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Review Articles

Advances in Hereditary Red Cell Enzyme Anomalies

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Introduction

Since the first discovery by Carson et al. (1956) of G6PD deficiency in patients with primaquine-induced hemolytic crisis, more than 20 different red cell enzymopathies with red cell dysfunction have been described. In addition, several metabolic diseases can be diagnosed through detection of a specific enzyme defect in red cells but do not alter red cell function and viability or normal oxygen carriage and delivery to the tissues. The main advances during recent years in knowledge of the enzyme disorders described earlier concern molecular and genetic mechanisms of the defects and the relationship between molecular anomalies, red cell dysfunction, and pathologic expression. In some instances, e.g., for G6PD, pyruvate kinase, and glucosephosphate isomerase, the association of secondary postsynthetic modifications with the genetic primitive alteration has been pointed out. Finally some new enzyme disorders with congenital nonspherocytic hemolytic anemia (CNSHA) have been described, and the percentage of CNSHA cases that are of unknown cause is continuously decreasing.

Since a considerable number of papers dealing with G6PD deficiency have been published, in particular in this Journal, we shall not cover this enzyme defect.

Moreover, we do not intend to be 'exhaustive,' and some enzymopathies will not be commented upon in this review, because there do not seem to have been any recent advances in our knowledge about them.

A) Enzyme Defects Involving the Embden-Meyerhof Pathway

I) Hereditary Deficiency in Red Cell Pyruvate Kinase Activity (PK; EC. 2.7.1.40)

This defect, first described by Valentine et al. (1961), represents the most frequent of the enzymopathies responsible for chronic congenital nonspherocytic hemolytic

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anemia. Probably more than 300 different observations of this congenital disease have been described so far. The defect is usually inherited as an autosomal recessive disease, but some heterozygotes suffer mild hemolytic symptoms (Hanel and Pedersen, 1972; Kahn et al., 1976b; Sachs et al., 1968). In the patients (homozygotes or heterozygotes for two different mutant alleles) the resulting hemolysis is usually severe. A striking finding in this hemolysis is that the deficient reticulocytes have a severely reduction of lifespan compared with the mature red cells (Mentzer et al., 1971); these young cells are mainly sequestered in the spleen, and splenectomy is therefore often accompanied by an improvement of the anemia and an increase in the reticulocyte count (Mentzer et al., 1971). From a metabolic point of view the main abnormalities in the deficient red cells are decreased ATP and increased 2,3-diphosphoglycerate (2,3-DPG) concentrations compared with normal red cells of the same age. It is commonly found that the ratio ATP:2,3-DPG is decreased by a factor of 2 (Buc et al., 1974). As for the molecular mechanisms of the defect, there is now general agreement that structural mutations are involved. It is possible in almost all cases to characterize the abnormal product(s) of the mutant genes.

The mutation seems to involve the structural gene coding for L-type PK: whenever it has been tested, patients' liver L-type PK has been shown to exhibit some of the molecular anomalies characterizing the defective red cell PK variant (Bigley and Koler, 1968; Imamura et al., 1973; Nakashima et al., 1974, 1977; Kahn et al., 1976b) while the other tissues and cells (expressing M_1 and M_2 isozymes) (Marie et al., 1976) were normal. However, no liver dysfunction has ever been documented.

Recent advances in this field have arisen from immunologic analysis of the defects and study of the postsynthetic processing of the mutant enzymes as compared with normal PK and the relationship between molecular alteration of the mutant protein and clinical symptoms.

1) Molecular Mechanism of the Defect

Although some authors have reported the hereditary defects of red cell PK to be due to secondary alterations of PK at the level of the SH groups (Van Berkel et al., 1973, 1974; Zanella et al., 1976) or other mechanisms (Guminska and Wazewska-Czyzewska, 1979), most groups working in this field consider that structural mutations are involved (Kahn et al., 1975b).

The main arguments in favor of this thesis are: heterogeneity of the defective enzymes, whose immunologic, electrophoretic, kinetic, and stability properties permit characterization of several 'variants'; detection of intermediate deficiency and qualitative anomalies in the heterozygotes; irreversibility of most of the molecular alterations of the defective enzymes; and finally, similar qualitative alteration of liver L-type and erythrocyte PK. The use of antierythrocyte PK antiserum has made it possible to check for antigenic differences between normal and mutant enzyme, and, if mutant and normal PK are antigenically identical with respect to the antiserum used (Kahn et al., 1977b; Marie et al., 1977b), to measure antigenic concentration of the mutant enzyme. By means of immunoinactivation tests Miwa et al. (1975a) and Nakashima (1974) reported that antigenicity of the

mutant PK variants was frequently modified. By contrast Black et al., by double immunodiffusion (1976) and Kahn et al. (1977b) and Marie et al. (1977b) by microcomplement fixation and double immunodiffusion, found no antigenic modification of the mutant PK variants. This antigenic identity made the determination of antigenic concentration of mutant enzymes with normal PK as standard valid (Kahn et al., 1977b).

The ratio of enzyme activity to antigenic concentration represents the catalytic efficiency of the mutant molecules; it is called 'immunologic specific activity' and is expressed as a percentage of the normal value. Application of these immunologic determinations to 23 different PK variants allowed us to range them into two classes.

In the first class both PK activity and antigen concentration were reduced in a parallel fashion; all 14 variants belonging to this group were unstable to heat and to urea, such that molecular lability rather than decreased enzyme synthesis in the erythroblasts explained this loss of PK antigen.

The nine variants in the second class were characterized by decreased immunologic specific activity, from 20%—60% of normal. This altered catalytic efficiency was mainly responsible for the deficiency in activity, antigenic concentration being slightly decreased, normal, or slightly increased (probably because of the high reticulocyte count found in the patients). Practically all these variants were stable in vitro, which accounted for the absence of drastic PK antigen deficiency.

In three of the 23 families studied in our laboratory, residual activity of the deficient red cells corresponded to a mixture of the isozyme L, normally present, and of the isozyme M_2, which is normally totally absent from the red cells (Kahn et al., 1975b).

In 1975 Miwa et al. reported the same results.

This abnormal expression of M_2 isozyme in some patients' red cells was demonstrated by electrophoresis in polyacrylamide slab gel (Miwa et al., 1975a) and (or) by immunoneutralization: PK activity was not totally neutralized by anti-L-type antiserum and was partially neutralized by anti-M_2-type antiserum (Kahn et al., 1975b; Marie et al., 1979). These observations should be compared with the situation encountered in PK deficient Basenji dog (Black et al., 1978), whose red cells contain only M_2-type PK and none of the normal L isozyme. In man, R. Rosa et al. (1978a) recently reported an observation of familial dominant persistance of M_2-type enzyme without deficiency but, on the contrary, an increase in PK activity. The pathologic consequences in this family were moderate polycythemia. Thus it appears that, in man as well as in animals, the normal switch-off of M_2-type gene in the erythroid cells can be impaired in some circumstances, particularly in the case of mutations affecting the L-type enzyme.

