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Glucose-6-phosphate dehydrogenase deficiency: a historical perspective

Ernest Beutler

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Glucose-6-phosphate dehydrogenase deficiency: a historical perspective

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Glucose-6-phosphate dehydrogenase deficiency serves as a prototype of the many human enzyme deficiencies that are now known. Since its discovery more than 50 years ago, the high prevalence of the defect and the easy accessibility of the cells that manifest it have made it a favorite tool of biochemists, epidemiologists, geneticists, and molecular biologists as well as clinicians. In this brief historical review, we trace the discovery of this defect, its clinical manifestations, detection, population genetics, and molecular biology. (Blood. 2008;111:16-24)

Introduction

The red blood cell has occupied a unique position in the study of human biology. Biochemists and cell biologists can readily purify 35 to 50 billion of these remarkably uniform cells from a single 10-cc blood sample. The fact that red cells are less complex than most other cells also has its advantages, and the study of red cells has been at the forefront of physiology, biochemistry, and even molecular biology (although the DNA must come from the white cells that were once discarded in the study of erythrocytes). The suitability of the red cell for study explains in part the fact that the hematologist has had the privilege of being at the vanguard of modern science for the past half century. Fifty years ago 3 enzyme deficiencies that produce disease in humans had been identified, all in human erythrocytes. These enzymes were catalase,[1] galactose-1-phosphate uridyltransferase,[2] and glucose-6-phosphate dehydrogenase (G6PD).[3] Although each of these deficiencies was discovered in red blood cells, only G6PD deficiency produces a hematologic disorder, namely hemolytic anemia, and it was as a result of investigation of hemolytic anemia that brought this common enzymatic deficiency to light. In 1973 it was estimated that 300 000 000 people worldwide were G6PD deficient.[4] Whatever the actual number was then, it is greater now. But one must beware of hyperbole in such matters. The number of affected individuals is large, but fortunately the clinical penetrance is exceedingly low.

Early history

G6PD deficiency was discovered as an outgrowth of an investigation of hemolytic anemia occurring in some individuals treated for malaria with 6-methoxy-8-aminoquinoline drugs. Cordes[5] reported the occurrence of acute hemolysis in such patients in 1926, but 3 decades passed before the mechanism of hemolysis could be understood. The discoveries that led to the recognition of G6PD deficiency were the result of several convergent events. First, the biochemical pathways through which red cells metabolize sugar were painstakingly unraveled by giants such as Warburg, Embden, and Meyerhof (reviewed in Beutler[6]). The tools available then seem incredibly primitive today, but by 1950, virtually every step in red cell glycolysis was established, an awesome intellectual accomplishment. Second, the development of isotopic methods permitting the accurate estimation of red blood cell survival was essential. Although in 1919 Ashby[7] had published her innovative method of measuring red cell survival by counting the inagglutinable cells, this method was too cumbersome and not sufficiently precise to have allowed solution of the problem. It was the ^{51}Cr method of labeling erythrocytes, first devised by Sterling and Gray[8] to measure the red cell mass and subsequently adapted for measuring red cell survival,[9] that set the stage for the studies that had to be performed. And finally, the circumstances surrounding World War II and the Korean War had created the necessity for the development of new, synthetic antimalarial drugs. The funding required to carry out the studies materialized from the United States Army.

It is worth noting that the clinical investigations needed to understand why 8-amino quinoline antimalarials cause hemolysis could not have been carried out without the participation of prisoner volunteers serving sentences in the Illinois State Penitentiary at Joliet. Contrary to common current belief of how research was conducted in the days before Institutional Review Boards (IRBs), these men were volunteers in the true sense of the word. There was no coercion, and all signed informed consents that spelled out in detail what was to be done. Today such studies could not be performed. If the idea that it was improper to study prisoner volunteers had been in vogue then as it is now, these men would have been deprived of making a contribution to society, and the discoveries that were to be made would have been held back for years. The discovery of G6PD deficiency is a clear example of how clinical studies carried out on prisoner volunteers can benefit society and save lives without harm to anyone.

Primaquine sensitivity is due to an intrinsic defect of the erythrocyte

The initial question that needed to be answered was straightforward. When normal volunteers were given 30 mg of primaquine daily, some developed acute hemolytic anemia; most did not. Did those who developed hemolytic anemia metabolize this 6-methoxy-8-amino quinoline antimalarial drug differently, or did their red cells differ in some manner? The development of the ^{51}Cr technique made possible an unequivocal answer. When ^{51}Cr-labeled cells from a primaquine-sensitive subject were transfused into a nonsensitive

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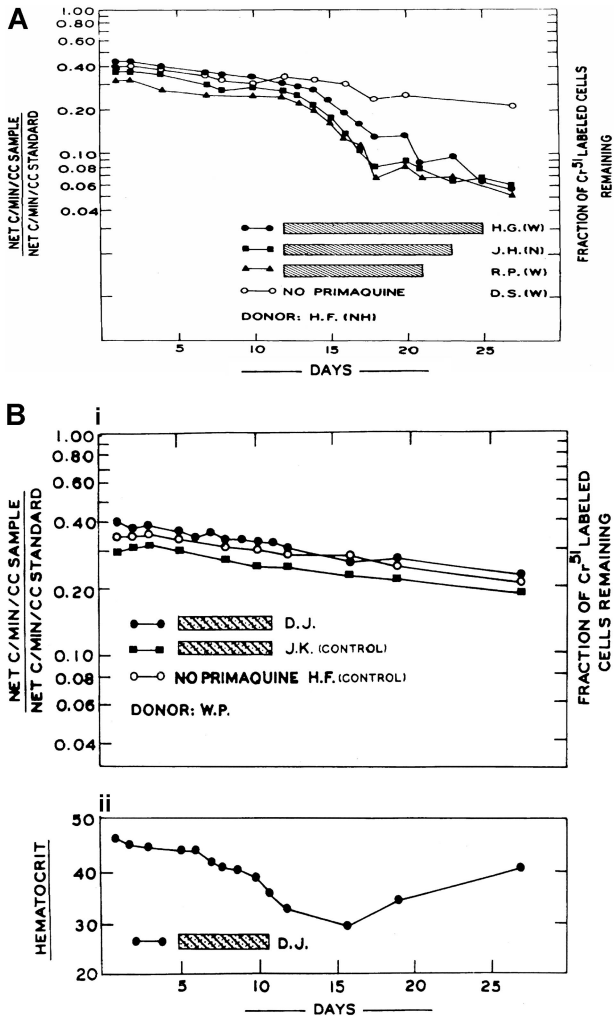

Figure 1. Chromium 51 studies showing that primaquine sensitivity was due to an intrinsic defect of the red blood cell. (A) Red cells from a primaquine-sensitive volunteer were labeled with chromium 51 and transfused into normal recipients. Three of these recipients were given 30 mg primaquine daily as shown by the bars. (B) Red cells from a primaquine-nonsensitive volunteer were labeled with chromium 51 and transfused into a normal and one primaquine-sensitive volunteer (D.J.). Two of the subjects were given primaquine, including D.J., who was primaquine sensitive. The fall of the hematocrit of D.J. is shown in the lower panel B. (Reprinted from Dern et al[67] with permission from Elsevier.)

