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Chemical Toxicity of Red Cells

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Exposure to toxic chemicals may result in alterations of red cell function. In certain cases, the toxic effect requires a genetic predisposition and thus affects only a restricted number of individuals; in other instances, the toxic effect is exerted on the hematopoietic system of every person.

Glucose-6-phosphate dehydrogenase deficiency is probably the most widespread genetic disorder. It is observed at highest frequency in populations from subtropical countries as a result of its selective advantage vis à vis falciparum malaria. The gene controlling this enzyme is located on the X-chromosome; thus, the defect is sex-linked. Individuals with a genetic defect of this enzyme are extremely susceptible to hemolysis, when exposed to oxidant drugs (such as certain antimalarials and sulfonamides) because of the inability of their red cells to regenerate NADPH.

Lead poisoning results in profound effects on the process of heme synthesis. Among the steps most sensitive to lead toxicity are the enzyme δ-aminolevulinic acid dehydratase and the intramitochondrial step that leads to the incorporation of iron into protoporphyrin. By these mechanisms, in severe lead intoxication there is an accumulation of large amounts of δ-aminolevulinic acid (a compound with inherent neurotoxicity), and there are abnormalities of mitochondrial function in all cells of the body. Individuals living in an industrialized society are unavoidably exposed to some environmental lead. Recent evidence indicates that, even at levels of exposure which do not increase the blood lead level above values presently considered normal, abnormalities of heme synthesis are clearly detectable.

The red cell is subject to a variety of toxic effects by chemicals. For some toxic effects the red cell is the primary target, but in other cases it acts as an innocent bystander, in the sense that, while the toxic effect of the chemical may be obvious by analysis of the red cell, its function and ability to survive remain largely unaffected. While some chemicals affect the red cells of all the individuals exposed, others exert their effects only in certain individuals.

Two examples of chemical toxicity to the red cells will be discussed in this report: the susceptibility to drug-induced hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficiency (*1*) and the hematological effects of lead poisoning (*2*). These two cases of chemical toxicity to the red cell have been the objects of studies in our laboratory and provide clear illustrations of different aspects of chemical toxicity to the red cell. The toxicity of some chemicals injures only the red cells and exclusively in G6PD-deficient individuals; on the other hand, the toxicity of lead involves practically all the tissues of all the individuals exposed. While toxic effects of lead are detectable in the red cell at extremely low levels of exposure (*3-5*), from a clinical point of view the damages to other tissues are more significant; in children, for example, neurological toxicity severe to the point of death may occur before the clinical effects on the red cell (anemia) become obvious.

G6PD Deficiency

This genetic defect is probably the most common one in humans; it has been estimated that it affects more than 300 million individuals (*1*). There are several mutants of G6PD which yield a variety of syndromes. This discussion, however, will focus only on the two most common ones, GdB^- and GdA^- (*6*). The clinical manifestations associated with the common types of G6PD deficiency consist of

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episodes of br [illegible] to a drug or other redox compound. The clinical history almost invariably reveals exposure either to drugs like primaquine, sulfonamides, or furadantin (to cite those most commonly used) or to chemicals such as naphthalene. Hemolysis may also occur during infections such as hepatitis (*7*) or during chemical imbalance of the internal milieu, such as diabetic acidosis (*8*). The toxic effects are direct on the circulating red cells, which literally burst in the circulation, releasing all of their hemoglobin content. Very rapidly, the hemoglobin binding protein, haptoglobin, is saturated, and hemoglobin is spilled in the urine.

The sequence of events at the cellular level can be reproduced easily in the laboratory, by exposing red cells of G6PD-deficient and normal individuals to an oxidant, such as acetylphenylhydrazine. While in all normal red cells there is some precipitation of hemoglobin, in the G6PD-deficient cells this is extreme (*9*). This phenomenon (called Heinz-body formation) is observed also *in vivo* during the acute hemolytic phase, when Heinz bodies can be seen in the circulation for a short period, before the cells burst and are cleared. The mechanism by which the normal red cell counteracts oxidant stimuli utilizes reduced glutathione as the hydrogen donor. The regeneration of reduced glutathione is assured by the chain of two enzymes: glutathione peroxidase and reductase, both of which are operative in G6PD deficiency. In the red cell, the exclusive source of NADPH, the substrate for glutathione reductase, is the reaction catalyzed by G6PD (an additional NADPH is generated by the subsequent step catalyzed by 6-phosphogluconate dehydrogenase, which utilizes 6-phosphogluconate, a product of G6PD). In G6PD-deficient red cells, the concentration of reduced glutathione becomes rapidly inadequate, as soon as the concentration of NADPH becomes rate-limiting (*10*). Thus, extensive peroxidation of the membrane and of hemoglobin itself results from oxidative insults. This leads to the formation of Heinz bodies *in vivo* and to consequent intravascular hemolysis.

