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age-dependent enzymes
erythrocyte
glucose-6-phosphate
metabolism
non-age-dependent enzymes

Red Cell Metabolic Alterations in Postnatal Life in Term Infants: Possible Control Mechanisms

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Summary

Red cell glycolytic intermediates and enzymes in term infants in the first year of life were correlated with the fetal hemoglobin concentration (%F), intra- and extracellular venous pH, plasma inorganic phosphorus (P_i) and pyruvate kinase (PK) activity. Changes in the non-age-dependent enzymes phosphoglycerate kinase, enolase, and phosphofructokinase correlated most significantly with the postnatal decline in %F ($P < 0.001$), not the age of the red cell population, as reflected in PK activity. The age-dependent enzymes, hexokinase and glucose-6-phosphate dehydrogenase, however, correlated well with PK activity ($P < 0.001$). The concentration of glucose-6-phosphate did not correlate significantly with the postnatal decline in %F ($P > 0.05$) or PK ($P > 0.10$), but correlated significantly with the plasma P_i concentration ($P < 0.001$). "Total triose phosphate" and 2,3-diphosphoglycerate did not correlate with P_i.

It appears from these studies that an extracellular factor, P_i, alters the pattern of glycolytic intermediates in term infants and that the postnatal changes in phosphoglycerate kinase, enolase, and phosphofructokinase are unique to the "fetal" red cell and reflect passage from fetal to "adult" erythropoiesis.

Speculation

It is proposed that the rise in the glucose-6-phosphate concentration in red cells from term infants previously reported is secondary to a combination of stimulation of hexokinase activity by plasma inorganic phosphorus and a relative block in glycolysis at the phosphofructokinase (PFK) step secondary to both decreased enzyme activity and decreased activation of PFK by plasma inorganic phosphorus. It is speculated from these studies that the relative block in glycolysis at the PFK step previously described in term infants is probably greater at the *in vivo* level than that predicted from enzyme activity under optimal *in vitro* conditions.

Prior studies of red blood cell enzymes in term infants on the first day of life have revealed that phosphoglycerate kinase (PGK) and enolase (ENO) activities are elevated out of proportion to the age of the red cell population and phosphofructokinase (PFK) activity is decreased when compared to both red cells of a similar mean age and those obtained from normal adults (11, 15). Recent studies in our laboratory have revealed that the activities of these enzymes remained relatively unchanged until 8 to 9 wk of life when there was a decrease in PGK and ENO levels and an initial rise in PFK activity (22). This occurred at a time when the two most age-dependent enzymes studied, hexokinase (HK) and pyruvate kinase (PK), transiently increased in value, which was consistent with active erythropoiesis by the infants' bone marrow and suggested that the developmental changes in PGK, ENO, and PFK during the first year of life represented passage from "fetal" to "adult" erythropoiesis.

Analysis of red cell glycolytic intermediates (21), however, revealed that the concentration of glucose-6-phosphate (G-6-P) continued to increase from day 1 to wk 3 to 4 despite PFK activity that remained essentially unchanged (22). This pattern of glycolytic intermediates was suggestive of a block in glycolysis at the PFK step that was modulated by factors other than enzyme activity alone.

It has been well established that changes in the extracellular concentration of inorganic phosphorus (P_i) can profoundly affect the metabolism of the human red cell (8, 12–14, 16, 17, 19, 23). Both hypo- (8, 13, 16, 23) and hyperphosphatemia (12, 14–17, 19) have been correlated with changes in the glycolytic rate and the pattern of red cell glycolytic intermediates and adenosine triphosphate (ATP) both *in vitro* and *in vivo*. The pattern of these changes in the intermediates is dependent on the pH of the medium (14). Because hyperphosphatemia is common in the newborn period (1, 3, 4, 7, 24) and acidosis has been reported, it would appear that both the plasma P_i concentration and intracellular pH may modify the metabolism and, hence, the pattern of glycolytic intermediates in neonatal red cells.

