Red Cell Enzymopathies in the Newborn
I. Evaluation of Red Cell Metabolism*

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ABSTRACT

In order to evaluate properly red cell metabolic data obtained in newborns with congenital hemolytic disorders, the unique metabolic characteristics and normal developmental changes that occur prenatally and postnatally are presented. The age-dependent red cell glycolytic enzymes (hexokinase, aldolase, pyruvate kinase) and glucose-6-phosphate dehydrogenase and most glycolytic intermediates are elevated at birth and at 11 to 12 months of age, consistent with the presence of a young red cell population the entire first year of life. However, certain red cell enzymes are elevated out of proportion to the age of the red cell population [phosphoglucose isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase (PGK), and enolase (ENO)] whereas others are decreased [phosphofructokinase (PFK), glutathione peroxidase, carbonic anhydrase, and others]. These metabolic characteristics are felt to be unique and representative of “fetal erythropoiesis.” Activities of PGK and ENO decrease and PFK increases toward normal adult values beginning at eight to nine weeks of age.

The concentration of glucose-6-phosphate steadily increases after birth and peaks at three to four weeks of age, at a time when PFK activity remains relatively unchanged, suggesting a relative block in glycolysis at the PFK step secondary to an enzyme with both decreased activity and altered kinetic properties (a “fetal” isozyme).

Thus, evaluation of red cell enzyme and glycolytic intermediate data obtained in the first year of life should be related to the knowledge that a young red cell population is present and the characteristic unique metabolic red cell alterations described in cord blood persist beyond the immediate neonatal period.

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Red cells from newborn infants are known to differ considerably from those of older children and adults. They are macrocytic and have a decreased life span.\(^5\) Thus, newborns have a relatively young red cell population, which results in higher activities of age-related enzymes.\(^6,10,14\) Macrophages similar to those observed in subjects with a red cell population of a similar mean age.\(^14,22,29,36,41\) However, not all of the metabolic characteristics observed in neonatal red cells can be attributed to a younger red cell population. The activities of certain enzymes such as phosphoglycerate kinase (PGK)\(^14,16,21,22,29,36,41\) and enolase (ENO)\(^14,21,22,29,36,41\) are disproportionately elevated when compared to cells of a similar mean age. Other enzymes, such as phosphofructokinase (PFK), are significantly reduced.\(^6,14,16,21,22,29,36,41\) Metabolically, these cells have been reported to consume less glucose than would be predicted for the age of the red cell population,\(^6,30\) and the relative deficiency of the regulatory enzyme, PFK, has been proposed as a possible cause of this metabolic handicap.\(^29,35\)

It would be anticipated that the pattern of red cell glycolytic intermediates might yield the most useful information concerning metabolic events at the in vivo level and the possible significance of decreased enzyme activity or altered kinetic properties, according to the "crossover theorem" of Chance.\(^7\) However, the pattern of glycolytic intermediates also differs in normal newborns when compared to both adults and subjects with a red cell population of a similar mean cell age.\(^29,34\)

The unique metabolic characteristics present in the red cells of infants persist beyond the immediate neonatal period.\(^6,34,36\) Thus, evaluation of red cell metabolic data obtained on infants must be related to the normal developmental changes that occur prenatally and postnatally. For these reasons, a discussion of red cell metabolic alterations characteristic of the newborn period will be presented prior to discussing the individual enzyme deficiencies in greater detail.

### Enzymes

The relatively young red cell population that is present in newborn infants consistently results in a significant increase in activity of the following glycolytic, hexose monophosphate (HMP) shunt and other red cell enzymes in cord blood.\(^6,10,14\)

1. **Glycolytic Pathway:** Hexokinase (HK), aldolase (ALD), triose phosphate isomerase (TPI), phosphoglycerate mutase, pyruvate kinase (PK), and lactate dehydrogenase.
2. **Hexose Monophosphate Shunt:** Glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), glutathione reductase (GR), and distal shunt.
3. **Others:** Galactokinase, galactose-1-phosphate uridyl transferase, glyoxalase I and II, and glutamic oxaloacetic transaminase.

