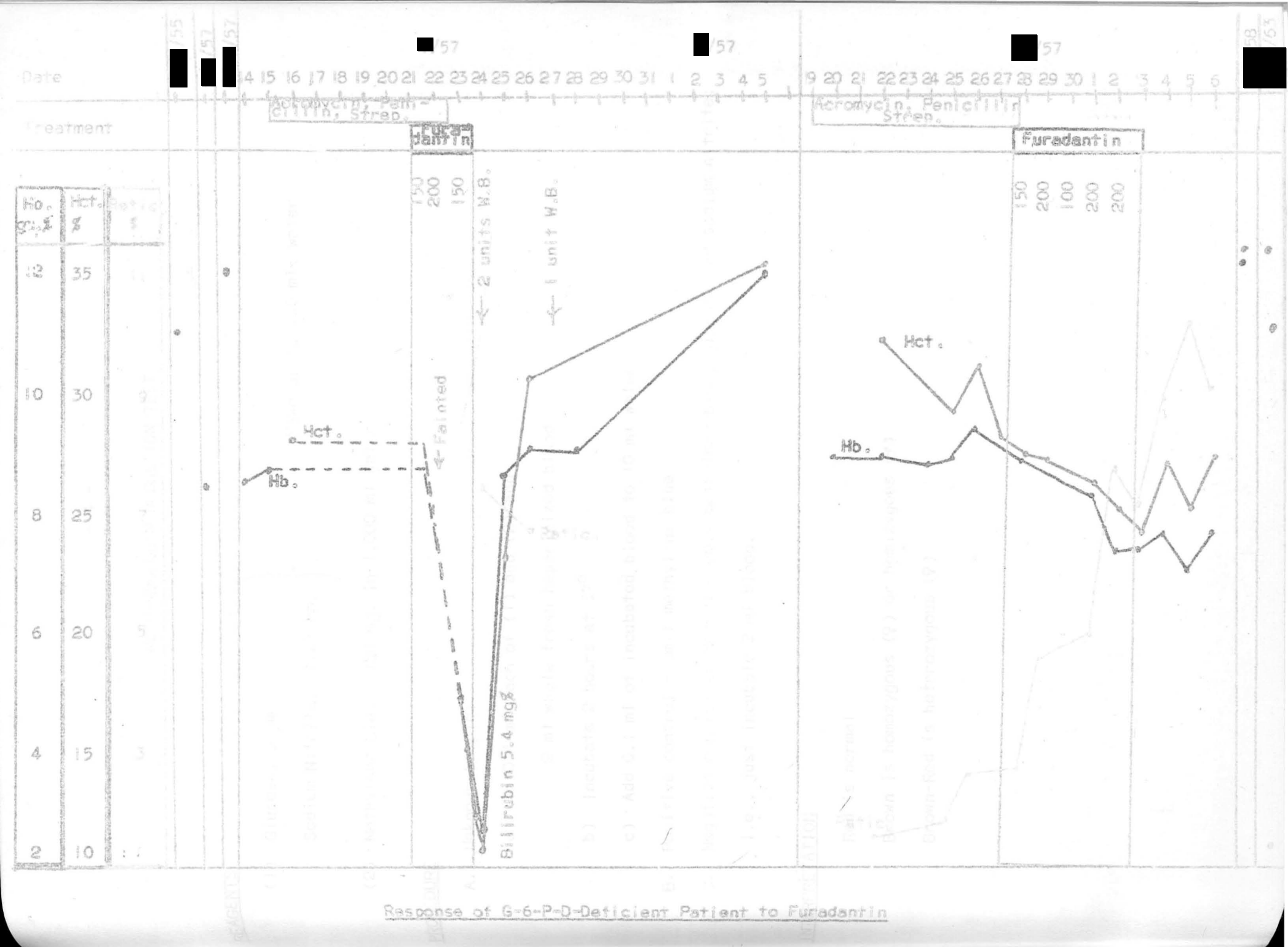
In the latter part of this pregnancy, she was placed on Kynex, 1 gm per day. On [redacted]-58 she was admitted for delivery of her term pregnancy. The blood pressure at that time was 148/110, 3+ albuminuria was noted and there were 1 to 3 white cells in her urine. The hematocrit was 33%. Because of the pre-eclampsia and the history of chronic pyelonephritis a tubal ligation was performed after delivery of a viable infant. The patient's serology was found to be positive, and she was treated after discharge with 10,000,000 units of Penicillin.