subject, primaquine administration resulted in rapid destruction of the labeled erythrocytes (Figure 1A). But when ^{51}Cr-labeled cells from a nonsensitive subject were transfused into a primaquine-sensitive subject, they survived normally even when the host's red cells were being rapidly destroyed (Figure 1B). These studies established clearly that sensitivity to the hemolytic effect of primaquine was due to an intrinsic defect of the erythrocyte. Further studies established that the hemolytic anemia was self-limited[10]; not all of the red cells of the primaquine-sensitive subjects were destroyed, even if drug administration was continued for weeks. Indeed, the hemoglobin levels of such volunteers returned to normal as they continued to receive the drug. Cohort labeling studies with ^{59}Fe demonstrated that the older red cells were the ones that were destroyed. Young red cells were resistant to destruction.[11]

Primaquine sensitivity is the result of a deficiency of red cell G6PD

But what was the defect that led to primaquine sensitivity? The fact that younger, metabolically more active red cells resisted the assault of the drug suggested the possibility that primaquine-sensitive cells had an intrinsic metabolic defect. There were few morphologic changes in the red cells of subjects undergoing primaquine-induced hemolysis, but Heinz bodies could be detected in the circulation before the onset of hemolysis. Heinz bodies can be induced in vitro by compounds such as phenylhydrazine or acetyl phenylhydrazine, and we were able to show that the pattern of in vitro Heinz body formation was quite different in sensitive and nonsensitive subjects (Figure 2).[12] This made it possible, for the first time, to detect primaquine sensitivity in vitro. More important, it provided a system in which metabolic inhibitors could be used to attempt to simulate primaquine sensitivity in vitro. The fact that iodoacetate and arsenite caused normal cells to behave like primaquine-sensitive cells[13] focused attention on the sulfhydryl groups of the erythrocyte. The amount of glutathione in the cells[13] was diminished, and the ability of erythrocytes to maintain glutathione levels in the face of an oxidative stress[14,15] was abnormal. Carson et al[3] then undertook a study of the reduction of glutathione by hemolysates. Fifty years ago nicotinamide adenine dinucleotide phosphate (NADPH; then known as TPNH) was very costly, and therefore an attempt was made to generate the reduced coenzyme in hemolysates from NADP and glucose-6-P through the G6PD reaction. When it was found that hemolysates from primaquine-sensitive men could not utilize glucose-6-phosphate to reduce glutathione, the more expensive NADPH was added as the hydrogen donor. This substrate could reduce glutathione in hemolysates from primaquine-sensitive men, making it apparent that the primary defect was in the glucose-6-P-utilizing enzyme, G6PD.

The mechanism of hemolysis

The appearance of Heinz bodies both in vivo and in vitro in G6PD-deficient cells and their inability to protect their GSH against drug challenge suggested that a major component of the hemolytic process was the inability of the erythrocytes to protect sulfhydryl groups against oxidative damage. Figure 3 illustrates a plausible scenario to explain the sequence of events that leads to damage of sulfhydryls in G6PD-deficient red cells. However, it has been shown that in mice, targeted disruption of the gene encoding glutathione peroxidase has little effect on oxidation of hemoglobin of murine cells challenged with peroxides.[16] If the murine model reflects what occurs in man, then different pathways requiring glutathione, such as the thioredoxin reaction, might also be involved.

The clinical spectrum of G6PD deficiency is broader than merely causing sensitivity to hemolysis by primaquine

Drug-induced hemolysis

It was obvious from the beginning that subjects sensitive to the hemolytic effect of primaquine were sensitive to some other drugs as well. After all, it had been known since Cordes's original publication[5] that other 6-methoxy 8-amino quinoline antimalarials were hemolytic in some individuals. But the spectrum of drugs that caused hemolysis was much broader than had been anticipated. Transfusing labeled red cells from a few primaquine-sensitive donors into a larger number of nonsensitive recipients and then challenging the sensitive cells with a variety of drugs made it possible to quantitate the extent to which each drug produced

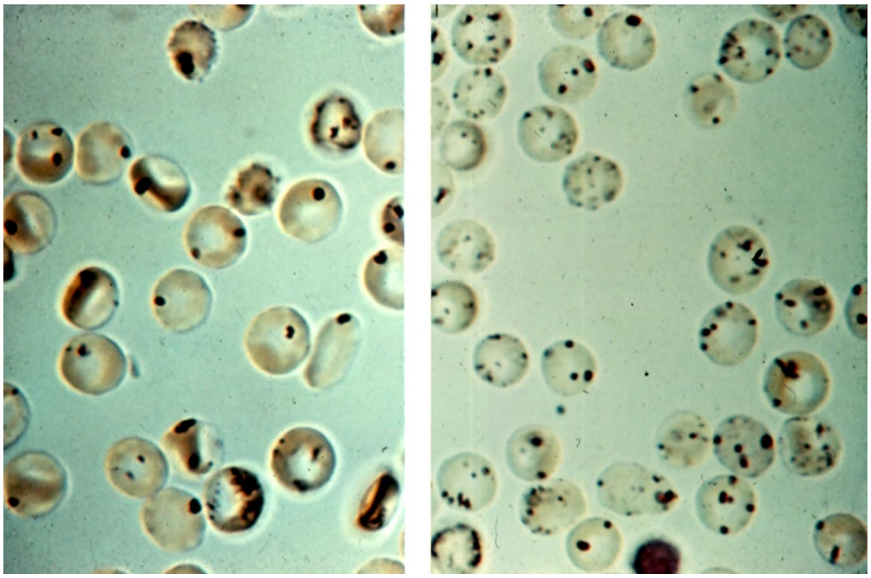

Figure 2. The Heinz body test for primaquine sensitivity (G6PD deficiency). The cells (right) are from a primaquine-sensitive (G6PD-deficient) donor; the cells in the left panel, from a normal control.

hemolysis[17] (Figure 4). Not only 8-aminoquinoline antimalarials but also other drugs, including sulfanilamide, acetanilid, and some sulfones, proved to be hemolytic. Using the same approach, these results were confirmed, and still other drugs also were clearly implicated.[18-20] However, there was soon considerable confusion regarding which drugs actually produced hemolytic anemia in G6PD-deficient persons. It was not initially recognized that G6PD-deficient persons sometimes developed hemolytic anemia when suffering from a febrile infection. It was simply assumed that the hemolysis that occurred in such persons was due to a drug that they had ingested. Accordingly, a careful drug history was taken, and some or all of the drugs to which they had been exposed were added to the list of proscribed substances. In addition, drugs that were shown to have a minor effect on red cell survival when given in large doses, such as aspirin, were listed. Table 1 lists drugs that have been shown to be capable of producing clinically significant hemolytic anemia in doses that are normally used. Table 2 indicates which drugs can be given safely to most patients with G6PD deficiency.

