THE CLINICAL EFFECTS of erythrocyte enzymopathies vary widely in humans. In some, such as glyoxalase II deficiency, there are no apparent clinical manifestations, hematologic or otherwise. The ability to convert methylglyoxal to lactate, therefore, may have little or no role in human metabolism. In other enzymopathies, there are no hematologic manifestations, but the red cell shares an abnormality that causes significant malfunction in other tissues. Examples include partial deficiency of hypoxanthine guanine phosphoribosyl transferase in one form of gout and that of adenosine deaminase in association with immunoincompetence. Certain inborn errors may result in hematologic disorders other than hemolytic anemia. Inherited deficiency of ribosephosphate pyrophosphokinase (PRPP synthetase), for example, is characterized by megaloblastic anemia as well as mental retardation and hypouricemia. This review, however, will be limited to enzymopathies associated with hemolytic syndromes accompanied by nonhematologic disease. The association of hemolysis with multisystem disease may point the way to the diagnosis of obscure disorders whose clinical features result from a single molecular lesion.

In a number of erythrocyte enzymopathies, the sole clinical manifestation is that of hemolytic anemia. This is true for deficiencies of hexokinase (HK), glucose-phosphate isomerase (GPI), and pyruvate kinase (PK). There are many reasons why genetically determined molecular lesions in important metabolic pathways may have sharply limited consequences at the clinical level. The mature human erythrocyte lacks a nucleus, organelles, and the capacity to carry out oxidative phosphorylation or protein or lipid synthesis. Other tissues may have access to mechanisms to avoid deleterious effects of single-point metabolic blocks. Unlike the erythrocyte, they can compensate for a catalytically active but unstable enzyme by ongoing synthesis. Some enzymatic reactions are catalyzed by a family of isozymes, each under separate genetic control but variably expressed in different tissues. In tissues largely dependent on one isozyme, a mutant gene product may have disastrous effects; in others more richly endowed, redundancy may afford protection against adverse consequences. Female heterozygotes for X chromosome-linked enzymopathies may manifest hemolysis, but may lack evidence of multisystem disease, which is prominent in male hemizygotes. The metabolic defect involves all cells of the male, but the female heterozygote has one population of defective cells and a second population capable of entirely normal metabolism. A given enzyme simply may not be important to the function of one tissue, yet crucial to that of another. Tissues can have differing capacities for proteolytic posttranslational modification of gene products. There are, however, many inborn errors that cause multisystem disease with a hemolytic syndrome as one component (Fig 1).

PHOSPHOFRUCTOKINASE (PFK) DEFICIENCY

PFK catalyzes the following reaction, which is essentially irreversible in vivo:

fructose-6-phosphate (F6P) + adenosine triphosphate (ATP) \[\text{PFK}_{\text{Mg}^+} \rightarrow \]
fructose-1,6-diphosphate (F1,6P) + adenosine diphosphate (ADP).
This allosteric enzyme catalyzes a major rate-limiting and regulatory reaction of anaerobic glycolysis, and its activity is modified in a complex manner by several metabolites, including ATP and 2,3-diphosphoglycerate (2,3-DPG). It exists as tetrameric isozymes composed of varying combinations of three different subunits, each under separate genetic control. These are associated with myopathy and congenital nonspherocytic hemolytic anemia by Tarui et al.\textsuperscript{15,16} and Layzer et al.\textsuperscript{17} some 23 additional cases in 20 unrelated families have been described.\textsuperscript{14} The majority exhibit the simultaneous presence of myopathy and hemolysis (the "classic" form, Tarui's disease, glycogenosis type VII). The myopathy has ranged from mild to severe and is characterized by muscle weakness, exercise intolerance, and sometimes pain and myoglobinuria resulting from rhabdomyolysis after strenuous exertion. Myopathic symptoms have sometimes escaped notice because of their infrequent occurrence in some subjects or because of the voluntary adoption of a sedentary lifestyle in others.\textsuperscript{18}