She has subsequently been seen occasionally in the Emergency Room and Out-patient Clinic for minor injuries and acute PID, but has otherwise been well.

In view of her history, the patient was recalled on [redacted]-63 and a drug history at that time revealed that she frequently took Anacin in a dosage of 3 tablets per day but was on no other medication. Her hemoglobin at this time was 11.1 gm.%, hematocrit 36, WBC 4,9000.

A glucose-6-phosphate dehydrogenase assay was carried out on her red cells, and the level of this enzyme was clearly depressed (TPNH-production in density units per minute per mg of hemoglobin was 0.23, normal control 1.90). The Methemoglobin Reduction Test of Brewer was likewise positive.

She was advised to take no medication without consulting a physician who is aware of her disease.



Response of G-6-P-D-Deficient Patient to Furadantin

METHEMOGLOBIN REDUCTION TEST

REAGENTS

(1) Glucose, 5 gm
Sodium Nitrite, 1.25 gm. } Make up to 100 ml. water

(2) Methylene Blue 150 mg. in 1,000 ml water

PROCEDURE

A. Unknown Sample:

a) To 0.1 ml each of (1) and (2) add
2 ml whole fresh heparinized blood

b) Incubate 2 hours at 37°

c) Add 0.1 ml of incubated blood to 10 ml water

B. Positive control - omit methylene blue

C. Negative control or Normal - omit methylene blue, glucose and sodium nitrite; i.e., just incubate 2 ml blood.

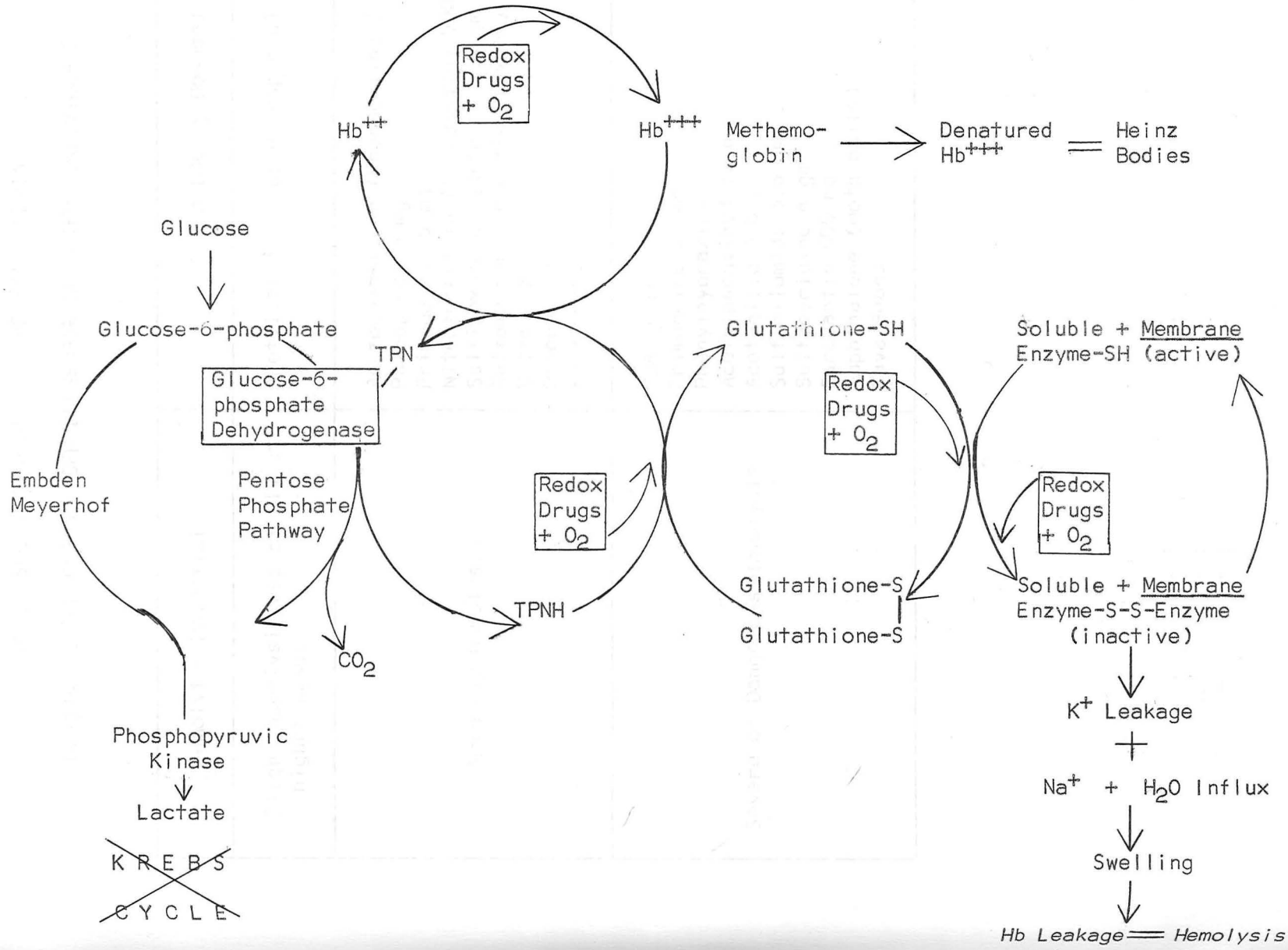
INTERPRETATION

Red is normal

Brown is homozygous (♀) or hemizygous (♂)

Brown-Red is heterozygous (♀)

BIOCHEMICAL LESIONS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY



DRUGS SHOWN TO PRODUCE HEMOLYTIC ANEMIA

IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT INDIVIDUALS

Hemolytic Potential	Drugs (Daily Dosage)
Slight hemolysis especially at higher doses	Acetylsalicylic acid (aspirin)