Up to now in this text, we have talked about 'the PK variants' without considering whether the patients' parents were really carrying the same mutation. It is only in this case, most frequently associated with consanguinity of the parents, that the defective enzyme corresponds to a given 'variant.' In most cases the parents are not consanguineous and the patients are heterozygous for two different mutant alleles. In this case different immunologic or kinetic anomalies

can be detected in each parent (Boivin, 1971; Paglia et al., 1976; Sprengers et al., 1978).

2) Postsynthetic Maturation of the Defective PK Variants

Discussion of this problem requires that the normal postsynthetic maturation of L-type PK from erythrocytes and liver be recalled (Kahn et al., 1978b); Marie et al., 1977a).

In the erythroblasts, L-type PK is synthesized as an L'_4 form (i.e., composed of four identical L′ subunits, with a molecular weight of about 62,000 daltons). Red cell maturation and aging is accompanied by a partial proteolysis of two subunits, such that L'_4 is transformed into the heterotetramer $L_2L'_2$; the molecular weight of the L subunits is about 58,000 daltons. In the adult liver the only L-type PK form is a homotetramer L_4. In vitro, a mild proteolytic attack of L'_4 results in the formation first of $L_2L'_2$ and then of L_4. This L_4 enzyme is similar to the liver enzyme in its electrophoretic mobility, antigenic properties, molecular weight, and kinetics. Therefore it may be proposed that in the liver the tetramer L_4 arises from L′ precursor subunits readily proteolyzed into L.

In vitro as well as in vivo, the maturation of L'_4 into L_4 is associated with important kinetic changes, which improve the regulatory properties of the enzyme. The Hill coefficient for phosphoenolpyruvate (PEP) is practically 1 for the L'_4 enzyme, and rises to 1.8—2 for L_4, which indicates that positive homotropic interactions appear when the L′ subunits are transformed into L. This transformation also results in a decrease of $K_{0.5}$ PEP, in the presence and in the absence of the allosteric activator fructose 1,6-bisphosphate (FDP), and in increase of the inhibition by ATP. The properties of $L_2L'_2$ are roughly intermediate between those of L'_4 and L_4.

In some patients with PK deficiency, the ability of the mutant PK variants to undergo a normal proteolytic maturation is impaired (Kahn and Marie, 1979; Marie et al., 1979). We distinguished three groups of PK variants according to their electrophoretic mobility and their sensitivity of trypsin. Group 1 was characterized by normal electrophoretic mobility and normal sensitivity to in vitro attack by trypsin; it included three cases. Group 2 included four variants with abnormal electrophoretic mobility but normal sensitivity to trypsin. Group 3, finally, included four variants characterized by their abnormal sensitivity to trypsin: the extent of the electrophoretic change induced by trypsin was less than for normal enzyme (Fig. 1). After total purification of the trypsin-treated mutant enzyme (by affinity chromatography on Blue Dextran-Sepharose; Kahn et al., 1978b), it was sometimes possible to demonstrate that the molecular weight of the mutant subunits was higher than in normal PK treated in the same way.

It is worth noting that the variants ranged in group 3 (i.e., those with abnormal sensitivity to trypsin as judged by electrophoresis) had kinetic properties that were not (or only slightly) changed by proteolytic attack (while the kinetics of the variants in groups 1 and 2 were changed in the same way as for normal PK). In addition, all these variants exhibited some common kinetic changes, namely stabilization in a 'T' allosteric conformation (with low affinity for the substrate phosphoenolpyruvate and for the allosteric activator fructose

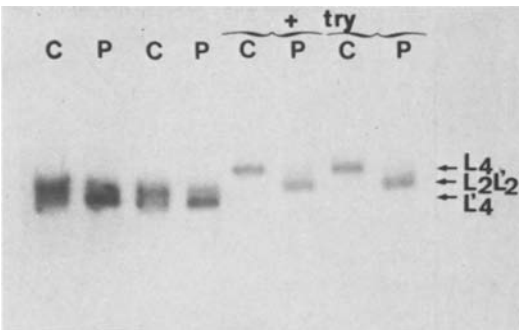

Fig. 1. Electrophoretic pattern of a mutant PK variant: Influence of trypsin. C, control erythrocyte PK; P, patient erythrocyte PK; + *try* indicates that the preparations have been treated with 0.1 mg trypsin/ml for 1 h at 37°C. The *arrows* (right of the figure) indicate the position of normal L'_4, $L_2L'_2$ and L_4 forms

1,6 P_2 and strong inhibition by the allosteric inhibitor ATP), normal in vitro stability, normal PK-related antigen concentration, and decreased immunologic specific activity. It is suggested that a family of mutations could shift allosteric equilibrium toward a T state, this conformation exhibiting abnormal sensitivity to proteolytic maturation. The kinetic anomalies of these variants could result from both primitive mutation and abnormal postsynthetic maturation of the mutant enzymes.

3) Relationship Between Molecular Anomalies of Mutant PK Variant and Hemolysis

The usual absence of any relationship between the degree of deficiency and the severity of hemolytic symptoms was reported several years ago (Boivin, 1971; Boivin et al., 1972; Miwa and Nishina, 1974). Some authors have interpreted this phenomenon as an indication that PK deficiency is only an 'epiphenomenon' associated with another basic metabolic disorder, located e.g., in the membrane (Zanella et al., 1978) or in the ATP-regenerating systems (Guminska and Wazewska-Czykewska, 1979; Schröter, 1972). Another possibility raised was that PK deficiency resulted from multigenic changes, PK deficiency being not the only cause of hemolysis (Zuelzer et al., 1968). More recently Schröter and Tillmann (1975) and Schröter et al. (1978) suggested that in the physiological conditions, about 4% of erythrocyte PK was bound to membranes; this membrane-bound enzyme could play a major role by directly providing ATP for membrane ATPase and other ATP-consuming membrane systems (Schröter et al., 1978). The severity of hemolysis of PK-deficient red cells could be related to the ratio of mutant PK bound to membrane (Schröter and Tillman, 1975). In our laboratory, however, we have been unable to reproduce these experiments and to find again such a relationship between the ratio of membrane-bound enzyme and clinical expression. As a matter of fact we do not find any noticeable amount of PK in ghost preparations. In contrast, it seems to us that there is a clear relationship between properties of the defective PK enzymes and the severity of the clinical symptoms. The main factors involved in clinical expression of red cell PK deficiency seem to be: (1) residual activity: PK deficiencies below 15%—20% of normal are always accompanied by marked hemolysis, whatever kinetic anomaly the defective enzymes exhibit; (2) kinetic anomalies: for residual activity ranging from 20%—

