Normal Metabolic Pathway and Recently Described Enzyme Deficiencies of Human Erythrocytes: A Brief Review

Shiro MIWA
3rd Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan
(Received November 7, 1974)

Knowledge about erythrocyte metabolic pathways has expanded greatly in a recent couple of years and several enzyme deficiencies of human erythrocytes have been described as well. Normal erythrocyte metabolic pathways with special emphasis on regulatory mechanisms will firstly be discussed followed by a brief review on recently described enzyme deficiencies of human erythrocytes.

METABOLIC PATHWAYS

In the mature erythrocyte, under normal physiologic conditions glucose is metabolized primarily via the Embden-Meyerhof glycolytic pathway to produce adenosine triphosphate (ATP) whereas about 10% of the glucose is metabolized by the hexose monophosphate oxidative pathway to produce dihydronicotinamide-adenine-dinucleotide phosphate (NADPH). Although glucose is the principal source of energy for the red cell under normal circumstances, it has the capacity also to utilize other substrates, such as inosine, fructose, mannose and galactose[1]. Recently it was found that erythrocyte can utilize dihydroxyacetone as well[2),3)]. The routes of utilization of these substrates are shown in Fig. 1.

Now I will discuss about how the hexose monophosphate pathway is regulated in physiological conditions. Hexose monophosphate pathway is mainly regulated by the first step, glucose 6-phosphate dehydrogenase (G6PD) reaction. Let us consider about the kinetic properties of the enzyme in the presence of *in vivo* concentrations of substrates and inhibitors (Fig. 2). Michaelis constant (Km) for the substrate glucose 6-phosphate (G6P) is 50 to 70 μM and the concentration of G6P in normal erythrocyte is estimated to be around 40μM. Even more remarkable is the fact that Km for the coenzyme NADP is 12.5μM, *in vivo* concentration of NADP is far below this level, being less than 2 μM. That means that

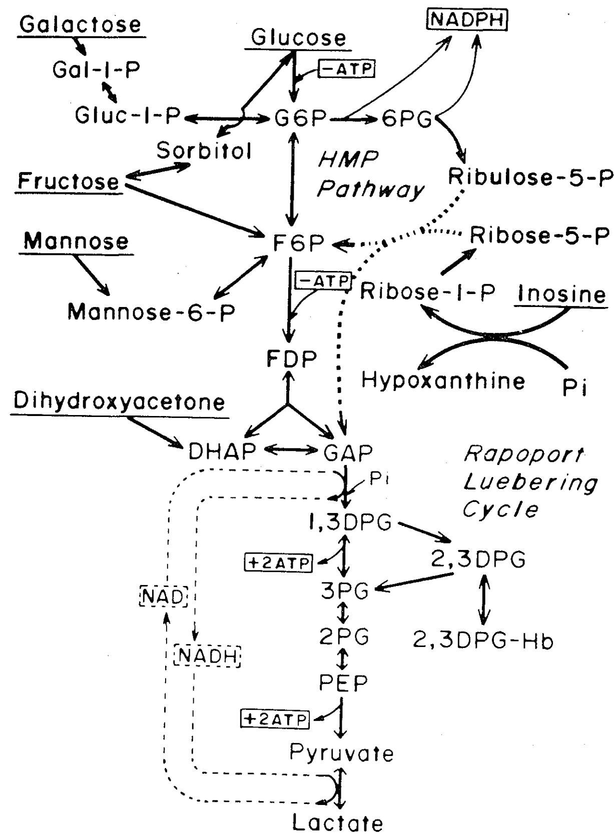
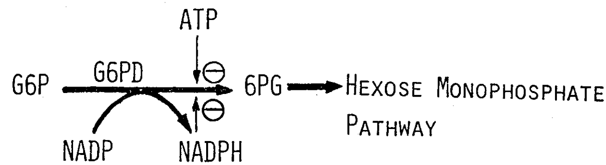


Fig. 1. Metabolic pathways of human mature erythrocytes, showing the routes or utilization of substrates.

G6P=glucose 6-phosphate; F6P=fructose 6-phosphate; FDP=fructose 1, 6-diphosphate; DHAP=dihydroxyacetone phosphate; GAP=glyceraldehyde 3-phosphate; 1, 3DPG=1, 3-diphosphoglycerate; 2, 3DPG=2, 3-diphosphoglycerate; 3PG=3-phosphoglycerate; 2PG=2-phosphoglycerate; PEP=phosphoenolpyruvate; 6PG=6-phosphogluconate; NADPH=dihydronicotinamide-adenine-dinucleotide phosphate; ATP=adenosine triphosphate; Pi=inorganic phosphate; NAD=nicotinamide-adenine dinucleotide; NADH=dihydronicotinamide-adenine dinucleotide; HMP Pathway=hexosemonophosphate pathway.