Moderate Hemolysis	Acetophenetiden (Phenacetin) 3.6 gm Pamaquine 10 mg Primaquine 15 mg Nitrofurantoin (Furadantin) 500 mg Sulfisoxazole (Gantrisin) 8 gm Sulfamethoxypyridazine (Kynex) 2 gm Vitamin K Probenecid Fava Beans
Severe or Dangerous Hemolysis	Pamaquine 20 mg Primaquine 30 mg Phenylhydrazine Acetylphenylhydrazine Acetanilid 3.6 gm Sulfanilamide 3.6 gm. Sulfapyridine 4 gm Furadantin 600 mg Naphthalene (moth balls) Fava Beans

MANIFESTATIONS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCIES

Syndrome	Race	RBC Hemolysis		G6P-D	
		Life Span Without Drugs	Sensitivity to Drugs	Activity	Enzyme Structure
Primaquine Sensitive Anemia	Negro	Slightly shortened (25%) (Compensated hemolysis)	Moderate (heterozygous) to severe (hemi- or homozygous). Not caused by chloramphenicol, PAS, or quinidine.	Mod. Depressed	Normal
	Caucasian	Slightly shortened.	Severe response to drugs including chloramphenicol, PAS and quinidine.	Very Depressed	Probably Normal.
Congenital Non-spherocytic Hemolytic Anemia (G6P-D-Deficient type)	Caucasian	Continuous hemolysis.	Severe response to small doses of drugs.	Almost Undetectable.	Abnormal

REFERENCES

Reviews of Primaquine Sensitive Hemolytic Anemia

1. Tarlov, A.R., Brewer, G. J., Carson, P.E. and Alving, A.S.: Primaquine Sensitivity. Archives. Int. Med. 109:209, 1962.
Best and most complete review of entire subject.

2. Kellermeyer, R. W., Tarlov, A. R., Brewer, G. J., Carson, P. E. and Alving, A.S.: Hemolytic Effect of Therapeutic Drugs, Clinical Considerations of the Primaquine-type Hemolysis. J.A.M.A. 180:388, 1962.
Good general review of clinical aspects of primaquine sensitivity.

3. Beutler, E.: The Hemolytic Effect of Primaquine and Related Compounds: A Review. Blood 14:103, 1959.
The most comprehensive review of the literature through 1957.