There is little or no increase in venous lactate or pyruvate when the ischemic exercise tolerance test\textsuperscript{19} is performed. Hemolysis is evidenced by reticulocytosis and diminished erythrocyte survival, but for reasons to be discussed subsequently, hemoglobin levels are often normal or even elevated.\textsuperscript{10,18} Early onset of hyperuricemia and gout is frequently noted.\textsuperscript{18,20}

Metabolic studies document essentially total lack of muscle PFK activity and a partial deficiency, usually about 50%, in red cells.\textsuperscript{15,17,21} Characteristically, only the L4 isozyme is found in erythrocytes, testifying to homozygosity for deficiency of the M subunit alone.\textsuperscript{18,22,23} Muscle biopsies demonstrate increased deposition of glycogen,\textsuperscript{10,15,17,24} and hence, the designation of glycogenosis type VII by Brown and Brown.\textsuperscript{25} Levels of hexosemonophosphates are increased behind the point of metabolic block,\textsuperscript{15,16,24} whereas F1,6P, triosephosphates, and especially 2,3-DPG\textsuperscript{10,18,24} are diminished beyond that point. Decreased 2,3-DPG results in unfavorable shifts in the oxygen dissociation curve of hemoglobin. The increased affinity for O\textsubscript{2} and diminished delivery of O\textsubscript{2} to tissues presumably stimulates erythropoietin production, accounting for the mild erythrocytosis or absence of anemia in the face of significant hemolysis. It has been postulated, but not proven, that 5-phosphoribosyl-1-pyrophosphate (PRPP) formation is increased as a result of enhanced pentose shunt metabolism, fostered by increased concentrations of hexosemonophosphates.\textsuperscript{18} Deficiency in muscle PFK could indicate lack of M subunit synthesis or synthesis of a mutant subunit resulting in extreme enzyme instability or catalytic inactivity. Heterogeneity of the molecular lesion is suggested by the reported presence of material cross-reacting immunologically
with highly specific anti-M antibodies in muscle or cultured skin fibroblasts in some instances\textsuperscript{8,26,27} and its absence in others.\textsuperscript{17,23}

PFK deficiency has also been documented in syndromes differing from Tarui's disease.\textsuperscript{7,18} In some, hemolysis has been reported as the sole clinical manifestation. However, on reinvestigation of certain syndromes, muscle PFK was found to be virtually absent, the ischemic exercise tolerance test results were positive, and mild myopathy was indeed present.\textsuperscript{18} In other cases, myopathic symptoms have been entirely absent and the exercise tolerance test results were normal.\textsuperscript{28,29} This could result from a catalytically active mutant isozyme with an unstable M subunit that is unrenewable in the anucleate red cell. Reports of myopathic PFK deficiency devoid of hemolysis require further documentation and may represent the classic disorder in which absence of hemolysis has been erroneously assumed solely on the basis of absence of anemia.\textsuperscript{18}

Subjects entirely devoid of myopathic or hemolytic features\textsuperscript{18,30} and with PFK activities approximately half normal by in vitro assay probably represent heterozygosity for either M or L subunit deficiency. This has been documented by Vora et al\textsuperscript{18} in a father and son heterozygous for an unstable mutant L subunit. It is apparent that different PFK isozymes have differing physiologic roles. M\textsubscript{4} is less sensitive to inhibition by ATP and 2,3-DPG than the L\textsubscript{4} isozyme\textsuperscript{31} and appears to be essential for adequate glycolytic flux in erythrocytes.\textsuperscript{18} Heterozygotes partially deficient in M or L still possess functional M-containing isozymes, which presumably protect against interruption of the smooth flow of glycolytic intermediates. In total deficiency of M subunit, the regulatory capabilities of residual L\textsubscript{4} enzyme are inadequate to prevent premature red cell destruction, even though in vitro assay indicates 50\% to 60\% of normal PFK activity. In rare instances, very severe progressive myopathy has been reported with PFK deficiency, but data are inadequate to permit final judgment as to whether the PFK deficiency was the sole effector of the syndrome.\textsuperscript{7,18}