G6PD is working far below the saturation of its substrates G6P and NADP. In addition, NADPH exerts profound product inhibition, inhibition constant (Ki) of which being 9 μM whereas *in vivo* concentration of NADPH appears to be 5 times more than this level. ATP also inhibits G6PD competitively with G6P but its physiological significance is probably much less than that of NADPH. *In vitro* experiments revealed that under the saturation of NADP and substrates, 10^{10} red cells could oxidize about 2.2 μmoles of G6P,



	G6PD B+	(PHYSIOL. CONC.)
KM G6P	50-70 μM	(40)
KM NADP	12.5 〃	(2)
KI NADPH	9 〃	(45)
KI ATP	1 mM	(1.5 mM)

ONLY 0.1-0.2 % OF VMAX

Fig. 2. Regulation of glucose 6-phosphate dehydrogenase (G6PD) reaction. NADP = nicotinamide-adenine dinucleotide phosphate; Vmax = velocity maximum.

producing 1.5 μmoles of CO_2 in 1 minute whereas only 2 to 3 nmoles of CO_2 are actually produced by 10^{10} red cells suspended in an isotonic solution containing glucose at 37°C[4),5)]. These facts clearly show that because of the strong inhibition of G6PD by physiologic concentration of NADPH and ATP, only 0.1 to 0.2% of its potential activity is estimated to be expressed in human red cells that contain low concentrations of NADP and G6P[6)].

If an oxidant drug such as nitrofurantoin is administered, its active derivative interacts with oxyhemoglobin, generating low levels of hydrogen peroxide. Hydrogen peroxide is inactivated by glutathione peroxidase reaction thus producing oxidized glutathione (GSSG). GSSG is reduced to GSH by glutathione reductase reaction which requires NADPH and produces nicotinamide-adenine-dinucleotide phosphate (NADP). NADPH decreases and NADP increases and thus release G6PD reaction from inhibition, resulting in increased flow in hexose monophosphate pathway.

Fig. 3 shows the mechanism of regulation of Embden-Meyerhof glycolytic pathway in mature human erythrocytes. In these steps three kinases, hexokinase, phosphofructokinase and pyruvate kinase are the rate limiting enzymes, and the rate of these enzyme catalized steps are influenced by several inhibitors and activators as well as allosteric effectors[7),8)]. Of these three enzymes, phosphofructokinase in particular plays the central role in regulating the rate of glycolysis.

I will discuss the regulatory mechanism of phosphofructokinase reaction. Inorganic phosphate accelerates this step markedly whereas decrease in pH inhibits it. It is to be noted that Beutler et al recently found that 2,3-diphosphoglycerate (2,3DPG) exhibits inhibitory effects on almost

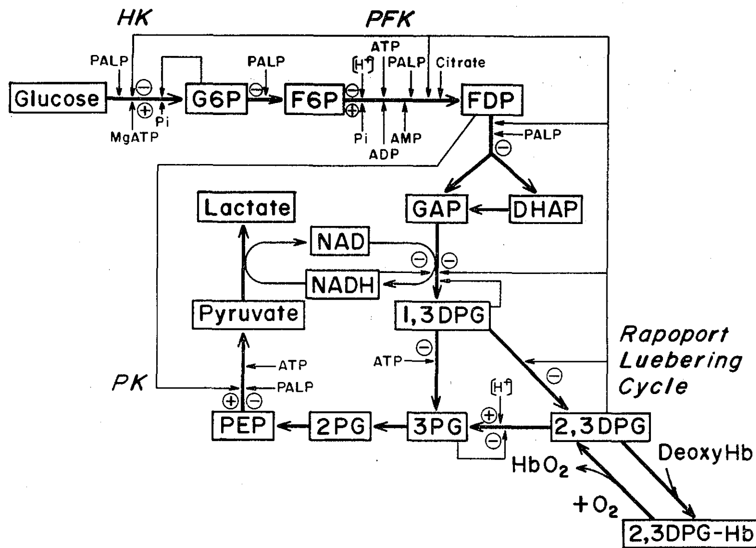


Fig. 3. Regulatory mechanisms of Embden-Meyerhof pathway and Rapoport-Luebering cycle.
HK=hexokinase; PFK=phosphofructokinase; PK=pyruvate kinase; PALP=pyridoxal phosphate; AMP=adenosine monophosphate; ADP=adenosine diphosphate.

all the proximal glycolytic steps, including phosphofructokinase[9),10),11)]. Srivastava and Beutler have also shown that pyridoxal phosphate inhibits phosphofructokinase[10)]. ATP is both a substrate and a strong allosteric inhibitor of phosphofructokinase. The inhibitory effects of ATP can be relieved by fructose 6-phosphate (F6P), inorganic phosphate (Pi) or adenosine diphosphate (ADP). It has been estimated that at the usual pH within the red cell and at the normal concentrations of F6P and ATP the enzyme may be operating at only 0.5% of its maximal capacity[1)]. Change in pH, intracellular F6P, ADP, ATP, Pi and intracellular magnesium may be expected to produce dramatic effects on phosphofructokinase control.