4. Beutler, E.: Drug-Induced Hemolytic Anemia (Primaquine Sensitivity). In The Metabolic Basis of Inherited Disease. Stanbury, Wyngaarden and Fredrickson. McGraw-Hill, 1960 p. 1031.
Another excellent review of the field.

5. Carson, P. E.: Glucose-6-phosphate Dehydrogenase Deficiency in Hemolytic Anemia. Fed. Proc. 19:995, 1960
Good general discussion of biochemistry of glucose-6-phosphate dehydrogenase deficiency.

6. Alving, A.S., Tarlov, A.R., Brewer, G. J., Carson, R.E., Kellermeyer, R. W. and Long, W. K.: Glucose-6-phosphate Dehydrogenase Deficiency: Some Biological Implications. Trans. Assoc. Am. Physicians 78:80, 1960.

Review of Red Cell Metabolism

7. Altman, K. I.: Some Enzymologic Aspects of the Human Erythrocyte. Am. J. Med. 27:936, 1959.
A very complete discussion of the details of red cell intermediary metabolism.

Early Observations that an Antimalarial Drug Pamaquin Would Produce Anemia.

8. Dimson, S. B. and McMartin, R. B.: Pamaquin Haemoglobinuria. Quart. J. Med. 15:25, 1946.
Careful review and description of 18 cases of hemolytic anemia following Pamaquin treatment of 18,000 cases of malaria in Indian Army. Hemolysis was not observed in British troops.

9. Earle, D. P. J., Bigelow, F.S., Zubrod, C. G. and Kane, C.A.: Studies on Chemotherapy of the Human Malarias. IX. Effect of Pamaquine on the Blood Cells of Man. J. Clin. Invest. 27:121, 1948.
Six out of 76 non-white, and 0 out of 81 white patients receiving Pamaquine developed acute anemia.

Hemotologic Studies of Primaquine-Sensitive Anemia

10. Hochwald, R. S., Arnold, J., Clayman, C. B.: Toxicity of Primaquine in Negroes. J.A.M.A. 149:1563, 1952.
Primaquine and Pamaquine caused dose dependant hemolysis in about 5% of Negro volunteers.

11. Dern, R. J., Beutler, E. and Alving, A.S.: Hemolytic Effect of Primaquine. The Natural Course of the Hemolytic Anemia and the Mechanism of its Self Limited Character. J. Lab. & Clin. Med. 44:171, 1954.
Despite continuing drug hemolysis stops and hemoglobin returns to normal. Demonstrates that the cells themselves become resistant to hemolysis.

12. Dern, R. J., Weinstein, I. M., LeRoy, G. V., Talmage, D.W., and Alving, A.S.: The Hemolytic Effect of Primaquine. I. The Localization of the Drug Induced Hemolytic Defect in Primaquine Sensitive Individuals. J. Lab. & Clin. Med. 43:303, 1954.
Labeled cells from primaquine-sensitive subjects injected into normal survive normally until primaquine is given but then are rapidly hemolyzed. Normal cells injected into primaquine-sensitive subject are not effected by subsequent primaquine.

13. Beutler, E., Dern, R. J., Alving, A.S.: The Hemolytic Effect of Primaquine. IV The Relationship of Cell Age to Hemolysis. J. Lab. & Clin. Med. 44:439, 1954.
Only the <u>older</u> cells are lysed by primaquine administration.

14. Brewer, C. J., Tarlov, A. R. and Kellermeyer, R. W.: The Hemolytic Effect of Primaquine. XII. Shortened Erythrocyte Life Span in Primaquine-Sensitive Male Negroes in the Absence of Drug Administration. J. Lab. & Clin. Med. 58:217, 1961.
In contrast to earlier studies, primaquine-sensitive patients were found to have a shorter red blood cell life span even without exposure to drugs.

Studies Elucidating the Defect in Primaquine Sensitive Anemia

15. Beutler, E., Dern, R. J., Flanagan, C. L. and Alving, A.S.: The Hemolytic Effect of Primaquine. VII. Biochemical Studies of Drug-Sensitive Erythrocytes. J. Lab. & Clin. Med 45:286, 1955.
First evidence of a biochemical defect in primaquine sensitive red blood cells. Glutathione decreased by 20-25%.