The peculiar racial distribution of G6PD deficiency, which is observed primarily among populations from subtropical areas, led to the suggestion by Motulsky that this genetic trait was maintained at such high levels by the selective pressure of falciparum malaria. The world maps of G6PD deficiency and of falciparum malaria appear, in fact, almost identical (*11*).

A series of classical pedigree studies by Child, Zinkam, et al., shortly after the discovery of the relationship between drug-induced hemolysis and G6PD deficiency, led to the demonstration that the [illegible] ome (*12*). This observation was further substantiated by the demonstration of a close linkage between G6PD deficiency and colorblindness, another gene whose location of the X-chromosome is well established (*13*). The location of the gene for G6PD on the X-chromosome has clarified the genetics of sex-linkage, to the point that nowadays detailed mappings of the X-chromosome are available (*14*).

It was soon noted that the degree of enzyme deficiency was different between Caucasians and Africans (*15*). Electrophoresis of the enzyme reveals that the common (wild) type moves slowly electrophoretically (this is designated as GdB^+) (*16*). Among Caucasian deficients, the defective enzyme was found to have identical electrophoretic mobility, but grossly decreased activity. [This defective mutant is designated GdB^- or Gd-Mediterranean (*6*), referring to its high frequency among Mediterranean populations]. Among African individuals, the majority have the common GdB^+ enzyme; however, many (in certain areas of Africa as many as 40%) exhibit an enzyme of faster electrophoretic mobility, designated GdA. Among these individuals, approximately half exhibited normal enzyme activity (GdA^+), while the other half exhibited reduced enzyme activity (GdA^-) (*6*). The two most frequently encountered types of G6PD-deficient mutant are therefore the GdB^- and GdA^-, among Caucasians and Africans, respectively. Many other mutants of G6PD have been described, some associated with decreased activity and some not; none of these, however, occurs at as high a frequency as GdB^- and GdA^-. These two common defective enzymes are different from each other not only in electrophoretic mobility, but also in the level of enzyme activity and its clinical consequences (*1*).

Table 1 summarizes the various possible genotypes, taking into consideration not only the differences between sexes, but also in the levels of activity between the GdB^- and GdA^- mutants.

In heterozygous females, whether of the B^- or A^- type, the reduced level of activity stems from the simultaneous presence in the circulation of two

Table 1. Possible genotypes of G6PD deficiency.

					B^-	A^-
Male						
XY	=	Hemizygote	=	Normal	100%	100%
X^-Y	=	Hemizygote	=	Abnormal	0-5%	5-15%
Female						
XX	=	Homozygote	=	Normal	100%	100%
X^-X	=	Heterozygote	=	Intermediate	20-80%	10-90%
X^-X^-	=	Homozygote	=	Abnormal	0-5%	5-15%

one defective, which can be identified by appropriate staining technique (*17*). This finding led to the development of the theory of gene dosage compensation (*18*), later confirmed by a variety of other techniques, which states that in any given cell of females only one of the two X-chromosomes is active. In the case of a woman heterozygous for G6PD deficiency, those red cells descending from a precursor cell in which the active chromosome carries the Gd^- gene are enzyme-deficient, while the remaining red cells are normal. It is then possible for a heterozygous-deficient woman to undergo hemolysis, despite a substantial enzyme level in her red cells (40-80% of normal), since the defective cells are just as susceptible to oxidative stress by chemicals or other agents as those from a homozygous-deficient man; however, her normal cells are resistant to hemolysis. Therefore, when a heterozygous female is exposed to an offending chemical, hemolysis can be abrupt, with hemoglobinuria and jaundice, but it is always self-limiting and ceases as soon as the entire subpopulation of deficient cells has been destroyed.

