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Red Cell Glycolytic Intermediates and Adenosine Triphosphate in Preterm Infants on the First Day of Life

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ABSTRACT. Red cell glycolytic intermediates and ATP were evaluated in 47 appropriate for gestational age preterm infants on the 1st day of life who were divided into three groups on the basis of gestational age: 28–30, 31–33, and 34–36 wk. The results were compared to those previously obtained in term infants. The concentrations of glucose-6-phosphate, total triose phosphates, and ATP were significantly higher than in term infants but appeared to be appropriately elevated for the young mean age of the red cell population. The concentration of red cell 2,3-diphosphoglycerate (2,3-DPG) was significantly decreased when compared to term infants and was lowest at 28–30 wk gestation. The content of red cell 3-phosphoglycerate was increased in term infants and was inappropriately elevated for the age of the red cell population at 28–30 wk gestation. This pattern of glycolytic intermediates was suggestive of a young red cell population metabolizing at an increased glycolytic rate with increased flow through the phosphoglycerate kinase step rather than the 2,3-DPG bypass in "normal" preterm infants. Two preterm infants of 28–30 wk gestation with low red cell intracellular pH were also evaluated and had markedly decreased concentrations of red cell 2,3-DPG and ATP and all phosphorylated intermediates distal to the phosphofructokinase reaction, indicative of a cross-over at the phosphofructokinase step secondary to acidosis. These studies demonstrate that the "normal" preterm infant has a decreased concentration of red cell 2,3-DPG in the steady state and in the presence of acidosis additional red cell metabolic perturbations occur which lead to a further fall in red cell 2,3-DPG and a decrease in the concentration of red cell ATP. (*Pediatr Res* 19: 117–121, 1985)

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Abbreviations

PFK, phosphofructokinase
G-6-P, glucose-6-phosphate
2,3-DPG, 2,3-diphosphoglycerate
TTP, total triose phosphates
P_i, inorganic phosphorus
AGA, appropriate for gestational age
F-6-P, fructose-6-phosphate
3-PG, 3-phosphoglycerate
2-PG, 2-phosphoglycerate
PEP, phosphoenolpyruvate
RBC, red blood cells
PK, pyruvate kinase
DPGM, diphosphoglycerate mutase
PGK, phosphoglycerate kinase

The pattern of glycolytic enzymes and intermediates in newborn red cells differs from that observed in subjects with a red cell population of a similar mean age (2, 4, 9, 10, 14, 15, 19, 31, 35). Metabolically, these cells appear to consume less glucose

than would be predicted for red cells of such a young mean cell age (22). The relative deficiency of the regulatory enzyme, PFK, that is characteristic of neonatal red cells, has been proposed as a possible cause of this metabolic handicap (19). It has also been suggested that there is a "fetal" PFK isozyme (11, 12, 34) in cord blood, and this isozyme is more inhibited by ATP (11) and may be less sensitive to pH activation (14) than the enzyme from adult red cells. Our laboratory has demonstrated that PFK has increased *in vivo* lability in cord blood (28), suggesting that the PFK deficiency in newborn red cells may be secondary to normal synthesis of an unstable enzyme.

It would be expected that the pattern of glycolytic intermediates might yield the most useful information regarding metabolic events at the *in vivo* level and the possible significance of the decreased enzyme activity and altered kinetic properties of PFK because the concentration of glycolytic intermediates at any particular time may reflect enzymatic flux, *in vivo*, according to the "cross-over theorem" of Chance *et al.* (3).

Red cell glycolytic intermediates have previously been evaluated in our laboratory in term infants (29). The concentration of G-6-P was elevated out of proportion to the age of the red cell population on the 1st day of life, continued to increase and peaked at 3–4 wk of age, at a time when the concentrations of red cell TTP and 2,3-DPG and the activity of PFK were similar to those observed at birth. Furthermore, the concentration of G-6-P correlated significantly with the plasma P_i concentration (20). These data suggested that the relative block in glycolysis at the PFK step and resulting pattern of glycolytic intermediates in term infants was not secondary to decreased PFK activity alone and that altered kinetic properties and/or modulation of PFK activity by extracellular factors such as P_i may also play a significant role at the *in vivo* level.