16. Flanagan, C. L., Beutler, E., Dern, R. J. and Alving, A.S.: Biochemical Changes in Erythrocytes during Hemolysis Induced by Analine Derivatives. J. Lab. & Clin. Med. 46:814, 1955.
Permaquine administered to normal individuals causes <u>no</u> change in glutathione. Permaquine sensitive individual causes marked drop in glutathione to 30 mg.% (Normal = 75 mg.%).

17. Beutler, E.: The Glutathione Instability of Drug Sensitive Red Cells. J. Lab. & Clin. Med. 49:84, 1957.
Unlike normal cells, primaquine sensitive red cells cannot maintain glutathione concentrations when incubated in vitro with primaquine or acetylphenylhydrazine.

18. Carson, P. E. and Flanagan, C. L., Ickes, C.F. and Alving, A.S.: Enzymatic Defects in Primaquine Sensitive Erthyrocytes. Science 124:484, 1956.
First demonstration that glucose-6-phosphate dehydrogenase is depressed in red blood cells of primaquine sensitive patients.

19. Gross, R. T., Hurwitz, R. F. and Marks, P. A. An Hereditary Enzymatic Defect in Erythrocyte Metabolism: Glucose-6-Phosphate Dehydrogenase Deficiency. J. Clin. Invest. 37:1176, 1958.
First to demonstrate glucose-6-phosphate dehydrogenase deficiency in uneffected population; i.e. prior to drug hemolysis. Incidence: Negroes 7.2%, Caucasians 1.3%. Glutathione "instability" was well correlated with glucose-6-phosphate dehydrogenase deficiency. Inheritance is sex linked.

Electron Microscopic Change in Glucose-6-phosphate Dehydrogenase Deficiency

20. Danon, D., Sheba, C.L. and Ramot, B. The Morphology of Glucose-6-phosphate Dehydrogenase Deficient Erythrocytes: Electron Microscopic Studies. Blood 17:229,1961.
Young red blood cells have "granulated" membranes in stroma. Old red blood cells have "smooth" membranes. In normal patients 3-30% of red blood cells are smooth. In glucose-6-phosphate dehydrogenase deficient patients 60-95% are smooth. Suggest disease is "progeria" of the RBCs.

Effect of Age on Red Blood Cell Glucose-6-phosphate Dehydrogenase

21. Marks, P.A., Johnson, A.B., Hirshberg, E.: Effect of Age on the Enzyme Activity in Erythrocytes. Proc. Nat. Acad. Sc. 44:529, 1958.
Older cells have as little as 1/20th the glucose-6-phosphate dehydrogenase of young RBCs.

Other Enzymes Altered in Primaquine-Sensitive Red Blood Cells

(1) Catalase decreased

22. Tarlov, A.R. and Kellermeyer, R. W. The Hemolytic Effect of Primaquine XI Decreased Catalase Activity in Primaquine Sensitive Erythrocytes. J. Lab. & Clin. Med. 58:204, 1961.

(2) Glutathione Reductase increased

23. Schrier, S.L., Kellermeyer, R. W., Carson. P.E., Ickes, C.E. and Alving, A.S. The Hemolytic Effect of Primaquine. IX Enzymatic Abnormalities in Primaquine Sensitive Erythrocytes. J. Lab. & Clin. Med. 52: 109, 1958.

(3) Aldolase increased.

24. Schrier, S.L., Kellermeyer, R. W., Carson, P.E., Ickes, C. F. and Alving, A.S. The Hemolytic Effect of Primaquine. X Aldolase and Glyceraldehyde-3-Phosphate Dehydrogenase Activity in Primaquine-Sensitive Erythrocytes. J. Lab. & Clin. Med. 54:232, 1959.

(4) Phosphomonoesterase decreased in Caucasians only

25. Oski, A., Shahidi, T. and Diamond, K.: Erythrocyte Acid Phosphomonoesterase and Glucose-6-Phosphate Dehydrogenase Deficiency in Caucasians. Science 139:409,1963.
Phosphomonoesterase deficiency was found in each of five Caucasian patients with glucose-6-phosphate dehydrogenase deficiency; level in Negro patients was normal.