In homozygous males, the severity of hemolysis induced by chemical toxicity can be very different, depending on whether the defect in enzyme is severe, as in GdB^- individuals, or moderate, as in GdA^- individuals. In GdB^- males the hemolysis upon exposure to a toxic chemical can be fulminating, with extreme anemia to levels as low as 1-2 g/dl of hemoglobin, and may be fatal. In these individuals, there may be a near-instantaneous intravascular destruction of all circulating erythrocytes, with massive hemoglobinuria. On the other hand, in GdA^- males, hemolysis is also abrupt and severe, often with hemoglobinuria, but the anemia never reaches extremely low values and it ceases in a few days, even when the exposure to the toxic chemical continues. This apparent paradox has been shown to result from the "age-dependency" of G6PD. Marks and Johnson first noticed that in individuals with reticulocytosis, the red cell G6PD activity was increased (*19*). Red cells can be separated into cohorts of progressively increasing age on discontinuous density gradients (*20*). Serial measurements of enzyme activity in red cell fractions of different ages have shown that G6PD activity decays exponentially with a $t_{1/2}$ of 60 days in normal red cells. However, in GdA^- red cells, the enzyme activity decays faster, with a $t_{1/2}$ of 16 days and in GdB^- red cells the enzyme activity decays extremely rapidly, with a $t_{1/2}$ of a few hours (*21*). Quantitative measurements of G6PD with these techniques have shown that in both GdA^- and GdB^- the enzyme activity of the youngest red cells. The enzymatic defect in the red cells in both cases, therefore, results from the synthesis of an unstable enzyme, and not from a failure of the synthetic rate. It is thus understandable how, in the case of GdA^- males, the anemia is self-limiting. In these individuals, exposure to oxidant chemicals, in fact, results in destruction only of the aged red cells. Once these are removed from the circulation, the remaining younger cells have adequate enzyme activity to resist hemolysis. Even if the exposure to the offending agent persists, the anemia improves, since the newly formed red cells have normal enzyme activity. In the case of the GdB^- males, in whom only a very small percentage of the young cells have adequate enzyme activity, hemolysis, which affects nearly all circulating cells, is fulminating, and it may be fatal unless prompt transfusion of non-G6PD-deficient blood is given (*22*).

The fact that G6PD deficiency stems from the formation of a highly unstable enzyme explains also why the enzyme defect (and the inherent susceptibility to chemical toxicity) is limited to the red cells and to the cells of the lens of the eyes. These cells lack a nucleus, and the ability of synthesizing protein; any loss of enzyme cannot therefore be compensated by an accelerated turnover. In all other cells of the body, the existence of the appropriate machinery for protein synthesis permits the maintenance of a normal level of enzyme (in the case of GdA^- individuals) or at least a substantial level (20-25% of normal, in the case of GdB^- individuals) (*15*).

Our knowledge of the genetics and biochemistry of G6PD deficiency has provided a clue to the different susceptibility of individuals, within the same ethnic group and even the same family, to the toxicity of some chemicals. In the case of this genetic defect, a rational scientific basis has been furnished for the centuries-old observation by physicians that there is an "individual variability" in the toxicity of certain chemicals.

Lead Poisoning

The chemical toxicity of lead results primarily from its affinity for the SH groups (*23*). Its effects are therefore extended to a variety of body functions, for which the integrity of SH groups in the proteins are essential. Additionally, lead is known to have an affinity for mitochondria and to inhibit a number of mitochondrial functions (*24*).

Lead is an ubiquitous contaminant of the modern environment as a result of its extensive mining and of the utilization of a variety of its compounds. The largest single source of lead contamination is in the

form of its alkyls, primarily used as gasoline additives (*24*).

To have an approximate idea of the degree of environmental contamination by lead, it is useful to look at the blood lead content of human populations. [Lead in the blood is almost exclusively present in the red cells (*25*).] The blood lead content represents that fraction of Pb which is present in the soft tissues; an additional fraction of the total body Pb is stored in the bones (*23*). Geophysical considerations indicated that the lead content in the blood of humans in the "natural" state (that is, without man-made contamination) should be on the order of 0.2 μg/dl of blood (*28*). Studies of one "unacculturated" population have recently revealed an average blood lead of 0.8 μg/dl, essentially of the same order of magnitude as predicted (*27*). A study from our laboratory of a remote Himalayan population has revealed a slightly higher, but still low, average blood Pb of 3.2 μg/dl (*28*). By contrast, in American populations, there is a gradient of blood Pb levels, with a progressive increase in average values from rural to suburban to urban populations. In urban populations, the average blood Pb level ranges between 15 and 25 μg/dl (*24*). The blood lead levels indicate the extent to which lead has permeated the human body, in the present society.