The conspicuous absence of organ injury, other than to muscle and red cells, in PFK deficiency caused by lack of M subunits is not surprising.\textsuperscript{18} Muscle alone exclusively expresses M subunits. While normally its energy requirements are met by oxidative phosphorylation and metabolism of fatty acid substrates, it is critically dependent on anaerobic glycolysis during severe exertion. During the latter, in the absence of an adequate glycolytic flux, muscle injury becomes inevitable. Energy needs of the red cell are essentially met entirely by glycolysis, the severe impairment of which cannot be adequately compensated. All three PFK loci are variably expressed in all fetal organs during early gestation, but adult isozyme patterns are expressed at birth.\textsuperscript{32} It appears that the proportion of subunits expressed in different organs after birth reflects the relative activity of glycolysis \textsuperscript{v} gluconeogenesis.\textsuperscript{18,32}

TRIOSEPHOSPHATE ISOMERASE (TPI) DEFICIENCY

TPI catalyzes the reversible isomerization of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P):

\[
\text{TPI} \quad \text{DHAP} \rightarrow \text{G3P}.
\]

Hemolytic anemia and multisystem disease caused by severe TPI deficiency have been reported in approximately 15 patients.\textsuperscript{31} In normal subjects, electrophoretically different forms of TPI have been shown to result from postsynthetic modifications involving deamidation of asparagines 15 and 71.\textsuperscript{14} TPI, however, is encoded by a single structural locus on chromosome 12.\textsuperscript{35,36} A strikingly high incidence of null alleles in the heterozygous state has been documented, with a suggested frequency of 0.3\% in whites and as high as 2.0\% in blacks.\textsuperscript{37-39} The large discrepancies between these and the number of clinically identified cases of severe TPI deficiency remain unexplained. In view of the facts that the syndrome has been recognized for some 18 years and its clinical manifestations often mandate hospitalization, it is unlikely that this is due simply to lack of recognition. It is possible, but unproven, that a subset of mutant alleles gives rise to gene products with sufficient residual TPI activity to permit survival, whereas homozygosity for truly null alleles is incompatible with life. Catalytically active but unstable, mutant TPI would provide one speculative example, for in some subjects, thermal instability of the mutant gene product has been a prominent feature.\textsuperscript{33}

The moderately severe hemolytic anemia is much less ominous than the inexorably progressive neurologic disease manifested by all cases thus far described.\textsuperscript{40-48} The neurologic disorder is difficult to classify in terms of conventional syndromes. It is not present in the neonatal period, appears in the first several months of life,\textsuperscript{41,47} and is neither clinically nor neurologically related to kernicterus. The first patient\textsuperscript{40} developed progressive spasticity, never walked, and was unable to hold her head erect. Generalized flaccidity without spasticity has been observed in other subjects. Only one patient lived to early adulthood.\textsuperscript{43} In this patient, there was no progression in neuromuscular deficits during the last several years of life, but neurologic findings at age 21 included diffuse...
weakness, unintelligible speech, facial diparesis, absent limb reflexes, fixed deformities of hands and feet, and bilateral hand tremor. There was no sensory impairment and intellect was normal.

Although a paucity of biopsy and autopsy material for study has limited investigations, there is strong reason to believe that TPI deficiency involves all body tissues. Activity is greatly reduced in muscle, plasma, spinal fluid, skin fibroblasts, and leukocytes.\textsuperscript{40,41,45,49} Sudden unexplained death, compatible with cardiac arrhythmias or arrest, has been described.\textsuperscript{41,42} Although no abnormalities in phagocytic or bactericidal capacities of granulocytes have been demonstrated,\textsuperscript{40,46} leukocytic TPI activity is low, and unusual susceptibility to infection has been prominent clinically in a number of patients. The most striking erythrocyte metabolic abnormality is the 20- to 60-fold increase in intracellular DHAP concentration.\textsuperscript{45,49}

PHOSPHOGLYCERATE KINASE (PGK) DEFICIENCY

Phosphoglycerate kinase catalyzes the reversible interconversion of 3-phosphoglycerate (3-PG) and 1,3-diphosphoglycerate, an important ATP-generating step in glycolysis:

\[
\text{PGK} \quad 1,3\text{-DPG} + \text{ADP} \rightarrow 3\text{-PG} + \text{ATP}. 
\]

It is bypassed if 1,3-DPG is mutated to 2,3-DPG and the latter returned to the mainstream of glycolysis through the action of a phosphatase (the Rapoport-Luebering shunt).