Table 1. Main kinetic characteristics of four mutant PK variants

Patients	Severe hemolytic process		Moderate hemolysis		Normal values (± 1 standard deviation)
	Sal[a]	Ca[a]	Otto[b]	Tor[c]	
Splenectomy	Yes	Yes	No	Yes	
Residual activity (%)	23	42	37	11	100
$K_{0.5}$ Phosphoenolpyruvate (mM)					
(– fructose 1,6 P_2)	6.3	4.4	0.83	1.46	1.46 ± 0.31
(+ fructose 1,6 P_2)	1.8	0.87	0.23	0.4	0.34 ± 0.13
ATP inhibition (mM)[d]	0.9	0.8	2.4	3.07	3.6 ± 0.55
Fructose 1,6 P_2 activation (μM)[e]	1.0	1.0	0.064	0.12	0.12 ± 0.02

[a] Patients Sal and Ca were suffering from severe forms of hemolysis, requiring frequent blood transfusions

[b] Patient Otto was practically not ill

[c] To had moderate anemia (hemoglobin concentration 10—12 g/dl), which did not require blood transfusions and was greatly improved after splenectomy

[d] ATP inhibition is expressed as mM ATP inhibiting PFK by 30% in the presence of 3 mM ADP

[e] Fructose 1,6 P_2 activation is expressed as μM/fructose 1,6 P_2 necessary for a 50% activation at 0.2 mM phosphoenolpyruvate

50% of normal, occurrence and severity of hemolytic anemia seem to depend on the association with functional alterations of the defective PK variants, the most important of which seem to be phosphoenolpyruvate affinity with and without fructose 1,6 P_2, ATP inhibition and fructose 1,6 P_2 affinity. Table 1 shows examples of two variants associated with very severe hemolytic disease and two others with well-tolerated hemolysis: it is clear that the severity of the disease is not related to the extent of deficiency, but rather to the kinetic alterations characterizing the two first variants, namely decreased phosphoenolpyruvate affinity with or without fructose 1,6 P_2, decreased fructose 1,6 P_2 affinity, and increased ATP inhibition. In the 23 different families so far studied in our laboratory we never found any strong contradiction between PK anomalies and severity of hemolysis.

Another point worthy of discussion is that it is possible to propose a tentative explanation for the special sensitivity to hemolysis of the PK-deficient reticulocytes. L'_4, the precursor form of L-type PK in red cells, is predominant in the reticulocytes; its characteristics are clearly defavourable in that phosphoenolpyruvate affinity is lower and ATP inhibition higher than for $L_2L'_2$ and L_4. These poor kinetic characteristics could aggravate the consequences of PK deficiency, leading to a more severely reduced lifespan of reticulocytes compared with mature cells. For the same reasons, hemolysis could be especially severe in patients whose defective PK exhibits abnormal proteolytic maturation and hence exists as a predominant L'_4 form. Patient Sal in Figure 1 and Table 1 illustrates this situation.

II) Glucose Phosphate Isomerase Deficiency

The congenital defect in glucose phosphate isomerase activity (GPI, EC. 5.3.1.9) represents the third most frequent enzymopathy in red cells. Since its discovery by Baughan et al. in 1967 at least 25 new cases have been described.

Glucose phosphate isomerase is a dimeric molecule (Detter et al., 1968); its structural gene is autosomal, and it is located on chromosome 19 (McMorris et al., 1973; Ruddle, 1973). A single genetic form of glucose phosphate isomerase is synthesized in all the cells of the organism (Arnold et al., 1974; Detter et al., 1968; Payne et al., 1972). Consequently, the structural mutation of this enzyme will be expressed in all the tissues. Since the mutations usually result in increased lability of the enzyme, however, the defect in activity will be maximum in the aged and anucleated cells, such as the red cells (Arnold et al., 1973a). Thus, the pathologic consequences of the enzyme deficiency will involve mainly the red blood cells, leading to chronic hemolysis. Sometimes, however, glucose phosphate isomerase deficiency can be responsible for some metabolic disturbances in various tissues, as demonstrated by Van Biervliet and Staal (1977) in the case of the patient with 'GPI Utrecht' who exhibited abnormal glycogen content in the liver.

The most recent work on GPI deficiency concerns the genetic mechanism of the defect, the mechanism of hemolysis of the GPI-deficient red cells, and the possibility of structural study of mutant GPI variants.

1) Genetic Mechanism of the Defect

In the observations of GPI deficiency, as in practically all the enzyme defects with autosomal recessive transmission, true 'homozygote' state is only probable if the patients' parents are consanguineous. In other cases most patients are heterozygous for two different mutant alleles. These mutant genes can either code for structurally modified GPI subunits or be 'silent', i.e., not synthesizing any protein enzymatically or immunologically recognized as GPI. This latter situation seems to be frequent; it was encountered in four of the seven families of GPI-deficient patients so far characterized in our laboratory (Kahn et al., 1976c, 1977a, 1978a; Isacchi et al., 1979).

Analysis of the genetic mechanism responsible for GPI deficiency in the patients requires a combination of enzymatic, immunologic, electrophoretic and, sometimes, stability methods applied to young (granulocytes) and old cells (red cells) from the patients and their parents.

Electrophoretically, Figure 2 shows an example of a family with GPI Nijmegen (Van Biervliet et al., 1975): only normal enzyme was detected in all the blood cells from the father, enzyme activity being 50% of normal.

The patient's blood cells contained only variant Nijmegen (with decreased isoelectric pH). In the mother, three forms were detected, corresponding to a normal homodimer, a heterodimer composed of one normal and one mutant subunit, and a mutant homodimer. Since GPI Nijmegen was unstable, the ratio of mutant homodimer and heterodimer was decreased in the mother's red cells, in the same way that residual enzyme activity was normal in her granulocytes and only 59% of normal in her red cells. From these data it was clearly apparent that the patient was heterozygous for a silent gene from the father and for the

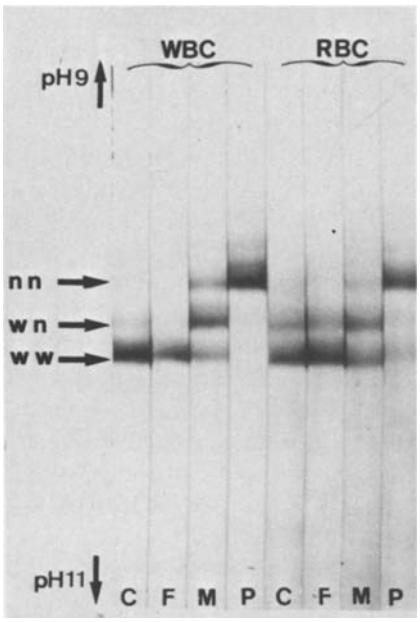

Fig. 2. Electrofocusing pattern of GPI Nijmegen. C, control; F, patient's father; M, patient's mother; P, patient; WBC, white blood cells; RBC, red blood cells. The *arrows* indicate position of normal homodimer (w-w), mutant homodimer (n-n), and heterodimer (w-n)

Nijmegen allele from the mother. As expected from this mechanism, enzyme activity was 50% of normal in the patient's granulocytes (young cells actively synthesizing proteins) and only 27% of normal in her red cells, because of instability of GPI Nijmegen. Immunologically, we found that the silent gene of the father did not code for any protein cross-reacting with anti-GPI antiserum: both enzyme activity and GPI-related antigen concentration were decreased by half.