The increase in activity of these enzymes is comparable to values obtained from subjects with a red cell population of a similar mean age.

Other glycolytic enzymes such as glucose phosphate isomerase (GPI),\(^10,22,41\) glyceraldehyde-3-phosphate dehydrogenase (G-3-PD),\(^22,41\) phosphoglycerate kinase (PGK),\(^14,21,22,29,36,41\) and enolase (ENO)\(^14,21,22,29,36,41\) are elevated out of proportion to the age of the red cell population. In contrast, certain enzymes such as PFK,\(^6,14,16,18,21,22,29,36,41,44\) glutathione peroxidase (GSH-Px),\(^43\) and others (table I) are decreased when compared to both red cells of a similar young mean age and red cells from normal adults. The developmental changes in the non-age-dependent enzymes PGK, ENO, and PFK and the age-dependent enzymes HK, ALD,
RED CELL ENZYMOPATHIES IN THE NEWBORN

| Increased Activity When Compared to Subjects with a Young Red Cell Population |
|-------------------|-------------------|-------------------|-------------------|
| Glucose phosphate isomerase  | Glyceraldehyde-3-phosphate dehydrogenase  | Phosphoglycerate kinase  | Enolase |

| Decreased Activity When Compared to Normal Subjects |
|-------------------|-------------------|-------------------|-------------------|
| Phosphofructokinase  | Glutathione peroxidas  | Glutathione synthetase | NADPH-methemoglobin reductase |
| Carbonic anhydrase  | Catalase | Ribosephosphate pyrophosphokinase | Acetylcholinesterase |

References: 14, 18, 21, 22, 26, 29, 36, 41

PK, and G-6-PD have been followed sequentially in our laboratory in term infants in the first year of postnatal life and compared with the concentration of fetal hemoglobin (%F) and the age of the red cell population using PK as an index of red cell age. (PK had been demonstrated previously to have a similar half-life in adult and cord red cells.)

The most significant change in the activities of PGK, ENO, and PFK towards normal adult values began between eight to nine weeks and 14 to 16 weeks of age and coincided with a transient increase in activity of the two most age-dependent glycolytic enzymes HK and PK (at eight to nine weeks), which was felt to represent resumption of active erythropoiesis by the infants' bone marrow. This suggested that developmental changes in these three enzymes are unique to the "fetal" red cell and represent passage from "fetal" to "adult" erythropoiesis. This relationship was more clearly demonstrated when it was shown that the sequential changes in the activities of PGK, ENO, and PFK during the first year of life correlated significantly with the postnatal decline in fetal hemoglobin, not the age of the red cell population. This interpretation is in agreement with the finding that "old" red cells from cord blood (the bottom layer or cells with the highest density) had a higher %F and demonstrated to a marked degree those characteristics attributed to whole blood, namely decreased activity of PFK and increased activity of PGK and ENO.

Other investigators have also described elevated levels of ALD and G-6-PD for most of the first year of life. PK activity has been reported as elevated the first two years of life and declined to normal adult levels by the third year. In contrast to the previously described study, PFK has been reported as decreased until 16 to 24 months of life. In that study, ENO activity decreased to the normal range by the age of one month.

Premature infants were studied on the first day of life in order to determine whether or not the increased activities of
PGK and ENO and decrease in PFK would be greater than in term infants. Preliminary results obtained in our laboratory in infants of 28 to 30 weeks gestation have revealed increased activity of the age-dependent enzymes HK, ALD, PK, and G-6-PD, indicative of a red cell population of a younger mean cell age than in term infants. Mean PGK was only slightly higher, but mean ENO activity was significantly increased when compared to values obtained from red cells in term infants. Mean PFK activity, however, was higher, not lower, in premature infants than in term infants, although the levels were still decreased when compared to cells of a similar mean cell age (table II). In contrast to term infants, PFK activity in prematures correlated significantly with the age of the red cell population, as reflected in PK activity. Thus, it appears that the young mean red cell population present in the premature infant on the first day of life significantly influences PFK activity resulting in higher enzyme levels than those anticipated at such a young gestational age. Elevation of PGK and ENO and normal activity of PFK (when compared to normal adults, not subjects with a young red cell population) have also been reported by other investigators in both premature infants and fetuses.