Thus, red cell intracellular and extracellular pH and the plasma P_i concentration were determined to evaluate the influence of these environmental (plasma) factors on red cell metabolism in the first year of life. In addition, red cell glycolytic intermediates and enzymes were contrasted with both the fetal hemoglobin concentration, to ascertain if the developmental changes correlated with the postnatal decline of fetal hemoglobin and are thus unique characteristics of the fetal erythrocyte, and the age-dependent enzyme, PK, because the level of several red cell glycolytic intermediates and enzymes is increased in young red cells and alterations observed may be a reflection of changes in the age of the red cell population.

MATERIALS AND METHODS

Blood was obtained from term infants on days 1 and 4; wk 3 to 4, 8 to 9, and 14 to 16; and months 5 to 6, 8 to 9, and 11 to 12 of life in tubes containing dried sodium heparin (Vacutainer; Becton-Dickinson and Co., Rutherford, NJ). There were 10 infants in each group. Extracts for glycolytic intermediates were precipitated immediately, neutralized, and assayed as previously described (15). Aliquots for determination of fetal hemoglobin (20) and intracellular and extracellular pH were removed when an adequate sample was obtained and rapidly processed. A hemoglobin 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 (6). The packed red cells were washed, filtered, and assayed for red cell glycolytic enzymes and glucose-6-phosphate dehydrogenase (G-6-PD) by methods previously described (22). Fetal hemoglobin determinations (%F) were obtained in 67 infants. Plasma P_i was obtained on all 80 infants evaluated in the study; 73 were used for P_i determination; seven were obviously hemolyzed specimens and were not evaluated.

Intracellular and extracellular pH were determined on venous blood by a freeze-thaw method (2). Detailed analysis of the glycolytic enzyme and intermediate data obtained in this study have been reported previously (21, 22).

Data were evaluated using standard linear regression analysis. The graphs were plotted using a Hewlett-Packard HP9830, and the data points were keyed in using a Hewlett-Packard programmable calculator. The data were checked using a TI-59 (Texas Instrument), and the correlation coefficients (r), slopes, and y intercepts were identical with both programs.

RESULTS

RED BLOOD CELL GLYCOLYTIC ENZYMES AND G-6-PD

PK activity was used as an index of red blood cell age in these studies. There was a significant positive correlation between the activities of the age-dependent enzymes, PK and HK ($n = 67$; $r = 0.39$; $P < 0.001$; Fig. 1) and PK and G-6-PD ($n = 67$; $r = 0.50$; $P < 0.001$; Fig. 2) in the first year of postnatal life. In contrast, in the 67 samples evaluated, there was not a significant correlation between PK and ENO levels ($r = 0.17$; $P > 0.1$) and PK and PFK activities ($r = 0.23$; $P > 0.05$) and a less significant correlation between PK and the activity of phosphoglycerate kinase (PGK) ($r = 0.26$; $P < 0.05$). ENO, PGK, and PFK activities correlated best with the postnatal decline in the %F ($P < 0.001$, Figs. 3 to 5), whereas PK activity did not correlate with the postnatal decline in the %F ($n = 67$; $r = -0.12$; $P > 0.10$).

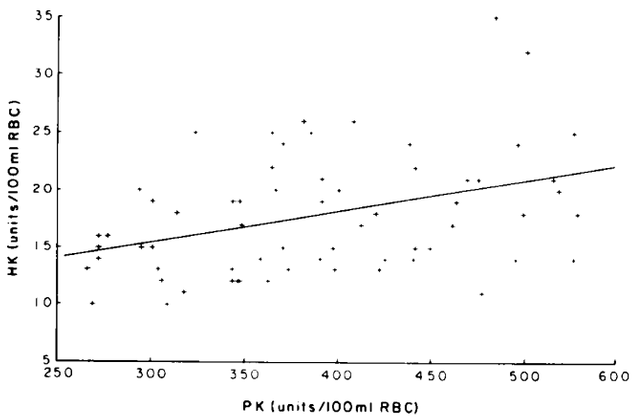

Fig. 1. Relationship between red blood cell PK and HK activities ($n = 67$; $r = 0.39$; $P < 0.001$).