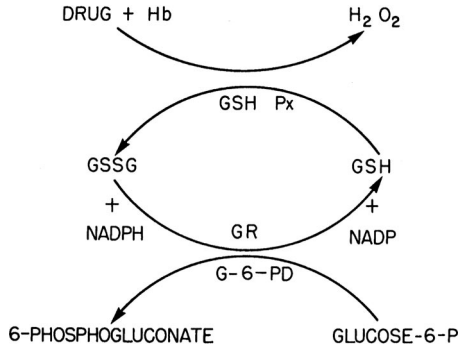

Figure 3. A plausible series of reactions that could explain the lack of resistance of G6PD-deficient red cells to oxidative stress. The interaction of drugs with red cells produces hydrogen peroxide, either directly or by way of reactive oxygen species. Glutathione removes the peroxide through the glutathione peroxidase reaction and continues to do so as long as the oxidized glutathione formed is able to be reduced through the glutathione reductase reaction. The latter reaction, requiring continual reduction of NADP to NADPH, is dependent on the G6PD reaction and fails in red cells deficient in this enzyme. (Reprinted from Beutler[68] with permission from FASEB.)

Infection-induced hemolysis

The role of infection, as noted in the previous paragraph, was not recognized immediately, but became apparent as clinical observations were made on G6PD-deficient patients who had not ingested any drugs. Indeed, a decade after the discovery of G6PD deficiency as the cause of drug-induced hemolysis, a survey of 73 episodes of hemolysis in a hospital in New York City suggested that infection was the most common precipitating factor of hemolytic anemia among G6PD-deficient patients.[21]

Favism

Ingesting fava beans has been known since antiquity to induce hemolytic anemia in some individuals. Pythagoras, the mathematician/philosopher who lived in the fifth century BCE, warned his disciples not to eat beans. An often-repeated story, very likely apocryphal, states that as he was being pursued by his enemies, Pythagoras halted at the edge of a field of beans saying that he would rather be taken than to enter the field. And so he met his fate.[22] All patients with favism are G6PD deficient, but many G6PD-deficient individuals can eat fava beans with impunity. Thus, the deficiency is a necessary but not sufficient cause of hemolysis. It has been suggested that the glycosides divicine and isouramil are the components of the bean responsible for a hemolysis,[23] but there is no rigorous proof that this is the case, and the additional factor that makes a minority of G6PD-deficient individuals develop hemolysis when they ingest the bean is still unknown, although it is probably inherited.[24]

Hereditary nonspherocytic hemolytic anemia

Another manifestation of G6PD deficiency was found to be hereditary nonspherocytic hemolytic anemia. This syndrome, first delineated by William Crosby in 1950[25] was of unknown origin. It was somewhat of a surprise then, when Newton and Bass[26] discovered that a 4-year-old Italian boy with this syndrome was G6PD deficient. It subsequently became apparent that some patients with hereditary nonspherocytic hemolytic anemia had a subset of G6PD variants that were functionally much more severe than the polymorphic mutations that had been studied until then.

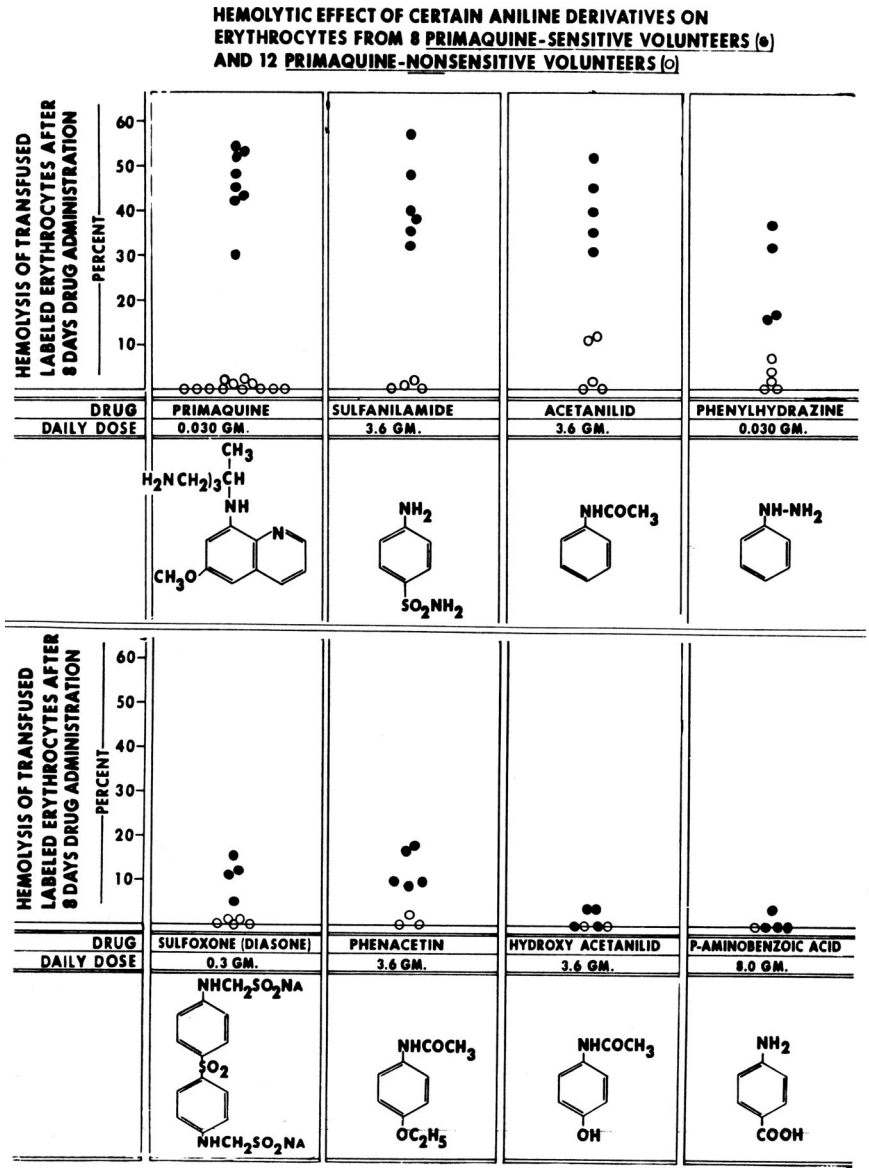

Figure 4. The effect of 8 different drugs on the survival of primaquine-sensitive red cells transfused into normal volunteers. Primaquine, sulfanilamide, acetanilid, phenylhydrazine, sulfoxone, and phenacetin in the doses shown cause significant hemolysis of the primiquine-sensitive cells. Hydroxyacetanilid and para-aminobenzoic acid did not prove to be hemolytic. (Reprinted from Dern et al[17] with permission from Elsevier.)