It has previously been reported that the concentration of G-6-P is higher and 2,3-DPG is lower in preterm than in term infants suggestive of a more profound block in glycolysis at the PFK step in the preterm infant (19), but our laboratory has recently demonstrated higher PFK activity in premature than in term infants and no significant differences in PFK activity between preterm infants of 28–30 and 34–36 wk gestation (S. F. Travis, L. Sacks, S. Kumar, M. Delivoria-Papadopoulos, unpublished data). Thus, glycolytic intermediates were measured in 49 preterm infants who were divided into three groups on the basis of gestational age: 28–30, 31–33, and 34–36 wk in order to investigate the possible influence of the maturational age of the infant on the pattern of glycolytic intermediates. These results were compared to those previously obtained in term infants and subjects with a young red cell population (29) in order to evaluate further the possible relationship between the gestational age of the infant and red cell metabolism and the influence, if any, of differences in red cell enzyme activity on the pattern of glycolytic intermediates.

MATERIALS AND METHODS

Glycolytic intermediates were evaluated in 49 preterm infants on the 1st day of life. They were all AGA and were divided into three groups based on gestational age as determined by Dubowitz score (6): 28–30, 31–33, and 34–36 wk, containing 12, 22, and 15 infants, respectively. Infants were studied as they became available which accounts for the different numbers of subjects in each group.

Extracts for glycolytic intermediates and ATP were prepared at the bedside: 2 ml of heparinized blood were immediately pipetted into 4 m of chilled 2 N perchloric acid, reextracted and neutralized by methods previously described (29). Blood was obtained via venipuncture or through umbilical catheters when samples were being taken for other purposes. Informed consent was obtained. Cord blood and blood obtained without a steady flow were deemed unsuitable due to changes that can occur in the pattern of glycolytic intermediates when blood cannot be obtained and processed fairly rapidly.

Aliquots for determination of fetal Hb (27) and intracellular and extracellular pH (1) were removed when an adequate sample was obtained and rapidly processed. A Hb and hematocrit were obtained on the remaining blood which was then centrifuged; the plasma was removed, recentrifuged, and frozen for later determination of P_i (8). Intracellular pH was obtained in 26 infants; P_i was determined on 42 samples; seven were obviously hemolyzed and were not evaluated. 2,3-DPG was determined spectrophotometrically by the Schroter and Heyden (26) modification of the technique of Krimsky (16). ATP was analyzed spectrophotometrically (17) by means of yeast HK (BMC Corp., Indianapolis, IN) and G-6-PD (BMC Corp.). Fructose diphosphate, glyceraldehyde-3-phosphate, and dihydroxyacetone phosphate were determined simultaneously as described by Keitt (13) and are referred to as TTP. G-6-P, F-6-P, 3-PG, 2-PG, and PEP were determined with modifications of the assay conditions described by Lowry *et al.* (18). G-6-P, F-6-P, TTP, 3-PG, 2-PG, and PEP were assayed fluorometrically with the use of an Eppendorf fluorometer (Brinkman Instruments) with a primary filter of 313 ± 366 nm and a secondary filter of 400 ± 3000 nm. PK and fetal Hb (%F) data were previously evaluated (S.F. Travis, L. Sacks, S. Kumar, M. Delivoria-Papadopoulos, unpublished data).

In these studies, as in prior studies in term infants (29), PK activity was used as an index of red cell age in order to compare results obtained in infants with appropriately age-matched controls. PK was believed to be a valid parameter of red cell age since PK from cord red cells in term infants had been demonstrated in our laboratory (28) to bear the same relationship between red cell age and density that had previously been demonstrated in red cells from adults (24, 25). In prior studies (29) there was wide variation in the mean age of the red cell population in subjects with similar degrees of reticulocytosis. They were thus divided into two groups based on PK activity: group I with a range of PK activity from 333.9 to 479.3 units/100 ml RBC representing a "moderately young" red cell population, and group II, that ranged from 518.4 to 820.9 units/100 ml RBC which represented a population of red cells with a very young mean cell age. By dividing subjects with reticulocytosis in this manner, it was believed that more accurate "age-matched" comparisons could be made between subjects with a young mean red cell age and term newborns.