Severe PGK deficiency, an X chromosome-linked disorder,\textsuperscript{50-52} has been documented in approximately nine unrelated kindreds.\textsuperscript{53,54} In hemizygous males, there is a concomitant neurologic disorder, characterized by seizures, variable mental retardation, emotional lability, speech impairment, and progressive extrapyramidal tract disease.\textsuperscript{50,53,55} The EEG may be abnormal.\textsuperscript{50} In some instances, neurologic involvement may be minimal.\textsuperscript{58}

In affected males, there is a variably severe, chronic, nonspherocytic hemolytic anemia, which sometimes requires repeated transfusion.\textsuperscript{50,60} In some cases, splenectomy has resulted in distinct benefit and virtual elimination of transfusion requirements. The hemolytic syndrome, however, is punctuated by crises, usually associated with intercurrent infections and with marked exacerbations in anemia, jaundice, and reticulocytosis.\textsuperscript{50,60} During crises, hemolysis may be intravascular with hemoglobinuria, and spherocytes may appear in large numbers on the stained blood film.\textsuperscript{60} Heinz bodies are not demonstrable. At the time of hemolytic exacerbation, neurologic crises can also supervene, with recurrent convulsions and even hemiplegia and coma. Recovery from the latter may be surprisingly good in some instances.\textsuperscript{50} Although clinical manifestations have been essentially confined to hemolytic anemia and neurologic defects, the activity of PGK is greatly reduced in leukocytes,\textsuperscript{50,59} muscle,\textsuperscript{62,63} lymphocytes, and platelets obtained from living subjects and in tissue samples of brain, skeletal and cardiac muscle, and liver obtained at autopsy.\textsuperscript{63}

Metabolic abnormalities in erythrocytes of affected hemizygous males include reduction in ATP and sharp increases in F1,6P, DHAP, and 2,3-DPG.\textsuperscript{50,56,57,60,64} Increased 2,3-DPG favorably shifts the oxygen dissociation curve of hemoglobin, promoting increased oxygen delivery to tissues. The relationship of PGK deficiency to the neurologic lesion is not defined. However, there is no reason to believe that neurologic defects are secondary to kernicterus, anoxia, or other nonspecific causes. Heterozygous females may have mild to moderate hemolysis\textsuperscript{50,64,65} due to a population of deficient cells coexisting with a normal cell population. They are usually asymptomatic, and neurologic deficits have not been described.

The primary structure of normal red cell PGK has now been identified.\textsuperscript{56,67} A single substitution among the 417 amino acids composing the enzyme protein has been documented in four variants.\textsuperscript{68-71} In each, the substitution was different, testifying to the fact that the usual cause of PGK deficiency involves a structural gene mutation, and that even in this rare disorder, there is marked polymorphism of the mutant genes and their products.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

X chromosome-linked G6PD catalyzes the following reaction in which reduced nicotinamide adenine dinucleotide phosphate (NADPH) is generated:

\[
glucose-6\text{-phosphate} + \text{NADP} \rightarrow \text{6-phosphogluconate} + \text{NADPH}. 
\]

