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A study of the relationship of glucose-6-phosphate dehydrogenase deficiency and bacterial infection in a hospitalized Iranian population

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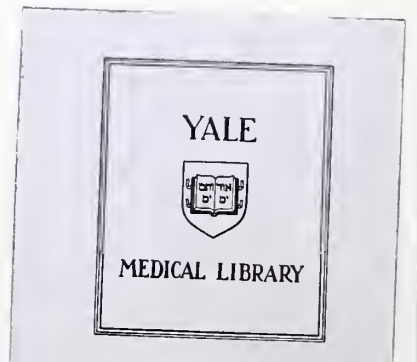
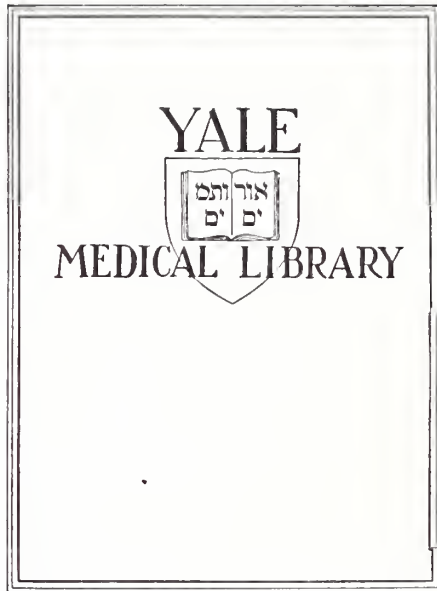


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A STUDY OF THE RELATIONSHIP OF G6PD
DEFICIENCY AND BACTERIAL INFECTION
IN A HOSPITALIZED IRANIAN POPULATION

MARCIA S. CLARK

1977



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Deficiency and Bacterial Infection in a Hospitalized " Iranian Population

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ABSTRACT

The subject of this paper is the relationship between glucose-6-phosphate dehydrogenase (G6PD) deficiency and bacterial infection. The relevant literature is reviewed in two parts. First, major biochemical and clinical aspects of G6PD deficiency are discussed. The second part covers neutrophil function and dysfunction; leukocyte G6PD and the implications of its deficiency are placed within the context of other neutrophil disorders. A hospitalized Iranian population comprised our study group. When we compared the rates of G6PD deficiency in infected and non-infected patients, the difference reached statistical significance under certain circumstances ($.05 > p > .01$). Our data also showed the G6PD deficient infected patients to be significantly younger than the G6PD normal infected patients ($.005 > p > .001$). This is consistent with the hypothesis that G6PD deficient patients, once infected, have a more severe clinical course. We conclude that there are significant interactions between G6PD deficiency and bacterial infection, although their exact nature remains to be defined. Mechanisms which would explain an association between these two conditions are discussed.

A STUDY OF THE RELATIONSHIP OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE
DEFICIENCY AND BACTERIAL INFECTION IN A HOSPITALIZED
IRANIAN POPULATION

by

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A Thesis Presented to the Faculty of Yale Medical School
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I

Definitions and History

Glucose-6-phosphate dehydrogenase (G6PD), the first enzyme in the pentose phosphate pathway, catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone while reducing NADP to NADPH [Figure 1]. The major practical importance of G6PD lies in its role in the red blood cell, where its deficiency can cause hemolytic anemia. Most commonly, the anemia is episodic and is related to one of a number of agents, but occasionally a chronic hemolytic anemia occurs. Although the former entity has been known for thousands of years, it was only recently that the link between G6PD deficiency and hemolytic anemia was established. In 1926, plasmoquin, an 8-aminoquinoline compound, was first used in treating syphilitics who had been inoculated with malaria; shortly thereafter, a series of reports appeared describing hemolytic anemia with administration of the drug [1]. Little progress was made in determining the etiology of the condition until 1950, when investigators received a new research tool in the form of primaquine, a compound related to but therapeutically more effective than plasmoquin. In 1954 Dern et al. [2], utilizing cross-transfusions between normal and primaquine-sensitive individuals, demonstrated that the basic abnormality was in the red blood cell. Other investigators found that the hemolytic susceptibility was restricted to the older erythrocytes and, since at

least some metabolic processes were known to decrease with aging, attention was directed towards a metabolic disorder [1]. In 1965, Carson and his collaborators [3] reported a decreased G6PD level in the erythrocytes of affected individuals.

Mechanisms of Hemolysis

Although it was soon generally accepted that the G6PD deficiency and hemolytic anemia were related, detailed understanding of the hemolytic mechanism was and still is lacking. An outline of events as they are known requires a brief digression into certain aspects of erythrocyte biochemistry.

As mentioned before, G6PD is one of the enzymes of the hexose monophosphate shunt (HMP) [Figure 1]. This pathway is very important to the red blood cell, even though it metabolizes a much smaller quantity of glucose than the Embden-Meyerhof pathway. The reduction of NADP to NADPH and the production of the nucleotide precursor ribose-5-phosphate take place via the HMP. The latter can also be formed by other means; in contrast, NADPH in red cells is supplied entirely by the HMS. A source of NADPH is critical because it acts as the coenzyme for the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH). The GSH prevents the oxidation of sulfhydryl groups of proteins such as hemoglobin, usually because GSH itself is instead oxidized to GSSG. In cases where the oxidant is H_2O_2 , GSH acts by reducing the H_2O_2 to H_2O [4]. A second important role of NADPH is to provide electrons for reduction and cleavage of GSH-hemoglobin complexes, which would otherwise be liable to precipitate.

It is believed that infections, fava beans, and other stimuli induce hemolysis by causing the production of various oxidizing substances such as H_2O_2 or other unstable radicals. Drugs themselves can also have oxidizing capacity. In the normal person, these substances can be handled by the production of NADPH from the HMS, but G6PD deficiency blocks this pathway. Consequently, the erythrocyte proteins, including hemoglobin, are not protected from oxidation. Oxidized hemoglobin can denature and precipitate as Heinz bodies, and by binding to and damaging the cell membranes, the Heinz bodies increase cell susceptibility to removal by the reticuloendothelial system [1,5].

Baehner et al. [6] have made a more specific proposal regarding the mechanism of hemolysis during infection; they suggest that H_2O_2 produced by phagocytizing leukocytes could serve as a partial source of the oxidant stress. Their hypothesis was supported by experiments in which phagocytizing leukocytes caused a fall in GSH in neighboring G6PD deficient red blood cells, a result associated with increased destruction of these cells in the liver and spleen. The effect was not present with normal erythrocytes, nor with separation of the G6PD deficient cells from the leukocytes. Pyrimidine aglycons have been implicated as the offending agent in favism [7], and naturally occurring substances such as ascorbate, pyruvate, and cysteine in "spontaneous" hemolysis [5].

G6PD Variants

During the course of these and other investigations, it became clear that G6PD deficiency varied enormously biochemically and clinically.

Over 80 variants have now been described in the 100 million or so people with the condition [8], and doubtless more remain to be found. Only a few of the major variants will be discussed here; complete compilations are available in the literature [9,10].

The normal G6PD enzyme, known as type B, has a molecular weight of 190,000 to 240,000 and has 3 to 6 similar or identical subunits, although the functioning enzyme is probably dimeric [1]. The biochemical properties, including substrate affinity, thermostability, optimal pH, and electrophoretic characteristics, are well known [Table 1]. One of the most common variants, called type A because it migrates faster than type B, is present in approximately 30 percent of American black males [5]. With the use of trypsin digests, it was found to differ from type B by only a single amino acid [11]. Type A has normal activity and so has little clinical importance, in contrast to Type A⁻ (which receives its name because it migrates with Type A, but has decreased activity). About 11 percent of American black males have Type A⁻ [5] and the gene exists in varying frequencies throughout Africa. The catalytic activity of each A⁻ enzyme molecule is close to normal, but there is a fall in the total number of molecules [1], and the decrease in activity between young and old cells is more marked than in normal cells [12,13]. Overall, the activity in the blood is usually 5-15 percent of normal [5]; very rarely, activity can be absent [14]. Affected individuals are generally asymptomatic except under conditions of stress, such as with certain drugs or infection [Table 2]. Because G6PD activity is low only in older cells, the hemolysis tends to be mild and self-limited; a typical

time course involves an onset 1 to 3 days after the inciting agent has been introduced, with recovery beginning in 4 to 6 days [5].

A more severe form of G6PD deficiency is found in the Mediterranean area, including Sardinia, Italy, Greece, Iran, Rumania, Israel, and perhaps some areas in the Far East [15]. It is possible that G6PD Mediterranean, as it is known, represents a heterogeneous group [11,16, 17,18] but the major biochemical characteristics listed in Table 1 are constant. The frequency of the gene ranges from less than 0.1 percent in Northern Europe to greater than 50 percent of Kurdish Jewish males [5]. The quantity of enzyme is reduced in very young red blood cells, and their catalytic efficacy is also lower [1]. Because all erythrocytes are affected, the G6PD activity of whole blood tends to be less than 1 percent, and the hemolytic episodes are often more severe and not self-limiting. The list of precipitating agents is similar to, but not identical with, that for G6PD A⁻; for example, chloramphenicol causes hemolysis in G6PD Mediterranean but not G6PD A⁻ [Table 2]. Fava beans, which can lead to an extremely rapid and severe hemolysis, also appear to affect mainly Caucasians [8]. Another factor may also be involved, since only some G6PD Mediterranean patients are affected by the fava beans.

A host of other G6PD variants have been described, ranging from G6PD Canton, similar in its properties to G6PD Mediterranean [15], G6PD Oklahoma, with a tremendous deterioration in activity with aging [19] and G6PD Hektoen, with increased activity [5]. The clinical manifestations depend on the characteristics of the enzyme; patients can be

asymptomatic, have mild or severe disease resembling that described above, or rarely, have a chronic nonspherocytic anemia. Seen with some of the rarer types of G6PD deficiency, as well as occasionally in G6PD Mediterranean, the degree of shortened erythrocyte lifespan is generally mild [5].

Leukocyte G6PD

G6PD deficiency is not necessarily limited to the red blood cells and articles have appeared describing decreased levels in leukocytes, lens tissue, kidney, adrenal tissue, platelets, saliva, liver and other tissues [1]; most of these studies were in Caucasians [8,20]. A complete analysis of these reports is not feasible here, and only the leukocyte levels will be discussed in some detail. While most people believe that the leukocyte and erythrocyte enzymes are the same [1], some investigators have claimed differences [1,19] and the issue has not been definitively settled. One published report even showed separate inheritance of the two defects, but the measurement techniques described are open to question [21]. Moreover, the findings have not been confirmed by other researchers. In any case, several different studies have demonstrated lowered leukocyte levels in association with some types of erythrocyte G6PD deficiency. Marks and Gross [12] reported that Caucasian, but not black, males with severe erythrocyte G6PD deficiency (less than 4.5 standard deviations below control values) have a leukocyte G6PD level approximately one third that of controls. A similar study carried out by Ramot et al. [22] showed leukocytes with 25 percent of normal activity. Affected Chinese males have also been

found to have decreased leukocyte G6PD levels [23]. The potential functional significance of lowered leukocyte G6PD levels to the anti-microbial activities of these cells will be discussed below.

Mode of Inheritance

The mode of inheritance of G6PD deficiency is well known to be X-linked. It was established by inheritance patterns, and with linkage studies of other traits such as color blindness and hemophilia A. The study of a patient with Turner's syndrome and fully expressed G6PD deficiency helped to rule out a sex-influenced process [7]. G6PD deficiency was, in fact, instrumental in confirmation of the Lyon hypothesis; Beutler et al. [24] studied females heterozygous for the trait and found two distinct populations of cells, rather than one with intermediate activity. It is a useful cell marker in other systems, for example the study of blood cell precursor development [25] and Down's syndrome [26].

G6PD Deficiency and Associated Conditions

In the short time that it has been known, G6PD deficiency has been associated with a number of other pathological processes. Its relationship to bacterial infection is complex, and leukocyte G6PD levels may well play a role in host defenses. This subject will be addressed in the following two sections of this paper; here, only a brief review of specific associated conditions, infectious and non-infectious, is presented.