It would be appropriate at this point to refer to the study of Yawata, Jacob and their colleagues on hyperalimentation hypophosphatemia[12)]. They reported that infusion of hyperalimentation solutions which lacked phosphates in starved dogs as well as in patients rapidly produced hypophosphatemia. Within 48 hours following the development of hypophosphatemia, hemolytic anemia becomes obvious. Exactly paralleling the decrease in serum phosphorus, ATP levels of red cells fall, and rigidity of these cells increases. If serum phosphorus is repleted by supplementation of the infusion solution with neutral phosphates, ATP levels and plasticity

of red cells normalized rapidly. Although they didn't measure the glycolytic rate of the red cells, it is quite likely that low inorganic phosphate in the red cell exerted inhibitory effects mainly on phosphofructokinase reaction as well as hexokinase and glyceraldehyde 3-phosphate dehydrogenase reactions, thus resulting in low glycolytic rate and low ATP level.

Now, let us consider about the 2,3-diphosphoglycerate cycle, which is also called Rapoport-Luebering cycle. The production of 2,3DPG is another important function of the Embden-Meyerhof pathway in human erythrocyte. 2,3DPG is an important regulator of the oxygen affinity of hemoglobin[13),14)]. Diphosphoglycercerate mutase is profundly inhibited by low concentration of its product 2,3DPG, Ki being only 0.85 μM, yet the normal red cell has a remarkably high concentration of 4 to 5 mM[1)]. The binding of 2,3DPG to hemoglobin reduces its affinity for oxygen, thus facilitating the release of oxygen to the tissue. The presence of hemoglobin within the cell provides a mechanism for relief of product inhibition of the diphosphoglycerate mutase, because the binding of 2,3DPG to deoxyemoglobin decreases the concentration of free 2,3DPG and thus releasing product inhibition.

Phosphoglycerate kinase has a very high affinity for 1,3-diphosphoglycerate (1,3DPG) and the activity of this enzyme appears to be determined by the ATP/ADP ratio within the cell. With high red cell ADP levels, 1,3DPG will be converted to 3-phosphoglycerate (3PG) and this step will resulted in the generation of ATP. On the other hand, with high ATP levels, phosphoglycerate kinase inhibition occurs and 1,3DPG, in the presence of deoxyhemogloben will be converted to 2,3DPG. It has been estimated that approximately 20% of the glucose is metabolized via the Rapoport-Luebering cycle[1),15)]

As it has been shown that 2,3DPG plays an important role in oxygen transporting function of hemoglobin in the red cell, how to keep the 2,3DPG level as well as the ATP level as high as possible in stored blood becomes an important practical investigative field. Recently incubation of ACD stored blood with inosine, Pi and pyruvate proved to restore 2,3DPG level remarkably well[16),17),18)]. As shown in Fig. 4, inosine and Pi produces glyceraldehyde 3-phosphate (GAP) via hexose monophosphate pathway. Sufficient nicotinamide-adenine dinucleotide (NAD) is provided because of the lactate dehydrogenase reaction in the presence of pyruvate in the media, thus facilitating the glyceraldehyde 3-phosphate dehydrogenase (GAPD) reaction together with Pi and finally 2,3DPG increases. This IPP (inosine, Pi and pyruvate) media may be applicable clinically particularly in patients who need massive blood transfusion. However it should be

Restoration of 2,3DPG Level (IPP media)
Inosine (10 mM) + Pi (50 mM) + Pyruvate (10 mM)

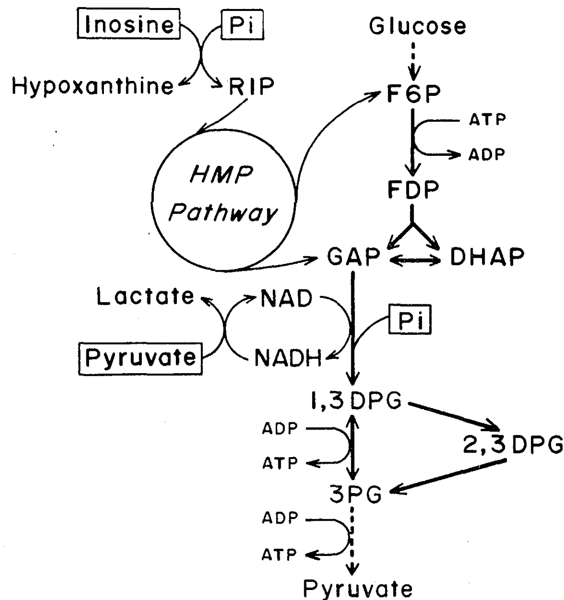
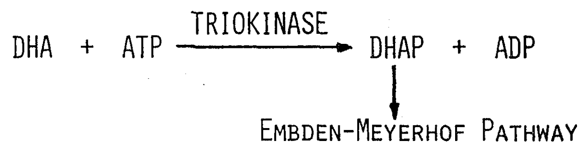


Fig. 4. Metabolic scheme showing the mechanisms for restoration of 2,3-diphosphoglycerate (2,3DPG) level by IPP media.

