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Foreword

The large part of this Congress devoted to enzymology shows once more the importance of this field in clinical medicine as well as in the area of experimental medicine. This volume contains the reports of two symposia and some free communications which concern either technical innovations for enzyme assays, or enzyme patterns in human pathology. One of the symposia was devoted to enzyme defects of erythrocytes, specifically hereditary defects and recent theories of aetiology. In the other symposium certain antiproteases were studied, primarily inhibitors of proteolytic enzymes involved in digestion or coagulation; the discussion centered around their determination, mode of action as well as their importance as metabolic regulators.

J. Frei.

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Inherited Abnormalities of Red Cell Glycolytic Enzymes

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Fifteen years ago, when an intensive research program was undertaken to explain primaquine-induced anemia, there was no inherited enzyme deficiency known to occur in the red cells. Now in 1969, there are at least 15 different enzyme deficiencies which have been defined as responsible for disorders of the red cell. They can be categorized according to the particular metabolic pathway which is involved. Because the mature erythrocyte has a drastically simplified metabolism, a classification of these inherited enzyme defects can be easily made.

I. The first category concerns the glycolytic pathway, also known as the Embden-Meyerhof pathway.

II. The second category concerns the hexose monophosphate shunt.

III. The third category comprises miscellaneous enzymes involved in glutathione metabolism and methemoglobin reduction.

A fourth category would be those enzymes whose deficiency is detectable in the red cells, but does not alter their function, shape or survival.

Our purpose is to consider the enzyme abnormalities belonging to category I, i.e. the glycolytic pathway (fig. 1). We will include in this review the by-pass pathway located between 1,3-diphosphoglycerate (1,3-DPG) and 3-phosphoglycerate (3-PG), often referred to as the Rapoport-Luebering cycle, in which a high energy phosphate bond is lost with production of 2,3-diphosphoglycerate. (2,3-DPG).

At present, on the glycolytic pathway, 9 different enzyme deficiencies have been defined, among which 6 are well-documented, whereas 3 others still need confirmation (table I).

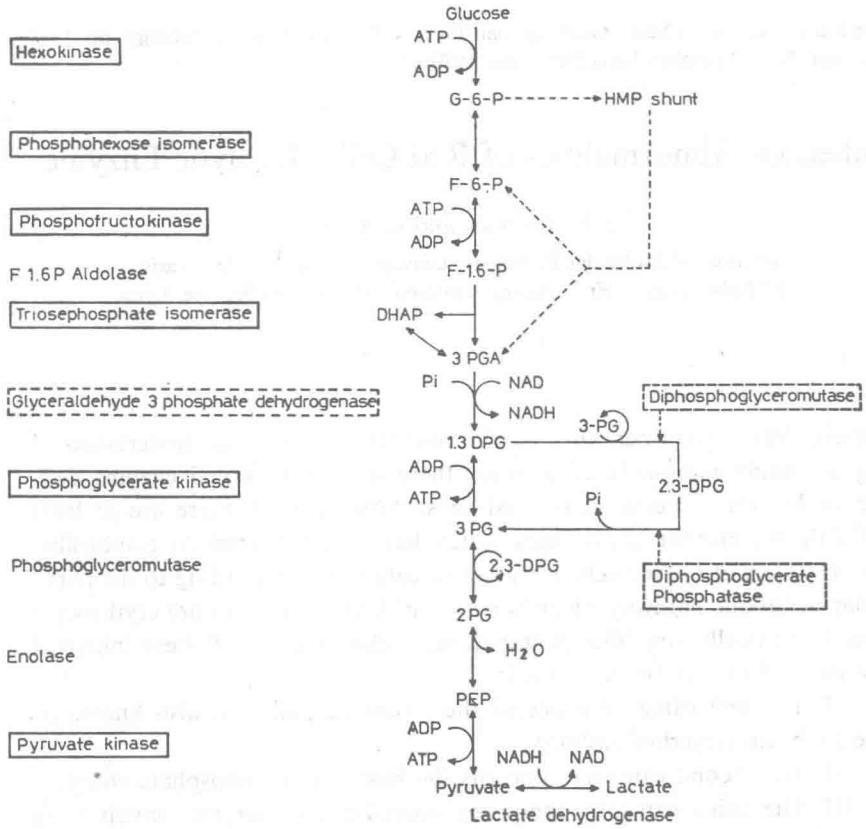

Fig. 1. Glycolysis in the red cell.

Well-established enzyme deficiencies.

Presumed enzyme deficiencies, requiring further investigation.