One area of great controversy concerns whether G6PD deficiency

might have a selective advantage in some circumstances. The fact that it is so common, and that the distribution to some extent parallels that of malaria, has led to the hypothesis that G6PD deficiency might confer a degree of protection against malaria. Support for this idea has been gathered in a series of experiments, most of which used G6PD A⁻ and Plasmodium falciparum; these experiments demonstrated decreased parasitization of the G6PD deficient red blood cells as compared to normal [27,28]. An explanation for this protection was proposed by Kosover [29]: the increased level of GSSG, a substance known to inhibit protein synthesis in rabbits, could act to retard parasite proliferation. These theories leave many points unexplained, including the fact that G6PD deficiency is so frequent and severe in areas where there is a fairly high selective disadvantage due to the popularity of fava beans. Huheey and Martin [30] suggested that favism may augment G6PD deficiency's protective effect against malaria, but this remains pure speculation. They also point out that G6PD Mediterranean may be particularly effective in combating P. vivax, which is more important than P. falciparum in Mediterranean areas. Their reasoning is that P. vivax has more exoerythrocytic stages than P. falciparum, and in G6PD Mediterranean many body tissues, including the liver, are G6PD deficient. Finally, as Carson and Fischer [7] have noted, there are other diseases, such as plague, which have a similar distribution and might also have played a selective role.

G6PD deficiency is a well known cause of neonatal jaundice, and in some parts of the world may be the most common one [19]. The process

seems to affect Caucasian, Indian, and Oriental infants [8,31,32]. Valaes et al. [33] studied groups of Greek newborns, confirming the link between G6PD deficiency and neonatal jaundice while raising additional, complicating issues. They found evidence for another icterogenic factor which accentuated the jaundice in normal and deficient patients; they also found that the degree of hyperbilirubinemia and anemia were not always parallel, as one might expect in a hemolytic state. Recently, Meloni et al. [34], discovering that barbiturates were therapeutically effective, postulated that transiently impaired liver function could also be partly responsible for the jaundice. An increased incidence and severity of jaundice has also been noted in black and Caucasian hepatitis patients with G6PD deficiency [35,36,37]. Doubtless this is partly due to hemolysis resulting from the oxidative stress of infection, but similar to neonatal jaundice, the evidence for hemolysis was not always entirely convincing, and other factors were believed to contribute. Jaundice has been associated with lobar pneumonia in African G6PD patients [38,39] and liver biopsies have shown changes consistent with cholestasis [40]. G6PD deficiency has been reported to predispose to typhoid in Ghana [41] and Thailand [42], but both studies could be questioned on grounds of bias in patient selection. A small study in India linked leprosy and G6PD deficiency [43], while TB was shown not to be related to G6PD deficiency in the Far East [44].

Regarding non-infectious diseases, several investigators have produced population studies showing an inverse relationship between G6PD deficiency and malignancy [45,46,47,48], but the study and control

groups differ in a number of ways and many other etiologies are equally or more plausible. Beaconsfield [45] did have an interesting suggestion of why G6PD deficiency and cancer might be related: lack of G6PD by depressing the pentose phosphate pathway interferes with pentose sugar and nucleic acid production and impedes rapid growth. More recently, Eaton [49] noted that catalase inactivation stimulates the phosphate shunt and some investigators have found decreased liver catalase with hepatomas, reinforcing the idea that the pentose phosphate pathway may be an important limiting factor in neoplastic (and possibly malarial) processes. Other diseases described as being related to G6PD deficiency include thalassemia [50], cataracts [51], regional enteritis, and granulomatous colitis [52,53], but the data is tenuous. Finally, a considerable amount has been written on the relationship of Hemoglobin S and G6PD deficiency [54,55,56,57,58]. While the controversy is not yet totally resolved, the prevailing opinion is that the genes are common in the same populations, but that the presence of one does not appreciably alter the clinical course of the other.

II

NORMAL NEUTROPHIL FUNCTION [5,8,59]

Production and Delivery

Neutrophils, the chief phagocytic cells in the blood, make up the myeloid series together with eosinophils and basophils. The first morphologic stage in their development is the myeloblast; whether there are distinct myeloblasts for each of the three lines of the myeloid series is unknown. Morphologically, myeloblasts are characterized by large nuclei with two to five nucleoli and even, diffuse chromatin, while the small quantity of basophilic cytoplasm contains numerous mitochondria and no granules. Myeloblasts give rise to promyelocytes, which can be distinguished by the increasingly prominent endoplasmic reticulum and, more importantly, the appearance of primary, azurophilic granules. These are formed on the concave surface of the Golgi body and are rounded or elongated, with crystalline contents of lysosomal hydrolases, myeloperoxidase, cationic proteins and others. Secondary granules, which appear first in the myelocyte stage, are made on the convex surface of the Golgi body and are variable in shape. Their homogeneous appearing contents include alkaline phosphatase and lactoferrin. This type of secondary granule is unique to neutrophils, and the myelocyte is thus the first stage at which differentiation of cell

lines can be made. Metamyelocytes have clearly indented nuclei lacking nucleoli, and moderately dense, sometimes clumped nuclear chromatin. The endoplasmic reticulum is much less prominent, as one would expect from the decrease in protein synthesis; under normal circumstances, metamyelocytes have probably lost their ability to divide and reproduce. The final two stages, the juvenile or band form and the mature polymorphonuclear leukocyte are usually distinguished on the basis of their nuclei; the former has partial separation of the nuclear lobes, while in the latter there are only thread-like connections. The nuclei of mature cells have coarse, condensed chromatin, and the cytoplasm contains both types of granules. In accord with the fact that their main source of energy is glycolysis, there are large stores of glycogen but few mitochondria. Precise understanding of the regulation of the relative numbers of cells in various stages of development in the marrow, the intravascular pool (both circulating and marginated) and the tissues is lacking at present. Several granulopoietic agents have been described. Other factors which may regulate the number of cells in different body pools include physiologic cycling (possibly at several different intervals); heat, exercise, emotional state, infection, and drugs [5,8].

The processes by which neutrophils are delivered to sites of inflammation are complex. For unknown reasons, capillary endothelium can change in such a way as to cause local granulocyte adherence followed by diapedesis through vessel walls [60]. This tends to occur in injured areas, and the white cells are further drawn to inflamed regions by two kinds of substances: cytotoxins, which act directly,

including most importantly by-products of complement activation, and cytotoxic agents, which generate cytotoxins and include lysosomes, endotoxins and immunologic complexes. These chemotactic factors do not increase the speed at which neutrophils move, but rather influence their direction [8]. It is known that while migrating, polymorphonuclear leukocytes develop a stiff rim of cytoplasm that contains actin polymers and glycogen particles, and that the ability to assemble microtubules is integral to responding with directed movement. Precise details of the events in migration are lacking, however.

Phagocytosis: Recognition and Ingestion

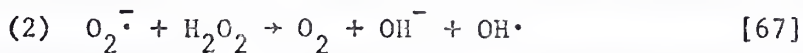
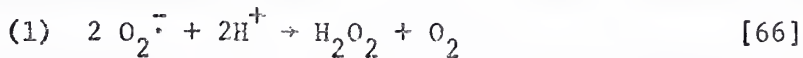
The susceptibility of many microbes to phagocytosis by neutrophils is enhanced by the process of opsonization. Both bacteria and polymorphonuclear leukocytes, particularly immature forms, have a net surface charge which is negative and they therefore naturally tend to repel each other. Opsonins are traditionally defined as agents in serum which enhance the palatability of particles [60]. They consist most importantly of heat-labile complement and heat stable antibody; specific receptors for the Fc fragment of IgG and for C3b may exist in neutrophils. Specific IgG antibody, therefore, can bind and opsonize directly, although in most physiological situations complement is probably also activated. In contrast, IgM must act via complement fixation since it has no specific receptors. In the absence of antibody some bacteria themselves can activate the alternate complement pathway [60,61]. It is possible that different bacteria require fixation of different complement products [62], and under some circumstances organisms

can be taken up if there is a suitable surface against which they can be trapped, so-called "surface phagocytosis" [60]. In order for the microbes to be ingested energy must be generated; as mentioned earlier, it is produced by glycolysis and oxygen is not required. This is probably advantageous, in view of the often anoxic inflammatory environment. During the act of attachment the hyaline ectoplasm, the outer cytoplasmic rim, forms filopodia which surround the particle. With phagocytosis the cellular membrane surrounding the particle is internalized, forming a phagosome whose walls are comprised of former plasma membrane.

Killing and Digestion

During ingestion, lysosomes fuse with the phagosomal membrane and discharge their contents into the vacuole; most likely the secondary granules fuse shortly before the primary. This process is known as lysosomal degranulation and the enzymes released mediate the oxygen independent microbicidal system. They have a variety of partially understood effects; for example, the hydrolases are believed to digest the bacteria and to prevent bacterial multiplication, lactoferrin binds iron, a necessary growth factor, and myeloperoxidase has a complicated bactericidal role to be discussed later. Many of the strongly cationic granular proteins also seem to be microbicidal, perhaps by binding to acidic groups on the target organisms and in some way preventing growth. In addition, lactate accumulation and carbonic acid production result in an acid environment in the phagocytic vacuole; this has an adverse effect on most organisms, as well as promoting the reactions described below.

In the normal sequence of events phagocytosis is followed by a burst of metabolic activity; the actual stimulus has been shown to be mediated by surface perturbation [63,64]. The oxidation of glucose via the hexose monophosphate shunt increases as does oxygen consumption, setting into motion the oxygen dependent bacterial mechanisms. Recent evidence has shown that the first event in the formation of bactericidal compounds is a one electron reduction of oxygen, resulting in superoxide anions, O_2^- [65] [Figure 2]. Not only is this agent intrinsically toxic, but it is an intermediate in the production of more such agents, H_2O_2 and OH^- by the following reactions:



These substances, together with other unstable intermediates (for example singlet oxygen) comprise the myeloperoxidase independent, oxygen dependent bactericidal system. In the myeloperoxidase mediated system, hydrogen peroxide and an oxidizable cofactor, usually a halide, are required. The hydrogen peroxide, provided by bacterial or leukocyte metabolism, forms a complex with the iron of the heme moiety delivered into the phagocytic vacuole by degranulation; this complex in turn forms antimicrobial substances by oxidizing the cofactor. The nature and mode of actions of these substances are known to vary with the cofactor but are not completely understood [67]. Figure 2 demonstrates some of the postulated interrelationships between phagocytosis-stimulated oxygen consumption, superoxide and peroxide formation and pathways for their

utilization in intact granulocytes.

NEUTROPHIL ABNORMALITIES

Disorders of Production and Delivery [5,8]

Neutrophil disorders can best be divided into those of production and delivery, recognition and ingestion, and killing and digestion. Granulocytopenia, here defined as a reduction in circulating neutrophils, can result from reduced granulopoiesis, impaired release from the marrow, or decreased cell survival. A variety of drugs and infections can produce granulocytopenia by any one or a combination of these mechanisms, and several hereditary syndromes exist which have been tentatively classified. Infantile genetic agranulocytosis is among the examples of reduced granulopoiesis. Inherited in an autosomal recessive pattern, the findings consist of marked neutropenia, moderate anemia, frequent severe infections and a bone marrow revealing maturation arrest at the myelocyte stage. Two forms of an autosomal dominant familial neutropenia exist: one in which there is moderate neutropenia, no clinical difficulties, and an essentially normal bone marrow, and a more severe type in which infections are very common and most of the granulocytes do not mature beyond myelocytes. An interesting report exists of two brothers with periodontal and throat infections whose granulocytes showed this maturation defect and whose plasma inhibited the differentiation of normal cells. A number of cases of cyclic neutropenia, involving approximately three week cycles of neutropenia and mild infection, have been described. It seems likely that the problem is one of mild marrow

failure and feedback mechanisms. Schwachman's syndrome consists of pancreatic insufficiency and neutropenia, the infections varying with the degree of the latter. Neutropenia can also be associated with immunoglobulin abnormalities, usually in the pediatric age group. Bone marrow examination again reveals myelocyte arrest, and the patients are subject to numerous infections. In another type of neutropenia, the entire granulocyte series is hypoplastic, in contrast to the normal erythropoiesis and thrombocytopoiesis. Classically, chronic dermatologic infections are apparent. Myelophthisis is another cause of reduced granulopoiesis, although pancytopenia is more likely to develop. Chronic benign neutropenia is believed to result from increased peripheral destruction, and therefore belongs in the second category. The bone marrow reveals bands but few mature forms, and most patients have a moderately increased incidence of pyogenic infection. A similar but clinically less severe form exists in adults. Two kinds of transient neonatal neutropenia have been reported, one in which the mothers themselves also were neutropenic and one, similar to red cell incompatibility, in which the mothers presumably had become sensitized to the fetus' leukocytes and transmitted agglutinins. Cases of leukocyte autoantibody and neutropenia often occur with connective tissue diseases, particularly systemic lupus erythematosus. The opposite of granulocytopenia, granulocytosis, can result from any one of a large number of conditions, for instance infections, inflammatory disorders, tumor, treatment with certain drugs (e.g., corticosteroids), emotional stimuli, and metabolic and hemotologic diseases. Hereditary forms, although extremely rare,

also exist. A number of mechanisms including increased production of cells in the marrow and release into the circulation shifts from marginating to circulating pools or decreased exit from the circulation can be invoked to explain granulocytosis in these states.