Table 1. Drugs and chemicals to be avoided by persons with G6PD deficiency

Acetanilid
Diaminodiphenyl sulfone
Furazolidone (Furoxone)
Glibenclamide
Henna (Lawsone)
Isobutyl nitrite
Methylene Blue
Naphthalene
Niridazole (Ambilhar)
Nitrofurantoin (Furadantin)
Phenazopyridine (Pyridium)
Phenylhydrazine
Primaquine
Sulfacetamide
Sulfanilamide
Sulfapyridine
Thiazolesulfone
Trinitrotoluene (TNT)
Urate oxidase

Further details concerning most of these drugs may be found in Beutler.[65,66]

Neonatal jaundice

The clinical consequences of drug-induced hemolysis, favism, or chronic nonspherocytic hemolytic anemia, are usually not devastating. All of these disorders can occasionally be serious. In the days before transfusion, favism was associated with a mortality rate of some 8% in children,[27] but nowadays deaths from favism are extremely rare. The most serious consequence of G6PD deficiency is not any of the aforementioned disorders, but rather neonatal jaundice, which may lead to kernicterus. Quite understandably, it is commonly believed that this is a consequence of hemolysis, but in reality the hemoglobin level and reticulocyte count of the infants are generally normal, and using modern techniques it is been shown that there is only a modest and inconsistent shortening of red cell lifespan, which may contribute to a limited extent to the jaundice.[28] The principal cause of neonatal icterus in G6PD-deficient infants is the inability of the liver to adequately conjugate bilirubin. This problem is compounded when the infant also inherits the UDP glucuronosyl transferase promoter polymorphism that is associated with Gilbert disease.[29]

Table 2. Drugs that probably can be safely given in normal therapeutic doses to G6PD-deficient patients without nonspherocytic hemolytic anemia

Acetaminophen (paracetamol, Tylenol, Tralgon, hydroxyacetanilide)
Acetophenetidin (phenacetin)
Acetylsalicylic acid (aspirin)
Aminopyrine (Pyramidon, aminopyrine)
Antazoline (Antistine)
Antipyrine
Ascorbic acid (vitamin C)
Benzhexol (Artane)
Chloramphenicol
Chlorguanidine (Proguanil, Paludrine)
Chloroquine
Colchicine
Diphenyldramine (Benadryl)
Isoniazid
L-Dopa
Menadione sodium bisulfite (Hykinone)
p-Aminobenzoic acid
p-Aminosalicylic acid
Phenylbutazone
Phenytoin
Probenecid (Benemid)
Procainamide hydrochloride (Pronestyl)
Pyrimethamine (Daraprim)
Quinine
Streptomycin
Sulfacytine
Sulfadiazine
Sulfaguanidine
Sulfamerazine
Sulfamethoxazole (Gantanol)
Sulfamethoxypyridazine (Kynex)
Sulfisoxazole (Gantrisin)
Tiaprofenic acid
Trimethoprim
Tripelennamine (Pyribenzamine)
Vitamin K

Further details may be found in Beutler. [65]

G6PD deficiency is X-linked and spawned the X-inactivation hypothesis

G6PD deficiency was first discovered in African-American subjects, and the fact that it seemed to be limited to one ethnic group made it seem likely that it had a genetic basis. The development of the glutathione stability test made possible testing of this hypothesis, and soon it was shown that transmission was generally from mother to son.[30] While rarely father-to-son transmission seemed to occur, such a pattern was sufficiently unusual that it was consistent with the idea that, since the defect was common, the father just happened to be affected but had not actually transmitted the genetic defect. Thus, it became apparent that G6PD deficiency was an X-linked disorder. But the family studies also raised an interesting question. While the putative heterozygotes often seemed to have intermediate degrees of glutathione stability, this was by no means always the case. Often the mothers seemed to be normal and sometimes fully affected.

It was at just this time that my friend and colleague, the late great biologist Susumu Ohno, had discovered that the 2 X-chromosomes of female mammals differed. One was condensed and formed the Barr chromatin body, while the other appeared the same as the autosomes.[31] Ohno's finding suggested to me that the reason that females had no more G6PD than males might be that only one of the 2 X-chromosomes was active (Figure 5). The pattern of inheritance suggested that it could not always be either the maternally derived or paternally derived X-chromosome that was inactivated, and that inactivation must occur early in embryogenesis, presumably when the Barr chromatin body (representing the condensed X-chromosome) appeared. We were able to show that female heterozygotes for G6PD deficiency had 2 red cell populations, normal cells and deficient cells, as would be predicted if only one of the 2 X-chromosomes was active in each precursor.[32,33] Based on Ohno's observations, the same hypothesis was developed independently by Lyon, based on X-linked mutant mouse coat color genes.[34]

X-activation is not only important biologically, and perhaps the first example of epigenetic modification of a group of genes, but it has been useful also in defining clonal characteristic of certain hematologic disorders such as polycythemia vera,[35] paroxysmal nocturnal hemoglobinuria,[36] and chronic granulocytic leukemia.[37]

The detection of G6PD deficiency

The Heinz body test (Figure 2) and the glutathione stability test distinguished, with some reliability, G6PD-deficient from normal individuals. But these tests were indirect. The Heinz body test, in particular, needed to be carried out under carefully controlled conditions with respect to oxygenation, and neither was simple to perform. Assaying G6PD by measuring the reduction of NADP to NADPH required an ultraviolet spectophotometer. The development of simple screening tests that could be carried out in the field facilitated population studies, and such tests were useful also for clinical purposes. Motulsky[38] described a procedure in which the reduction of NADPH, invisible to the naked eye, was linked to the reduction of the visible dye brilliant cresyl blue. Subsequently, other simple tests, using as visual endpoints the reduction of substances such as methylene blue, MTT tetrasodium, dichloroindophenol, or methemoglobin, were developed.[39] These tests have been superseded by the fluorescent spot test.[40] In this procedure the reduction of NADPH is observed directly by virtue of its fluorescence, instead of linking the reduced pyridine nucleotide to a dye.

Screening procedures are quite robust in the detection of the fully developed defect in males, but they fall short in the ascertainment of female heterozygotes and in patients with relatively mild forms of G6PD deficiency, such as those with the A− variants, who are undergoing hemolysis. The correct diagnosis of patients who have recently undergone hemolysis may be obscured by the large proportion of young cells in the circulation. It has been proposed that harvesting the most dense cells after centrifugation may allow a more accurate diagnosis,[41] but simply waiting for a week or 2 is usually sufficient to establish the correct diagnosis. The ascertainment of females is difficult because their blood contains markedly varying proportions of normal and deficient cells. Diagnostic accuracy is improved by methods in which each red cell acts as an independent metabolic unit,[39] as contrasted with methods in which a hemolysate serves as the sample examined, but there is no biochemical method that is entirely reliable in the detection of heterozygotes.[42] Only DNA analysis serves this purpose.