Very high activity of G-6-PD that fell with maturation, decreased activity of 6-PGD that increased with gestational age, and similar activity of GR in fetuses 12 to
28 weeks of age have been described when values were compared to term infants. Since PFK has decreased activity in cord blood and is a regulatory enzyme, it has been extensively studied. It has been suggested that the relative deficiency of PFK characteristic of the newborn period is secondary to an unstable enzyme or fetal isozyme,\textsuperscript{18,33,40} since there is a disproportionate loss of PFK activity in “old” red cells as compared to “young” cells in cord blood as compared to adults.\textsuperscript{14,18,21,33} Kahn et al\textsuperscript{18,19} have reported that PFK from premature infants and “old” red cells from term infants are relatively deficient in the muscle-type (M) subunits. Vora and Piomelli\textsuperscript{40} have demonstrated that PFK from adult blood purified in DEAE-Sephadex elutes as one peak, but cord blood elutes as two peaks, one identical to the adult heterotetramer of muscle and liver (M and L) subunits and the other, an unstable liver homotetramer (L4). The accumulation of the L4 homotetramer represents a “fetal” isozyme pattern and the instability of this isozyme probably accounts for both the previously described increased lability of cord blood PFK and the relative deficiency of this enzyme in the newborn period.

Chen et al\textsuperscript{9} studied the isozyme patterns of 26 enzymes by starch gel electrophoresis in hemolysates obtained from human fetuses 65 to 138 days gestation and compared them to normal adults. The zymograms of 16 enzymes were identical in fetal and control red cells. The staining intensity of isozyme zones of PFK differed from controls and was attributed to a possible decrease in M-type subunits. Other red cell enzymes with isozymes of different staining intensities in cord blood when compared to adult controls were HK, ENO, lactate dehydrogenase, guanylate kinase, and nucleoside phosphorylase. Mitochondrial forms of isocitric dehydrogenase and glutamic oxaloacetic transaminase were present in fetal red cells, but not red cells from adults. Phosphoglyceromutase-type 3 (PGM-3) was detected in fetal cells whereas only PGM-1 and PGM-2 are readily seen in red cells from adults. The isozyme pattern of HK has been investigated by others, with conflicting results.\textsuperscript{17,32} Carbonic anhydrase levels, especially isozymes B and C, are markedly decreased in cord blood. This enzyme is also decreased in juvenile chronic myelocytic leukemia.\textsuperscript{42} This disease entity provides a possible example of “fetal erythropoiesis” in the older child since it is characterized by a marked increase in fetal hemoglobin. Carbonic anhydrase isozymes B and C progressively decrease to levels observed in cord blood\textsuperscript{42} and the activities of red cell ENO, G-3-PD and G-6-PD are elevated out of proportion to the age of the red cell population and are similar to values obtained from 24 week old fetuses,\textsuperscript{13} and to those previously described in cord blood.

**Glycolytic Intermediates**

The pattern of red cell glycolytic intermediates (table III) in term infants in the first year of life has been investigated in our laboratory.\textsuperscript{34} With the exception of phosphoenolpyruvate (PEP), the concentrations of all glycolytic intermediates are elevated at birth when compared to normal adults, consistent with the presence of a young mean red cell population. Mean levels of glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), and “total triose phosphate” [simultaneous measurement of fructose diphosphate (FDP), dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P)] were increased on day 1 of life when compared to both normal adults and subjects with a similar young mean red cell age. The concentration of G-6-P steadily increased, peaked at three to four weeks of
<table>
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<tr>
<th>Subjects* with a Young Mean Cell Age</th>
<th>3-4 Weeks</th>
<th>8-9 Weeks</th>
<th>5-6 Months</th>
<th>11-12 Months</th>
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<td>Day 1</td>
<td>Day 4</td>
<td>3-4 Weeks</td>
<td>8-9 Weeks</td>
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<tr>
<td>G-6-P</td>
<td>53.3±8.6</td>
<td>79.8±14.5</td>
<td>92.3±35.7</td>
<td>59.7±19.4</td>
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<td>16.6±1.1</td>
<td>25.2±4.4</td>
<td>28.3±10.5</td>
<td>21.1±8.5</td>
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<tr>
<td>TTP</td>
<td>19.2±2.6</td>
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<td>2,3-DPG</td>
<td>4.69±383</td>
<td>54.3±587</td>
<td>4.679±947</td>
<td>4.559±648</td>
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<tr>
<td>3-PG</td>
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<td>PEP</td>
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<td>PK = Pyruvate kinase</td>
<td>TTP = Total triose phosphate</td>
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</table>