Glucose-6-Phosphate Dehydrogenase Deficiency in Tissues Other than Red Blood Cells

26. Ramot, B., Fisher, S., Szeinberg, A., Adam, A., Sheba, C. and Gafni, D.: A Study of Subjects with Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency. II. Investigation of Leukocyte Enzymes. J. Clin. Invest. 38:2234, 1959.
Leukocytes of Caucasian patients with glucose-6-phosphate dehydrogenase deficiency also show depression of

See also Marks Ref. No. 55

27. Ramot, B., Szeinberg, A., Adam, A., Sheba, C. and Gafni, D. A Study of Subjects with Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency: Investigation of Platelet Enzymes. J. Clin. Invest. 38:1659, 1959.
Enzyme is definitely depressed (5x) in platelets of patients with defect in red blood cells.

28. Ramot, B., Sheba, C., Adam, A., Ashkenasi, I. Erythrocyte Glucose-6-Phosphate Dehydrogenase-deficient Subjects: Enzyme-level in Saliva. Nature 185:931, 1960.
Glucose-6-phosphate dehydrogenase is present in normal saliva and in decreased amounts in saliva from patients with G-6P D deficiency in red blood cells.

Mechanism of Hemolysis

29. Jacob, H. S. and Jandl, J. H. Effects of Sulfhydryl Inhibition on Red Blood Cell. I. Mechanism of Hemolysis. J. Clin. Invest. 41:779, 1962.
An ingenious study demonstrating that the SH groups of red cell membranes are responsible for membrane integrity.

30. Jacob, H. S. and Jandl, J. H. Effects of Sulfhydryl Inhibition on Red Blood Cells. II. Studies In Vivo. J. Clin. Invest. 41:1514, 1962.
Blockage of SH groups on RBC membrane causes cells to be immediately (T50 = 8 min-2 hrs) destroyed by the spleen.

31. Jandl, J. H., Engle, L.K. and Allen, D. W. Oxidative Hemolysis and Precipitation of Hemoglobin. I. Heinz Body Anemias as an Acceleration of Red Cell Aging. J. Clin. Invest. 39:1818, 1960.
Demonstrates that Heinz Bodies are precipitated, oxidized hemoglobin. Excellent discussion of mechanism by which drugs produce alteration in hemoglobin and relation to normal cell aging.

32. Allen, D. W. and Jandl, J. H. Oxidative Hemolysis and Precipitation of Hemoglobin. II. Role of Thiols in Oxidative Drug Action. J. Clin. Invest. 40:454, 1961
Sequence of events leading to Heinz body formation demonstrated. Oxidized glutathione probably becomes bound to hemoglobin in the process but reduced glutathione protects hemoglobin from oxidation.

Tests for Glucose-6-Phosphate Dehydrogenase Deficiency

The Methemoglobin Test

33. Brewer, J., Tarlov, A. R., and Alving, A.S. Methaemoglobin Reduction Test: A New, Simple, in Vitro Test for Identifying Primaquine-Sensitivity. Bull. Wld. Hlth. Org. 22:633, 1960.

34. Brewer, G.J., Tarlov, A.R., Alving, A.S.: The Methemoglobin Reduction Test for Primaquine-Type Sensitivity of Erythrocytes. A Simplified Procedure for Detecting A Specific Hypersusceptibility to Drug Hemolysis. J.A.M.A. 180:386, 1962.

Modified Motulsky-Dye Test

35. Bernstein, R.E.: A Rapid Screening Dye Test for the Detection of Glucose-6-Phosphate Dehydrogenase Deficiency in Red Cells. Nature 194:192, 1962.

Direct Enzyme Assay

36. Marks, P.A.: Red Cell Glucose-6-Phosphate and 6-Phosphogluconic Dehydrogenases and Nucleoside Phosphorylase. Science 127:1338, 1958.

See also discussion following Ref. 53 for difficulties.

Population and Genetic Studies

37. Szeinberg, A., Sheba, C., Adam, A. Selective Occurrence of Glutathione Instability in Red Blood Corpuseles of Various Jewish Tribes. Blood 13:1043, 1958.