DIHYDROXYACETONE (DHA) METABOLISM
& TRIOKINASE (BEUTLER & GUINTO 1973)



TRIOKINASE: KM DHA 6 μM (VERY LOW !)
KM ATP 20 μM

DHA MAY BE USEFUL IN SHOCK, CORONARY ARTERY DISEASE, CONGESTIVE HEART FAILURE, ETC.

Fig. 5. Dihydroxyacetone metabolism and triokinase in human mature erythrocytes. DHA=dihydroxyacetone.

noted that the large amount of uric acid formed from inosine will be an important limiting factor in the use of this combination.

In this regard, an important finding was recently reported by Beutler and Guinto[2),3)]. Human erythrocytes can utilize dihydroxyacetone rather efficiently by virtue of the triokinase activity and produce dihydroxyacetone phosphate (DHAP) which is a normal glycolytic intermediate and thus dihydroxyacetone can be normally metabolized by the Embden-Meyerhof pathway (Fig. 5). Km for dihydroxyacetone and also Km for ATP of this enzyme are both remarkably low, and so red cells can utilize dihydroxyacetone very efficiently. Dihydroxyacetone may prove to be useful in the maintenance of 2,3DPG levels during blood storage and also in the *in vivo* manipulation of red cell 2,3DPG levels.

RECENTLY DESCRIBED ENZYME DEFICIENCIES OF HUMAN ERYTHROCYTES

Fig. 6 shows the Embden-Meyerhof glycolytic pathway and Rapoport-Luebering cycle in which the blocking steps by the known enzyme deficiencies are indicated. It is remarkable that enzyme deficiencies have so far been described in 12 out of 13 Embden-Meyerhof pathway and Rapoport-Luebering cycle enzymes, although in a few of which either detailed description is lacking or cause and effect relationship is not

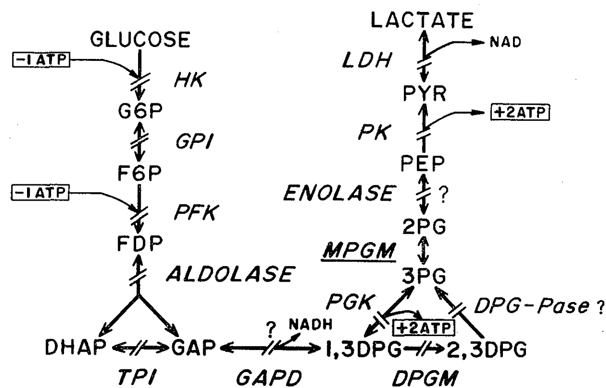


Fig. 6. Embden-Meyerhof pathway and Rapoport-Luebering cycle. Blocking steps by known erythrocyte enzyme deficiencies are indicated.
GPI = glucosephosphate isomerase; TPI = triosephosphate isomerase;
GAPD = glyceraldehyde 3-phosphate dehydrogenase;
DPGM = diphosphoglyceromutase; DPG-Pase = diphosphoglycerate phosphatase;
PGK = phosphoglycerate kinase; MPGM = monophosphoglycerate mutase;
LDH = lactate dehydrogenase; PYR = pyruvate.

clear[19].

Aldolase Deficiency

Among these glycolytic enzyme deficiencies, aldolase deficiency was quite recently found by Beutler et al[20]. Erythrocyte aldolase deficiency shows hereditary hemolytic anemia. The propositus was a 4 year-old boy. The parents were first cousins. At six weeks he was hospitalized with pallor, 5 cm hepatomegaly and the spleen tip was felt. Liver biopsy showed a modestly elevated glycogen the structure of which was normal, and the propositus had once been considered to belong type VI glycogen storage disease. He had mental retardation as well. Erythrocyte aldolase was found to be decreased, being 16% of normal, and electrophoretic as well as kinetic studies have disclosed no structural abnormality so far.

Three types of electrophoretically distinct and tissue specific aldolases are known. The isozymes in the liver and the erythrocyte are different. The mildly elevated glycogen in the liver in this case could be explained on the basis of diversion of dietary glucose and fructose from the peripheral tissues, which are aldolase deficient to the liver where aldolase is under separated control.

Pyrimidine 5′-Nucleotidase Deficiency

Valentine et al reported quite recently pyrimidine 5′-nucleotidase deficiency associated with hereditary hemolytic anemia[21]. Four members in 3 kindreds were found. The syndrome was characterized by marked increase in red cell basophilic stippling, total nucleotides and GSH, and by a fairly severe deficiency of ribosephosphate pyrophosphokinase, and patient erythrocytes uniquely contained large amounts of pyrimidine 5′-nucleotides. In earlier studies, these were erroneously considered to be adenosine phosphates[22],[23]. They therefore erroneously called the syndrome "high ATP syndrome"[24].