All G6PD deficiency is not the same

With the development of the glutathione stability test and soon after rapid screening tests[38] for G6PD, it became apparent that this disorder did not only occur exclusively among people of African

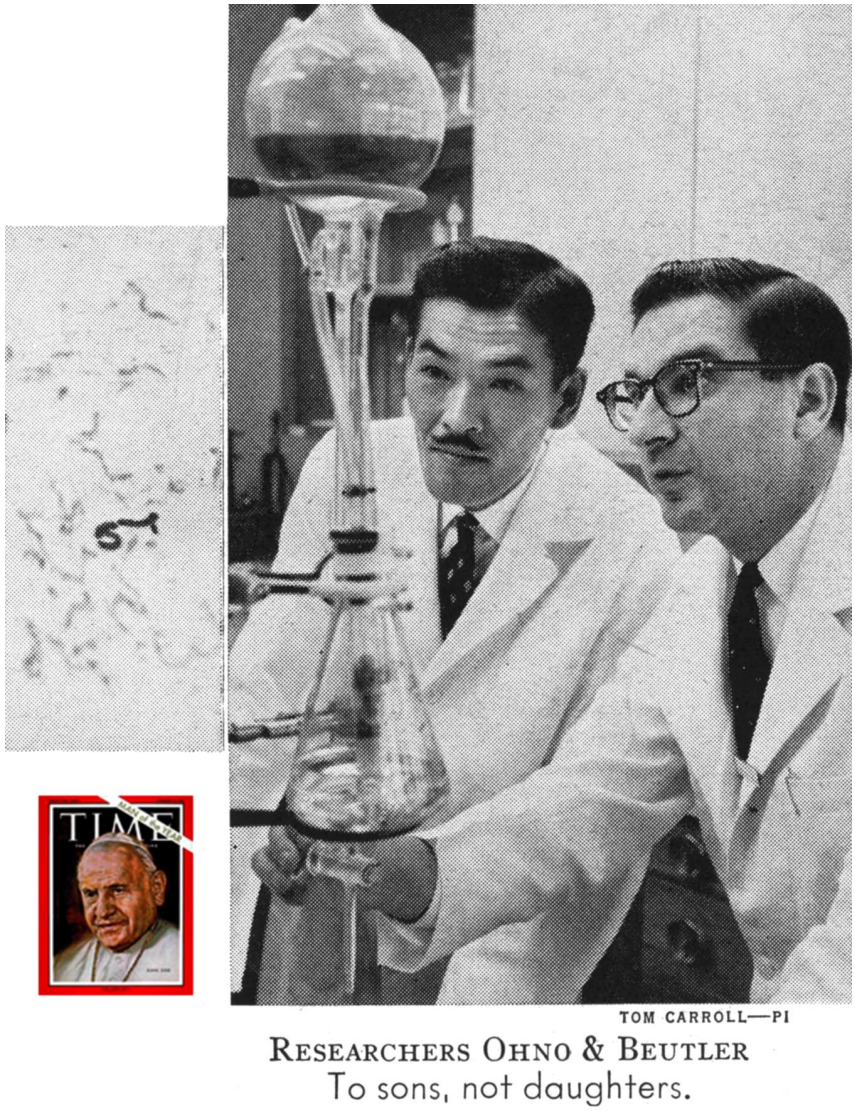

Figure 5. The author and Dr Susumo Ohno (left), whose original observations of the morphology of the mammalian X-chromosome provided the author with the idea that only one of the 2 X-chromosomes was active, the other undergoing inactivation in early embryogenesis. This photograph was taken in my laboratory at the City of Hope Medical Center and published in *Time Magazine*, January 4, 1963, to accompany a feature article entitled, "Research Makes It Official: Women Are Genetic Mosaics." The insert shows the hyperchromatic X-chromosome of a female. The photographer paid no heed to the author's insistence that the burette with which we are posed had nothing to do with X-inactivation.

origin, but also in Southern Europe and the Middle East. Later studies showed that the distribution was even wider. In the 1950s it was not generally appreciated that many different mutations could strike a gene. Thus, it was initially assumed that G6PD deficiency was a single disorder, but the studies of Marks and Gross[43] showed that G6PD deficiency in Mediterranean peoples was much more severe than among African-Americans. With the development of electrophoretic methods for measuring the mobility of the enzyme on starch gel[44] and for studying the kinetic properties of the residual enzyme,[45] much greater heterogeneity became apparent. These methods were standardized by an expert committee convened by the World Health Organization (WHO), and these standard methods were used by most investigators.[46] In a subsequent WHO publication[47] a classification of variants according to severity of the phenotype was introduced: class 1: severe enzyme deficiency with chronic nonspherocytic hemolytic anemia; class 2: severe enzyme deficiency (less than 10% of normal); class 3: moderate-to-mild enzyme deficiency (10%-60% of normal); class 4: very mild or no enzyme deficiency (60%-100% of normal); and class 5: increased enzyme activity (more than twice normal).

There appeared to be 2 types of mutations among Africans: G6PD A, a normally active enzyme with rapid electrophoretic mobility, and G6PD A−, an enzyme with the same mobility as G6PD A, but with diminished activity. The enzyme among Mediterranean subjects was designated as G6PD B− but subsequently renamed G6PD Mediterranean. But G6PD Mediterranean was by no means the only mutant enzyme found in the Mediterranean region; other enzyme variants such as G6PD Seattle were common as well. In Asia even more heterogeneity was found. By 1988 more than 370 variants had been described.[48] But were they all really different? The methods for differentiating variants from one another were not precise, and there was obviously a professional advantage for an investigator to describe a new variant rather than having rediscovered an old one. Thus, minor differences, due to technical variation, might be the basis of describing the same variant under several different names. Whether this was the case had to await the molecular cloning of G6PD.

The population genetics of G6PD deficiency

The prevalence of G6PD deficiency is very high in some parts of the world. Its distribution is largely tropical and paralleled the distribution of malaria, a disease with high morbidity and mortality, and therefore a powerful selective force in human populations. The hypothesis that this infection was responsible for a balanced polymorphism, maintaining a high frequency of G6PD-deficiency

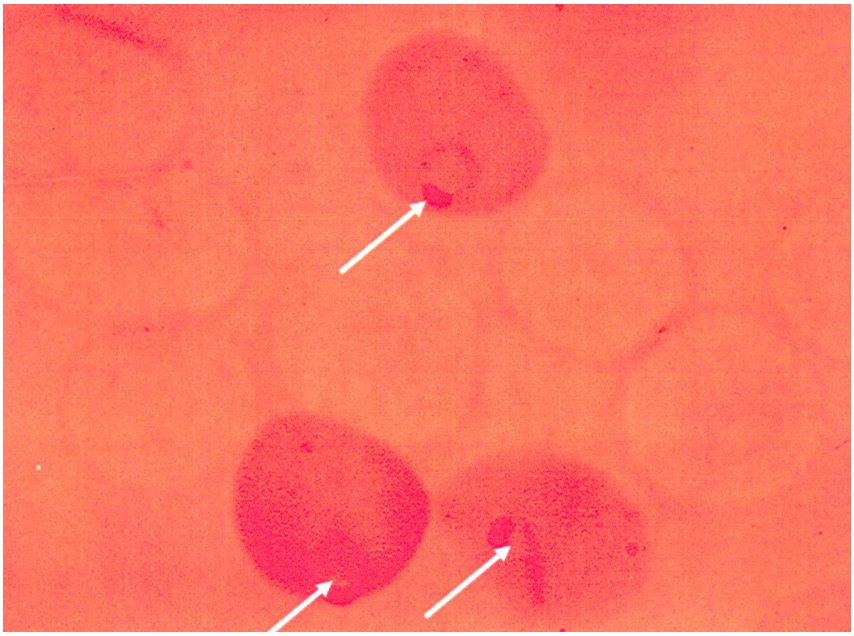

Figure 6. An indirect histochemical stain of erythrocytes from a heterozygote for G6PD deficiency who was suffering from naturally acquired malaria. The more pigmented cells are those that contain normal G6PD activity. They contain the highest percentage of malaria parasites, as shown by the arrows. (Courtesy Professor Lucio Luzzatto.)