In this case the deficiency in the patient's red cell was due to the silent gene from the father (accounting for 50% of this deficiency) and to instability of the mutant enzyme.

A similar mechanism was found for the three other variants associated with a silent gene (GPI Barcelona, Utrecht, and Kortrijk), here associated with lowered catalytic efficiency of the mutant GPI: immunologic specific activity was 75%, 75%, and 65% of normal, respectively (Kahn et al., 1977a).

Migration of GPI Utrecht being normal, genetic analysis of this case required the use of other methods than electrophoresis. Heat stability analysis provided the means of demonstrating the presence of two components in the mother's granulocytes (one stable, the other unstable), while in the father, who was heterozygous for a silent gene, there was a single component with normal stability (Fig. 3).

In spite of the relatively high frequency of the mutations responsible for a silent GPI structural gene, GPI deficiency due to the inheritance of two silent genes has never been described. It can be speculated that the homozygous state of such a mutation, which would lead to zero enzyme activity in all the tissues, could be lethal. As in most cases of genetic disorders due to silent genes, the nature of

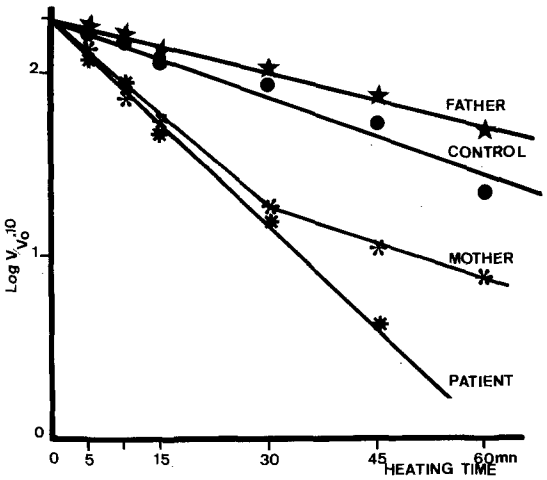

Fig. 3. Heat stability of leukocyte GPI from the patient with GPI Utrecht, her mother, and her father. Temperature 48°C

the mutation involved is unknown. A deletion, a polar mutation, a structural mutation leading to a highly labile product, and a *cis*-dominant regulator mutation are the different genetic hypotheses that could be put forward.

2) Mechanism of Hemolysis of the GPI-Deficient Red Cells

If the molecular lability of the deficient GPI variants, leading to a very low enzyme activity in the oldest red cells, partly explains the decreased viability of the GPI-deficient red cells (Arnold et al., 1973a), the metabolic bases of this hemolysis are imperfectly known. Several hypotheses have been presented by various authors to explain it: (1) Feedback inhibition of hexokinase by a high G6P concentration; (2) As a consequence of the hypothetical inhibition of hexokinase and of the metabolic block at the level of GPI, decrease of the metabolic outflow through the Embden-Meyerhof pathway, which would result in a decreased ATP synthesis; (3) Impairment of the recycling of F6P into G6P, leading to a reduced maximum capacity of the hexose monophosphate pathway. The shunt might be operating near maximum capacity as judged by the production of $^{14}CO_2$ from glucose labeled in the first carbon position, but would be unable to recycle F6P into G6P, which would result in a decreased maximum production of NADPH by the pentose phosphate pathway.

In our observations (Kahn et al., 1978a) G6P concentration and G6P:F6P ratio were not significantly different from those found in patients suffering from other types of hemolytic anemia, so the feedback inhibition of hexokinase can hardly be suggested. This result differs from those reported by Miwa et al. (1975b) and Beutler et al. (1974), but is similar to the data of Van Biervliet et al. (1975) and Arnold et al. (1973b). In our laboratory (Kahn et al., 1978a) we found that the ATP concentration of GPI-deficient red cells was either subnormal or only slightly decreased compared with that of patients with autoimmune hemolytic

anemia, such that lowering of the ATP concentration can hardly account for the severity of the hemolysis observed. This finding agrees with the data of authors who report ATP concentration to be not significantly altered in some patients with GPI defects (Baughan et al., 1968; Miwa et al., 1975b; Paglia et al., 1975a). By contrast, other patients with GPI defects have been reported to have a notably lowered ATP concentration in the red cells (Beutler et al., 1974; Oski and Fuller, 1971).

A functional impairment of the hexose monophosphate pathway can be evoked from the low reduced glutathione concentration found in the red cells from several patients (Arnold et al., 1973b; Beutler et al., 1974; Miwa et al., 1975b; Vives-Corrons et al., 1975). In addition, Heinz bodies were found after incubation of red cells with acetylphenylhydrazine in two cases reported by our group (Kahn et al., 1978a) and in two observations reported by other authors (Van Biervliet, 1975; Vives-Corrons et al., 1975). In the patient with GPI 'Utrecht,' reduced glutathione was unstable during incubation of the red cells with acetylphenylhydrazine (Van Biervliet, 1975).

We have recently reported the first observation of a mutant GPI variant (GPI Paris) with markedly altered kinetics (Kahn et al., 1978a), while all other variants described so far have exhibited normal affinity for substrates and inhibitors (Paglia and Valentine, 1974). GPI Paris was kinetically characterized by a doubled Michaelis Constant (Km) for fructose-6P and a Km for glucose-6P that was decreased to one-quarter: the inhibition constant by 6-phosphogluconate with respect to fructose-6P was also decreased to one-quarter, while the inhibition constant with respect to the other substrate glucose-6P was increased fivefold. From these changes it was expected that for a similar deficiency, GPI Paris ensures a more efficient isomerization of glucose-6P into fructose-6P than variants without kinetic modifications. The clinical symptoms, however, were as severe in this patient as in those with other variants.

This discrepancy between kinetic changes, which are thought to attenuate the blockage of G6P isomerization (and thus the impairment of the Embden-Meyerhof pathway) and the absence of any favorable influence on the hemolytic symptoms does not support the exclusive role of the reduction of the metabolic outflow through the Embden-Meyerhof pathway in the reduced lifespan of the GPI-deficient red cells.