In earlier studies, Valentine and his colleagues measured adenosine monophosphate (AMP), ADP and ATP by the conventional enzymatic procedure described by Minakami et al[25]. The assay procedure had been considered reliable and specific, since normally 97% or more of the nucleotides of human red cells are adenine nucleotides, and pyrimidine-containing nucleotides are present in negligibly small amounts. In the assay procedures, however, non-adenosine nucleotides would react, but at a relatively slow rate. In their recent investigations of a new patient, they became aware of the probable presence of significant concentrations of such "slow reacting" nucleotides.

Total nucleotides in patient cells were present in amount 3 to 6 times greater than normal, and more than 80% were pyrimidine-containing,

Table 1.
Pyrimidine 5′-nucleotidase deficiency.

	Total Nucleotide (μmol/10^{10}rbc)	Total Pyrimidine (%)	Pyrim. 5′-Nucleotidase (% of Normal)
Patient R.	8.5	86	11
Tr.	8.8	83	6
Control	2.0	<3	100

whereas normal red cells contained less than 3% (Table 1). The activity of pyrimidine 5′-nucleotidase in the patients' red cells were only 6 to 11% of that of normal subjects.

The ultraviolet spectral curves of deproteinized red cell extracts exhibited a shift in maximum absorbance from the usual 256–257 nm to approximately 266–270 nm. This spectral characteristic of red cell extracts provides the basis of an easily performed screening procedure which does not require rather sophisticated red cell enzyme assay.

The pyrimidine 5′-ribonucleotides are presumed to be derived from RNA degradation, and, not being diffusible, accumulate when the enzyme catalyzing their dephosphorylation is deficient. It is postulated that the prominent basophilic stippling results from retarded ribosomal RNA degradation secondary to accumulation of degradation products, namely pyrimidine 5′-ribonucleotides. Ribosephosphate pyrophosphokinase deficiency is considered to be an epiphenomenon. The mechanism responsible for increased red cell GSH is unknown. The mechanism by which a hemolytic syndrome is produced must be complex and unknown at the moment. Several mechanisms can only be speculated. First of all, the presence of uridine and cytidine di- and triphosphates, might act as competitive co-factors capable of occupying binding sites of enzymes such as hexokinase, pyruvate kinase and phosphoglycerate kinase, where ADP and ATP are preferred and much more efficient. The possibility of deleterious feedback inhibitions, conceivably mediated by cytidine and uridine nucleotides, had been virtually unexplored.

Adenosine Deaminase Deficiency

In 1972, Giblett and her colleagues reported erythrocyte adenosine deaminase deficiency associated with severe combined immunodeficiency disease and not associated with hemolytic anemia[26),27),28)]. The first patient was a 22-month-old girl, the child of a consanguineous mating. Severe T-lymphocyte deficiency was manifested from birth by recurrent respiratory infections, candidiasis, and marked lymphopenia. She has shown no

delayed hypersensitivity response to candida, mumps, and could not be sensitized to dinitrochlorobenzene. Her lymphocytes responded poorly to phytohemagglutinin. Immunoglobulin levels were very low. Although her blood-group was 0, she failed to develop anti-A and anti-B. It was finally found that her red cells contained no measurable adenosine deaminase activity. Since adenosine deaminase of the red cell type predominates in the lymphocytes, it was proposed that the enzyme difeicency in the lymphocyte was probably a causal agent in the disease. The precise way in which adenosine deaminase deficiency impairs the immune response is not known with certainty. However, the work of Green and Chan[29] had shown that an increased level of adenosine, and thus of ADP and ATP, which could occur in adenosine deaminase deficient cells causes "pyrimidine starvation" due to a blockage of *de novo* uridine synthesis, at the level of orotic acid production (Fig. 7). Thus, adenosine deaminase deficient

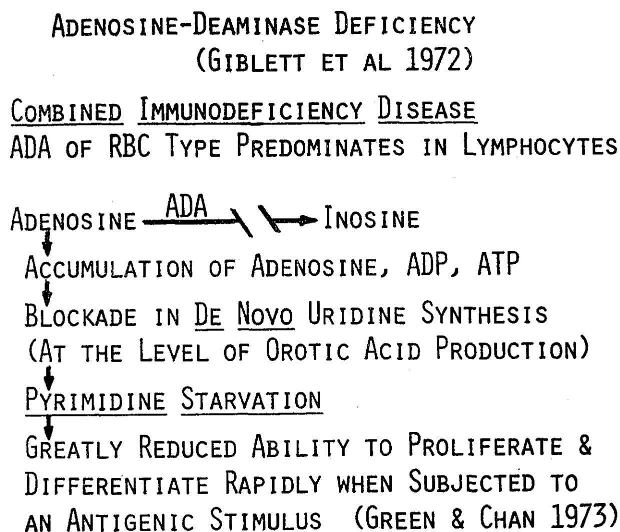


Fig. 7. Adenosine-deaminase deficiency.

lymphocytes may have greatly reduced activity to proliferate and differenciate rapidly when subjected to an antigenic stimulus. Combined immunodeficiency diseases are a heterogeneous group of severe congenital defects involving both cellular and humoral immunity. The discovery of erythrocyte adenosine deaminase deficiency associated with combined immunodeficiency disease certainly reperesent a major contribution for the elucidation of these syndromes and their underlying immunobiology.