Results obtained in preterm infants were compared to those previously obtained in term infants, normal subjects, and subjects with a young red cell population (29). Data were analyzed using the one-way analysis of variance (F statistic).

RESULTS

G-6-P, F-6-P, and TTP (Table 1). The concentrations of G-6-P and TTP were significantly higher in premature infants than in term infants at 28–30, 31–33, and 34–36 wk gestation. The increase in F-6-P was not statistically significant. There was no significant difference between the concentrations of G-6-P, F-6-P, or TTP at 28–30 and 34–36 wk gestation. The concentrations of G-6-P, F-6-P, and TTP were significantly higher than in subjects with a moderately young red cell population (group I) but not when compared to subjects with a very young red cell population (group II).

2,3-DPG and ATP (Table 1). 2,3-DPG was significantly lower in preterm than in term infants at 28–30 and 31–33 wk but not at 34–36 wk gestation. The mean concentration of 2,3-DPG tended to decrease as the prematurity of the infants increased but those differences did not reach statistical significance.

In contrast, the mean red cell ATP concentration was higher in preterm infants than in term infants. This increase was significant at 28–30, and at 31–33 wk, but not at 34–36 wk gestation. Mean ATP concentration was higher at 28–30 than at 34–36 wk gestation but the difference was not significant. The ATP concentration at 28–30 wk was significantly higher than group I, but not group II.

Table 1. *Glycolytic intermediates and ATP (mean ± SD) (nmol/ml RBC)*

		Subjects with reticulocytosis			Premature infants, day 1–2 gestational age (wk)		
	Normal adults	Group I	Group II	Term infants Day 1	28–30	31–33	34–36
(*n*)	(10)	(10)	(10)	(10)	(12)	(22)	(15)
PK (units)	240.8 ± 42.3	392.7 ± 51.2	665.4 ± 112.6	385.6 ± 57.6	536.9 ± 122.2	481.8 ± 93.5	474.2 ± 69.8
(*n*)				(10)	(10)	(10)	(10)
G-6-P	25.4 ± 3.2	36.3 ± 7.4	66.5 ± 23.1	53.3 ± 8.6	77.3 ± 11.4[a, **, c, **, d, †]	72.3 ± 18.4[a, **, c, **, d, †]	75.7 ± 20.1[a, **, b, †, c, **, d, †]
F-6-P	7.3 ± 1.5	10.4 ± 1.9	19.5 ± 9.0	16.6 ± 3.1	18.3 ± 6.3[a, †, c, **, d, †]	17.2 ± 5.4[a, †, c, **, d, †]	19.7 ± 7.8[a, †, b, †, c, **, d, †]
TTP	9.1 ± 3.0	12.7 ± 2.6	22.9 ± 5.3	19.2 ± 6.9	27.7 ± 10.8[a, *, c, **, d, †]	27.7 ± 11.3[a, *, c, **, d, †]	29.4 ± 10.8[a, *, b, †, c, **, d, †]
2,3-DPG	4423 ± 1907	4586 ± 529	5052 ± 602	4691 ± 383	3567 ± 618[a, **]	3714 ± 864[a, **]	4105 ± 919[a, †, b, †]
3-PG	52.3 ± 6.4	51.2 ± 8.5	55.5 ± 7.5	58.1 ± 7.0	68.0 ± 10.2[a, *, c, **, d, **]	62.5 ± 14.8[a, †, c, *, d, †]	64.1 ± 13.6[a, †, b, †, c, **, d, †]
2-PG	4.60 ± 1.3	6.97 ± 2.36	9.65 ± 3.24	6.78 ± 3.44	4.59 ± 1.25[a, *]	4.61 ± 1.83[a, *]	4.89 ± 1.74[a, †, b, †]
PEP	14.1 ± 2.0	17.0 ± 2.1	19.9 ± 3.4	13.7 ± 4.7	12.3 ± 1.6[a, †]	10.5 ± 3.3[a, *]	9.7 ± 2.1[a, *, b, **]
ATP	1024 ± 100	1147 ± 222	1320 ± 231	1056 ± 144	1365 ± 220[a, **, c, *, d, †]	1212 ± 230[a, *, c, †, d, †]	1263 ± 307[a, †, b, †, c, †, d, †]
Pi (mg/dl)	4.2 ± 0.9			5.95 ± 0.75	4.65 ± 1.18[a, *]	4.79 ± 0.872[a, **]	4.76 ± 0.561[a, **, b, †]
(*n*)				(8)	(12)	(15)	(15)
Intracellular pH	7.170 ± 0.007			7.156 ± 0.019	7.192 ± 0.070[a, †]	7.150 ± 0.083[a, †]	7.166 ± 0.035[a, †]
(*n*)				(4)	(10)	(9)	(7)
Fetal Hb (%)				65.6 ± 17.0	82.2 ± 3.4	81.8 ± 3.5	78.2 ± 6.2[b, †]
(*n*)				(10)	(10)	(10)	(10)