In conclusion, the major metabolic cause of hemolysis of the GPI-deficient red cells remains rather unclear, the exclusive responsibility of the impairment of Embden-Meyerhof pathway being, at least in some observations, uncertain. The role played by the impairment of hexose monophosphate pathway, due to the markedly decreased recycling (F6P→G6P) capacity, should be considered further.

Purification and Structural Studies of Mutant GPI Variants. We have seen in the section concerned with PK deficiency that it is now possible to obtain pure mutant PK variants using affinity chromatography. This is also true for the GPI variants, which are relatively easy to purify by chromatography on phosphocellulose (or another cation exchanger resin) and affinity elution with ligands (glucose 6P, fructose 6P or 6 phosphogluconate) (Bertrand et al., 1976; Tilley et al., 1974). Tilley et al. (1974) have already determined the structural modification of a nondeficient GPI variant, GPI Singh. It is expected that recent progress in

miniaturization of the protein chemistry techniques will make structural analysis of deficient mutant GPI variants possible in the near future.

III) Triose Phosphate Isomerase Deficiency

Triose phosphate isomerase (TPI, EC. 5.3.1.1) catalyzes the interconversion of glyceraldehyde phosphate and dehydroxyacetone phosphate in the glycolytic pathway. Its deficiency is known since 1965 (Schneider et al., 1965, 1968). The number of known cases is only about 15. This defect is inherited as an autosomal recessive disease. Enzyme deficiency is generalized such that the clinical symptoms include a severe hemolytic anemia and generalized, systemic manifestations involving particularly the central nervous system and muscles. The patients seldom survive for more than a few years, death being due usually to heart failure and infections. The molecular mechanisms of TPI deficiency are not yet completely understood. Electrophoresis of extracts of normal tissues reveals the presence of several bands of TPI activity. Such isozymes may reflect the expression of more than one gene (Krietsch et al., 1971; Sawyer et al., 1972) or may represent postsynthetic modifications (Peters et al., 1973). A study was recently made by Skala et al. (1977) and Vives-Corrons et al. (1978b) of three patients with TPI deficiency. The following results were obtained: (a) Activity in red cells and white cells was less than 10% of normal. In cultured fibroblasts of two patients of American extraction the activity was 2% and 10% of controls. In the third patient's cells, a Spanish child, it reached 50% of normal. (b) Thermal stability was strikingly decreased in extracts from leukocytes and from fibroblasts of the three patients. (c) Electrophoresis of red cells showed the normal three-banded pattern with a predominance of the most cathodal band, as previously described by Kaplan et al. (1968). The electrofocusing pattern was normal. The starch gel electrophoretic picture of fibroblast extract was abnormal: the fast, anodic band described by Rubinson et al. (1971, 1973) was absent. (d) An immunologic study based on Mancini's immunodiffusion technique did not demonstrate the presence of cross-reacting material. Two types of conclusions could be drawn from this study: (a) In all three cases the TPI deficiency seemed to result not from a reduced catalytic efficiency of the mutant enzyme molecule, but from a marked instability of the enzyme. The qualitative abnormalities (electrophoretic pattern, thermolability) were identical in the three cases. (b) By contrast, residual activity in fibroblast cell cultures was very variable.

IV) Phosphoglycerate Kinase Deficiency

Hereditary deficiency of phosphoglycerate kinase (PGK, EC. 2.7.2.3) associated with hemolytic anemia was first described in 1968 by Kraus et al. Since this date about ten unrelated observations have been described. The PGK structural gene is unique in all the cells; the X chromosome carries it (Valentine et al., 1969; Chen et al., 1971). Consequently the deficiency can be expressed in all the tissues; in particular, PGK activity is decreased in leukocytes as well as in red cells. The main clinical manifestations of this defect are a severe form of chronic hemolytic anemia, usually associated with mental retardation and neurologic disorders (Valentine et al., 1969; Hjelm and Wadman, 1970; Miwa et al., 1972a; Konrad et

al., 1973). Sometimes the neurologic symptoms are very mild (Boivin et al., 1974). Recently Krietsch et al. (1977) described a PGK variant (PGK München) with moderate enzyme deficiency (21% of normal in red cells) and without any clinical symptoms. The mode of inheritance is recessive and X-linked.

We recently studied an observation of PGK deficiency (10% of residual activity in the blood cells) and found the PGK-related antigen concentration normal in both leukocytes and red cells. The partially inactive cross-reacting material corresponding to the mutant PGK variant was more stable than the normal enzyme to heat and urea denaturation; it was also very stable to purification (Kahn et al., 1976a). In this observation it was clear that abnormal catalytic efficiency was the only cause of the deficiency observed, synthesis and stability of the mutant enzyme being normal or increased.

PGK is easy to purify, especially by means of affinity elution (Cottreau et al., 1976; Scopes, 1977) or affinity chromatography (Kuntz et al., 1978). Since PGK-related antigen concentration can be normal and the enzyme variant can be stable (Kahn et al., 1976a), some defective PGK variants should be relatively easy to purify and could therefore be subjected to structural analysis. It should be noted that Yoshida et al. (1972) have already performed such analysis for a nondeficient variant and have been able to suggest, in this case, an asparagine→threonine mutation.

V) Erythrocyte Phosphofructokinase Deficiency

In man, erythrocyte phosphofructokinase (PFK, EC. 2.7.1.11) is a composite enzyme with two types of subunits: M (or muscle-type) and L (or liver-type) (Karadsheh et al., 1977; Kaur and Layzer, 1977; Layzer and Rasmussen, 1974; Tarui et al., 1965, 1969; Kahn et al., 1979 Cottreau et al., 1979).

Tarui et al. (1965) and then Layzer et al. (1967) reported observations in patients with total muscle PFK deficiency and partial red cell PFK deficiency. Immunologically it has been shown that the red cells of such patients are deficient in muscle type subunits (Layzer and Rasmussen, 1974; Layzer et al., 1967; Tarui et al., 1969).

From a clinical point of view these patients were suffering muscle symptoms similar to those of muscle phosphorylase deficiency (i.e., McArdle's disease, with muscle fatigue, cramping, muscle pain with exercise and intermittent episodes of myoglobinuria). In addition, moderate chronic hemolytic anemia was constantly found.

More recently Boulard et al. (1974), Miwa et al. (1972b), and Waterbury and Frenkel (1972) described some observations of partial or pronounced red cell PFK deficiency with chronic hemolysis but without muscle symptoms. In none of these observations was muscle PFK activity assayed.

We have personally had the opportunity of studying such an observation of partial red cell PFK deficiency associated with chronic hemolysis, but without muscle disease (Etiemble et al., 1976; Kahn et al., 1975a). Defective red cell PFK was less neutralized by anti-M PFK antiserum and more neutralized by anti-L-type antiserum than was normal enzyme, which supported the view that the red cells were deficient in M-type subunits, residual activity being due to the only L-type subunits.