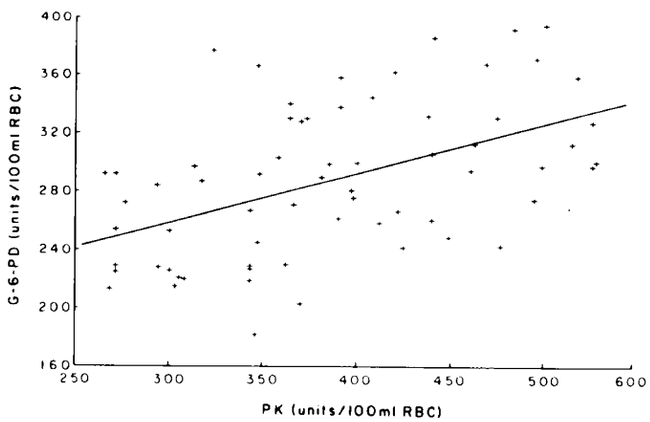

Fig. 2. Relationship between red blood cell PK and G-6-PD activities ($n = 67$; $r = 0.50$; $P < 0.001$).

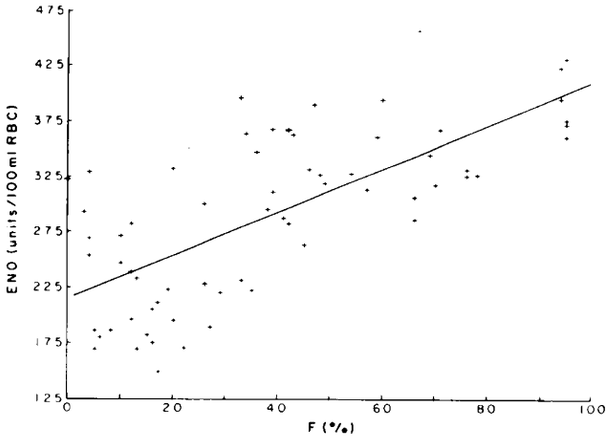

Fig. 3. Relationship between red blood cell ENO activity and %F ($n = 67$; $r = 0.70$; $P < 0.001$).

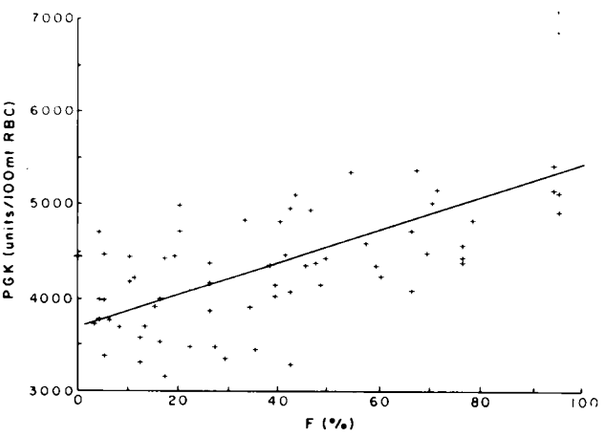

Fig. 4. Relationship between red blood cell PGK and %F ($n = 67$; $r = 0.66$; $P < 0.001$).

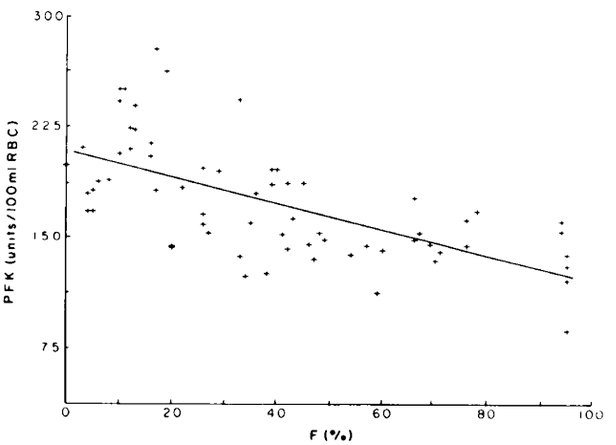

Fig. 5. Relationship between red blood cell PFK and %F ($n = 67$; $r = -0.64$; $P < 0.001$).