The Pelger-Huët anomaly is an example of another type of production disorder, that of a predominantly morphologic abnormality. It is a dominant condition which, in the heterozygous state, causes leukocyte nuclei to have a decreased number of lobes, so called "pince-nez" nuclei. That, together with the persistence of nucleoli and the coarse nuclear chromatin, are suggestive of an abnormality of nuclear maturation. While these cells function normally, the homozygous state is felt to be lethal. Interestingly, similar cells are seen in pseudo or acquired Pelger-Huët anomaly, which is associated with various malignancies, metabolic diseases, infections, and so forth. A generally benign condition with hypersegmented nuclei is also known, and can mimic the morphology seen in vitamin B₁₂ or folate deficiency. The May-Hegglin anomaly is a disease in which the granulocytes have basophilic inclusions but apparently function normally; the platelets, however, also have inclusions and sometimes abnormal bleeding occurs. Alder-Reilly leukocytes, associated with polysaccharide disorders such as Hunter's and Hurler's syndromes, also contain giant granules but function normally. The Chediak-Higashi syndrome and other granule abnormalities will be discussed as disorders of phagocytosis as they have important functional significance.

The well-named lazy leukocyte syndrome exemplifies impaired

neutrophil delivery; interestingly, both random movement and chemotaxis are impaired. This presumably causes the peripheral neutropenia and poor response to infection. Other tests of phagocytosis, bactericidal capacity, and humoral and cellular immunity are normal. The two children with the disease had presented with recurrent stomatitis, otitis, gingivitis, and low grade fevers [68].

The constellation of findings known as Job's syndrome was first described in two red haired, fair skinned girls with recurrent cold staphylococcal abscesses [69]. Laboratory tests on these patients [69,70] revealed no abnormalities, although two similar patients reportedly failed to reduce nitroblue tetrazolium [71] (see below for discussion of this test). More recently, however, a paper has appeared in which the leukocytes were found to have defective chemotaxis and extremely high IgE levels. Random migration, phagocytosis and bactericidal activity against staphylococcus and E. coli were normal. The suggestion was made that patients with Job's syndrome lack the ability to develop an early inflammatory response and are therefore extremely susceptible to staphylococcus, the most common bacterium on the skin. Some experimental support is available in animals, in whom it has been shown that infection will be suppressed only if an appropriate inflammatory response takes place in two to four hours. The authors' explanation for the lack of classic signs of inflammation associated with the abscesses was intriguing, although pure speculation: the leukocytes, spending more time in the systemic circulation and there being exposed to an increased histamine level, might therefore release

fewer inflammatory mediators in local sites [72]. Clark et al. [73] reported a case of an eleven year old girl with recurrent pyogenic and mucocutaneous candida infections. Examination revealed normal production of chemotactic factors, but impaired migration; cellular immunity also was abnormal. One possible etiology of abnormal mobility was suggested by a brief report of an infant with bacterial skin and visceral infections, neutropenia, and decreased migration. Electron microscopy showed a defect of contractile proteins comprising cellular microfilaments, perhaps of actin polymers [74]. Other factors may also be involved; for example, cirrhotic patients have impaired chemotaxis associated with both a serum inhibitor and a deficit of complement [75].

Disorders of Recognition and Ingestion [59]

Several examples of recognition and ingestion defects are known. Defective opsonization may be partly responsible for the increased susceptibility of newborns to infection. IgM, which may be useful in the opsonization of gram negative organisms, is not transported across the placenta and lack of it may account for some of this [76]. Levels of both IgG [77] and complement [78] are related to birth weight, which may lead to additional difficulties for low birth weight infants. It has been shown that serum from sickle cell patients does not enhance pneumococcal phagocytosis as well as normal serum, and there is some evidence that this is due to a defect in the alternate complement pathway [79]. An infant with eczema, diarrhea, and recurrent infections with staphylococci and gram negative organisms was reported to have a dysfunction of C5. Although no decrease in C5 could be found, the

defect was repaired by the addition of purified C5 [62,80]. Defects of other complement components, resulting in a wide range of clinical findings, have also been reported [81]. Another type of syndrome is termed tuftsin deficiency. Tuftsin is a tetrapeptide covalently bound to leukokinin, a leukophilic gamma globulin. It is cleaved off by an enzyme called leukokininase and is thought to act directly on the phagocyte, not the opsonized target. Tuftsin is probably synthesized in the spleen, as it is absent in splenectomized individuals. Four patients with rash, lymphadenopathy, and pulmonary disease, caused by staphylococcus, streptococcus, candida, and perhaps other organisms, have been reported. They were found to have an inactive tuftsin mutant, and clinically they responded to the administration of gamma globulin [82].

Disorders of Killing and Digestion [59] (See Figure 2)

Among the causes of impaired killing and digestion are disorders which affect the neutrophil granules. Chediak-Higashi syndrome appears to result from abnormal granule formation in cells throughout the body, but the primary defect is not known. Histochemically, the granules contain normal constituents for the cell line in which they appear; in neutrophils, they are primary granules. Patients with the disease have light coloring, due to the decreased number of melanin granules, and an increased incidence of infection. A study by Root et al. [83] showed that Chediak-Higashi neutrophils have defective bactericidal defences against catalase positive and catalase negative organisms, secondary to a delay and a decrease in the transfer of granule enzymes. Phagocytosis

as well as the metabolic response to phagocytosis were found to be normal, but the authors note that neutropenia and an impaired chemotactic response have also been demonstrated in this disease. A second type of primary granule abnormality is myeloperoxidase (MPO) deficiency. As described above, MPO is required for part of the oxygen-dependent bactericidal system of leukocytes. It may also help regulate hexose monophosphate shunt (HMP) metabolism, and help protect neutrophils from the toxic effects of H_2O_2 [84]. In the presence of azide and cyanide, which inhibit MPO by forming complexes with the iron in the heme moiety, killing of Lactobacillus acidophilus, Staphylococcus aureus, and Candida tropicalis is impaired [85]. MPO deficiency, which appears to be an autosomal recessive trait, has been described in five patients. Of these, only one had an increased susceptibility to infection; he had disseminated candidiasis, but it should be noted that the patient had diabetes mellitus as well. Study of the neutrophils revealed normal phagocytosis, decreased killing of serratia and S. aureus, and absent killing of candida. However, the fact that the man did not present until middle age, and that the other cases are clinically well, speaks against MPO being an essential part of defense mechanisms [84]. Perhaps the polymorphonuclear leukocytes have a great overkill capacity, or perhaps they are able to compensate with an increase in their non MPO dependent bactericidal mechanisms. The latter suggestion is supported by Klebanoff's [85] demonstration that MPO deficient leukocytes are more efficient than azide treated normal leukocytes in killing ingested bacteria. It is interesting that one case of anomalous specific,

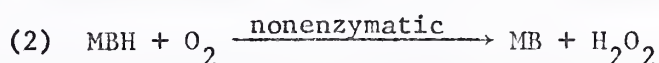
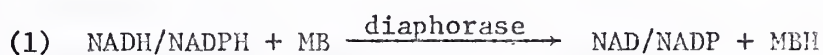
secondary granules has been reported. A 14 year old boy with recurrent staphylococcal infections of the skin and respiratory tract had neutrophils with bilobed nuclei and decreased bactericidal activity against staphylococcus. A complete lack of leukocyte alkaline phosphatase was found and may have been related in some way to the killing disorder [86], although it should be noted that the low-leukocyte alkaline phosphatase in chronic myelogenous leukemia is not associated specifically with impaired microbicidal activity.

A second category of defective bactericidal mechanisms is that in which the neutrophils do not have the appropriate metabolic response after phagocytosis: they do not increase their oxygen consumption and glucose metabolism to produce H_2O_2 . While all aerobic bacteria produce H_2O_2 by their own metabolic pathways, some contain catalase to break it down. Catalase negative organisms, however, provide a source of H_2O_2 which is apparently sufficient to allow normal bactericidal mechanisms to proceed; hence, patients with this sort of abnormality are more prone to infection with catalase positive bacteria only [87]. The large number of reactions involved in hydrogen peroxide production and catabolism implies that any one of a number of different enzymatic defects could produce this picture (see Figure 2). In chronic granulomatous disease of childhood (CGD), perhaps the best known example of this type of disease, recent work has shown a deficiency of NADPH oxidase activity [87]. The function of this enzyme is to generate the highly reactive reduction product of oxygen superoxide. Hydrogen peroxide is then formed by a dismutation reaction in which one molecule

of O_2^- is oxidized and the other reduced producing H_2O_2 and O_2 . The defect in CGD is probably a failure of activation rather than an absence of the enzyme [88]. Much of the earlier confusion may stem from the fact this deficiency can be detected only in phagocytizing cells, whereas resting cells appear normal. The failure in pentose shunt activity is believed to be secondary to the failure of H_2O_2 production. Classically, these patients are males whose disease is inherited in an X-linked recessive pattern. Catalase positive bacteria which are ingested, but usually not killed, are transported to the reticuloendothelial system, where they gradually are released and rephagocytized by macrophages which may be similarly defective [89]. The granuloma formation which follows can be compared to that of tuberculosis and brucellosis, where live organisms also survive intracellularly for long periods of time [90]. The typical clinical picture is one of widespread reticuloendothelial involvement (lymphadenopathy, hepatomegaly, splenomegaly) as well as numerous infections (pustular dermatitis, conjunctivitis, osteomyelitis, subcutaneous abscesses, pneumonia). Laboratory workup should include a nitroblue tetrazolium (NBT) test as part of an evaluation of immune competence. Normal leukocytes reduce this soluble yellow dye to an insoluble purple formazan which is easily visualized [91]. CGD leukocytes, for reasons probably related to decreased superoxide anion production [66], are incapable of reducing the dye. In a few instances, patients who appear to have CGD have been found to lack glutathione peroxidase rather than NADPH oxidase. This has been noted mainly in females [92], although

one case of a Japanese male has been recorded [93]. CGD, therefore, may not be a single disease entity.

Glucose-6-phosphate dehydrogenase appears to be intimately involved in hydrogen peroxide formation and catabolism. Through the activity of G6PD, the NADPH needed to reduce oxygen to superoxide is produced. Hydrogen peroxide is then derived from H_2O_2 by dismutation as shown in Figure 2. On this basis, one might predict that a lack of leukocyte G6PD would cause a lack of hydrogen peroxide formation and affect cellular metabolism and function in a way mimicking CGD. A comparison of the two types of cells has been made, and it was found [94] that leukocyte G6PD levels less than 5 percent of normal resulted in defective killing of catalase positive bacteria. Similar to CGD leukocytes, there is no post phagocytic respiratory burst with H_2O_2 production, and there is failure of NBT reduction. Both will reduce NBT if cells are disrupted and NADH or NADPH is added. The addition of methylene blue (MB) differentiates the two types of cells by the following reactions:



MB added to CGD cells will stimulate normal pentose shunt activity and improve microbicidal activity [94]. G6PD deficient cells which have decreased levels of NADH and NADPH will exhibit no such response on exposure to MB. Further study of the G6PD deficient cells revealed normal phagocytosis, granule formation, and degranulation. Leukocytes

with G6PD activity of 20-50 percent of normal had no functional defects; as mentioned before, only at 1-5 percent levels were bactericidal abnormalities detected [94].