Clinically significant deficiency of G6PD results from an enormous heterogeneity of variant enzymes. It is expressed at the clinical level in hemolytic syndromes that are similarly polymorphic. These vary from episodic hemolysis, induced by drugs and other oxidant stresses, to severe, chronic, nonspherocytic hemolytic anemia.\textsuperscript{72} Although a single structural gene encodes the enzyme in all tissues, including leukocytes, clinical manifestations of deficiency in all but rare instances are limited to hemolysis. While 20% residual activity in granulocytes is sufficient to permit entirely normal metabolic and bactericidal capability,\textsuperscript{73} defec-
tive bacterial killing has been documented in five subjects with complete or nearly complete deficiency of leukocyte G6PD. Normal phagocytosis and destruction of ingested microorganisms by granulocytes is accompanied metabolically by a "respiratory burst." This is initiated by activation of a plasma membrane-bound flavoprotein oxidase, and NADPH is oxidized to NADP with production of superoxide (O₂⁻). The latter partially dismutates to H₂O₂, and other highly reactive oxidant radicals are subsequently formed. Measurable events accompanying the respiratory burst include increased oxygen consumption, production of O₂⁻ and H₂O₂, intense stimulation of the hexosemonophosphate (HMP) shunt, and oxidation of C¹⁺ and O¹⁻ in a reaction catalyzed by myeloperoxidase. In the most common form of X chromosome-linked chronic granulomatous disease, impaired bactericidal activity results from inability to activate the O₂⁻-forming NADPH oxidase. The result is recurrent infection with certain catalase-positive microorganisms, lack of the normal respiratory burst, but normal capacity to kill catalase-negative bacteria capable of generating H₂O₂ on their own. In the rare instances of bactericidal impairment in granulocytes with essentially total lack of G6PD, the failure to generate the respiratory burst results from inability to maintain NADPH, the substrate for the oxidase. Although clinical manifestations in these subjects appeared less severe than in classical chronic granulomatous disease, one subject died of sepsis with Escherichia coli, and a second unrelated individual had chronic granulomatous lymphadenitis due to Staphylococcus aureus.

DEFIENCIES IN GSH SYNTHESIS

The tripeptide glutathione (GSH), the most abundant of intracellular thiols, is synthesized by the following energy-requiring reactions:

(1) L-glutamate + L-cysteine + ATP
    \[ \rightarrow L-\gamma\text{-glutamyl-L-cysteine} + ADP + P_i \]
(2) L-\gamma\text{-glutamyl-L-cysteine} + glycine + ATP
    \[ \rightarrow \text{glutathione} + ADP + P_i \]

Severe deficiencies of both \( \gamma \)-glutamyl cysteine synthetase and glutathione synthetase are documented.

In two siblings with moderate lifelong hemolytic anemia and neurologic abnormalities, GSH levels were only 2% to 3% of normal. GSH synthetase activity was normal, but that of \( \gamma \)-glutamyl cysteine synthetase was nearly lacking. GSH was reduced in leukocytes to about 40% of normal and in muscle to about 25%. There was no evidence of myopathy, and no impairment of granulocyte bactericidal capacity was demonstrable. Both siblings had progressive spinocerebellar ataxia, which was initially diagnosed in adulthood. By age 35, the woman had mild ataxia and impaired coordination in upper and lower extremities. Her brother, at age 37, exhibited muscle weakness, ataxia, absent deep tendon reflexes in the lower limbs, and impaired vibratory and position sensation in all extremities, together with dysmetria and dysdiadochokinesia. Later developments included irregular staccato speech and painful myoclonic spasms of the right calf and foot. Both siblings also had monobasic and diatomic aminoaciduria. Otherwise unexplained spinocerebellar degeneration in two affected siblings, when all other family members were normal, established a strong presumption that the neurologic and hematologic syndromes had a common etiology in the enzyme deficiency. Although a precise role for \( \gamma \)-glutamyl cysteine synthetase in neural tissue cannot be defined, the GSH content of normal brain is relatively high, and the enzymes of the \( \gamma \)-glutamyl cycle are widely distributed.

Glutathione synthetase deficiency exists in two forms, one with hemolytic anemia as the sole manifestation, and one with hemolysis, massive 5-oxoprolinuria, severe metabolic acidosis, and often, but not invariably, neurologic disease. In GSH deficiency associated with hemolysis alone, the hemolytic anemia has been modest, the in vitro Heinz body test has been strongly positive, and erythrocytes are exquisitely sensitive to damage by chromium, precluding use of chromium 51 labeling in erythrocyte survival studies. Because GSH is essential for the normal function of the HMP shunt, as in G6PD deficiency, oxidant stresses, including fava bean ingestion, are expected to exacerbate hemolysis of GSH-deficient erythrocytes.