Carbonic Anhydrase B Deficiency

Quite recently Shapira et al[30] reported an inactive mutant form of red cell carbonic anhydrese B in three members of a large kindred who manifest infantile renal tubular acidosis and nerve deafness. Both antigenic and electrophoretic properties of the mutant enzyme were said to be identical to those of the normal form. Since two major isozymes, carbonic anhydrases B and C exist, and are distributed in varying proportions in different body tissues, the inactive form of these patients suggests that carbonic anhydrase B may have a major role in renal tubular acidification.

Glucose 6-Phosphate Dehydrogenase (G6PD) Deficiency

More than 100 G6PD variants are now known to exist[31],[32], which have been identified according to the recommendation of WHO scientific group[33]. G6PD "Kyoto"[34],[35] and G6PD Heian[36] are the variants found in Japanese.

Yoshida and Lin[36] recently made an very important finding. It has been a puzzling fact that some G6PD variants that are associated with severe enzyme deficiency, such as G6PD Union and G6PD Mediterranean, have no chronic hemolytic anemia, whereas several variants with less severe enzyme deficiency, such as G6PD Manchester and G6PD Tripler, are associated with chronic hemolytic anemia. Examination of the enzymes from these variant subjects under simulated physiologic conditions revealed that; (1) enzymes from the hemolytic variant subjects are strongly inhibited by a physiologic concentration of NADPH due to their high Km for NADP or low Ki for NADPH. Thus, these variant enzymes presumably cannot

Table 2.

Several typical G6PD variants which either associated with or without chronic hemolytic anemia.

	Chron. Hem. An.	Activity (%)	Km NADP (μM)	Ki NADPH (μM)	Heat Stability
B^+	−	100	12.5	9	Normal
A^-	−	8-20	8	13	Normal
Mediterranean	−	<5	3.8	9.5	Low
Union	−	<3	8.2	37	Low
Manchester	+	25-30	18.5	0.8	Low
Tripler	+	35	80	2.6	Very Low
Physiological Concentation			<2	50	

generate NADPH in red cells to maintain an adequate level of GSH (Table 2). (2) The nonhemolytic variant enzymes are far less sensitive to the inhibition by NADPH, because of their low Km for NADP and high Ki for NADPH. These variants are also more resistant to the inhibition by ATP.

Pyruvate Kinase (PK) Deficiency

Owing to the development in the polyacrylamide gel electrophoresis method for pyruvate kinase isozyme studies by Imamura and Tanaka[37], identification of pyruvate kinase variants became feasible in our laboratory[38],[39]. So far eight pyruvate kinase variants, PK Tokyo I, PK Nagasaki, PK Sapporo, PK Kiyose, PK Tokyo II, PK Ube, PK Maebashi and PK Tsukiji and so-called classical type PK deficiency have been identified in our hands, using several parameters such as urea-stability test, neutralization test using anti-human-erythrocyte-PK serum, Km for phosphoenolpyruvate, velocity maximum (Vmax), nucleotide specificity as well as polyacrylamide gel electrophoresis (Fig. 8). Classical type PK deficiency lacks red-cell-PK isozyme but instead, M_2-type PK are weakly present probably by compensative mechanism.

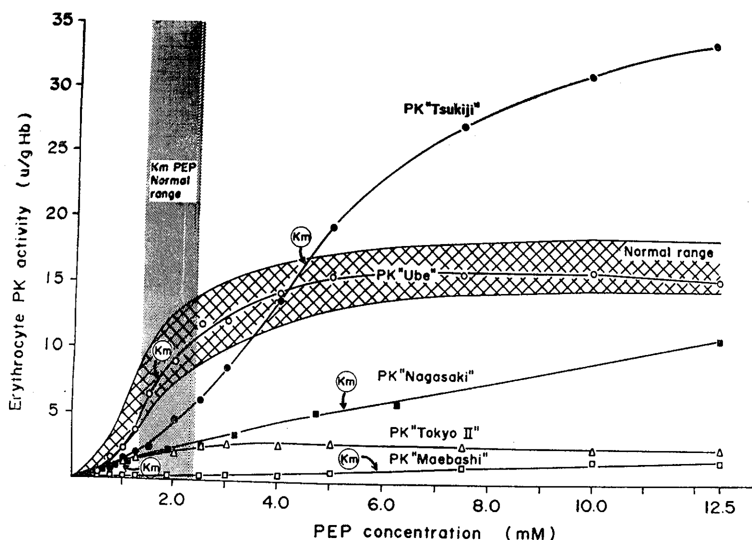


Fig. 8. Pyruvate kinase kinetic curves of several pyruvate kinase variants. Km = Michaelis constant.