See Beutler Ref. 3

38. Siniscalco,M. and Bernini, L.: Favism and Thalassaemia in Sardinia and Their Re-Lationship to Malaria. Nature 190:1179, 1961.
Up to 35% of population in Sardinian Villages have glucose-6-phosphate dehydrogenase deficiency. Correlates well with prev. of malaria

Relation to Falciparum Malaria

39. Allison, A.C.: Glucose-6-Phosphate Dehydrogenase Deficiency in Red Blood Cells of East Africans. Nature 186:531, 1960.
Good correlation between incidence of trait and endemic malaria.

40. Allison, A.C., Clyde, D.F. Malaria in Africal Children with Deficient Glucose-6-Phosphate Dehydrogenase in Erythrocyte. Brit.Med.J. 1:1346,1961.
Parasite rates in children with glucose-6-phosphate dehydrogenase deficiency are significantly lower than in child without defect.

41. Allison, A.C. Malaria and Glucose-6-Phosphate Dehydrogenase Deficiency. Nature 197:609, 1963.
Brief review and summary of arguement that Glucose-6-Phosphate Dehydrogenase Deficiency protects against Falciparum Malaria.

Inheritance

42. Childs, B. and Zinkham, W., Browne, E. A., Kimbo, E. I., Torbert, J. V.
A Genetic Study of a Defect in Glutathione Metabolism in the Erythrocyte.
Bull. J. Hopkins Hospital 102:21, 1958.
Glucose-6-Phosphate Dehydrogenase Deficiency is probably inherited as sex linked gene with incomplete dominance.

43. See also Marks et. al. reference. 19

44. Adam, A. Linkage between deficiency of Glucose-6-Phosphate Dehydrogenase and colour-blindness. Nature 189:686, 1961.
The two conditions (Glucose-6-Phosphate Dehydrogenase Deficiency color blindness) if separate in a single family remain separate. If "linked" remain "linked."

? Catatonic Schizophrenia

45. Dern, R. J., Glynn, M. F. and Brewer, G. J.: Studies on the Influence of Hereditary Glucose-6-Phosphate Dehydrogenase Deficiency in the Expression of Schizophrenic Patterns. Clin. Res. 10:80, 1962.
53% of female catatonic and 25% of male catatonic schizophrenic patients were reported to have Glucose-6-Phosphate Dehydrogenase Deficiency.

Drugs Which Produce Hemolysis in Glucose-6-Phosphate Dehydrogenase Deficient Individuals

46. Kellermeyer, R. W , Tarlov, A. R., Schrier, S. L. and Alving, A. S.: Hemolytic Effect of Commonly Used Drugs in Erythrocytes Deficient in Glucose-6-Phosphate Dehydrogenase. J. Lab. Clin. Med. 52:827, 1958.
Brief evaluation of toxic doses of various drugs in Primaquine-sensitive individuals.

See also Kellermeyer, Ref. 2.

47. Kimbro, W.L., Sachs, M.V. and Torbert, J.V. Mechanism of Hemolytic Anemia Induced by Nitrofurantoin Furadantin. Bull. J. Hopkins Hospt. 101:245,1957.
Furadantin hemolysis is shown to be due to G-6-PD deficiency.

48. Zinkham, W. H. and Childs, B. Effect of Vitamin K and Naphthalene on Glutathione Metabolism of Erythrocytes from Normal Newborns and Patients with Naphthalene Hemolytic Anemia. A.M.A. Dis. Child. 94:420, 1957.
Vitamin K anemia in newborns and naphthalene hemolytic anemia is probably due to G-6-PD deficiency.

49. Szeinberg, A., Sheba, C., Hirshorn, N. and Bodony, E.: Studies on Erythrocytes in Cases with Past History of Favism and Drug-incuded Acute Hemolytic Anemia. Blood 13:603, 1957.
Showed glutathione deficiency in RBC's and cross sensitivity between Fava beans and sulfa drugs in patients with favism.

Factors which Enhance Hemolytic Anemia

Infection

50. Szeinberg, A., Kellerman, J., Adam, A, Sheba, C. and Ramot, B. Haemolytic Jaundice Following Aspirin Administration to a Patient with a Deficiency in Glucose-6-Phosphate Dehydrogenase in Erythrocytes. Acta Haemat. 23:58, 1960.
Severe hemolysis following 1.5 gm of aspirin in man with severe viral infection.