One group of investigators [95] postulated that G6PD deficiency might be responsible for some cases of classic CGD; they observed an increased rate of decay of G6PD activity in three male CGD patients. A 16 month old girl with CGD, defined by a compatible clinical history and failure to reduce NBT, was found to have a white cell G6PD level approximately half of normal. Of note is the fact that her parents were first cousins [96]. While the clinical significance of these reports can be questioned, at least two patients with complete absence of leukocyte G6PD and impaired microbicidal activity are known. One was a middle-aged Caucasian female with hemolytic anemia and fatal E. coli sepsis [97]. Studies of her leukocytes, whose G6PD activity was completely nondetectable, revealed normal bacterial ingestion, normal destruction of S. faecalis (catalase-negative), and abnormal destruction of S. aureus, E. coli and S. marcescens (catalase-positive). As one would predict, an NBT test revealed no reduction. Measurements of leukocyte NADH and NADPH oxidase were not different from controls. The patient's post-transfusion erythrocyte G6PD level was 50 percent of normal; four brothers, three sisters and one son all had normal red and white cell G6PD and no increased incidence of infection. Genetically, it is possible that the patient was homozygous for an autosomal recessive gene which in some way affects G6PD activity, or that she was heterozygous for the X-linked G6PD gene. In the latter case, one must invoke

the Lyon hypothesis and postulate that most of the cells with the normal gene were inactivated [97]. A family was described by Gray et al. [98] in which the male propositus had chronic nonspherocytic hemolytic anemia, pneumonia, and recurrent granulomatous staphylococcal infections since puberty. Leukocyte function abnormalities were similar to, although less marked than the previously described patient. Both erythrocyte and leukocyte G6PD were nondetectable, even by immunologic studies. Of two brothers with the same G6PD finding, one had had a single episode of cervical lymph adenitis and one had no history of unusual infections. The father had low normal and the mother intermediate levels of red and white cell G6PD. Metabolic activity post phagocytosis (glucose oxidation, formate oxidation and iodination) was nearly identical in the propositus and his brother with lymphadenitis; these measurements were not made in the symptom-free brother, although his leukocytes had not reduced NBT. Conceivably two X-linked genetic defects, G6PD deficiency and a form of CGD, are present in this family, but it seems more likely that the G6PD deficiency is responsible for the clinical symptomatology.

A disorder termed lipochrome histiocytosis bears some resemblance to the disorders of bactericidal function just described and some feel it is a type of CGD. Three sisters with the syndrome have been reported, of whom one failed to reduce NBT. All had impaired post phagocytic respiration and HMP activity. Other clinical and laboratory features included pulmonary infiltrates, splenomegaly, rheumatoid arthritis, increased susceptibility to infection, hypergammaglobulinemia and

defective staphylococcal destruction; pathologically, the histiocytes had lipochrome pigmentation. Unlike CGD, there was no evidence of granuloma formation, in spite of the bactericidal defect presumably resulting in prolonged intracellular survival of bacteria [99,100].

Miscellaneous Disorders

A number of other, for the most part, poorly understood syndromes exist which have in common defective killing of staphylococci; some may turn out to be the same as one of the above. A female patient with recurrent staphylococcal infections had normal chemotaxis, phagocytosis, and NBT reduction, but an unstable leukocyte pyruvate kinase. Other leukocyte enzymes, as well as red blood cell and lymphocyte pyruvate kinase, were normal [101]. Another young woman with staphylococcal facial infections, pneumonia and bacteremia was found to have normal leukocyte bactericidal activity against E. coli but defective activity with staphylococcus. The fact that the parents were related and that there was a rather high number of infant deaths in the family pedigree led the author to suggest an autosomal recessive disorder, but its nature is not known [102]. A girl with fatal phycomycosis whose leukocytes showed decreased bactericidal capacity against staphylococcus and a negative NBT test might have had a form of CGD, but her sex and benign clinical course until the age of 13 is extremely atypical [103]. A family in which two, possibly three siblings of both sexes suffered from oral, respiratory and skin infections has been reported; unlike in CGD, the neutrophils exhibited a normal respiratory burst with phagocytosis of IgG coated particles, but not with plain latex particles

[104]. Finally, it should be pointed out that not all of these syndromes are necessarily related to neutrophil dysfunction. A potential example is a male with repeated staphylococcal infections culminating in death at age 19. Although his immunoglobulins were normal, he was found to lack specific antibodies to the staphylococcal F and S antigens of leukocidin. The hypothesis was made that the patient may have become tolerant of staphylococcal antigens during the second trimester of pregnancy when his mother had impetigo [105]. Such speculation is interesting but wild, and is perhaps an indication of the great amount of work that remains in the field of host defense mechanisms.

III

Introduction

In the preceding section neutrophil function and dysfunction were discussed, and the role of G6PD in bactericidal mechanisms reviewed in the context of other abnormalities. Leukocytes with a G6PD level less than 5 percent of normal were shown to have defective killing of catalase positive bacteria; several cases of individuals with G6PD deficient leukocytes and increased infection susceptibility were cited. Other ways in which G6PD deficiency might be related to infection have been proposed, for example, via the hemolysis which so often occurs. Kaye and his collaborators [106,107] have shown that acute hemolysis in mice lowers resistance to S. typhimurium, E. coli, and S. aureus, but not to D. pneumoniae. They theorized that macrophages may become temporarily overloaded with erythrocyte products, and may be unable to sustain their usual bacterial defenses as well. The possibility also exists that free hemoglobin itself can increase infection susceptibility, by providing an easily accessible source of iron for invading microbes [108]. In addition, there is some evidence that G6PD deficiency is associated with pneumococcal and salmonella infections, suggesting a defect in opsonization similar to that in sickle cell disease [42].

That G6PD deficiency can adversely affect host defense against bacterial infection in isolated individuals has been fairly well

established as described above, but whether it also plays a role in large populations is not known. In order to answer this question, we decided to study a population in which severe G6PD deficiency and bacterial infections were both common, to see whether infected patients had a higher rate of G6PD deficiency. Although it was not feasible for us to measure leukocyte G6PD levels in addition to those in the erythrocytes, we chose a Caucasian population in which deficiency of the latter is known to be associated with deficiency of the former. All bacterial infections were included, and where possible, subgroups were analyzed, to allow for the different mechanisms that may be operative.

Materials and Methods

(a) Patient selection

The project was carried out in Shiraz, Iran at Nemazee and Saadi Hospitals, two teaching hospitals affiliated with Pahlavi University Medical School. All subjects were Iranian males, and all except one were inpatients at one of these hospitals. Approximately three times per week charts were reviewed of all adult patients in Nemazee Hospital, and from the medical, orthopedic and neurosurgical wards in Saadi Hospital. During the last month of the three month period, pediatric charts were also reviewed. Patient data were gathered from the charts and when necessary, from responsible medical personnel. The data collected included age, place of origin, history of present illness, past medical history, family history, physical examination findings, laboratory data, procedures performed, therapy received, and hospital

course. All male inpatients with bacterial infections and no known predisposing reason to infection other than possible G6PD deficiency were studied as the infected group. Infections were diagnosed by one or more of the following methods: cultures, titers, biopsies, x-rays, diagnostic taps, and antibiotic response. In cases where the etiologic agent was definitively established by cultures, titers, or biopsies, the patient was categorized as Class I. In other cases the presence of a bacterial infection was well established by a combination of clinical symptoms, x-rays, examination of body fluids, biopsies and antibiotic response, but proof of the etiologic agent was not obtained and the patient was categorized as Class II. Patients in whom an etiologic agent was highly suspected, but not proven (for example, a chest x-ray with cavitary lesions in a person with clinical signs of tuberculosis) were also placed in the latter category. The one outpatient in the infected group was seen at Nemazee Outpatient Clinic, brucellosis titers were diagnostic, and it was elected to give him antibiotics at home rather than in the hospital. Those patients free of diseases known to be associated with infection or G6PD deficiency were selected as control "A". As far as possible, all individuals with systemic diseases were avoided. Thus, to isolate G6PD deficiency as a potential cause for bacterial infections, patients who had the following conditions or treatments were excluded from the infected and the major control groups: steroid therapy, malignancy, diabetes mellitus, cirrhosis, chronic renal or cardiac failure, sickle cell anemia, Cooley's anemia, malaria, hydatid cyst, open trauma, opium

abuse, viral infections, tetanus, vasculitis or renal stones, and those in whom no diagnosis could be made. Most patients identified with these conditions were not tested, since these disorders in and of themselves can predispose to secondary bacterial infections. In some situations G6PD testing was performed on individuals with these conditions before all clinical data was available, and they serve as control "B". This provided us with two control groups; control "A" is a group selected for a lack of predisposing factors for bacterial infection, and control "B" a group with a variety of potentially predisposing factors to infection, or syndromes which mimicked bacterial infections in some of their features.

(b) Assay

Measurement of erythrocyte G6PD activity was carried out as follows: for each assay approximately 5 cc. of blood was drawn into a syringe, transferred immediately to a Vacutainer tube containing EDTA, and mixed well. Bloods were refrigerated at 4°C within 15 minutes and were tested within three days with the semiquantitative method developed by Sigma (Technical Bulletin No. 400). Kit instructions were followed exactly, including appropriate compensation for the degree of anemia present. In this assay, erythrocytes are lysed by water to release G6PD. This solution is then added to glucose-6-phosphate and NADP in the presence of phenazine methosulfate, an electron carrier and dichlorophenol indophenol, a blue dye. The NADPH formed during the oxidation of glucose-6-phosphate to 6-phosphogluconate reduces the dye to a colorless form, and the rate of the reaction can be followed

visually. The tubes are observed for a six hour period. For a minority of patients the quantitative test for G6PD activity performed in the Nemazee Hospital Laboratory (Brilliant Cresyl Blue Reduction, Dade, Miami, California) was used. In these cases, only samples with 0% activity were considered deficient.

(c) Grouping of data and statistical analysis

Statistical significance of differences in rates of G6PD deficiency were determined by the chi-square method of analysis. Infected patients were compared with control "A" and control "A" plus control "B". No separate comparisons with control "B" were made, because of the small number of subjects. Subgroups of patients matched exactly for age and origin were analyzed in a similar way. Those infected patients in whom an etiologic agent was established were divided according to whether the agent was catalase positive or negative. The catalase positive group was compared with controls, but the catalase negative group was too small to permit statistical analysis. The unpaired "t" test was used to analyze the mean age difference between the G6PD normal and the G6PD deficient infected groups. With both the chi-square and the unpaired "t" test, $p < .05$ is considered statistically significant.

Results

(a) Assay validity

Before conducting the studies in Iran assays were run at Yale University School of Medicine using blood samples tested quantitatively by the Yale-New Haven Hospital laboratories. The age of the samples

ranged from two hours to eleven days. Samples of all ages with normal G6PD activity decolorized within one hour, and samples with 10 percent activity decolorized in two to two and a half hours, consistent with data obtained by Sigma.

In Iran, an effort was made to study all infected patients as soon as possible after admission, usually within a few days. It was not always feasible to carry out the assays when no medications were being given; however, a complete list of drugs that each patient was receiving was compiled, and no agents that might have affected the assay were present in the study group. Fourteen of our seventeen abnormal samples had not decolorized at 6 hours, indicating very severe deficiency. Two of the remaining three took 3 hours but when repeated took 4 hours or more, and the third took $4\frac{1}{2}$ hours but could not be repeated. Of the 14 that took more than 6 hours, 5 were randomly repeated and all confirmed the original result; in 2 cases the assay was performed within 2 hours of the time the blood was drawn. Three of the normal assays were repeated and confirmed.

(b) Patient characteristics

The demographic characteristics of all patients are presented in Table 3. The average age of the 55 infected patients was 31 years; the G6PD normal group differed substantially from the G6PD deficient group, 34 years compared to 22. This difference was statistically significant ($.005 > p > .001$). There were 65 control "A" patients, with an average age of 34 years, and 33 control "B" patients, with an average age of 38 years. In these two groups there was no marked difference between

G6PD normal and G6PD deficient patients. The distribution between those from villages and from major cities was relatively even, although a somewhat greater proportion of the control "A" group came from the cities. Of the city patients, a total of seven came from places other than Shiraz (three infected patients and three control "B" patients, one each from Yazd, Teheran, and Mashad, and one control "A" patient from Yazd). It seems reasonable to assume that villagers are less likely to travel, and that therefore an even smaller percentage is from outside the Shiraz area.