INTRACELLULAR AND EXTRACELLULAR pH AND PLASMA P_i (TABLE 1)

Intracellular and extracellular venous pH were similar to those obtained from normal adults in term infants from days 1 to 11 to 12 months of life.

Plasma P_i was elevated on day 1 of life, continued to increase, and peaked at 3 to 4 wk of age at a mean concentration of 9.69

Table 1. *Plasma Pi and intracellular and extracellular venous pH*

	Normal adults	Term infants									
		Day 1	Day 4	3–4 wk	8–9 wk	14–16 wk	5–6 mos.	8–9 mos.	11–12 mos.
Pi									
n	10	8	9	9	10	8	10	10	9
Mean ± S.D.	4.2 ± 0.9	5.95 ± 0.75	8.84 ± 1.27	9.69 ± 1.74	6.35 ± 1.38	7.39 ± 1.87	6.10 ± 0.94	6.57 ± 1.49	6.37 ± 1.67
pH									
n	10	4	4	5	7	8	9	9	5
Extracellular Mean ± S.D.	7.345 ± 0.008	7.348 ± 0.022	7.351 ± 0.021	7.341 ± 0.036	7.344 ± 0.024	7.329 ± 0.025	7.330 ± 0.025	7.357 ± 0.028	7.364 ± 0.028
Intracellular Mean ± S.D.	7.170 ± 0.007	7.156 ± 0.019	7.134 ± 0.024	7.144 ± 0.034	7.161 ± 0.040	7.127 ± 0.070	7.143 ± 0.030	7.165 ± 0.034	7.143 ± 0.038

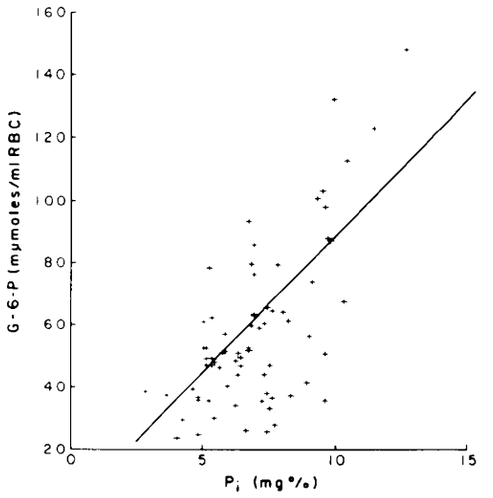

Fig. 6. Relation of red blood cell G-6-P concentration to plasma P_i ($n = 73$; $r = 0.66$; $P < 0.001$).

± 1.74 mg %. Plasma P_i decreased after 3 to 4 wk, but remained elevated throughout the first year of life.

RED BLOOD CELL GLYCOLYTIC INTERMEDIATES

There was a significant positive correlation between the concentration of red cell G-6-P and the plasma P_i concentration in the first year of postnatal life in the 73 samples evaluated ($r = 0.66$; $P < 0.001$; Fig. 6), and no correlation with PK activity ($r = 0.16$; $P > 0.1$). In contrast to red cell PGK, ENO, and PFK activities, there was not a significant correlation between the red blood cell concentration of G-6-P and the %F ($n = 67$; $r = 0.22$; $P > 0.05$). There was not a significant correlation between plasma P_i and %F in 67 samples evaluated ($r = 0.21$; $P > 0.05$).

In the 73 samples studied, there was not a significant correlation between the RBC concentration of "total triose phosphate" and plasma P_i ($r = 0.10$; $P > 0.10$) or the level of red blood cell 2,3-diphosphoglycerate (2,3-DPG) and plasma P_i ($r = 0.15$; $P > 0.10$) in the first year of postnatal life.