In GSH synthetase deficiency associated with 5-oxoprolinuria, the reduction in activity appears to be generalized and is readily demonstrable, for example, in placenta and cultured skin fibroblasts. It has been postulated that clinical manifestations limited to the erythrocyte may result from mutations that primarily affect enzyme stability, the concept being that other tissues can renew enzyme activity by continued synthesis, whereas the red cell cannot. By the same token, generalized GSH deficiency would be inevitable if the mutation resulted in no gene product, a catalytically inactive gene product, or one with extreme instability.

In the generalized deficiency, there is massive urinary excretion of 5-oxoproline, markedly increased levels of 5-oxoprolin in blood and spinal fluid, severe metabolic acidosis usually requiring ongoing treatment with bicarbonate, and often evidence of defective central nervous system function. The first case, reported in 1970, was that of a 19-year-old boy who had been
mentally retarded since childhood and who had evidence of severe organic brain damage. Neurologic status progressively deteriorated until death at age 28. At autopsy, there was selective atrophy of the granular cell layer of the cerebellum and focal lesions in the frontoparietal cortex, visual cortex, and thalamus. It is not possible to distinguish clearly the effects of GSH synthetase deficiency, the normal feedback inhibition by GSH of γ-glutamylcysteine synthesis is eliminated, and the dipeptide is formed in abnormally large amounts. It is metabolized to 5-oxoprolinone, an intermediate of the cycle, which accumulates at a rate outstripping the ability of oxoprolinase to degrade it. This accounts for the massive 5-oxoprolinemia and 5-oxoprolinuria and consequent metabolic acidosis.

RED CELL ENZYMOPATHIES POSSIBLY ASSOCIATED WITH MULTISYSTEM DISEASE

Hereditary hemolytic anemia associated with red cell aldolase deficiency, many dysmorphic features, and mental and growth retardation was first reported by Beutler et al.96 However, two cases in a Japanese kindred exhibited only hemolytic anemia.97 Additional data are therefore required to determine whether the enzymopathy was fortuitously or etiologically related to multisystem disease in Beutler’s patient. Aldolase catalyzes the interconversion of F1,6P and the two trioses, DHAP and 3-PG.

Pyrimidine-5'-nucleotidase deficiency is associated with hemolytic anemia.98 The enzyme catalyzes the dephosphorylation of uridine and cytidine monophosphates,98 but not that of the monophosphates of thymidine and deoxyuridine.99,100 In six unrelated families, Beutler et al.101 observed mild mental retardation in three index cases. In one family, the IQs of three siblings with hemolytic anemia ranged between 67 and 72, while the one unaffected sibling tested had an IQ of 90. In approximately ten kindreds studied in our laboratory, one index case had mental retardation requiring institutionalization (unpublished observations). It is not yet clear whether nervous system manifestations are coincidental or are caused by the enzymopathy. Heterogeneity in respect to presence or absence of neurologic involvement is well established for GSH synthetase deficiency98 and for congenital methemoglobinemia due to cytochrome b5 reductase deficiency.

In conclusion, a variety of genetically determined hemolytic syndromes are accompanied by multisystem disease caused by the same inherited lesion. Their recognition permits an etiologic diagnosis in a number of obscure disorders. The heterogeneity of clinical manifestations often associated with variants of a single gene emphasizes the enormous polymorphism of even infrequently encountered inherited disorders in humans. Their investigation provides insight into modes of expression in different tissues and information that could be helpful in understanding normal metabolic functions and their regulation.

REFERENCES

(PFKP) to chromosome 10p: Novel use of polyspecific rodent antiserum to localize human enzyme genes. Hum Genet 63:374, 1983
34. Yuan PM, Talent JM, Gracy RW: Molecular basis for the accumulation of acidic isozymes of triosephosphate isomerase on aging. Mech Ageing Dev 17:151, 1981


55. Hjelm M, Wadman B: Nonpheroporphic haemolytic anemia with phosphoglycerate kinase deficiency. XIII International Congress of Hematology, Munich, 1970, p 121 (abstr)


Erythrocyte enzymopathies, hemolytic anemia, and multisystem disease: an annotated review

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