The criteria by which infections were established are listed in Table 4, and the diagnoses themselves in Table 5. Tables 6a and 6b contain the diagnoses of control "A" and control "B" respectively. Some of the patients had a history of a disease other than that for which they were admitted to the hospital. Of the infected patients, three had a past history of typhoid (the present illnesses were two with pneumonia and one with osteomyelitis), one had psoriasis (shigellosis), and one had hypertension (meningitis). Of the control "A" patients, one had had typhoid (snake bite), one had had malaria (upper gastrointestinal tract bleed), and one had an ulcer (low back pain); of control "B" patients, one had rheumatoid arthritis (and was on steroid therapy) and one had had smallpox (diagnosis of present illness not made).

The results of the G6PD assays are in Table 7. Twenty two percent of the infected patients were G6PD deficient, compared to twelve percent of control "A" and six percent of control "B" (Table 7a).

Tables 7b, 7c, and 7d show the rates of G6PD deficiency in three subgroups, one with matched ages, one with matched origins (villagers only), and one selected on the basis of whether the etiologic agent was catalase positive or catalase negative. Matching for age was important from two aspects: older patients might have a lower frequency of G6PD deficiency if the gene causes an increase in mortality rates and older patients have a naturally higher rate of infection for a variety of reasons. Matching for origin was important to account for genetic differences in rates of G6PD deficiency, and genetic and environmental differences in rates of infection. We looked at whether infections were caused by catalase positive or catalase negative organisms, because of the previously cited work showing the G6PD deficient leukocytes may be similar to CGD leukocytes in demonstrating impaired killing of catalase positive organisms [92].

For Tables 7a, 7b, and 7c the differences in rates of G6PD deficiency between the infected group and control "A" were analyzed by the chi-square method, and in all cases the differences were found not to be statistically significant ($p > .10$). Comparisons were also made between each infected group and control "A" plus control "B". The results are as follows:

<u>Table</u>	<u>Patient Group</u>	<u>χ^2</u>	<u>P</u>
7a	all	3.86	.05 > p > .01
7b	age-matched	3.67	.10 > p > .05
7c	origin-matched	1.79	p \approx .10

Thus, when controls "A" and "B" together are considered as the control

group, the differences in rates of G6PD deficiency reached statistical significance. No separate comparisons with control "B" were made, owing to the small number of subjects. For Table 7d, the catalase positive group was compared with all control "A" patients (Table 7a), and with all control "A" patients plus control "B" patients (Table 7a). The differences were not statistically significant ($p > .10$). The catalase negative group had no G6PD deficiency, but statistical analysis was not carried out because of the small size of the group.

Mortality data was collected but is incomplete because it was not possible to follow patients remaining in hospital after termination of the study. As far as is known, none of the 12 G6PD deficient infected patients and 2 of the 43 G6PD normal infected patients expired. There was no mortality in the control "A" group of 65 patients; in control "E", one of the 2 G6PD deficient and 3 of the 31 G6PD normal patients failed to survive.

Discussion

The frequency of G6PD deficiency in Iran has been studied by a number of other investigators (see Table 8), and our results are in rough agreement. We did not have enough geographic distribution to confirm or refute Beaconsfield's [110] finding of a higher incidence in previously malaria-infested areas.

Although the rates of G6PD deficiency were greater in infected than in control groups, the differences were not significant when the control group was matched as closely as possible for age, origin, and underlying diseases. When we included a secondary control group with

a variety of conditions that were not present in the infected groups, the differences reached significance (p as low as $.05 > p > .01$, in patients matched exactly for age). However, the secondary control group included patients whose susceptibility to infection might have been increased due to disease (other than G6PD deficiency) or therapy, thus it was not strictly comparable with the infected patient group. One might expect an artificially low rate of G6PD deficiency in control "B" as the combination of G6PD deficiency and infection could lead to an increased mortality. The same bias could occur if patients with G6PD deficiency are less liable to develop the types of disorders present in control "B". Either of these possibilities would explain why the differences in rate of G6PD deficiency between the infected and control groups increase when the secondary control group is included. Some studies have shown that G6PD deficient individuals are more susceptible only to catalase positive organisms (see previous section), but our comparison of the group infected with catalase positive organisms and the controls showed no statistically significant differences in the frequency of G6PD deficiency. Although the rate of G6PD deficiency in the catalase negative group was zero, the number of cases was too small to permit meaningful statistical analysis. Neither did we have enough subjects with any one disease to analyze separately, and so we cannot make a definite statement on this issue. However, the G6PD deficient patients did seem to have a disproportionate share of staphylococcal septicemia, pneumonia, and brucellosis. We also compared the demographic characteristics of the G6PD deficient infected

group with the G6PD normal infected group. The average age of the former was 22 years, and the latter 34 years (difference significant at the $.005 > p > .001$ level). One interpretation of this observation is that the combination of G6PD deficiency and infection leads to hospitalization at an earlier age than infection alone. This is consistent with the hypothesis that G6PD deficient patients, once infected, have a more severe clinical course. Data on mortality rates would be useful in further testing of this hypothesis, but our data are insufficient for this purpose.

It is important to point out what we feel are some potential weaknesses in our study. The number of patients sampled was relatively small, and conceivably some of the observed trends might become significant only in a large population study. Lack of optimal facilities and culturing techniques prevented us from establishing every diagnosis of infectious disease by culture, and sampling error might have been involved in those that were. It was not possible for us to fully document previous antibiotic therapy, and this would be important in altering the course of infectious diseases. We were not able to measure leukocyte G6PD levels, which would have made our study more precise. Exact data on leukocyte G6PD activity would be particularly relevant in establishing the clinical significance of defective pentose phosphate metabolism in the cells. Finally, the method of patient selection might be faulted in several ways. Specifically, ongoing hemolysis caused by infection or its treatment can lead to hospital admissions. By concentrating only on patients with infections severe enough to result in

hospitalization, a true picture of a population's susceptibility to infection is not obtained. Rather, one has only an estimate of the prevalence of serious infections, and this estimate does not differentiate between infections made more severe by G6PD deficiency (with or without hemolysis) and the reverse. Close monitoring of hemolytic parameters before hospitalization would be required to determine whether this is a source of bias, as well as perhaps producing information on the relationship of hemolysis and infection; it would also provide a more accurate index of the incidence of infection in normal and G6PD deficient subjects. By selecting mainly adult patients, we automatically minimized the chances of detecting very serious effects of G6PD deficiency on host resistance, which could result in early mortality. Some of the information we attempted to collect may not have been reliable and complete. Thorough medical records were not always available and some relevant data may have been deleted including precise statements concerning ethnic background, nutritional status, and means of referral.

Another population study of the relationship of G6PD deficiency and infection has been published by Lampe et al. [42]. They compared rates of G6PD deficiency in hospitalized Thai children with S. typhi, H. influenza b, pneumococcus, staphylococcus, and tuberculous infections with outpatient controls. Taking only male patients, their data show a difference in rate of G6PD deficiency between the infected group and the controls approaching significance ($p \approx .05$), a finding rather similar to ours. Their criteria for diagnosis of infectious disease were more rigorous than ours, but their definition of G6PD deficiency is vague,

and it is not clear how well the patients and controls were matched for exact age and origin. Furthermore, like our study, their method of patient selection precludes an estimate of the relative incidence rates for infection in normal and G6PD deficient subjects.

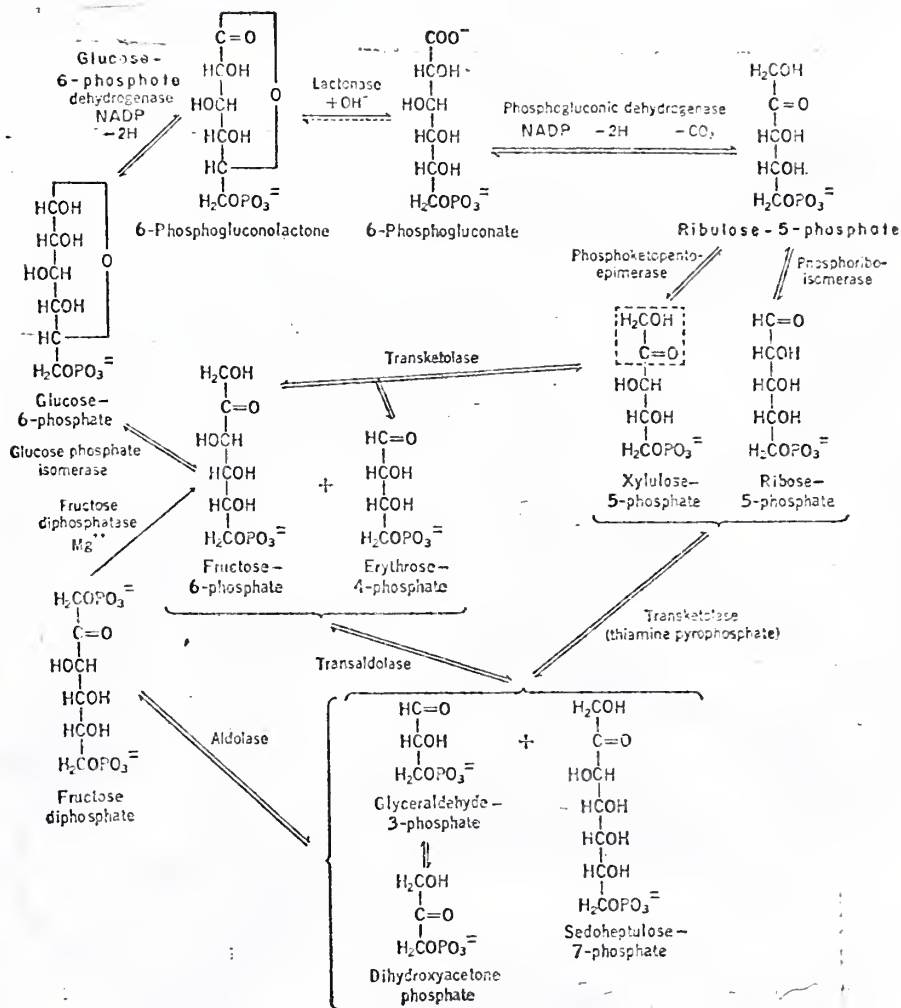
Thus, while we can offer no final conclusions regarding the relationship of G6PD deficiency and bacterial infections, a reasonable interpretation of data from these studies is that the two conditions do have meaningful interactions. This statement is justified by our data showing the G6PD deficient infected group to be significantly younger than the G6PD normal infected group, indicating the adverse effect of having both G6PD deficiency and infection. Although a rise in the rate of G6PD deficiency in an infected hospital population versus a non-infected population was demonstrated both by Lampe and ourselves, this rise approaches statistical significance only under certain circumstances. Therefore, whether it is real remains to be evaluated.

There are several mechanisms which would explain an association between G6PD deficiency and bacterial infection. First, as we have suggested above, the oxidant stress of infection or its treatment may lead to hemolysis and a weakening of host defenses against infection as well as hospitalization. Second, if the leukocyte G6PD level is less than 5 percent of normal, bactericidal activity against catalase positive organisms can be impaired. On the basis of published information, it is more likely that this occurs on an individual basis, rather than in all patients with reduced leukocyte G6PD. A thorough prospective analysis of large groups is required to pick up the more subtle

influences which might increase infection rates as well as severity. In such a study, the importance of other factors, such as hemolysis and abnormal opsonization, could be evaluated. This project would be potentially useful in providing additional knowledge of host defense mechanisms, as well as having clinical importance to the millions of people with G6PD deficiency.