mutations as a defense against malaria, was examined in numerous epidemiologic studies in Africa and elsewhere.[49,50] The most convincing evidence for an effect of G6PD deficiency on malarial infections came from ingenious observations made by Luzzatto et al.[51] Since heterozygous females contained in their circulation a mixture of normal and G6PD-deficient cells, it was possible to examine the percentage of each type of cell parasitized in such individuals, and it was shown that the deficient cells were spared in comparison of the normal ones (Figure 6).

The cloning of G6PD and definition of mutations

In the 1960s and 1970s the techniques for sequencing large proteins were developed, and the sequencing of the hemoglobin molecule and "fingerprinting" of many hemoglobin variants provided insight into how many different changes in the amino acid structure of the protein existed. But hemoglobin could be purified from red cells in gram quantities; the situation was quite different for G6PD. Two liters of blood contained only about 30 mg of enzyme protein,[52] about 1/10 000 of the amount of hemoglobin, and one could hardly expect to purify such a small amount with a high yield. Nonetheless, as early as 1967, Yoshida was able to purify and fingerprint G6PD A+ and G6PD B and to determine that they differed in a single amino acid substitution of asparagine to aspartic acid.[53] Obtaining the entire sequence of the enzyme by protein sequences eluded even this master protein chemist, however. Fifteen years later, at the dawn of the time when determining the sequence of a protein by examining DNA was more facile than protein sequencing, the sequence data[54] that were available bore little relationship to the sequence of the protein as we now know it. Nonetheless, using this partial, and largely inaccurate, sequence, Persico et al[55] succeeded in isolating cDNA clones of G6PD and thereby in deducing its primary structure. This achievement opened the door to understanding the polymorphisms of the G6PD gene in a much more precise fashion than had been possible when differentiation of variants depended on enzyme kinetics, heat stability, and electrophoretic mobility.

It was possible to show quite rapidly that Yoshida's deduction that G6PD A was a result of a substitution of aspartic acid for asparagine had been quite correct. Interestingly, it became apparent that G6PD A− is not a single variant but a group of variants that had in common the same mutation as G6PD A+, together with one additional mutation, usually c.202 G→A (V68M).[56]

Modern structural studies

Conventional protein biochemical methods had shown that G6PD was composed of identical subunits 53 kDa in size and that it

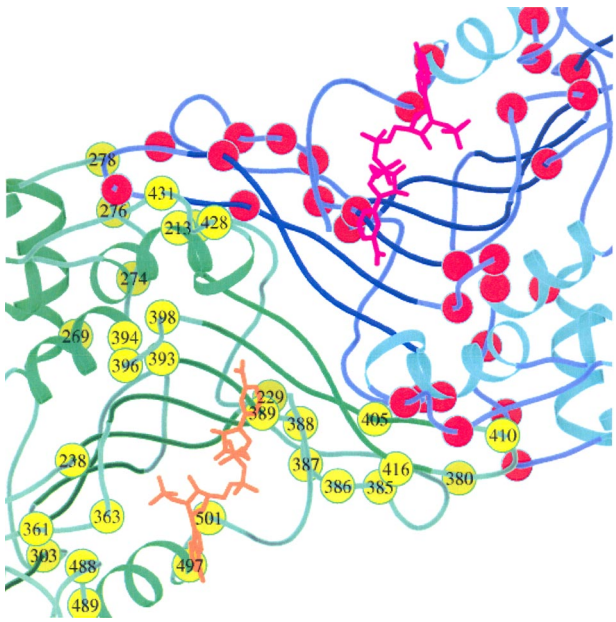

Figure 7. The region of the human G6PD molecule in which the subunits form contacts. The numbers indicate the amino acids that are altered to produce type I variants, those that cause hereditary nonspherocytic anemia. Two subunits are shown. The NADP bound by one subunit is shown in orange; the other NADP is shown in purple. (Courtesy Drs Margaret Adams, Sheila Gover, and Shannon Au.)

existed as monomers, dimers, and tetramers.[57] It also had been demonstrated that association of these subunits was NADP dependent.[58,59] It was possible to create hybrid G6PD molecules by dissociating subunits by removing NADP, mixing the monomers that formed, and reassociating them by adding NADP.[60] The finding that 4 moles of NADP were very tightly bound to the enzyme tetramer and that there was, in addition, more loosely bound NADP gave rise to the concept that there may be "structural" and catalytic NADP in the enzyme.[61]

The detailed crystal structure of G6PD has been solved.[62] As had been proposed on the basis of earlier biochemical studies, there are bound "structural" molecules of NADP and catalytic NADP molecules. As shown in Figure 7, the mutations that cause the most severe disease phenotype, hereditary nonspherocytic hemolytic anemia, tend to occur in the regions of the subunit contacts near the tightly bound "structural" NADP.

What remains to be done?

Much has been learned about G6PD deficiency and its effects in the past 50 years, but as in every field of science there is still much to learn. Here I can only mention a few of the problems that I consider to be important now.

The development of simple means of testing new drugs in vitro to determine whether they will cause hemolysis in patients has eluded us. Simply incubating drugs with red cells is unreliable, at least in part because it is usually drug metabolites, not the original drug, that are hemolytic. Several different model systems have been proposed, but none have been extensively validated.[63] Perhaps the most attractive of these is the use of plasma from a normal subject ingesting the drug and determining whether it increases metabolism by way of the hexose monophosphate shunt.[64]

Another area of importance is the most devastating result of G6PD deficiency: kernicterus. There has been a resurgence of severe neonatal jaundice and kernicterus in recent years, and G6PD deficiency is one of the causes. Should all newborns be screened? Is the human and the financial cost worth it? Should the screening be limited to ethnic groups who are known to have high gene frequencies, and if so, how will they be identified? Or would it be better simply to monitor bilirubin levels of newborns more closely, thus saving infants from the tragedy of kernicterus, regardless of cause?

Concluding thoughts

It is a rare privilege for a scientist to be able personally to follow a field for 50 years—from the clinical problems, through the initial glimmerings of understanding, to the development of a comprehensive picture including pathophysiology, biochemical, and then molecular genetics, population genetics, and fine structure of the molecule that is at the root of the whole problem. Many have contributed to this understanding, and although it is the nature of science to raise new questions as old ones are answered, G6PD deficiency has taught us a great deal in many disciplines. It has provoked us to alter our thinking in some as we strive to better understand nature.