Lead toxicity is particularly dangerous to children whose nervous system is more vulnerable than that of adults, at comparable levels of exposure. Besides being more susceptible to its toxicity, children retain and absorb more lead, through a higher respiratory rate. Additionally, children ingest more lead, through their normal hand-to-mouth (thumb-sucking) activities, which result in the intake of lead-containing dust (*29*). A particularly dangerous source of lead for children is peeling paint, which in pre-World War II housing has a very high content of lead. Since all sources of lead are additive, it is not surprising that inner-city children, who are subject to higher pollution and are most likely to live in old and run-down housing, are at the greatest risk of lead intoxication. This is compounded by the fact that the simultaneous nutritional deficit increases both lead absorption and lead toxicity (*30*).

The hematological effects of lead toxicity are obvious in the circulating red cells and result both from a direct effect of lead and from its toxicity in the bone marrow at the time of red cell production. Two steps in the pathway of heme synthesis are very sensitive to lead: (1) the cytoplasmic enzyme, δ-aminolevulinic acid dehydratase, which converts two molecules of δ-aminolevulinic acid into porphobilinogen, and (2) the mitochondrial enzyme ferrochelatase, which inserts an atom of Fe at the center of the ring of protoporphyrin IX. It must be noted that abnormalities of heme synthesis are easily detectable in the red cells, because of all tissues this is the easiest to sample. However, the inhibition of heme synthesis is not unique to the erythropoietic tissue, since heme is the universal respiratory pigment and a constituent of the respiratory chain of all cells, the cytochrome system.

The enzyme δ-aminolevulinic acid dehydratase is exquisitely sensitive to the effects of lead. Hernberg and co-workers have demonstrated an inhibition of this enzyme at the blood lead levels well below those considered "normal" for Western populations (*3*). Their studies indicate that there is no threshold blood lead level for this inhibitory effect. The inhibitory effect is so pronounced that for instance at blood lead levels of 16 μg/dl, the enzyme activity is already reduced to 50% of its potential. [The maximum potential of the enzyme in any given individual can be established by exposing its red cells to an SH reagent such as dithiothreitol (*31*).]

Inhibition of δ-aminolevulinic acid dehydratase results in an accumulation of its substrate, δ-aminolevulinic acid (a compound associated with a variety of neurotoxic effects), and it is reflected by an increased urinary excretion. As the activity of the enzyme declines exponentially with an increasing blood Pb level (*3*), this is paralleled by an exponential rate of increase of δ-aminolevulinic acid in the urine (*5*) (Fig. 1).

The inhibition of the enzyme ferrochelatase by lead probably results from the effect of lead on the transmitochondrial transport of iron, more than from a direct effect on the enzyme itself (*32*). Whatever the precise mechanism of this manifestation of lead toxicity, during erythropoiesis it produces an accumulation of protoporphyrin (a pigment nonfunctional from the respiratory point of view) in the heme pocket of the hemoglobin. Protoporphyrin is initially formed as the free-base, but during the circulation of the red cell, rapidly binds Zn (*33*) to form Zn-protoporphyrin (*34*). The fluorescence of protoporphyrin (*35*) and Zn-protoporphyrin (*36*) has been utilized to devise simple techniques that permit its detection and thus the rapid screening of children for lead poisoning (*36*).

Currently, the definition of the Center for Disease Control recommends medical attention for lead poisoning for all children with a blood Pb level > 30 μg/dl, accompanied by a red cell protoporphyrin level > 50 μg/dl. Initial studies, which demonstrated the exponential increase of protoporphyrin with a linear increase in blood Pb level, focussed on children with a high (> 40 μg/dl) blood Pb level (*35*).