As a preliminary point, it is interesting to emphasize that they all give rise to a hematological pattern of congenital non spherocytic hemolytic disease (CNHD), with a variable degree of severity.

Table I. Inherited abnormalities of red cell glygolytic enzymes

Enzyme deficiency	First report	Year	Incidence	Comments
Pyruvate kinase	VALENTINE *et al.*	1961	Not rare	Polymorphism suspected
Diphosphoglycero-mutase	PRANKERD	1961	Rare	Indirect evidence
Diphosphoglycerate phosphatase	JACOBASH *et al.*	1964	Rare	Needs confirmation
Triosephosphate isomerase	SCHNEIDER *et al.*	1965	Rare	Generalized disease
Phosphofructokinase	TARUI *et al.*	1965	Rare	A form of muscular glycogenosis (Type VII)
Glyceraldehyde 3-phosphate dehydrogenase	HARKNESS	1966	Rare	Needs confirmation
Hexokinase	VALENTINE *et al.*	1967	Rare	
Phosphohexose isomerase	BAUGHAN *et al.*	1968	Rare	
Phosphoglycerate kinase	VALENTINE *et al.*	1968	Rare	Sex linked trait

I. Enzymes of the Direct Embden-Meyerhof Pathway

A. Pyruvate Kinase

Pyruvate kinase (PK) deficiency was reported by VALENTINE *et al.* in 1961 [58, 65]. Since this first report, over 100 cases have appeared in the literature [60]. It now appears that pyruvate kinase deficiency ranks as the second most common cause of CNHD, behind glucose-6-phosphate dehydrogenase (G6PD) deficiency, but far ahead of the other enzyme deficiencies presently characterized. This recessive autosomal condition has been described in various countries and ethnic groups but seems to be more common in people of North European origin [59]. The clinical pattern is variable. The hemolytic syndrome can be mild and the condition discovered at the adult age. Conversely it can be severe, giving symptoms of hemolytic anemia in early infancy. Several cases of neo-natal hemolytic syndrome have been described. Usually there are no cytological abnormalities of the red cells, except a high degree of reticulocytosis. However, cases with distortion of

the erythrocyte shape have been reported [43]. Splenectomy seems to bring a definite improvement in these severe forms with an important shortage of red cell life span. It is noteworthy that after splenectomy there is an increase in reticulocyte counts, suggesting a sieving effect of spleen on these cells [31]. In most cases there is an increase of *in vitro* auto-hemolysis not corrected by adenosine triphosphate (ATP). Thus pyruvate kinase deficiency belongs to the type II group of chronic hemolytic anemias as defined by SELWYN and DACIE on the basis of auto-hemolysis [57]. Actually this test, which had at one time an operational value for classification of CNHD, now appears to be almost obsolete. Its significance has been questioned many times. The puzzling correcting effect of ATP, a compound which cannot cross the erythrocyte membrane, has been recently reinvestigated by DACIE's group [21]. It was shown that ATP is only active through an acidification of the medium, and that the correction of increased auto-hemolysis is no longer observed when neutralized ATP is used.

The diagnosis of pyruvate kinase deficiency is ascertained by the enzyme assay in hemolysed red cells. This can be done by spectrophotometric measurement of NADH disappearance in the lactic dehydrogenase (LDH) coupled reaction [60]. Special care must be taken to avoid any contamination of red cells by leukocytes, since the pyruvate kinase activity of these cells is 300 times higher than that of normal red cells, and is not affected in pyruvate kinase deficiency [58]. The pyruvate kinase level in deficient red cells from homozygous subjects is usually diminished to 5 to 20% of the normal level [59]. In the heterozygotes, the residual activity is around 50%. These subjects do not display any clinical or hematological abnormality. It is noteworthy that there is no apparent correlation between the enzyme level and the degree of hemolysis in the patients. Two screening tests have been described. The first [13] is based upon the increase of pH which occurs during the pyruvate kinase reaction. A coloured pH indicator is used. The second [4] is much more specific because the consumption of NADH by the endogenous LDH coupled reaction is followed by examination of dry spots under UV light. The defluorescence rate parallels the pyruvate kinase activity. This test permits diagnosis of both homozygous and heterozygous states [50].

Metabolic abnormalities of pyruvate kinase deficient red cells have been extensively studied [14, 16, 20, 31, 59, 73]. Conflicting results have been reported as far as the ATP level is concerned [31, 59, 64], although a lowered ATP level would be expected since pyruvate kinase catalyses one of the two ATP producing steps in the red cell. As emphasized by KEITT [31]