These recently described hereditary enzyme deficiencies of human erythrocytes are valuable examples of the investigation into the biochemical nature of disease, and points out the vast areas still yet to be investigated in the field of red cell metabolism.

This paper was presented at the XVth International Congress of Hematology, Jerusalem, Israel, September, 1974.

REFERENCES

1) Oski, F.A.: Normal red cell metabolism and the influences of age and the plasma environment. *In: Blood Cells as a Tissue.* Holmes, W.L. (ed.) Plenum Press, New York, 1970, pp. 99-129.
2) Beutler, E. and Guinto, E.: Dihydroxyacetone metabolism by human erythrocytes: Demonstration of triokinase activity and its characterization. *Blood,* **41** : 559-568, 1973.
3) Beutler, E. and Guinto, E.: The metabolism of dihydroxyacetone by intact erythrocytes. *J. Lab. Clin. Med.,* **82** : 534-545, 1973.
4) Luzzatto, L. and Afolayan, A.: Genetic variants of human erythrocyte glucose 6-phosphate dehydrogenase. II. *In vitro* and *in vivo* function of the A⁻variant. *Biochemistry,* **10** : 420-423, 1971.
5) Yoshida, A.: Hemolytic anemia and G6PD deficiency. *Science,* **179** : 532-537, 1973.
6) Yoshida, A. and Lin, M.: Regulation of glucose-6-phosphate dehydrogenase activity in red blood cells from hemolytic and nonhemolytic variant subjects. *Blood,* **41** : 877-891, 1973.
7) Minakami, S. and Yoshikawa, H.: Studies on erythrocyte glycolysis. II. Free energy changes and rate limitings steps in erythrocyte glycolysis. *J. Biochemistry,* **59** : 139-144, 1966.
8) Jacobasch, G., Minakami, S. and Rapoport, S.M.: Glycolysis of the erythrocyte. *In: Cellular and Molecular Biology of Erythrocytes.* Yoshikawa, H. and Rapoport, S.M. (eds.), University of Tokyo Press, Tokyo, 1974, pp. 55-92.
9) Beutler, E.: 2,3-Diphosphoglycerate affects enzymes of glucose metabolism in red blood cells. *Nature, New Biology,* **232** : 20-21, 1971.
10) Srivastava, S.K. and Beutler, E.: The effect of normal red cell constituents on the activities of red cell enzymes. *Arch. Biochem. Biophys.,* **148** : 249-255, 1972.
11) Beutler, E. Matsumoto, F. and Guinto, E.: The effect of 2,3-DPG on red cell enzymes. *Experientia,* **30** : 190-191, 1974.
12) Jacob, H., Yawata, Y., Craddock, P., Hebbel, R., Howe, R. and Silvis, S.: Hyperalimentation hypophosphatemia: Hematologic, neurologic dysfunctions due to ATP depletion. *Trans. Assoc. Amer. Physicians,* **86** : 143-153, 1973.
13) Chanutin, A. and Curnish, R.R.: Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. *Arch. Biochem. Biophys.,* **121** : 96-102, 1967.
14) Benesch, R. and Benesch, R.E.: The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. *Biochem. Biophys. Res. Comm.,* **26** : 162-167, 1967.
15) Duhm, J.: 2,3-Diphosphoglycerate metabolism of erythrocytes and oxygen transport function of blood. *In: Erythrocytes, Thrombocytes, Leukocytes. Recent Advances in Membrane and Metabolic Research.* Gerlach, E., Moser, K., Deutsch, E. and Wilmans, W. (eds.) Georg Thieme, Stuttgart, 1973, pp. 149-157.
16) Oski, F.A., Travis, S.F., Miller, L.D., Delivoria-Papadopoulos, M. and Cannon, E.: The in vitro restoration of red cell 2,3-diphosphoglycerate levels in banked blood. *Blood,* **37** : 52-58, 1971.
17) Deuticke, B. and Duhm, J.: Accumulation of organic phosphates in human erythrocytes. A method of modifying the intracellular conenctration of non-penetrating anions. *In: Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status.* Rørth, M. and Astrup, P. (eds.) Munksgaard, Copenhagen, 1972, pp. 576-582.
18) Sugerman, H.J., Pollock, T., Rosato, E.F., Delivoria-Papadopoulos, M., Miller, D. and Oski, F.A.: Experimentally induced alterations in affinity of hemoglobin for oxygen. II. In vivo

effec of inosine, pyruvate, and phosphate on oxygen-hemoglobin affinity in rhesus monkey. *Blood*, 39 : 525-529, 1972.