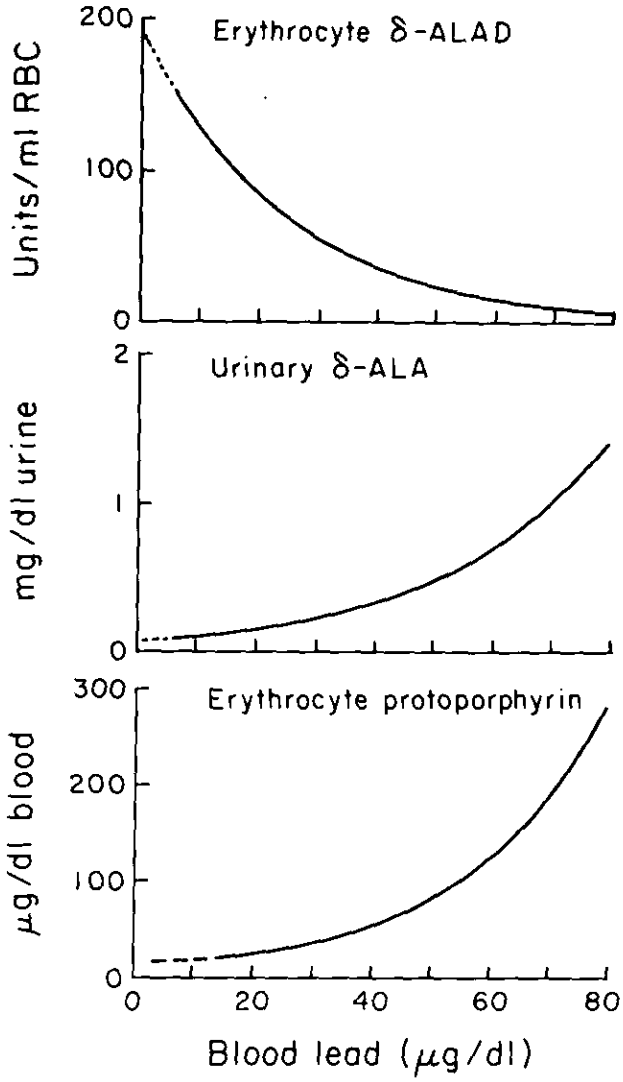

FIGURE 1. Relationship between blood Pb and parameters of heme synthesis. (Top) Erythrocyte δ-aminolevulinic acid dehydratase (δ-ALAD). Exponential decline of activity determined by Hernberg, et al., (*3*). No measurements were obtained below blood Pb 5 μg/dl; the dotted part of the line is the extrapolation to blood Pb = 0 (log δ-ALAD ≈ 2.274 − 1.018 blood Pb). (Middle) Urinary δ-aminolevulinic acid (δ-ALA). Exponential increase in urinary excretion determined by Selander and Cramer (*5*). No measurements were obtained below blood Pb 5 μg/dl; the dotted part of the line is the extrapolation to blood Pb = 0 (log δ-ALA = −1.0985 + 0.0157 blood Pb). (Bottom) Erythrocyte protoporphyrin (EP). Exponential increase of erythrocyte protoporphyrin determined by Piomelli, et al., (*28*). Measurements were obtained from blood Pb = 2μg/dl. The dashed line indicates lack of increase below blood Pb = 14.5 μg/dl (log EP = 1.014 + 0.018 blood Pb).

30 μg/dl (i.e., in the range accepted as normal) have shown that the threshold blood Pb level for the increase in red cell protoporphyrin is between 14 and 17 μg Pb/dl (*4*). These values are well below the average blood Pb level of most populations of urban children. These observations indicated that damage of an essential mitochondrial function, to a degree which results in the accumulation of a substrate, occurs at previously unsuspected low levels of exposure to lead. Since heme synthesis is a general function of all cells, detection of its abnormality in the red cell reflects a widespread injury to other tissues.

As techniques of detection become more refined, it is clear that the level of exposure to lead which induces demonstrable significant damage will become progressively lower. Apart from biochemical alterations of the red cells, it has recently been shown that children may exhibit neuropsychological disturbances at levels of exposure lower than previously suspected (*33*). As our knowledge of the biochemical basis of pathology deepens, alterations of specific enzyme systems are progressively identified as the specific mechanism of clinical symptoms. For instance, the "saturnine gout" reflects the inhibition of guanine aminohydrolase (*38*); the "peripheral neuropathy" of lead workers reflects the inhibition of aminocyclase (*39*); the "Monday morning colic" reflects the defect in cytochrome P-450, an essential component of the mixed liver oxidase (*40*). As these correlations between pathology and its biochemical basis become more apparent, it becomes difficult to assume that the increased blood lead level of urban populations and the detectable inhibition of essential biochemical systems is an unavoidable and harmless result of industrialization.

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