(*n*) refers to the number of subjects in each group.
[a] Premature *vs* term infants.
[b] 28–30 *vs* 34–36 wk.
[c] Premature infants *vs* group I.
[d] Premature infants *vs* group II.
** $p \leq 0.005$.
* $p \leq 0.05$.
† Not significant ($p > 0.05$).

Table 2. *Red cell glycolytic intermediates* in two premature infants with a low red cell intracellular pH*

Gestational age	A 28–30 wk	B 28–30 wk	Controls† 28–30 wk (mean ± SD)
G-6-P	65.8	60.2	77.3 ± 11.4
F-6-P	19.5	10.8	18.3 ± 6.3
TTP	5.3	6.0	27.7 ± 10.8
2,3-DPG	2281	1882	3567 ± 618
3-PG	46.4	20.6	68.0 ± 10.2
2-PG	2.2	4.2	4.59 ± 1.25
PEP	8.0	7.2	12.3 ± 1.6
ATP	650	750	1365 ± 220
Intracellular pH	6.866	7.010	7.192 ± 0.070
PK activity (units/100 ml RBC)	479.4	556.5	536.9 ± 122.2

* nmol/ml RBC.
† $n = 12$.

3-PG, 2-PG, and PEP. The mean concentration of red cell 3-PG was higher in preterm than in term infants, but this increase was only statistically significant at 28–30 wk gestation, not at 31–33 or 34–36 wk gestation. Red cell 3-PG was significantly higher than group I at 28–30, 31–33, and 34–36 wk gestation. The 3-PG concentration was also significantly higher than group II at 28–30 wk, but not at 31–33 or 34–36 wk gestation. There was no statistical difference in the 3-PG concentration between 28–30 and 34–36 wk or 28–30 and 31–33 wk gestation.

The concentration of red cell 2-PG was significantly higher in term than in preterm infants of 28–30 and 31–33 wk but not at 34–36 wk gestation. There was no significant difference in the 2-PG concentration between 28–30 and 31–33 wk or 28–30 and 34–36 wk gestation.

The PEP concentration was significantly lower in preterm than in term infants at 34–36 and 31–33 wk but not at 28–30 wk gestation. There was a statistically significant difference in the PEP concentration between 28–30 and 34–36 wk, but not between 28–30 and 31–33 wk and 31–33 and 34–36 wk gestation.

P_i and intracellular pH (Table 1). Mean P_i was lower in preterm than in term infants and this difference was significant at 28–30, 31–33, and at 34–36 wk, but there was not a significant difference between 28–30 and 34–36 wk gestation.

Intracellular pH was not significantly different in preterm and term infants.

Red cell glycolytic intermediates in two preterm infants with a low red cell intracellular pH (Table 2). Samples from two infants of 28–30 wk gestation with low red cell intracellular pH of 6.866 and 7.010 were inadvertently obtained. The concentrations of red cell 2,3-DPG and ATP and all phosphorylated intermediates distal to the PFK reaction (TTP, 3-PG, 2-PG, and PEP) were markedly decreased. The levels of red cell G-6-P and F-6-P were nearly normal for the age of the red cell population (as estimated using red cell PK activity as an index of mean red cell age).