FIGURE 1

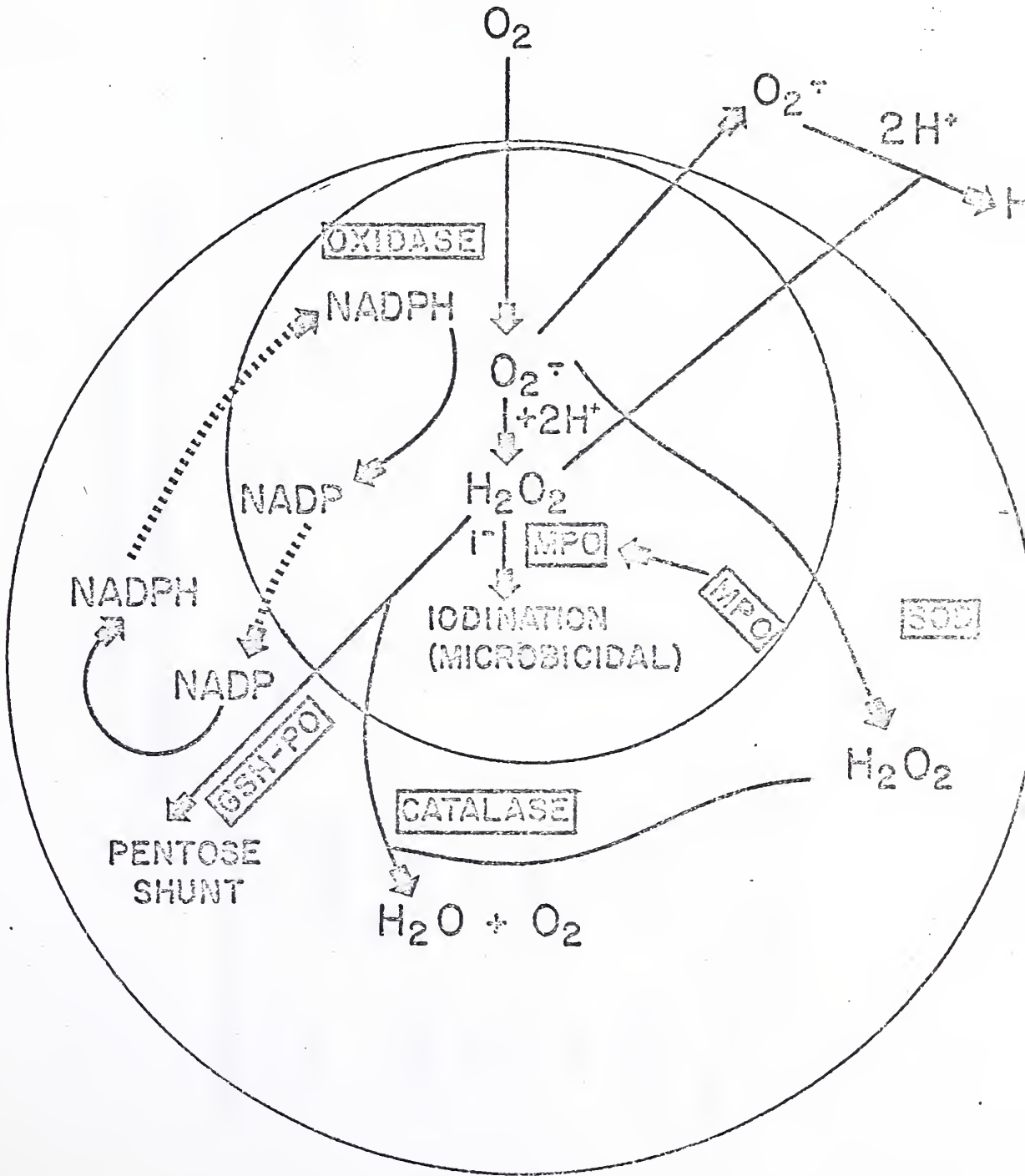
HEXOSE MONOPHOSPHATE SHUNT



(From Reference 1)

FIGURE 2

METABOLIC PATHWAYS FOR OXYGEN CONSUMPTION DURING PHAGOCYTOSIS IN PMNS



MPO: Myeloperoxidase

SOD: Superoxide Dismutase

GSH-PO: Glutathione Peroxidase

(Figure by Dr. Richard Root)

TABLE 1 (from Reference 1)
 BIOCHEMICAL CHARACTERISTICS OF G6PD VARIANTS

Variant	RBC Activity % normal	Electrophoretic mobility, % normal	K, G6P μM	K, NADP μM	2dG6P Utilization, % normal	Heat Stability	pH Optima
Normal B	100	100	50-78	2.9-4.4	<4	Normal	Normal
A ⁺	88	110	Normal	Normal	<4	Normal	Normal
A ⁻	8-20	110	Normal	Normal	<4	Normal	Normal
Med	0-7	100	19-26	1.2-1.6	23-27	Decreased	Biphasic
Hektoen	400-500	100 ¹	51	3.0	3	Normal	Normal

¹129 (PO₄ - pH6.7)

TABLE 2 (from Reference 1)

COMPOUNDS KNOWN TO HAVE INDUCED HEMOLYSIS OF
GLUCOSE-6-PHOSPHATE DEHYDROGENASE-DEFICIENT RED CELLS

Analgesics:

Acetanilid
Acetylsalicylic acid*
Acetophenetidin (phenacetin)*
Antipyrine
Pyramidone

Sulfonamides and sulfones:

Sulfanilamide
Sulfapyridine
Diaphenylsulfone
N₂-Acetylsulfanilamide
Sulfacetamide
Sulfisoxazole (Gantrisin)*
Thiazolsulfone
Salicylazosulfapyridine (Azulfadine)
Sulfoxone*
Sulfamethoxypyridazine (Kynex)

Antimalarials:

Primaquine
Pamaquine
Pentaquine
Quinocide
Quinacrine (Atabrine)

Nonsulfonamide antibacterial agents:

Furazolidone
Furmethonol
Nitrofurantoin (Furadantin)
Nitrofurazone
Chloramphenicol‡
Paraaminosalicylic acid
Neoarsphenamine

TABLE 2 (continued)

Miscellaneous:

Naphthalene
Trinitrotoluene
Methylene blue*
Nalidixic acid
Dimercaprol (BAL)*
Phenylhydrazine
Quinine†
Quinidine†
Mestranol

* Slightly hemolytic in Negroes, or only in very large doses.

† Hemolytic in Caucasians, but not in Negroes.

‡ Possibly hemolytic in Caucasians, but not in Negroes or Orientals.

TABLE 3

PATIENT DEMOGRAPHIC DATA

C A T E G O R Y	Number of Patients	Average Age (Range)	O R I G I N				
			Village	City	Nomad	Not Known	
INFECTED							
G6PD n1	43	34 (8-85)	22	14	1	6	
G6PD def	12	22 (11-35)	8	3	0	1	
Total	55	31 (8-85)	30	17	1	7	
CONTROL							
"A"							
G6PD n1	57	34 (14-70)	28	28	0	1	
G6PD def	8	37 (22-55)	6	1	1	0	
Total	65	34 (14-70)	34	29	1	1	
CONTROL							
"B"							
G6PD n1	31	38 (15-80)	18	10	0	3	
G6PD def	2	39 (18-60)	2	0	0	0	
Total	33	38 (15-80)	20	10	0	3	

¹ Shiraz, Teheran, Isfahan, and Mashad

TABLE 4

DIAGNOSTIC CRITERIA IN INFECTED PATIENTS

CLASS I:	DIAGNOSIS	CRITERION FOR DIAGNOSIS ¹
	Pneumococcus	Culture
	Staphylococcal sepsis	Culture
	Streptococcal meningitis	Culture
	Shigellosis	Culture
	Anthrax	Culture
	Brucellosis	Titer
	Typhoid	Titer
	Tuberculosis	Biopsy
	A. israelii abscess	Biopsy
CLASS II:	DIAGNOSIS	CRITERIA FOR DIAGNOSIS ¹
	Pneumonia	CXR and response to antibiotics
	Meningitis	LP and response to antibiotics
	Osteomyelitis	X-ray or biopsy
	Tuberculosis	CXR
	Erysipelas	Response to antibiotics
	Brain abscess	Pus and air in CSF
	Pyopneumothorax	Pus in pleural fluid

¹In all cases, clinical symptoms were also compatible.

TABLE 5
LIST OF INFECTED PATIENTS BY DIAGNOSIS

Diagnosis	Number of Patients	Number Deficient
Osteomyelitis	12	2
Tuberculosis	9	1
Pneumonia ¹	8	4
Brucellosis	6	2
Staphylococcal septicemia	3	2
Meningitis ²	3	1
Typhoid	3	0
Anthrax	3	0
Shigellosis	3	0
Erysipelas	1	0
Pyelonephritis	1	0
Pyopneumothorax	1	0
Brain abscess	1	0
Extradural abscess	1	0
	55	12

¹In 2 cases, one of which was G6PD deficient, the etiologic agent was determined by culture to be pneumococcus.

²In 1 case, which was G6PD normal, the etiologic agent was determined by culture to be β hemolytic streptococcus.

TABLE 6a
 LIST OF CONTROL "A" PATIENTS BY DIAGNOSIS

DIAGNOSIS	Number of Patients	Number Deficient
Closed trauma	25	4
UGI bleed ¹	11	0
Disc herniation	10	2
Low back pain	4	1
Hernia	3	0
Minor congenital defect	3	0
Snake bite	3	1
Poisoning	3	0
Myocardial infarction	2	0
Burn contracture	1	0
	65	8

¹Malignancy ruled out.

TABLE 6b

LIST OF CONTROL "B" PATIENTS BY DIAGNOSIS

DIAGNOSIS	Number of Patients	Number Deficient
Malignancy	6	1
Pneumonia ¹	4	1
No diagnosis	4	0
Viral infection	3	0
Steroid therapy ²	3	0
Open trauma	2	0
Diabetes mellitis	2	0
Cooley's anemia	1	0
Sickle cell anemia	1	0
CRF	1	0
CHF	1	0
Cirrhosis	1	0
Rheumatic heart disease	1	0
Tetanus	1	0
Renal stones	1	0
Opium abuse	1	0
	<u>33</u>	<u>2</u>

¹Underlying causes (malignancy, hydatid cyst, TB) not ruled out.

²For rheumatoid arthritis, vasculitis unknown etiology, and Henoch-Schönlein purpura.

TABLES 7a-7d COMPARISONS OF G6PD DEFICIENCY RATES

<u>Category</u>	<u>Number of Patients</u>	<u>Number Deficient</u>	<u>% Deficient</u>
ALL PATIENTS:			
Infected	55	12	22
Control "A"	65	8	12
Control "B"	33	2	6
<u>Category</u>	<u>Number of Patients</u>	<u>Number Villagers</u>	<u>% Deficient</u>
MATCHED FOR AVERAGE AGE: (31 Years)			
Infected	55	30 (55%)	22
Control "A"	59	31 (59%)	12
Control "B"	27	18 (67%)	7
<u>Category</u>	<u>Number of Patients</u>	<u>Average Age</u>	<u>Number Deficient</u>
MATCHED FOR ORIGIN: (Villagers Only)			
Infected	30	27	8
Control "A"	34	35	6
Control "B"	20	36	2
<u>Category</u>	<u>Number of Patients</u>	<u>Average Age</u>	<u>% Deficient</u>
MATCHED FOR ORIGIN: (Villagers Only)			
Infected	30	27	27
Control "A"	34	35	18
Control "B"	20	36	10

BY ETIOLOGIC
AGENT (Infected,
Class I Only):

<u>Category</u>	<u>Number of Patients</u>	<u>Average Age</u>	<u>Number Deficient</u>	<u>% Deficient</u>
Catalase +	22	31	5	23
Catalase -	4	50	0	0

Catalase + : Staphylococcus, Shigella, Salmonella, Brucella,
Anthrax, Mycobacterium tuberculosis

Catalase - : Pneumococcus, Streptococcus, Actinomyces israelii

TABLE 8

G6PD DEFICIENCY IN IRANIAN MALES, PUBLISHED STUDIES

STUDY	% Deficient	Total Number Subjects	Subject Characteristics	Geographical Origin	Religion
Bowman 1959 [109]	9.8	358	hospital staff	275 from Shiraz, others scattered	Moslem
Beaconsfield 1968 [110]	2.5 25 19-25 9	192		Yazd Caspian littoral area Kermanshah Isfahan	Moslem Moslem Moslem Moslem
Hedayat 1969 [111]	7 9.9 12	142 557 108	Blood donors and hospital staff	Oman littoral area Teheran Teheran	Moslem Moslem Jewish
Frischer 1973 [112]	9.8	409			

A blank space signifies that the information was not available.