In muscle, PFK activity was normal but was very unstable to heat, to urea, and to the only dilution without protective agents. Muscle PFK was an enzyme variant with fast electrophoretic mobility. On the grounds of these results we suggested that there was no muscle PFK deficiency because of the active protein synthesis in this nucleated tissue. In contrast, erythrocytes no longer synthesize proteins and are relatively old cells; therefore, deficiency of an unstable enzyme can be detected.

VI) Red Cell Hexokinase Deficiency

Hereditary deficiency of red cell hexokinase (HK, EC. 2.7.1.1) associated with congenital nonspherocytic hemolytic anemia is a very rare disease with autosomal recessive inheritance (Valentine et al., 1967; Necheles et al., 1970; Keitt, 1969; Moser et al., 1970; Goebel et al., 1972; Rijksen and Staal, 1978; Board et al., 1978). The observations reported in 1965 by Löhr et al. seemed to be different, in that HK deficiency was associated with Fanconi's disease and not with chronic hemolysis.

The level of HK deficiency in red cells is usually moderate compared with normal erythrocyte populations, but more severe than in reticulocyte-rich red cells. In physiological conditions two genetically different forms of HK exist in the red cells, HK-1 (highly predominant) and HK-3. In the case described by Necheles et al. (1970), HK-3 was absent, while a recent observation by Rijksen and Staal (1978) described anomalies of HK-1: abnormal electrophoretic pattern, decreased inhibition by glucose 1,6 P_2, and insensitivity to inorganic phosphate. Rijksen and Staal hypothesized that in normal controls there are two noninterconvertible forms of HK-1, one with high affinity for glucose 1,6 P_2, inhibition of which is regulated by inorganic phosphate, and the other with lower affinity for glucose 1,6 P_2, which is insensitive to inorganic phosphate. In patients only the latter form was found. The normal transition of one form to the other could be age-dependent, and the defective HK-1 found in the patient could be an 'old-before-its-time' enzyme. According to this hypothesis the kinetic changes of defective HK are presumably a consequence of an accelerated molecular aging (Rijksen and Staal, 1978).

VII) 2,3-Diphosphoglycerate Mutase/Phosphatase Deficiency

The Rapaport-Luebering or 2,3-diphosphoglycerate (2,3-DPG) shunt is an important alternate metabolic pathway of glycolysis in the human erythrocyte. In the first reaction of this pathway, 1,3-DPG is irreversibly converted to 2,3-DPG by the enzyme 2,3-DPG mutase (DPGM). 3-Phosphoglycerate is a necessary cofactor:

$$\text{1,3-DPG} \xrightarrow[\text{3-phosphoglycerate}]{\text{DPGM}} \text{2,3-DPG.}$$

2,3-DPG combines with deoxyhemoglobin and reduces the affinity of hemoglobin for oxygen, thereby shifting the oxygen-hemoglobin dissociation curve to the right. A deficiency of 2,3-DPGM would thus be expected to result in a decreased concentration of 2,3-DPG and a consequent increase in the affinity of hemoglobin for oxygen.

Important developments in this field were provided by two recent discoveries: (a) Diphosphoglyceromutase (DPGM, EC. 2.7.5.4) and diphosphoglycerate phosphatase (DPGP, EC. 3.1.3.13) are carried by a single molecule, which in addition contains some monophosphoglycerate mutase activity (MPGM, EC. 2.7.5.3) (Rosa et al., 1973, 1975a and b). (b) Two cases of complete DPGM deficiency have been described, allowing a better evaluation of the role of DPGM in red blood cells metabolism (Rosa et al., 1978; Peterson, 1978).

This type of defect is probably very rare. In the three first observations (Bowdler and Prankerd, 1964; Alagille et al., 1964; Löhr and Waller, 1963), DPGM deficiency was based on indirect evidence of a low 2,3-DPG concentration and inability to accumulate this compound when the glycolytic pathway was blocked distal to DPGM reaction; all these cases were described in patients with chronic hemolysis. Well-compensated chronic hemolysis was also the clinical symptom noted in the observations of partial enzyme deficiency (about 50% of normal) reported by Travis et al. (1978), while Cartier et al. (1972) and Labie et al. (1970) described a similar partial deficiency in two related subjects without any clinical symptoms. Three homozygous DPGM-deficiency observations have so far been reported. The first one, described by Schröter (1965) in a child with severe chronic hemolysis, is only tentative, since frequent blood transfusions have made it impossible to assay DPGM activity in the index patient. The parents, however, seemed to be heterozygotes (50% of normal activity); they were asymptomatic. In two cases complete DPGM deficiency has been proved (Rosa et al., 1978; Peterson, 1978) in patients with moderate symptoms. The case described by Rosa et al. was the only one to be extensively studied on a biochemical and functional point of view; the patient was characterized by a complete absence of DPGM and DPGP activities, and a 50% decrease of MPGM. Electrophoresis on cellulose acetate confirmed the lack of bands for the two missing enzymes and showed the lack of one of the three normal isozymes of MPGM.

2,3-DPG was down to 3% of normal values in the homozygote, and to 60%—70% in the heterozygotes. The consequence of the decreased 2,3-DPG-concentration was a shift to the left of the oxygen dissociation curve and a lowering of the P_{50}. This explains the major clinical symptom of the patient, which was a ruddy cyanosis with a hemoglobin level of 19 g/dl.

The observation that a nearly complete absence of 2,3-DPG in red cells is well tolerated, and is not necessarily a cause of hemolysis, is remarkable.

The clinical polymorphism of the disease is still not understood, since in other families a partial deficiency is accompanied by hemolysis or absence of clinical symptoms; furthermore, the child described by Schröter (1965) seemed to be homozygous and was suffering from severe hemolysis. It may be, then, that the decrease in 2,3-DPG concentration is not equally well tolerated in different individuals, or that DPGM deficiency is not the real cause of hemolysis in the patients displaying this clinical symptom. The molecular mechanism of the defect was recently determined through the use of specific antisera (Peterson, 1978; R. Rosa et al., personal communication); in both observations of complete DPGM deficiency so far described, inactive cross-reacting material was determined by double immunodiffusion; immunoelectrophoresis showed the inactive

cross-reacting material to exhibit abnormal migration. These data firmly establish that structural mutations are involved here, as in most cases of red cell enzyme defects.

B) Oxido-Reduction Systems

I) Glutathione Synthetase Deficiency

Congenital nonspherocytic hemolytic anemia with deficiency of reduced glutathione (G-SH) was first described by Oort et al. (1961). Prins et al. demonstrated in 1966 that impaired G-SH synthesis was involved. Boivin and Galand (1965) and Boivin et al. (1966) were able to locate the defect at the level of glutathione synthetase. In addition to moderate chronic hemolysis, this metabolic disease includes some drug-induced hemolytic crises; inheritance is autosomal recessive.