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References

1. Takahara S, Miyamoto H. Three cases of progressive oral gangrene due to lack of catalase in the blood. Nippon Jibiinkoka Gakkai Kaiho. 1948;51:163.
2. Isselbacher KJ, Anderson EP, Kurahashi E, Kalckar HM. Congenital galactosemia, a single enzymatic block in galactose metabolism. Science. 1956;123:635-636.
3. Carson PE, Flanagan CL, Ickes CE, Alving AS. Enzymatic deficiency in primaquine-sensitive erythrocytes. Science. 1956;124:484-485.
4. Luzzatto L. New developments in glucose-6-phosphate dehydrogenase deficiency. Isr J Med Sci. 1973;9:1484-1498.
5. Cordes W. Experiences with plasmochin in malaria. In: Anonymous. 15th Annual Report. Boston, MA: United Fruit Co; 1926:66-71.
6. Beutler E. The red cell: a tiny dynamo. In: Wintrobe MM, ed. Blood Pure and Eloquent. New York, NY: McGraw-Hill, Inc; 1980:141-168.
7. Ashby W. The determination of the length of life of transfused blood corpuscles in man. J Exp Med. 1919;29:267-281.
8. Sterling K, Gray SJ. Determination of the circulating red cell volume in man by radioactive chromium. J Clin Invest. 1950;29:1614-1619.
9. Necheles TF, Weinstein IM, LeRoy GV. Radioactive sodium chromate for the study of survival of red blood cells, I: the effect of radioactive sodium chromate on red cells. J Lab Clin Med. 1953;42:358-367.
10. Dern RJ, Beutler E, Alving AS. The hemolytic effect of primaquine, II: the natural course of the hemolytic anemia and the mechanism of its self-limited character. J Lab Clin Med. 1954;44:171-175.
11. Beutler E, Dern RJ, Alving AS. The hemolytic effect of primaquine, IV: the relationship of cell age to hemolysis. J Lab Clin Med. 1954;44:439-442.
12. Beutler E, Dern RJ, Alving AS. The hemolytic effect of primaquine, VI: an in vitro test for sensitivity of erythrocytes to primaquine. J Lab Clin Med. 1955;45:40-50.
13. Beutler E, Dern RJ, Flanagan CL, Alving AS. The hemolytic effect of primaquine, VII: biochemical studies of drug-sensitive erythrocytes. J Lab Clin Med. 1955;45:286-295.
14. Beutler E. The glutathione instability of drug-sensitive red cells: a new method for the in vitro detection of drug-sensitivity. J Lab Clin Med. 1957; 49:84-95.
15. Beutler E, Robson M, Buttenwieser E. The mechanism of glutathione destruction and protection in drug-sensitive and non-sensitive erythrocytes: in vitro studies. J Clin Invest. 1957;36:617-628.
16. Johnson RM, Goyette G, Ravindranath Y, Ho YS. Red cells from glutathione peroxidase-1–deficient mice have nearly normal defenses against exogenous peroxides. Blood. 2000;96:1985-1988.
17. Dern RJ, Beutler E, Alving AS. The hemolytic effect of primaquine, V: primaquine sensitivity as a manifestation of a multiple drug sensitivity. J Lab Clin Med. 1955;45:30-39.
18. Chan TK, Chesterman CN, McFadzean AJS, Todd D. The survival of glucose-6-phosphate dehydrogenase–deficient erythrocytes in patients with typhoid fever on chloramphenicol therapy. J Lab Clin Med. 1971;77:177-184.
19. Chan TK, Todd D, Tso SC. Red cell survival studies in glucose-6-phosphate dehydrogenase deficiency. Bull Hong Kong Med Assoc. 1974;26:41-48.
20. Zail SS, Charlton RW, Bothwell TH. The haemolytic effect of certain drugs in Bantu subjects with a deficiency of glucose-6-phosphate dehydrogenase. S Afr J Med Sci. 1962;27:95-99.
21. Burka ER, Weaver Z III, Marks PA. Clinical spectrum of hemolytic anemia associated with G 6 PD deficiency. Ann Intern Med. 1966;64:817-825.
22. Arie THD. Pythagoras and beans. Oxf Med School Gaz. 1959;11:75-81.
23. Mager J, Chevion M, Glaser G. Favism. In: Anonymous. Toxic Constituents of Plant Foodstuffs. New York, NY: Academic Press, Inc; 1980:265-294.
24. Stamatoyannopoulos G, Fraser GR, Motulsky AG, Fessas P, Akrivakis A, Papayannopoulou T. On the familial predisposition to favism. Am J Hum Genet. 1966;18:253-263.
25. Crosby WH. Hereditary nonspherocytic hemolytic anemia. Blood. 1950;5:233-253.
26. Newton WA Jr, Bass JC. Glutathione sensitive chronic non-spherocytic hemolytic anemia. Am J Dis Child. 1958;96:501-502.