DISCUSSION

Analysis of glycolytic intermediates in preterm infants of 28–30, 31–33, and 34–36 wk gestation has revealed a significantly increased concentration of G-6-P and TTP when compared to term infants and subjects with a moderately young red cell population (group I), but the increased mean concentrations of G-6-P and TTP were not statistically significant when compared to subjects with a very young red cell population (group II). PK activity is also significantly increased in preterm infants (32) and it is likely that the increased concentrations of G-6-P and TTP in preterm infants are consistent with a red cell population of a younger mean red cell age.

The mean concentrations of red cell 3-PG and ATP were also higher in preterm than in term infants, but this increase was significant only in the most premature infants (28–30 wk gestation). The ATP concentration in preterm infants of 28–30 wk gestation was significantly higher than in group I, but not group II, whereas the 3-PG concentration was significantly higher in all three groups of preterm infants than in group I and was also significantly greater than group II at 28–30 wk gestation. These data suggest that the concentration of red cell 3-PG was elevated out of proportion to the age of the red cell population. The concentrations of red cell ATP and 3-PG were highest in the most premature infants although the differences between 28–30 and 34–36 wk gestation were not statistically significant.

In contrast, the mean concentration of red cell 2,3-DPG was lower in preterm than in term infants; this decrease was significant at 28–30 and 31–33 wk gestation but not at 34–36 wk. Mean 2,3-DPG tended to be lowest in the most immature infants, but differences in the red cell 2,3-DPG concentration at 28–30 and 34–36 wk gestation did not reach statistical significance.

Although there were no significant differences in the concentrations of red cell 2,3-DPG, ATP, and 3-PG between 28–30 and 34–36 wk gestation, the most significant changes occurred at 28–30 wk gestation (*i.e.* increased concentrations of 3-PG and ATP and decreased 2,3-DPG).

The finding of an increased concentration of red cell G-6-P, F-6-P, TTP, 3-PG, and ATP in association with a decreased concentration of 2,3-DPG and a young mean red cell age appears to be most compatible with red cells metabolizing at an increased glycolytic rate with increased flow through the PGK reaction, which is an ATP generating step, rather than the 2,3-DPG bypass. A relative block in glycolysis at the PFK reaction could also contribute to the decreased concentration of 2,3-DPG and increased concentration of G-6-P as previously proposed (19), but the concentration of TTP in premature infants was significantly increased, whereas a decrease in the TTP concentration would be anticipated if there were a cross-over at the PFK reaction. Thus, the relative deficiency of PFK known to exist in newborns would be an unlikely contributing factor to the decreased concentration of red cell 2,3-DPG observed in preterm infants. The markedly increased activity of PGK may have contributed to the elevated 3-PG concentration, but the 3-PG concentration was higher in the red cells of preterm infants than in term infants, despite similar PGK activity, which suggests that increased PGK activity alone was not responsible for the increased concentration of 3-PG found in the red cells of preterm infants.

Alternatively, it could be postulated that the increased concentration of 3-PG was secondary to a metabolic block at or distal to the mutase step, especially since the concentrations of 2-PG and PEP were decreased. A metabolic block distal to the 2,3-DPG bypass, however, should result in an increase in the 2,3-DPG concentration, not the decrease that was observed. In addition, the activities of red cell enolase and pyruvate kinase have been measured in our laboratory (S.F. Travis, L. Sacks, S. Kumar, M. Delivoria-Papadopoulos, unpublished data) and both enzymes are significantly increased in preterm infants.