51. Kellermeyer. See Ref. 2.

Diabetes

52. Gant, F.L. and Winks, G.F. Primaquine-Sensitive Hemolytic Anemia Complicating Diabetic Acidosis. Clin. Res. 9:27, 1961.
Three patients developed acute hemolytic anemia during diabetic acidosis. Two had G-6-PD deficiency.

Possible Differences in Expression of Enzyme Deficiency in Negro and Caucasian Individuals

53. Kirkman, H. N. "Primaquine Sensitivity in Molecular Genetics and Human Disease." L. I. Gardner, (ed). Charles C. Thomas, Springfield, Ill., 1961, P.106-133.
Discusses the mechanisms by which G-6-PD deficiency or alteration can be produced at a genetic level. No difference in characteristics of enzyme from normal and effected persons could be found.

54. Marks, P.A., Szeinberg, A. and Banks, J. Erythrocyte Glucose-6-Phosphate Dehydrogenase of Normal and Mutant Human Subjects. J. Biol. Chem. 236:10,1961.
No qualitative difference in the G-6-PD of normal and primaquine-sensitive patients could be detected.

55. Marks, P.A. and Gross, R. T.: Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency: Evidence of Differences between Negroes and Caucasians with Respect to this Genetically Determined Trait. J. Clin. Invest. 38:2253, 1958.
Caucasian men have a more severe deficiency in G-6-PD than do Negro men, and leukocytes are definitely involved in the former.

56. Ramot, B., Ashkenasi, I, Adam, A. and Sheba, C. Activation of Glucose-6-Phosphate Dehydrogenase of Enzyme Deficient Subjects. II. Properties of the Activator and the Activation Reaction.
Red blood cell stroma from normal patients have an "activator" which will partially activate the G-6-PD of defective cells but not of normal cells. (?already maximally activated). Primaquine-sensitive cell-stroma lack this activating factor.

Congenital Non-spherocytic Hemolytic Anemia - Glucose-6-Phosphate Dehydrogenase Type

57. Zinkham, W. H. and Lenhard, R. E.: Observations on the Significance of Primaquine-Sensitive Erythrocytes in Patients with Congenital Nonspherocytic Hemolytic Anemia. A.M.A. J. Dis. Child. 98:22, 1959.
Four of six patients with nonspherocytic hemolytic anemia had severe G-6-PD deficiency.

58. Kirkman, H. N., Riley, H.D., Crowell, B.B.: Different Enzymic Expressions of Mutants of Human Glucose-6-Phosphate Dehydrogenase. Proc. Nat. Acad. Sc. 46:938, 1960.
Presents evidence that the G-6-PD from RBCs of patients with congenital nonspherocytic hemolytic anemia is a qualitatively different protein than G-6-PD from normal or primaquine-sensitive patients.
First evidence that gene may have two enzymic expressions.

59. Kirkman, H. N. and Riley, H. D.: Congenital Nonspherocytic Hemolytic Anemia. A.M.A. J. Dis.Child 102:313, 1961
Studied family with congenital nonspherocytic hemolytic anemia. Mode of inheritance is probably identical to that of primaquine-sensitive anemias.

Nonspherocytic Hemolytic Anemia in Phosphopyruvic Kinase Deficient Type

60. de Gruchy, G.C., Santamaria, J.N., Parsons, I.C., and Crawford, H.: Nonspherocytic Congenital Hemolytic Anemia. Blood 16:1371, 1960.
One type of nonspherocytic congenital hemolytic is reversible with ATP.

61. Tanaka, R., Valentine, W.N. and Miwa, S.: Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia. Blood 19:267, 1962.
Reports 7 patients with nonspherocytic macrocytic hemolytic anemia with marked deficiency of phosphopyruvic kinase.

Treatment

Intermediate Use of Drug

62. Alving, A.S., Johnson, C.F., Tarlov, A.R., Brewer, G.J., Kellermeyer, R.W. and Carson, P.E.: Mitigation of the Haemolytic Effect of Primaquine and Enhancement of its Action against Exoerythrocytic Forms of the Chesson Strain of Plasmodium vivax by Intermittent Regimens of Drug Administration. Bull. Wld. Hlth Org. 22:621, 1960

Nicotinic Acid

63. Tarlov - quoted in Carson Ref. 5.
Nicotinic acid given for 3-4 months gave 40% protection against primaquine-sensitive anemia.