BIBLIOGRAPHY

1. Stanbury, J.B., Wyngaarden, J.B., Frederickson, D.S. The Metabolic Basis of Inherited Disease, 3rd ed. New York: McGraw-Hill, 1972.
2. Dern, R.J., Weinstein, I.M., LeRoy, G.V., Talmage, D.W., 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).
3. Carson, P.E., Flanagan, C.L., Ickes, C.E., Alving, A.S. "Enzymatic Deficiency in Primaquine-Sensitive Erythrocytes," Science 124: 484 (1956).
4. Beutler, E. "Abnormalities of the Hexose Monophosphate Shunt," Sem Hem 8: 311 (1971).
5. Williams, W.J., Beutler, E., Erslev, A.J., Rundles, R.W. Hematology. New York: McGraw-Hill, 1972.
6. Baehner, R.L., Nathan, D.G., Castle, W.B. "Oxidant Injury of Caucasian G6PD-Deficient Red Blood Cells by Phagocytosing Leukocytes During Infection," JCI 50: 2466 (1971).
7. Carson, P.E., Frischer, H. "G6PD Deficiency and Related Disorders of the Pentose Phosphate Pathway," Am J Med 41: 744 (1966).
8. Wintrobe, M.W., Lee, G.R., Boggs, D.R., Bithell, T.C., Athens, J.W., Foerster, J. Clinical Hematology, 7th ed. Philadelphia: Lea & Febiger, 1974.
9. Yoshida, A., Beutler, E., Motulsky, A. "Human G6PD Variants," Bull WHO 45: 243 (1971).
10. Livingstone, F.B. Abnormal Hemoglobins in Human Populations. Ann Arbor, Michigan: Anthropological Institute of the University of Michigan, 1973.
11. Yoshida, A. "A Single Amino Acid Substitution (Asparagine to Aspartic Acid) Between Normal (B+) and the Common Negro Variant (A+) of Human G6PD," Proc Nat Acad Sci 57: 835 (1967).
12. Marks, P.A., Gross, R.T. "Erythrocyte G6PD Deficiency: Evidence of Differences Between Negroes and Caucasians with Respect to This Genetically Determined Trait," JCI 38: 2253 (1953).

13. Piomelli, S., Corash, L.M., Davenport, D.D., Miraglia, J., Amrosi, E.L. "In vivo Lability of G6PD in Gd A- and Gd Mediterranean Deficiency," JCI 47: 940 (1968).
14. Owusu, S.K. "Absence of G6PD in Red Cells of an African," Br Med J 4: 25 (1972).
15. McCurdy, P.R. "Clinical Manifestations of Variant G6PD Enzymes," Ann Clin Lab Sci 1: 184 (1971).
16. Kirkman, H.N., Schettini, F., Pickard, B.M. "Mediterranean Variants of G6PD," J Lab Clin Med 63: 726 (1964).
17. Bonsignore, A., Fornaini, G., Leoncini, G., Fantoni, A. "Electrophoretic Heterogeneity of Erythrocyte and Leucocyte G6PD in Italians from Various Ethnic Groups," Nature 211: 876 (1966).
18. Stamatoyannopoulos, G., Voigtlander, V., Kotsakis, P., Akrivakis, A. "Genetic Diversity of Mediterranean G6PD Deficiency Phenotype," JCI 50: 1253 (1971).
19. Luzzatto, L. "Inherited Haemolytic States: G6PD Deficiency," Clin Haem 4: 83 (1975).
20. Bonsignore, A., Fornaini, G., Fantoni, A., Segni, P. "Characterization of Leukocyte G6PD in Sardinian Mutants," JCI 45: 1865 (1966).
21. Sabine, J.C., Jung, E.D., Fish, M.B., Pestanar, L.C., Rankin, R.E. "Observations on the Inheritance of G6PD Deficiency in Erythrocytes and in Leucocytes," Brit J Haem 9: 164 (1963).
22. Ramot, B., Fisher, S., Szeinberg, A., Adam, A., Sheba, C., Gafni, D. "A Study of Subjects with Erythrocyte G6PD Deficiency. II. Investigation of Leukocyte Enzymes," JCI 38: 2234 (1959).
23. Chan, T.K., Todd, D., Wong, C.C. "Tissue Enzyme Levels in Erythrocyte G6PD Deficiency," J Lab Clin Med 66: 937 (1965).
24. Beutler, E., Yeh, M., Fairbanks, V.F. "The Noram Human Female as a Mosaic of X-Chromosome Activity: Studies Using the Gene for G6PD Deficiency as a Marker," Proc Nat Acad Sci 48: 9 (1962).
25. Gandini, E., Gartler, S.M. "G6PD Mosaicism for Studying Development of Blood Cell Precursors," Nature 224: 599 (1969).
26. Phillips, J., Herring, R.M., Goodman, H.O., King, J.S. Jr. "Leukocyte Alkaline Phosphatase and Erythrocyte G6PD in Down's Syndrome," J Med Genet 4: 268 (1967).

27. Kruatrachue, M., Klengkumnuanhara, K., Harinasnta, C. "Infection-rates of Malarial Parasites in Red Blood Cells with Normal and Deficient G6PD," Lancet 1: 404 (1966).
28. Gilles, H.M., Fletcher, K.A., Hendrickse, R.G., Linder, R., Reddy, S., Allan, N. "G6PD Deficiency, Sickling and Malaria in African Children in Southwestern Nigeria," Lancet 1: 138 (1967).
29. Kosower, N.S., Kosower, E.M. "Molecular Basis for Selective Advantage of G6PD Deficient Individuals Exposed to Malaria," Lancet 2: 1343 (1970).
30. Huheey, J.E., Martin, D.L. "Malaria, Favism, and G6PD Deficiency," Experientia 31: 1145 (1975).
31. Sharma, A., Singh, H., Dhatt, P.S., Gupta, H.L., Arya, R.K. "G6PD Deficiency in the Newborn," Indian Ped 12: 393 (1975).
32. Das, B.N., Bhakoo, O.N., Jolly, J.G. "Neonatal Hyperbilirubinemia Associated with G6PD Deficiency," Indian Ped 11: 645 (1974).
33. Valaes, T., Karaklis, A., Stravarakakis, D., Bavela-Stravarakakis, K., Perakis, A., Doxiadis, S.A. "Incidence and Mechanism of Neonatal Jaundice Related to G6PD Deficiency," Ped Res 3: 448 (1969).
34. Melloni, T., Cagnazzo, G., Dore, A., Cutillo, S. "Phenobarbital for Prevention of Hyperbilirubinemia in G6PD Deficient Newborn Infants," J Peds 82: 1048 (1973).
35. Choremis, C., Kattamis, C.A., Kyriazakou, M., Gavriilidou, E. "Viral Hepatitis in G6PD Deficiency (letter)," Lancet 1: 269 (1966).
36. Morrow, R.H. Jr., Smetana, H.F., Sai, F.T., Edgcomb, J.H. "Unusual Features of Viral Hepatitis in Accra, Ghana," Ann Int Med 68: 1250 (1968).
37. Kattamis, C.A., Tjortjatou, F. "The Hemolytic Process of Viral Hepatitis in Children with Normal or Deficient G6PD Activity," J Peds 77: 422 (1970).
38. Owusu, S.K. "G6PD Deficiency in Jaundice Associated with Lobar Pneumonia," (letter), Lancet 2: 325 (1973).
39. Tugwell, P. "G6PD Deficiency in Nigerians with Jaundice Associated with Lobar Pneumonia," Lancet 1: 968 (1973).

40. Williams, A.O., Tugwell, P., Edington, G.M. "G6PD Deficiency and Lobar Pneumonia, Fine Structure of the Liver," Arch Path Lab Med 100: 25 (1976).
41. Owusu, S.K., Foli, A.K., Konotey-Ahulu, F.I.D., Janosi, M. "Frequency of G6PD Deficiency in Typhoid Fever in Ghana," (letter), Lancet 1: 320 (1972).
42. Lampe, R.M., Kirdpon, S., Mansuwan, P., Benenson, M. "G6PD Deficiency in Thai Children with Typhoid," J Peds 87: 576 (1975).
43. Banait, P.P., Junnarkar, R.V. "Study of Erythrocyte G6PD Deficiency in Leprosy," Int J Lep 39: 168 (1971).
44. Saha, N. "Incidence of G6PD Deficiency in Patients of Three Different Ethnic Groups Suffering from Pulmonary Tuberculosis," J Med Genet 6: 292 (1969).
45. Beaconsfield, P., Rainsbury, R., Kalton, G. "G6PD Deficiency and Cancer," Oncologia 19: 11 (1965).
46. Sulis, E., Spano, G. "Osservazioni preliminari sull' incidenza neoplastica e sul comportamento enzimatico e proliferativo del tessuto tumorale negli individui carenti di glucosio-6- fosfato deidrogenasi," Boll Ital Biol Speri 44: 1246 (1968).
47. Naik, S.N., Anderson, D.E. "G6PD Deficiency and Cancer," (letter) Lancet 1: 1060 (1970).
48. Sulis, E. "G6PD Deficiency and Cancer," (letter), Lancet 1: 1185 (1972).
49. Eaton, J.W. "Catalase Activity and Cancer," (letter), Lancet 2: 46 (1972).
50. Misra, R.C., Basu, A.K., Swarup Mitra, S. "Erythrocyte G6PD Deficiency in Thalassemia Syndromes--A Cytochemical Study," J Indian M A 65: 40 (1975).
51. Harley, J.D., Agar, N.S., Gruca, M.A., McDermid, M.E., Kirk, R.L. "Cataracts with a G6PD Variant," (letter), Br Med J 2: 86 (1975).
52. Sheehan, R.G., Necheles, T.F., Lindeman, R.J., Meyer, H.J., Patterson, J.F. "Regional Enteritis and Granulomatous Colitis Associated with Red Blood Cell G6PD Deficiency," NEJM 277: 1124 (1967).
53. Katsaros, D., Truelove, S. "Regional Enteritis and G6PD Deficiency," NEJM 281: 295 (1969).

54. Petrakis, N.L., Weisenfield, S.L., Sams, B.J. Collen, M.F., Cutler, J.L., Siegelau, A.B. "Prevalence of Sickle-Cell Trait and G6PD Deficiency. Decline with Age in Frequency of G6PD Deficient Negro Males," NEJM 282: 787 (1970).
55. Piomelli, S., Reindorf, C.A., Arzanian, M.T., Corash, L.M. "Clinical and Biochemical Interactions of G6PD Deficiency and Sickle Cell Anemia," NEJM 287: 213 (1972).
56. Steinberg, M.H., Dreiberg, B.J. "G6PD Deficiency in Sickle-Cell Anemia," Ann Int Med 80: 217 (1974).
57. Beutler, E., Johnson, C., Powars, D., West, C. "Prevalence of G6PD Deficiency in Sickle-Cell Disease," NEJM 290: 826 (1974).
58. Bienzle, U., Sodeinde, O., Effiong, C.E., Luzzatto, L. "G6PD Deficiency and Sickle Cell Anemia: Frequency and Features of the Association in an African Community," Blood 46: 591 (1975).
59. Baehner, R.L. "Microbe Ingestion and Killing by Neutrophils: Normal Mechanisms and Abnormalities," Clin Haemat 4: 609 (1975).
60. Stossel, T.P. "Phagocytosis: Recognition and Ingestion," Sem Hemat 12: 83 (1975).
61. Murphy, P. The Neutrophil. New York: Plenum Medical Book Company, 1976.
62. Miller, M.E., Nillson, U.R. "A Familial Deficiency of the Phagocytosis-Enhancing Activity of Serum Related to a Dysfunction of the Fifth Component of Complement (C5)*," NEJM 282: 354 (1970).
63. Goldstein, I.M., Roos, D., Kaplan, H.B., Weissmann, G. "Complement and Immunoglobulins Stimulate Superoxide Production by Human Leukocytes Independently of Phagocytosis," JCI 56: 1155 (1975).
64. Goldstein, I.M., Cerqueira, M., Lind, S., Kaplan, H.B. "Evidence That the Superoxide Generating System of Human Leukocytes is Associated with the Cell Surface," JCI in press.
65. Babior, B.M., Curmutte, J.T., McMurrich, B.J. "The Particulate Superoxide-Forming System from Human Neutrophils," JCI 58: 989 (1976).
66. Baehner, R.L., Murrman, S.K., Davis, J., Johnston, R.B. Jr.. "The Role of Superoxide Anion and Hydrogen Peroxide in Phagocytosis-Associated Oxidative Metabolic Reactions," JCI 56: 571 (1975).