27. Luisada L. Favism: singular disease affecting chiefly red blood cells. Medicine. 1941;20:229-250.
28. Kaplan M, Hammerman C. Glucose-6-phosphate dehydrogenase deficiency: a hidden risk for kernicterus. Semin Perinatol. 2004;28:356-364.
29. Kaplan M, Renbaum P, Levy-Lahad E, Hammerman C, Lahad A, Beutler E. Gilbert syndrome and glucose-6-phosphate dehydrogenase deficiency: a dose-dependent genetic interaction crucial to neonatal hyperbilirubinemia. Proc Natl Acad Sci U S A. 1997;94:12128-12132.
30. Browne EA. The inheritance of an intrinsic abnormality of the red blood cell predisposing to drug-induced hemolytic anemia. Johns Hopkins Med J. 1957;101:115-118.
31. Ohno S, Kaplan WD, Kinosita R. Formation of the sex chromatin by a single X-chromosome in liver cells of rattus norvegicus. Exp Cell Res. 1959;18:415-418.
32. Beutler E, Yeh M, Fairbanks VF. The normal human female as a mosaic of X-chromosome activity: studies using the gene for G-6-PD deficiency as a marker. Proc Natl Acad Sci U S A. 1962;48:9-16.
33. Beutler E, Baluda MC. The separation of glucose-6-phosphate dehydrogenase–deficient erythrocytes from the blood of heterozygotes for glucose-6-phosphate dehydrogenase deficiency. Lancet. 1964;1:189-192.
34. Lyon MF. Gene action in the X-chromosome of the mouse (*mus musculus l.).* Nature. 1961;190:372-373.
35. Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF, Prchal JT. Discrimination of polycythemias and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-E response to erythropoietin. Blood. 2003;101:3294-3301.
36. Oni SB, Osunkoya BO, Luzzatto L. Paroxysmal nocturnal hemoglobinuria: evidence for monoclonal origin of abnormal red cells. Blood. 1970;36:145-152.
37. Fialkow PJ, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. Proc Natl Acad Sci U S A. 1967;58:1468-1471.
38. Motulsky AG, Campbell-Kraut JM. Population genetics of glucose-6-phosphate dehydrogenase deficiency of the red cell. In: Blumberg BS, ed. Proceedings of the Conference on Genetic Polymorphisms and Geographic Variations in Disease. New York, NY: Grune & Stratton;1961:159-180.
39. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: diagnosis, clinical and genetic implications. Am J Clin Pathol. 1967;47:303-311.
40. Beutler E, Mitchell M. Special modifications of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency. Blood. 1968;32:816-818.
41. Herz F, Kaplan E, Scheye ES. Diagnosis of erythrocyte glucose-6-phosphate dehydrogenase deficiency in the negro male despite hemolytic crisis. Blood. 1970;35:90-93.
42. Fairbanks VF, Fernandez MN. The identification of metabolic errors associated with hemolytic anemia. JAMA. 1969;208:316-320.
43. Marks PA, Gross RT. Erythrocyte glucose-6-phosphate dehydrogenase deficiency: evidence of differences between Negroes and Caucasians with respect to this genetically determined trait. J Clin Invest. 1959;38:2253-2262.
44. Boyer SH, Porter IH, Weilbacher RG. Electrophoretic heterogeneity of glucose-6-phosphate dehydrogenase and its relationship to enzyme deficiency in man. Proc Natl Acad Sci U S A. 1962;48:1868-1876.
45. Kirkman HN, Riley HD Jr, Crowell BB. Different enzymic expressions of mutants of human glucose-6-phosphate dehydrogenase. Proc Natl Acad Sci U S A. 1960;46:938-944.
46. Betke K, Beutler E, Brewer GJ, et al. Standardization of procedures for the study of glucose-6-phosphate dehydrogenase: report of a WHO scientific group. WHO Tech Rep Ser. 1967;366.
47. Yoshida A, Beutler E, Motulsky AG. Human glucose-6-phosphate dehydrogenase variants. Bull WHO. 1971;45:243-253.
48. Beutler E, Yoshida A. Genetic variation of glucose-6-phosphate dehydrogenase: a catalog and future prospects. Medicine. 1988;67:311-334.
49. Luzzatto L. Genetics of red cells and susceptibility to malaria. Blood. 1979;54:961-976.
50. Tishkoff SA, Varkonyi R, Cahinhinan N, et al. Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. Science. 2001;293:455-462.
51. Luzzatto L, Usanga EA, Reddy S. Glucose 6-phosphate dehydrogenase deficient red cells: resistance to infection by malarial parasites. Science. 1969;164:839-842.
52. Yoshida A. Glucose-6-phosphate dehydrogenase of human erythrocytes, I: purification and characterization of normal (B+) enzyme. J Biol Chem. 1966;241:4966-4976.
53. Yoshida A. A single amino acid substitution (asparagine to aspartic acid) between normal (B+) and the common negro variant (A+) of human glucose-6-phosphate dehydrogenase. Proc Natl Acad Sci U S A. 1967;57:835-840.
54. Beutler E. Glucose-6-phosphate dehydrogenase deficiency. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS, eds. The Metabolic Basis of Inherited Disease. New York, NY: McGraw-Hill, Inc; 1983:1629-1653.
55. Persico MG, Viglietto G, Martino G, et al. Isolation of human glucose-6-phosphate dehydrogenase (G6PD) cDNA clones: primary structure of the protein and unusual 5′ non-coding region. Nucl Acids Res. 1986;14:2511-2522.
56. Hirono A, Beutler E. Molecular cloning and nucleotide sequence of cDNA for human glucose-6-phosphate dehydrogenase variant A(−). Proc Natl Acad Sci U S A. 1988;85:3951-3954.
57. Cohen P, Rosemeyer MA. Subunit interactions of glucose-6-phosphate dehydrogenase from human erythrocytes. Eur J Biochem. 1969;8:8-15.
58. Kirkman HN, Hendrickson EM. Glucose-6-phosphate dehydrogenase from human erythrocytes, II: subactive states of the enzyme from normal persons. J Biol Chem. 1962;237:2371-2376.
59. Chung AE, Langdon RG. Human erythrocyte glucose-6-phosphate dehydrogenase, I: isolation and properties of the enzyme. J Biol Chem. 1963;238:2309-2316.
60. Beutler E, Collins Z. Hybridization of glucose-6-phosphate dehydrogenase from rat and human erythrocytes. Science. 1965;150:1306-1307.
61. De Flora A, Morelli A, Giuliano F. Human erythrocyte glucose 6-phosphate dehydrogenase: content of bound coenzyme. Biochem Biophys Res Commun. 1974;59:406-413.
62. Kotaka M, Gover S, Vandeputte-Rutten L, Au SW, Lam VM, Adams MJ. Structural studies of glucose-6-phosphate and NADP(+) binding to human glucose-6-phosphate dehydrogenase. Acta Crystallogr D Biol Crystallogr. 2005;61:495-504.
63. Horton HM, Calabrese EJ. Predictive models for human glucose-6-phosphate dehydrogenase deficiency. Drug Metab Rev. 1986;17:261-281.
64. Gaetani GD, Mareni C, Ravazzolo R, Salvidio E. Haemolytic effect of two sulphonamides evaluated by a new method. Br J Haematol. 1976;32:183-191.
65. Beutler E. Hemolytic Anemia in Disorders of Red Cell Metabolism. New York, NY: Plenum Press, Inc; 1978.
66. Beutler E. Disorders of red cells resulting from enzyme abnormalities. In: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal J, eds. Williams Hematology. New York, NY: McGraw-Hill; 2006:603-631.
67. Dern RJ, Weinstein IM, Le Roy GV, Talmage DW, Alving AS. The hemolytic effect of primaquine, I: the localization of the drug-induced hemolytic defect in primaquine-sensitive individuals. J Lab Clin Med. 1954;43:303-309.
68. Beutler E. Drug-induced anemia. Fed Proc. 1972;31:141-146.

I was always interested in science as a child, and reading Paul De Kruif's *Microbe Hunters* and Sinclair Lewis' *Arrowsmith* focused my attention on medicine. In medical school and throughout my house staff training at the University of Chicago, I found many fascinating and challenging problems in all of the specialties. But hematology was unique. One could secure the tissue needed and could quantitate it, features that had attracted the finest academically oriented physician/scientists into the field. Thus, I felt privileged to be allowed to become a member of the Hematology division. It was headed by Leon Jacobsen, whose qualities as a compassionate physician and imaginative, productive scientist I greatly admired, and who was my mentor in those early years.

As my vistas broadened, I realized that worldwide there were many other great biomedical scientists in hematology, scientists who also were to serve as role models for me as I tried to make my own contributions to the field. Because of the type of person attracted to our field and because of our unique access to blood cells, hematology has been at the vanguard of many of the important developments in biology and medicine over the past 50 years. Once I became a hematologist, I never had any regrets about my choice and never looked back.