DISCUSSION

This study has demonstrated that the postnatal changes in the activities of the red cell enzymes PGK, ENO, and PFK correlate well with the postnatal decline in fetal hemoglobin, not the age of the red cell population, which suggests that developmental changes in these enzymes during the first year of life probably represent the passage from fetal to adult erythropoiesis and are unique to the fetal erythrocyte. This interpretation is in agreement with the prior finding that "old" red cells in cord blood from term infants had a high %F (9) and demonstrated to a marked degree those characteristics attributed to whole blood, namely decreased red cell PFK activity and increased activity of red cell PGK and ENO (10). These "older" red cells probably represent cells produced earlier in gestation. In contrast, the activities of the age-dependent enzymes HK and G-6-PD correlated well with PK activity and developmental changes in these enzymes during the first year of postnatal life reflect the age of the red cell population.

Plasma P_i was elevated the entire first year of life which has been reported previously (1, 3, 7). However, the mean values for plasma P_i on day 4 and 3 to 4 wk of age in the present study are significantly higher than those reported in the early literature (1, 3). This increase in mean plasma P_i is partially explained by five samples with plasma P_i levels of 10 mg % or higher (10, 10.4, 10.5, 11.5, and 12.7 mg %). In a more recent study (7), however, on the influence of diet on plasma P_i in the first wk of life, a range of 4 to 11 mg % at 2 days of age and 4 to 13 mg % at 3 to 8 days was reported in infants receiving evaporated milk and a range of 5 to

9 mg % was observed at 5 days of life in infants fed whole milk with vitamin D. These values are more comparable to those obtained in the present study, and it is possible that the higher plasma P_i values observed in our subjects are partially due to dietary differences. In addition, hemolysis of red cells can elevate plasma P_i. Samples that exhibited obvious hemolysis were not evaluated, but it is possible that mild hemolysis was present in the samples in which the plasma P_i was markedly increased. In any event, it is the relationship between plasma P_i and red cell G-6-P, not the absolute values of plasma P_i, that is relevant to this study.

The concentration of G-6-P correlated well with the plasma P_i level, not the age of the red cell population or the %F. The concentration of plasma P_i has been reported to influence the metabolism of red cells *in vivo* and *in vitro* (8, 12–14, 16, 17, 19, 23). An increase in the P_i concentration of the medium *in vitro* is associated with an increased glycolytic rate and an accumulation of triose phosphates and fructose diphosphate (total triose phosphate), 2,3-DPG, and ATP (12, 14, 17–19). These changes are dependent on the pH of the medium (14) and are greatest at pH 8.0, where the glycolytic rate is almost maximal. The increased glycolytic rate at pH 8.0 is associated with a fall in the concentration of G-6-P and an accumulation of total triose phosphate and other phosphorylated intermediates distal to the PFK step. At pH 7.0, the opposite occurs. The concentration of G-6-P increases, and there is no detectable increase in total triose phosphate. This pattern of glycolytic intermediates is attributed to activation of PFK activity at pH 8.0, secondary to release of ATP inhibition by P_i; at pH 7.0, there is stimulation of HK without enhancement of PFK activity by P_i due to the stong inhibition of PFK by ATP at low pH. At physiolgoic pH 7.4 *in vitro*, there appears to be balanced stimulation of HK and PFK with no accumulation of G-6-P (14, 19) and an increased concentration of total triose phosphates and other phosphorylated intermediates beyond the PFK step. The increase in total triose phosphates appears to be an *in vitro* artifact secondary to the inability of the red cell to maintain the ratio of oxidized to reduced pyridine nucleotides (NAD/NADH ratio) in the test tube (18). This can be prevented by adding pyruvate to the medium which is converted to lactate and restores the NAD/NADH ratio. This marked accumulation of total triose phosphate would not be anticipated at the *in vivo* level due to the availability of pyruvate in the plasma environment which is freely diffusable across the red cell membrane. At the *in vivo* level, studies of red cells from uremic patients with elevated serum P_i have revealed an increased concentration of 2,3-DPG and ATP; total triose phosphate and G-6-P were not evaluated.