67. Klebanoff, S.J. "Antimicrobial Mechanisms in Neutrophilic Polymorphonuclear Leukocytes," Sem Hemat 12: 117 (1975).
68. Miller, M.E., Oski, F.A., Harris, M.B. "Lazy Leucocyte Syndrome. A New Disorder of Neutrophil Function," Lancet 1: 665 (1971).
69. Davis, S.D., Schaller, J., Wedgwood, R.J. "Job's Syndrome. Recurrent, 'Cold', Staphylococcal Abscesses," Lancet 1: 1013 (1966).
70. White, L.R., Ianetta, A., Kaplan, E.L., Davis, S.D., Wedgwood, R.J. "Leucocytes in Job's Syndrome," (letter), Lancet 1: 630 (1969).
71. Bannatyne, R.M., Skowron, P.N., Weber, J.L. "Job's Syndrome--A Variant of Chronic Granulomatous Disease," J Peds 75: 236 (1969).
72. Hill, H.R., Ouie, P.G., Pabst, H.F., Ochs, H.D., Clark, R.A., Klebanoff, S.J., Wedgwood, R.J. "Defect in Neutrophil Granulocyte Chemotaxis in Job's Syndrome of Recurrent 'Cold' Staphylococcal Abscesses," Lancet 2: 617 (1974).
73. Clark, R.A., Root, R.K., Kimball, H.R., Kirkpatrick, C.H. "Defective Neutrophil Chemotaxis and Cellular Immunity in a Child with Recurrent Infections," Ann Int Med 78: 515 (1973).
74. Boxer, L., Hedley-White, T., Glader, B., Stossel, T. "A Primary Defect in Neutrophil Motility," Clin Res 22: 384A (1974).
75. DeMeo, A.N., Andersen, B.R. "Defective Chemotaxis Associated with a Serum Inhibitor in Cirrhotic Patients," NEJM 286: 735 (1972).
76. Dossett, J.H., Williams, R.C. Jr., Ouie, P.G. "Studies on Interaction on Bacteria, Serum Factors and Polymorphonuclear Leukocytes in Mothers and Newborns," Peds 44: 49 (1969).
77. Forman, M.L., Stiehm, E.R. "Impaired Opsonic Activity but Normal Phagocytosis in Low Birth Weight Infants," NEJM 281: 926 (1969).
78. McCracken, G.H. Jr., Eichenwald, H.F. "Leukocyte Function and the Development of Opsonic and Complement Activity in the Neonate," Amer J Dis Child 121: 120 (1971).
79. Johnston, R.B. Jr., Newman, S.L., Struth, A.G. "An Abnormality of the Alternate Pathway of Complement Activation in Sickle Cell Disease," NEJM 288: 803 (1973).
80. Miller, M.E., Seals, J., Kaye, R., Levitsky, L.C. "A Familial, Plasma-Associated Defect of Phagocytosis. A New Cause of Recurrent Bacterial Infections," Lancet 2: 60 (1968).

67. Klebanoff, S.J. "Antimicrobial Mechanisms in Neutrophilic Polymorphonuclear Leukocytes," Sem Hemat 12: 117 (1975).
68. Miller, M.E., Oski, F.A., Harris, M.B. "Lazy Leucocyte Syndrome. A New Disorder of Neutrophil Function," Lancet 1: 665 (1971).
69. Davis, S.D., Schaller, J., Wedgwood, R.J. "Job's Syndrome. Recurrent, 'Cold', Staphylococcal Abscesses," Lancet 1: 1013 (1966).
70. White, L.R., Ianetta, A., Kaplan, E.L., Davis, S.D., Wedgwood, R.J. "Leucocytes in Job's Syndrome," (letter), Lancet 1: 630 (1969).
71. Bannatyne, R.M., Skowron, P.N., Weber, J.L. "Job's Syndrome--A Variant of Chronic Granulomatous Disease," J Peds 75: 236 (1969).
72. Hill, H.R., Ouie, P.G., Pabst, H.F., Ochs, H.D., Clark, R.A., Klebanoff, S.J., Wedgwood, R.J. "Defect in Neutrophil Granulocyte Chemotaxis in Job's Syndrome of Recurrent 'Cold' Staphylococcal Abscesses," Lancet 2: 617 (1974).
73. Clark, R.A., Root, R.K., Kimball, H.R., Kirkpatrick, C.H. "Defective Neutrophil Chemotaxis and Cellular Immunity in a Child with Recurrent Infections," Ann Int Med 78: 515 (1973).
74. Boxer, L., Hedley-White, T., Glader, B., Stossel, T. "A Primary Defect in Neutrophil Motility," Clin Res 22: 384A (1974).
75. DeMeo, A.N., Andersen, B.R. "Defective Chemotaxis Associated with a Serum Inhibitor in Cirrhotic Patients," NEJM 286: 735 (1972).
76. Dossett, J.H., Williams, R.C. Jr., Ouie, P.G. "Studies on Interaction on Bacteria, Serum Factors and Polymorphonuclear Leukocytes in Mothers and Newborns," Peds 44: 49 (1969).
77. Forman, M.L., Stiehm, E.R. "Impaired Opsonic Activity but Normal Phagocytosis in Low Birth Weight Infants," NEJM 281: 926 (1969).
78. McCracken, G.H. Jr., Eichenwald, H.F. "Leukocyte Function and the Development of Opsonic and Complement Activity in the Neonate," Amer J Dis Child 121: 120 (1971).
79. Johnston, R.B. Jr., Newman, S.L., Struth, A.G. "An Abnormality of the Alternate Pathway of Complement Activation in Sickle Cell Disease," NEJM 288: 803 (1973).
80. Miller, M.E., Seals, J., Kaye, R., Levitsky, L.C. "A Familial, Plasma-Associated Defect of Phagocytosis. A New Cause of Recurrent Bacterial Infections," Lancet 2: 60 (1968).

81. Johnston, R.B. Jr., Stroud, R.M. "Complement and Host Defense Against Infection," J Peds 90: 169 (1977).
82. Constantopoulos, A., Najjar, V.A., Smith, J.W. "Tuftsin Deficiency: A New Syndrome with Defective Phagocytosis," J Peds 80: 564 (1972).
83. Root, R.K., Rosenthal, A.S., Balestra, D.J. "Abnormal Bactericidal, Metabolic and Lysosomal Functions of Chediak-Higashi Syndrome Leukocytes," JCI 51: 649 (1972).
84. Lehrer, R.I., Cline, M.J. "Leukocyte Myeloperoxidase Deficiency and Disseminated Candidiasis: Role of Myeloperoxidase in Resistance to Candida Infection," JCI 48: 1478 (1969).
85. Klebanoff, S.J. "Myeloperoxidase: Contribution to the Microbicidal Activity of Intact Leukocytes," Science 169: 1095 (1970).
86. Strauss, R.G., Bove, K.E., Jones, J.F., Mauer, A.M., Fulginitis, V. "An Anomaly of Neutrophil Morphology with Impaired Function," NEJM 290: 478 (1974).
87. Hohn, D.C., Lehrer, R.I. "NADPH Oxidase Deficiency in X-Linked Chronic Granulomatous Disease," JCI 55: 707 (1975).
88. McPhail, L.C., DeChatelet, L.R., Shirley, P.S., Wilfert, C., Johnston, R.B. Jr., McCall, C.E. "Deficiency of NADPH Oxidase Activity in Chronic Granulomatous Disease," J Peds 90: 213 (1977).
89. Johnston, R.B. Jr., Baehner, R.L. "Chronic Granulomatous Disease: Correlation Between Pathogenesis and Clinical Findings," Peds 48: 730 (1971).
90. Holmes, B., Quie, P., Windhorst, D., Good, R. "Fatal Granulomatous Disease of Childhood: An Inborn Abnormality of Phagocytic Function," Lancet 1: 1225 (1966).
91. Baehner, R.L., Nathan, D.G. "Quantitative Nitroblue Tetrazolium Test in Chronic Granulomatous Disease," NEJM 278: 971 (1968).
92. Holmes, B., Park, B.H., Malawista, S.E., Quie, P.G., Nelson, D.L., Good, R.A. "Chronic Granulomatous Disease in Females. A Deficiency of Leukocyte Glutathione Peroxidase," NEJM 283: 217 (1970).
93. Matsuda, I., Oka, Y., Taniguchi, N., Furuyama, M., Kodama, S., Arashima, S. "Leukocyte Glutathione Peroxidase Deficiency in a Male Patient with Chronic Granulomatous Disease," J Peds 38: 581 (1976).

94. Baehner, R.L., Johnston, R.B. Jr., Nathan, D.G. "Comparative Study of the Metabolic and Bactericidal Characteristics of Severely G6PD Deficient Polymorphonuclear Leukocytes and Leukocytes from Children with Chronic Granulomatous Disease," RES 12: 150 (1972).
95. Bellanti, J.A., Cantz, B.E., Sclegel, R.J. "Accelerated Decay of G6PD Activity in Chronic Granulomatous Disease," Ped Res 4: 405 (1970).
96. Corberand, J., deLarrard, B., Vergnes, H., Bildstein, M., Carriere, J.P. "Maladie granulomateuse chronique avec déficit en glucose-6-phosphate déshydrogénase leucocytaire. Observation d'une fillette de 16 mois," Nouv Presse Med 5: 868 (1976).
97. Cooper, M.R., DeChatelet, L.R., McCall, C.E., LaVia, M.F., Spurr, C.L., Baehner, R.L. "Complete Deficiency of Leukocyte G6PD with Defective Bactericidal Activity," JCI 51: 769 (1972).
98. Gray, G.R., Klebanoff, S.J., Stamatoyannopoulos, G., Austin, T., Naiman, S.C., Yoshida, A., Kliman, M.R., Robinson, G.C.F. "Neutrophil Dysfunction, Chronic Granulomatous Disease, and Non Spherocytic Haemolytic Anaemia Caused by Complete Deficiency of G6PD," Lancet 2: 530 (1973).
99. Ford, D.K., Price, G.E., Culling, C.F.A., Vassar, P.S. "Familial Lipochrome Pigmentation of Histiocytes with Hyper Globulinemia, Pulmonary Infiltration, Splenomegaly, Arthritis, and Susceptibility to Infection," Am J Med 33: 478 (1962).
100. Rodey, G.E., Park, B.H., Ford, D.K., Gray, B.H., Good, R.A. "Defective Bactericidal Activity of Peripheral Blood Leukocytes in Lipochrome Histiocytosis," Am J Med 49: 322 (1970).
101. Burge P.S., Johnson, W.S., Hayward, A.R. "Neutrophil Pyruvate Kinase Deficiency with Recurrent Staphylococcal Infections: First Reported Case," Br Med J 1: 742 (1976).
102. Mandell, G.L. "Staphylococcal Infection and Leukocyte Bactericidal Defect in a 22 Year Old Woman," Arch Int Med 130: 754 (1972).
103. Bruun, J.N., Solberg, C.O., Hamre, E., Janssen, C.J. Jr., Thunold, S., Eide, J. "Acute Disseminated Phycomycosis in a Patient with Impaired Granulocyte Function," Acta path microbiol scand 84: 93 (1976).
104. Van der Meer, J.W.M., van Zwet, T.L., van Furth, R., Weemaes, C.M.R. "New Familial Defect in Microbicidal Function of Polymorphonuclear Leukocytes," Lancet 2: 630 (1975).

105. Davis, W.C., Douglas, S.D., Fudenberg, H.H. "A Selective Neutrophil Dysfunction Syndrome: Impaired Killing of Staphylococci," Ann Int Med 69: 1237 (1968).
106. Kaye, D., Hook, E. "The Influence of Hemolysis or Blood Loss on Susceptibility to Infection," J Imm 91: 65 (1963).
107. Kaye, D., Gill, F., Hook, E. "Factors Influencing Host Resistance to Salmonella Infections: The Effects of Hemolysis and Erythrophagocytosis," Am J Med 254: 205 (1967).
108. "Iron and Resistance to Infection," (editorial), Lancet 2: 325 (1974).
109. Bowman, J.E., Walker, D.G. "Glutathione Stability of the Erythrocytes in Iranians," (letter), Nature 184: 1325 (1959).
110. Beaconsfield, P. "G6PD Deficiency and Physiopathologic Processes," (letter), NEJM 278: 914 (1968).
111. Hedayat, Sh., Amirshahy, P., Khademy, B. "Frequency of G6PD Deficiency Among Some Iranian Ethnic Groups," Trop geogr Med 19: 163 (1969).
112. Frischer, H., Bowman, J.E., Carson, P.E., Reickmann, K.H., Willerson, D.J. Jr., Colwell, J. "Erythrocytic Glutathione Reductase, Glucose-6-Phosphate Dehydrogenase and 6-Phosphogluconic Dehydrogenase Deficiencies in Populations of the United States, South Vietnam, Iran, and Ethiopia," J Lab Clin Med 81: 603 (1973).

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