New aspects in this field have been recently provided by the discovery of the relationship between G-SH synthetase deficiency and pyroglutamic aciduria (or oxiprolinuria): this latter disease, which is responsible for chronic metabolic acidosis in children, is associated with G-SH synthetase deficiency and inconstant, moderate hemolysis (Jellum et al., 1970; Marstein et al., 1976).

The γ-glutamyl cycle, first described by Meister (1974a and b), offers an explanation for this association (Fig. 4).

In the normal conditions γ-glutamyl cysteine (γ-Glu-CySH), synthesized from γ-glutamic acid and cysteine by γ-Glu-CySH-synthetase, is transformed into G-SH by the addition of glycine; this latter reaction is catalyzed by GSH-synthetase in the presence of ATP. G-SH is an allosteric inhibitor of γ-Glu-CySH synthetase, such that deficiency of G-SH synthetase results in deficiency of G-SH, de-inhibition of γ-Glu-CySH synthetase, and massive accumulation of γ-Glu-CySH dipeptide.

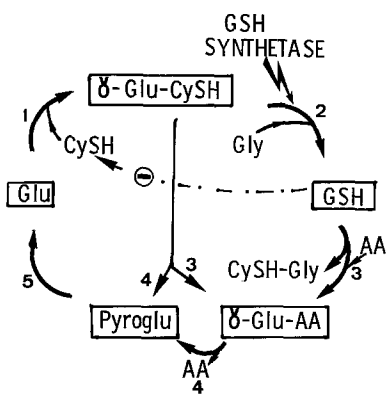

Fig. 4. The γ-glutamyl cycle (adapted from Meister, 1974a and b). Reactions occurring in the normal cycle are shown by *solid broad lines*, and those occurring in G-SH synthetase deficiency by *thin lines*. The enzymes involved are: 1, γ-glutamylcysteine synthetase; 2, glutathione synthetase; 3, γ-glutamyl transpeptidase; 4, γ-glutamyl cyclotransferase; and 5, pyroglutamate hydrolase. *Pyroglu* represents pyroglutamate, AA any aminoacid or peptide. The *dotted line* plotted from G-SH indicates the normal allosteric inhibition of γ-glutamyl cysteine synthetase by G-SH

This dipeptide can be directly transformed into pyroglutamic acid (by γ-glutamyl-cyclotransferase), or into another γ-glutamyl-aminoacid secondarily transformed into pyroglutamic acid.

The reasons for the two distinct clinical patterns associated with this defect probably correspond to the now classic mechanism already discussed for PFK deficiency: when deficiency is severe only in red cells, because it is related to unstable enzyme variants, clinical symptoms are limited to hemolytic anemia. By contrast, when deficiency is generalized (leukocytes, fibroblasts and, probably, kidney and liver), clinical symptoms include pyroglutamic acidemia and hemolytic disease (Spielberg et al., 1978).

This latter type of defect could be ascribed to decreased synthesis of the enzyme, synthesis of an inactive enzyme, or high lability of GSH synthetase.

II) Cytochrome b_5 Reductase (NADH-Methemoglobin Reductase) Deficiency (EC. 1.6.2.2)

Recessive congenital methemoglobinemia (RCM) was shown in 1959 to be due to red cell NADH-diaphorase (or NADH-methemoglobin reductase) deficiency (Scott and Griffith, 1959).

In homozygous subjects the accumulation of methemoglobin in the red cells (15%—30%) is usually well tolerated with no other symptom than chronic cyanosis, which is easily controlled by ascorbic acid or methylene blue. However, in a significant proportion of cases the methemoglobinemic syndrome is associated with severe mental retardation (Jaffé et al., 1966). This clinical heterogeneity was poorly understood until it was postulated that the severe form with encephalopathy was due to generalized deficiency of the so-called NADH-methemoglobin reductase, whereas the enzyme defect was restricted to the red cells in the benign type of disease (Kaplan et al., 1970). The so-called NADH-methemoglobin reductase was indeed found to be ubiquitously distributed in every tissue (Leroux and Kaplan, 1972). This surprising finding was consistent with the discovery by Hultquist and Passon (1971) that the reduction of methemoglobin was promoted in normal RBC by a cytochrome b_5-cytochrome b_5 reductase system. In the red cells, which are devoid of endoplasmic reticulum, these two proteins therefore appeared to represent the soluble counterpart of components already known as part of the ubiquitous microsomal electron transport system (Strittmatter, 1963). The identity between cytochrome b_5 reductase and methemoglobin reductase was further demonstrated on immunologic grounds by three independent groups (Goto-Tamura et al., 1976; Kuma et al., 1976; Leroux et al., 1977). Since the red cell cytochrome b_5 reductase is soluble, whereas it is mainly bound to the endoplasmic reticulum in the other cells, there are two possibilities: (i) both entities are coded by a single gene and the soluble form derives from the microsomal enzyme by posttranslational modification; (ii) the soluble and the microsomal enzymes are coded by two separate genes. Hypothesis (i) appears now to be the only valid one, since in several cases of congenital recessive methemoglobinemia with encephalopathy and athetosis both entities were found to be deficient (Kaplan et al., 1974; Leroux et al., 1975; Beauvais et al., 1976; Vives-Corrons et al., 1978a). Recently the corresponding gene locus (DIA_1) was

assigned to chromosome 22 (Fischer et al., 1977; Junien et al., 1978). From the comparison of 14 patients with RCM without encephalopathy (type I) and 8 patients with RCM with encephalopathy (type II), it appears clearly that in type I the cytochrome b_5 reductase deficiency affects only or mainly the erythrocytes, whereas in type II the defect is systemic, involving both the soluble and the microsomal forms of cytochrome b_5 reductase (Beauvais, Leroux and Kaplan, in preparation). In the latter category the defect was found in leukocytes, fibroblasts, liver, muscle, and brain. Different mutations at a single locus might produce either a defect of the red cell soluble cytochrome b_5 reductase (type I) or a generalized deficiency (type II). In the latter type the absence of membrane-bound cytochrome b_5 reductase might block some essential microsomal metabolic pathway, such as fatty acid desaturation (Holloway and Katz, 1972). This hypothesis remains purely speculative in our present state of knowledge.

Whereas in type II the treatment of methemoglobinemia by conventional means (methylene blue or ascorbic acid) is without effect on the neurologic syndrome, it is successful and justified in type I, allowing the subjects to lead a normal and active life. We have recently experienced the effectiveness of riboflavine (10-30 mg daily) in two patients (Kaplan and Chirouze, 1978). The rationale for this treatment is that riboflavine is reduced in the red cells by a newly discovered flavine reductase (Yubisui et al., 1977) and then provides direct reduction of methemoglobin. Riboflavine seems to be a valuable and safe substitute for ascorbic acid, which can induce hyperoxaluria upon long-term administration of large doses (Tiselius and Almgärd, 1977).