Glycolytic Intermediates and ATP (mumoles/ml RBC) During the First Year of Life in Term Infants* +

*Ten subjects in each group.
†Mean±S.D.
age at levels 2.5 times greater than values observed in subjects with a red cell population of a similar mean age, and then progressively decreased in concentration. The total triose phosphate (TTP) concentration peaked on day 4 of life at a level 1.9 times that observed in red cells of a similar mean age and then decreased significantly by three to four weeks of age. The mean level of 2,3-diphosphoglycerate (2,3-DPG) was normal for cell age on day 1 of life, increased on day 4 and then declined by three to four weeks of age to normal values similar to results previously reported. At five to six months of age 2,3-DPG increased. Adenosine triphosphate (ATP) followed a pattern similar to 2,3-DPG.

Although the mean PEP concentration was decreased for red cell age on day 1, this decrease was not felt to be statistically significant in our laboratory. Mean concentrations of 3-phosphoglycerate (3-PG) and 2-phosphoglycerate (2-PG) were normal for mean red cell age on day 1 of life. The mean level of 2-PG increased at three to four weeks of age and remained elevated for cell age at 11 to 12 months of life, but this increase was not considered statistically significant. There was no significant change in 3-PG during the entire first year of life. At one year of age, all red cell glycolytic intermediates and ATP were elevated when compared to red cells from normal controls. They were comparable to those observed in subjects with a red cell population of a similar mean cell age, consistent with the persistence of a relatively young red cell population throughout the first year of life.

The progressive increase in the concentration of red cell G-6-P that occurred between day 1 and three to four weeks of life appeared to be independent of cell age, using PK activity as an index of red cell age, because mean PK activity did not increase during that time period (table III). The increased level of red cell TTP was transient and had decreased significantly by three to four weeks of age, at a time when the mean G-6-P concentration reached its highest level. This pattern of glycolytic intermediates was highly suggestive of a relative block in glycolysis at the PFK step. During this period, however, PFK activity remained relatively unchanged and HK activity decreased, resulting in a decrease in the HK/PFK ratio which should have led to a decrease in the concentration of G-6-P, not the increase that was observed. These data suggested that the relative block in glycolysis at the PFK step that led to continued accumulation of G-6-P despite stable PFK activity was not due to decreased PFK activity alone, but also secondary to altered kinetic properties of the enzyme.

Further investigation revealed that changes in the concentration of red cell G-6-P in the neonatal period demonstrated a significant positive correlation with the plasma inorganic phosphorus (Pi) concentration; however, the 2,3-DPG levels did not correlate with Pi. These results suggested that the accumulation of G-6-P and the lack of a sustained increase in ATP and 2,3-DPG in the presence of increased plasma Pi was due to a combination of imbalanced stimulation of HK and PFK (HK > PFK) by Pi in red cells from term infants and a relative block in glycolysis at the PFK step. The block at the PFK step is probably secondary to both a relative deficiency of PFK and an enzyme that is less sensitive to Pi stimulation than PFK from adults. Kahn et al. have demonstrated that PFK from fetal red cells is lacking in the M-type subunit and is more inhibited by ATP than PFK in red cells from adults. This enhanced ATP inhibition of PFK may be responsible for the apparent decreased activation of PFK by increased plasma Pi since Pi stimulates PFK activity by relief of ATP inhibition of the enzyme. Although Bentley et al. reported that prematures and term infants metabolized glucose at the same rate as young red cells
from adults in the presence of high Pi, there was no predictable stoichiometry in cord blood whereas the adults consistently produced two moles of lactate per mole of glucose consumed. Glycolytic intermediates were not measured, but there appeared to be a relative block in glycolysis. It is possible that HK was stimulated more than PFK by Pi, and the relative block was at the PFK step.