19) Miwa, S.: Hereditary hemolytic anemia due to erythrocyte enzyme deficiency. *Acta Haem. Jap.*, 36 : 573-615, 1973.
20) Beutler, E., Scott, S., Bishop, A., Margolis, N., Matsumoto, F. and Kuhl, W.: Red cell aldolase deficiency and hemolytic anemia: A new syndrome. *Trans. Assoc. Amer. Physicians*, 86 : 154-166, 1973.
21) Valentine, W.N., Fink, K., Paglia, D.E., Harris, S.R. and Adams, W.: Hereditary hemolytic anemia with human erythrocyte pyrimidine 5′-nucleotidase deficiency. *J. Clin. Invest.*, 54 : 866-879, 1974.
22) Valentine, W.N., Anderson, H.M., Paglia, D.E., Jaffé, E.R., Konrad, P.N. and Harris, S.R.: Studies on human erythrocyte nucleotide metabolism. II. Nonspherocytic hemolytic anemia, high red cell ATP, and ribosephosphate pyrophosphokinase (RPK, E. C. 2. 7. 6. 1) deficiency. *Blood*, 39 : 674-684, 1972.
23) Valentine, W.N., Bennett, J.M., Krivit, W., Konrad, P.N., Lowman, J.T., Paglia, D.E. and Wakem, C.J.: Nonspherocytic haemolytic anaemia with increased red cell adenine nucleotides, glutathione and basophilic stippling and ribosephosphate pyrophosphokinase (RPK) deficiency: Studies on two new kindreds. *Brit. J. Haemat.*, 24 : 157-167, 1973.
24) Valentine, W.N.: Deficiencies associated with Embden-Meyerhof pathway and other metabolic pathways. *Seminars in Hematology*, 8 : 348-366, 1971.
25) Minakami, S., Suzuki, C., Saito, T. and Yoshikawa, H.: Studies on erythrocyte glycolysis. I. Determination of the glycolytic intermediates in human erythrocytes. *J. Biochemistry*, 58 : 543-550, 1965.
26) Giblett, E.R., Anderson, J.E., Cohen, F., Pollara, B. and Meuwissen, H.J.: Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet*, 2 : 1067-1069, 1972.
27) Chen, S., Scott, R. and Giblett, E.R.: Adenosine deaminase: Demonstration of a "silent" gene associated with combined immunodeficiency disease. *Amer. J. Human Genet.*, 26 : 103-107, 1974.
28) Yount, J., Nichols, P., Ochs, H.D., Hammar, S.P., Scott, C.R., Chen, S., Giblett, E.R. and Wedgwood, R.J.: Absence of erythrocyte adenosine deaminase associated with severe combined immunodeficiency. *J. Pediat.*, 84 : 173-177, 1974.
29) Green, H. and Chan, T.: Pyrimidine starvation induced by adenosine in fibroblasts and lymphoid cells: Role of adenosine deaminase. *Science*, 182 : 836-837, 1973.
30) Shapira, E., Ben-Joseph, Y., Eyal, F.G. and Russell, A.: Enzymatically inactive red cell carbonic anhydrase B in a family with renal tubular acidosis. *J. Clin. Invest.*, 53 : 59-63, 1974.
31) Yoshida, A., Beutler, E. and Motulsky, A.G.: Human glucose-6-phosphate dehydrogenase variants. *Bull. Wld. Hlth. Org.*, 45 : 243-253, 1971.
32) Beutler, E. and Yoshida, A.: Human glucose-6-phosphate dehydrogenase variants: A supplementary tabulation. *Ann, Hum. Genet.*, 37 : 151-155, 1973.
33) WHO Scientific Group on the Standarization of Procedures for the Study of Glucose-6-Phosphate Dehydrogenase. *Wld. Hlth. Org. Techn. Rep. Ser.*, No. 366, 1967.
34) Kojima, H.: Congenital nonspherocytic hemolytic anemia due to glucose-6-phosphate dehydrogenase deficiency in a Japanese child. Clinical and metabolic studies. *Acta Haem. Jap.*, 35 : 20-31, 1972.
35) Kojima, H.: Congenital nonspherocytic hemolytic disease (CNHD) due to a G-6-PD variant: G-6-PD Kyoto. *Acta Haem. Jap.*, 35 : 32-38, 1972.
36) Nakai, T. and Yoshida, A.: G6PD Heian, a glucose-6-phosphate dehydrogenase variant associated with hemolytic anemia found in Japan. *Clin. Chim. Acta.*, 51 : 199-203, 1974.
37) Imamura, K. and Tanaka, T.: Multimolecular forms of pyruvate kinase from rat and other

mammalian tissues. I. Electrophoretic studies. *J. Biochemistry*, 71 : 1043-1051, 1972.
38) Nakashima, K., Miwa, S., Oda, S., Tanaka, T., Imamura, K. and Nishina, T.: Electrophoretic and kinetic studies of mutant erythrocyte pyruvate kinases. *Blood*, 43 : 537-548, 1974.
39) Miwa, S., Nakashima, K., Ariyoshi, K., Shinohara, K., Oda, E. and Tanaka, T.: Four new pyruvate kinase (PK) variants and a classical type PK deficiency. *Brit. J. Haemat.*, 29 : 135-147, 1975.