It has been demonstrated that red cells from neonates have greater 2,3-DPG instability (21, 33, 36) than red cells from adults *in vitro* when incubated under identical conditions. Thus, 2,3-DPG instability could also conceivably have contributed to the decreased 2,3-DPG concentration and increase in the concentrations of 3-PG and ATP. This 2,3-DPG instability was believed to be secondary to increased 2,3-DPG breakdown by Trueworthy and Lowman (33) and Oski and Komazawa (21), but Zipursky *et al.* (36) believed that it was due to decreased 2,3-DPG synthesis. Decreased 2,3-DPG synthesis in neonatal red cells has been previously demonstrated *in vitro* (23). Maximal stimulation of red cell glucose consumption at pH 8.2 resulted in a greater increase in red cell 2,3-DPG in adults and subjects with a young red cell population than in neonates, suggestive of decreased flow through the 2,3-DPG bypass. It has also been reported (20) that red cells from adults, but not neonates, demonstrated a rapid increase in 2,3-DPG when incubated under nitrogen, although red cell glycolysis was stimulated in both adults and newborns.

It was believed that the affinity of 2,3-DPG for deoxy fetal Hb was insufficient to facilitate the generation of additional 2,3-DPG, whereas in the adult, 2,3-DPG preferentially binds deoxy Hb A, thus relieving the end product inhibition of DPGM by 2,3-DPG and allowing further synthesis of 2,3-DPG. It was suggested that under circumstances of hypoxia, neonatal red cells may have preferential flow through the PGK reaction whereas metabolism in red cells from adults flows preferentially through the DPGM reaction (7).

The pattern of red cell glycolytic intermediates obtained in the present study suggests that similar mechanisms may be operative at the *in vivo* level as well, and that the higher fetal Hb concentration present in preterm infants, which tends to increase with decreasing gestational age, may have led to preferential flow through the PGK reaction rather than the 2,3-DPG bypass, resulting in the observed decrease in the concentration of red cell 2,3-DPG and increase in red cell 3-PG and ATP.

P_i and intracellular pH were also evaluated in preterm infants on the 1st day of life since acidosis can result in a decrease in 2,3-DPG (5) and increased P_i was correlated with an elevated red cell G-6-P concentration in term infants (30). P_i was slightly higher than in adults but was lower than in term infants; intracellular pH was normal, suggesting that P_i and intracellular pH do not contribute to the differences observed in the pattern of glycolytic intermediates in "normal" preterm infants on the 1st day of life. However, samples from two preterm infants of 28–30 wk gestation with a very low intracellular pH of 6.87 and 7.01 were inadvertently obtained and evaluated. They were found to have markedly decreased levels of 2,3-DPG, ATP and all phosphorylated intermediates distal to the PFK reaction and near normal levels of G-6-P and F-6-P. This pattern of glycolytic intermediates revealed a cross-over at the PFK reaction and represents an *in vivo* demonstration of decreased glycolysis at low pH secondary to inhibition of PFK activity.

Thus, our studies of red cell glycolytic intermediates in preterm infants were believed to be most compatible with a young red cell population and an increased glycolytic rate (leading to an increased concentration of G-6-P, F-6-P, and TTP) with preferential flow through the PGK reaction resulting in a significantly decreased concentration of 2,3-DPG and an increase in 3-PG and ATP in normal preterm infants. It is possible that decreased 2,3-DPG stability may also have contributed to the decrease in 2,3-DPG and increase in the 3-PG and ATP concentrations. These changes were most marked at 28–30 wk gestation. Red cell PFK did not appear to contribute significantly to the decreased concentration of red cell 2,3-DPG in "normal" preterm infants. However, two acidotic infants had markedly decreased concentrations of 2,3-DPG and ATP and all phosphorylated intermediates distal to the PFK reaction secondary to inhibition of PFK activity at low pH.

It is apparent from these studies of red cell glycolytic intermediates that the "normal" preterm infant has a decreased concentration of 2,3-DPG secondary to either decreased synthesis of 2,3-DPG and/or increased 2,3-DPG instability and the level of 2,3-DPG can decrease further in the presence of hypoxemia and/or acidosis. This is particularly pertinent in the smallest preterm infants who not only have a high fetal Hb, lower concentration of red cell 2,3-DPG, and more left shifted oxygen-hemoglobin dissociation curve than the term infant under "normal" circumstances but are also at greatest risk for developing respiratory distress syndrome, infections, and other problems which are associated with hypoxia and acidosis which may then lead to further red cell metabolic perturbations.

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