It would thus be anticipated from *in vitro* and *in vivo* studies of the effects of P_i on red cell metabolism that increases in plasma P_i at physiologic pH should result in balanced stimulation of HK and PFK *in vivo* without any alteration in the concentration of G-6-P and an increase in the concentration of 2,3-DPG and ATP. Studies of term infants in this laboratory have demonstrated that despite a normal venous pH, the concentration of G-6-P was elevated out of proportion to the age of the red cell population at birth and continued to increase until 3 to 4 wk of age when it peaked at a value 2.5 times greater than that observed on day 1 of life. Total triose phosphate, 2,3-DPG, and ATP also increased between days 1 and 4 of life, but this increase was transient and by 3 to 4 wk of age had decreased to normal values for the age of the red cell population. The pattern of glycolytic intermediates at 3 to 4 wk was suggestive of a relative block in glycolysis at the PFK step, but PFK activity remained essentially unchanged from birth to 8 to 9 wk of age (22), suggesting factors other than enzyme activity alone were responsible for this "block."

The G-6-P level at any time reflects the balance between HK and PFK activities, and an increase in the concentration of G-6-P could result from increased activity of HK, unbalanced stimulation of HK and PFK (HK > PFK), a block in glycolysis at the PFK step, or combined mechanisms. We have previously demonstrated that HK activity decreases between day 4 and wk 3 to 4 and PFK activity remains fairly constant during this time period resulting in a decrease in the HK/PFK ratio (22). Thus, an increased level of HK is not responsible for the marked accumulation of G-6-P in the presence of increased plasma P_i.

The concentration of red cell G-6-P in term infants correlated significantly with the plasma P_i but the concentrations of total triose phosphate and 2,3-DPG did not. It is felt that the increase in the concentration of G-6-P was partially due to stimulation of HK activity secondary to release of G-6-P inhibition of HK by plasma P_i. The continued accumulation of G-6-P and lack of a sustained increase in the concentration of 2,3-DPG and ATP suggested a combination of imbalanced stimulation of HK and PFK (HK > PFK) by P_i in red cells from term infants and a relative block in glycolysis at the PFK step. This block at the PFK step is probably secondary to both a relative deficiency of PFK and an enzyme that is less sensitive to P_i stimulation than PFK from adults. The lack of correlation between plasma P_i and red cell 2,3-DPG in the present study is at variance with the previously reported finding by Card and Brain (4) of a statistically significant positive correlation ($P < 0.01$) between plasma P_i and red cell 2,3-DPG in children over one year of age. This difference in results is probably secondary to both the influence of elevated levels of fetal hemoglobin on the oxygen hemoglobin equilibrium curve in children less than 6 months of age (5) and the relative block in glycolysis at the PFK step secondary to decreased stimulation of PFK by P_i in red cells from term infants as discussed above. In Card and Brain's study (4), children less than a year of age were not evaluated. Thus, the effects of both fetal hemoglobin and fetal PFK on the concentration of red cell 2,3-DPG were not contributory factors.

It has previously been demonstrated that old red cells from cord blood which are presumably those produced early in gestation did not accumulate total triose phosphate when allowed to stand (10), whereas red blood cells from normal adults invariably demosntrated a marked increase in these compounds. The increase in total triose phosphate *in vitro* in adult cells is secondary to a rise in intracellular pH and activation of PFK, which is suggestive that PFK in fetal red blood cells is also relatively insensitive to pH activation. Kahn *et al.* (9) have demonstrated that fetal red blood cells are relatively deficient in the M-type of PFK and are more inhibited by ATP than PFK from adult red cells. This enhanced ATP inhibition of PFK might have functional significance at the *in vivo* level since it may be responsible for the apparent decreased activation of PFK by increased plasma P_i because P_i stimulates PFK activity in red cells from adults by counteracting ATP inhibition of the enzyme.

These studies suggest that the decreased glycolytic rate and other metabolic alterations observed in red cells from newborn infants may not only be secondary to decreased PFK activity as previously proposed (10, 15), but it is also probable that altered kinetic properties of the enzyme exist which may result in a greater "metabolic handicap" at the *in vivo* level than that predicted from enzyme activity alone.

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