It has previously been demonstrated that old red cells from cord blood which are presumably those produced early in gestation did not accumulate TTP when allowed to stand, whereas red cells from normal adults invariably demonstrate a marked increase in these compounds and reciprocal fall in the level of G-6-P. This increase in TTP and fall in G-6-P in red cells from adults is secondary to an increase in intracellular pH and activation of PFK, which suggests that PFK in fetal red cells may also be relatively insensitive to pH activation.

Fetal hemoglobin does not interact well with 2,3-DPG, unlike adult hemoglobin. Fetal anemia does not cause an increase in the concentration of 2,3-DPG whereas anemia and hypoxia regularly cause an increase in 2,3-DPG in the adult. As the infant matures, the %F decreases and the hemoglobin A concentration increases, causing the oxygen-hemoglobin equilibrium curve to normalize.

Measurement of Red Cell Enzyme Activity and Glycolytic Intermediates

It is ideal to evaluate an infant with a suspected red cell enzyme deficiency using standard techniques to measure all of the red cell glycolytic, HMP shunt, and other enzymes and all of the red cell glycolytic intermediates. Problems can arise, however, in obtaining the necessary quantities of blood to perform all of these studies. Cord blood is ideal for evaluating red cell enzyme activity since relatively large quantities can be obtained with no risk to the infant. Unfortunately, it is rare that a congenital enzyme deficiency is suspected at birth; thus, cord blood is either not available when diagnostic studies are to be performed or is clotted. In addition, the specialized techniques necessary to perform all of the enzyme assays are generally not available in routine laboratories. However, screening tests for G-6-PD, PK, TPI, GPI, GR, and NADPH-methemoglobin reductase are available and easily performed. The glutathione (GSH) level and glutathione stability test reflect the integrity of the HMP shunt and very low levels of GSH suggest glutathione synthetase or glutamyl-cysteine synthetase deficiency. Moderate reductions in GSH are found in G-6-PD, GPI deficiency, and in subjects with unstable hemoglobins. A normal screening test does not rule out an enzyme deficiency since PK kinetic mutants exist with normal activity and Blacks deficient in G-6-PD (A-) may have a normal screen during a hemolytic episode. Quantitation of enzyme activity in the latter instance will reveal normal G-6-PD activity when it should be elevated owing to the presence of a young red cell population.

The measurement of glycolytic intermediates and ATP may reveal a “crossover” point that will suggest a specific enzyme deficiency. In this manner, the number of enzymes assayed can be minimized. However, blood drawn for the analysis of glycolytic intermediates should be handled by experienced personnel. If blood is allowed to stand, the intracellular pH rises and an alteration in the pattern of glycolytic intermediates can occur with an increase in FDP, DHAP and G-3-P and fall in G-6-P and fructose-6-phosphate, owing to activation of PFK. For these reasons, the blood must be drawn as quickly as possible, preferably without a tourniquet, and must be deproteinized immediately at the bedside.
a task that is not easily performed on a newborn infant.

However, the measurement of the red cell 2,3-DPG concentration can help clarify the site of the enzymatic defect. A decrease in 2,3-DPG suggests a block in glycolysis at, or prior to, the 2,3-DPGM step. An increased 2,3-DPG concentration is suggestive of a defect at the level of, or distal to the PGK reaction. This assay can be easily performed using a micro-technique obtaining 0.1 ml of blood by heel puncture. Red cell ATP concentration is variable, but a markedly elevated concentration in a hemolytic disorder is suggestive of pyrimidine-5'-nucleotidase deficiency. The rise in ATP is spurious, however, since it is pyrimidine nucleotides that are truly increased. It must be remembered to relate values obtained for red cell enzymes and intermediates to the unique metabolic characteristics previously described in cord blood persist beyond the immediate neonatal period.

References