C) Anomalies of Purine and Pyrimidine Metabolism

I) Pyrimidine 5′ Nucleotidase Deficiency

Although its real cause has been only recently recognized by Valentine et al. (1974), the 'history' of this enzymopathy is more ancient. It was initially described as an increased intra-erythrocytic ATP concentration associated with phosphoribosylpyrophosphate synthetase deficiency (Valentine et al., 1972, 1973). It is worth noting that, unsatisfied with their first results, the same authors have reconsidered these observations and have succeeded in both characterizing the primary defect of their patients and describing a new enzyme, pyrimidine 5′ nucleotidase (P5′N).

Pyrimidine 5′ nucleotidase ensures dephosphorylation of the pyrimidine nucleotides, for instance cytosine monophosphate and uridine monophosphate; this reaction constitutes the basis of the assay methods.

From a clinical point of view, the affected patients suffered moderate, chronic hemolysis characterized by the presence of basophilic stippling. This situation is rather similar to that encountered in lead intoxication; indeed, specific inhibition of P5′N is thought to be a major cause of the red cell toxicity of lead, and decreased P5′N activity can be used as a sensitive index of lead exposure (Buc and Kaplan, 1978; Paglia et al., 1975 b; Valentine et al., 1976). Inheritance is autosomal recessive. This disease does not seem to be exceptional, since about 14 observations have been described in four years.

The main metabolic anomaly of red cells is a very important increase (3—6 times) of total nucleotides, 80% of which contain pyrimidine (while in normal conditions, adenosine nucleotides account for 97% of the total). As a consequence of this change in nucleotide composition, the UV absorbance spectrum of deproteinized red cells exhibited a shift in maximum absorbance from the usual 256—257 nm to approximately 266—270 nm. Reduced glutathione concentration is constantly increased, the cause of this anomaly being unknown.

Deficiency of P5'N is always severe (less than 15% of normal), with intermediate values in the heterozygous parents.

Activity of phosphoribosylpyrophosphate synthetase is about 20% of normal in the patients: this deficiency is thought to be a secondary phenomenon.

Hemolysis is expected to be a consequence of both the basophilic stippling (leading to decreased deformability of red cells and membrane damage) and perhaps the accumulation of pyrimidine nucleotides acting as competitive inhibitors (or poor substrate analogs) for AMP/ADP/ATP-dependent enzymes. It seems probable that the basophilic stippling represents undegraded RNA, secondary to feedback inhibition resulting from the high concentration of pyrimidine nucleotides.

The molecular mechanism of the deficiency of enzyme activity remains undetermined. Some qualitative anomalies of residual enzyme have been proved by means of kinetic and electrophoretic studies (Rosa et al., 1977). However, it is not yet known whether this residual enzyme activity is supported by a mutant enzyme or by another minor isozyme masked by major P5'N in the controls. Some recent data by H. A. Buc et al (personal communication) seem to indicate that the residual activity in a deficient patient might in fact represent unspecific acid phosphatase activity still active at the pH used for the P5'N determination.

II) Increase of Red Cell Adenosine Deaminase Activity and Hemolytic Anemia

The fundamental importance of the enzyme adenosine deaminase (ADA, EC. 3.5.4.4), in the development of cellular immunity has been established since the initial work of Giblett et al. (1972) on the consequence of ADA deficiency. It is now demonstrated that a lack of activity of ADA and other enzymes of purine metabolism (purine nucleoside phosphorylase, EC. 2.4.2, Giblett et al., 1975, ecto-5'nucleotidase, EC. 3.1.3.5, Edwards et al., 1978) may cause immunodeficiencies. ADA deficiency (as well as PNP deficiency) can be recognized in erythrocytes. The concentration of ATP is increased in red cells (Schmalstieg et al., 1976) as well as in lymphocytes. There is, however, no hemolysis, and erythrocyte lifespan is normal. Detailed description of this syndrome is therefore beyond the scope of this review.

In contrast to ADA deficiency, an increase of ADA activity in red cells has been recently described in patients with well-tolerated chronic hemolysis and normal immune function. These observations had been reported previously by Paglia et al. (1970) as chronic hemolytic anemia with dominant inheritance characterized by a lowered ATP concentration in the erythrocytes. In 1977 Valentine et al. demonstrated that 11 of 23 members of this family have the same hemolytic syndrome: ATP and total adenine nucleotides are less than 50% of

those in patients with comparable reticulocyte counts. The major enzymatic abnormality was a 45- to 70-fold elevation of adenosine deaminase activity. Another quite similar family was described by Miwa et al. (1978). Apparent Km for adenosine, Ki for the ADA inhibitor guanylurea sulfate, electrophoretic mobility, thermal stability, and molecular specific activity were indistinguishable from those of controls. In the observation reported by Miwa et al. (1978) it was shown that immunologic properties and aminoacid composition were similar for normal and increased enzyme. Thus, by all criteria so far employed, the greatly increased ADA represents overproduction of normal enzyme rather than a mutationally altered catalytic protein. It should be noted that this anomaly only exists in the erythroid cells: ADA activity is normal in leukocytes and cultured fibroblasts (Miwa et al., 1978).

The decrease in ATP concentration associated with ADA hyperactivity is to be compared with the increased ATP found in erythrocytes and lymphocytes of patients with ADA deficiency. Adenosine is a substrate for adenosine kinase, for entering the salvage pathway. Adenosine kinase may be at a disadvantage in a competition for the common substrate, adenosine, from the manifold increased activity of ADA.

The considerable overproduction of apparently normal ADA in a dominant disorder is difficult to explain. A defect in feedback regulation of ADA in the nucleated precursor of erythrocytes has been hypothesized; modifications of transport of adenosine at specific membrane sites exhibiting both abnormal saturability and high affinity have also been postulated. In this hypothesis, increased ADA synthesis would be secondary to the increased influx of adenosine in the erythroblasts.

Conclusions

From the above report we can observe that recent advances in hereditary disorders of red cell enzymes concern molecular mechanisms of the defect and relationship between molecular anomalies and pathologic consequences. Moreover, congenital nonspherocytic hemolytic anemias of undetermined cause are becoming fewer.

It seems to us that the perspectives opened in this field could develop in two ways. Firstly, the current possibility of obtaining homogeneous mutant enzymes (for pyruvate kinase, glucose phosphate isomerase, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase) should enable the study of the structure-function relationship, as was done for hemoglobin. Secondly, the recent progress in genetic analysis and genetic engineering could provide a direct approach of the nature of the genetic defect at the DNA level. This should be fundamental to understand the nature of some enzyme deficiencies without any detectable abnormal product of a mutant gene (e.g., glucose phosphate isomerase deficiencies with silent gene and M-type phosphofructokinase deficiency).

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