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Fructose 2,6-bisphosphate and glucose 1,6-bisphosphate levels in erythrocytes with high and low 2,3-bisphosphoglycerate content during postnatal development

Carme Gallego and José Carreras

Departament de Ciències Fisiològiques Humanes i de la Nutrició, Unitat de Bioquímica, Facultat de Medicina, Universitat de Barcelona, Zona Universitària de Pedralbes, 08028 Barcelona, Spain

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In rabbit and sheep erythrocytes the concentrations of 2,3-bisphosphoglycerate, fructose 2,6-bisphosphate and glucose 1,6-bisphosphate suffer important changes after birth, which differ in both species. The changes of fructose 2,6-bisphosphate and glucose 1,6-bisphosphate correlate with the changes in the levels of the enzymatic activities involved in their synthesis. The change of 2,3-bisphosphoglycerate levels in rabbit but not in sheep erythrocytes could be explained by the changes of the phosphofructokinase/pyruvate kinase and 2,3-bisphosphoglycerate synthase/2,3-bisphosphoglycerate phosphatase activity ratios.

Bisphosphoglycerate, 2,3-; Fructose 2,6-bisphosphate; Glucose 1,6-bisphosphate; (Erythrocyte, Rabbit, Sheep)

1. INTRODUCTION

Fructose 2,6-bisphosphate (Fru-2,6-P_2) and glucose 1,6-bisphosphate (Glu-1,6-P_2), which have been implicated in the regulation of carbohydrate metabolism in liver and other tissues [1-3], could also play a relevant role in erythroid cells. We have already shown that the concentrations of both bisphosphorylated hexoses vary in chicken erythrocytes during development [4] and that the concentration of Fru-2,6-P_2 in rabbit erythroid cells decreases during differentiation [5]. We report now the postnatal changes of the levels of Fru-2,6-P_2, Glu-1,6-P_2 and their synthesizing enzymes in erythrocytes of the rabbit and the sheep, species which differ in the way the respiratory function of the blood is adapted to the transition from fetal to extrauterine existence. Rabbit erythrocytes, like rat, mouse and pig erythrocytes, possess hemoglobin with intrinsically high oxygen affinity. Adaptation to postnatal respiratory requirements is accomplished by increasing the concentration of 2,3-bisphosphoglycerate (2,3-BPG) which interacts with hemoglobin and decreases its affinity for oxygen [6-8]. In sheep, as in goat, embryonic hemoglobin is replaced first by fetal hemoglobin, which has intrinsically high oxygen affinity, and then by adult or pre-adult hemoglobins, which have low affinity for oxygen. In sheep, unlike in the rabbit, 2,3-BPG does not bind appreciably to either fetal or adult hemoglobin. However in lamb erythrocytes a transitory postnatal rise in 2,3-BPG occurs which decreases blood oxygen affinity by the Bohr effect until erythrocytes with adequate amounts of adult sheep hemoglobin are present [6,8-11].

Correspondence address: C. Gallego, Departament de Ciències Fisiològiques Humanes i de la Nutrició, Unitat de Bioquímica, Facultat de Medicina, Universitat de Barcelona, Zona Universitària de Pedralbes, 08028 Barcelona, Spain

2. MATERIALS AND METHODS

Enzymes and biochemical reagents were obtained from either Boehringer or Sigma. New Zealand White rabbits and domestic sheep were used. Blood was obtained from the external jugular vein of the newborn and adult sheep, by heart puncture of

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newborn rabbits and from the lateral ear vein of adult rabbits. The blood was drawn into 1 vol. of ice-cold 150 mM NaCl, containing 15 mM sodium citrate and 5 mM glucose, pH 7.2. Red blood cells were quickly washed 3 times with the same medium without citrate at 0–3°C.

Cell extracts were prepared as described in [4]. Methods previously reported were used to measure hemoglobin [16], 2,3-BPG [12], Fru-2,6-P_2 [5], aldohexoses 1,6-bisphosphate (glucose 1,6-P_2 + mannose 1,6-P_2) [13], 2,3-BPG synthase (BPGS) and 2,3-BPG phosphatase (BPGP) [14], 6-phosphofructo-2-kinase (PFK-2) [5], Glu-1,6-P_2 synthase [15], hexokinase (HK), 6-phosphofructo-1-kinase (PFK-1), phosphoglycerate mutase (PGM) and pyruvate kinase (PK) [16].

3. RESULTS AND DISCUSSION

As shown in fig.1, whereas the concentration of 2,3-BPG at birth is higher in rabbit erythrocytes than in sheep erythrocytes, the concentration of Fru-2,6-P_2 and aldohexoses 1,6-bisphosphate are similar in both species, being the levels of Fru-2,6-P_2 three orders of magnitude lower than those of aldohexoses 1,6-bisphosphate.

As reported by others [7,17,18], the concentration of 2,3-BPG in rabbit erythrocytes gradually increases during postnatal development, reaching an adult level after 30 days which is about 6 times greater than that of neonatal erythrocytes. The concentration of both Fru-2,6-P_2 and aldohexoses 1,6-bisphosphate also increases during postnatal life, although adult levels are only 2 times greater than those of neonatal red blood cells.

In agreement with the results of others [9–11,18], the concentration of 2,3-BPG in lamb erythrocytes

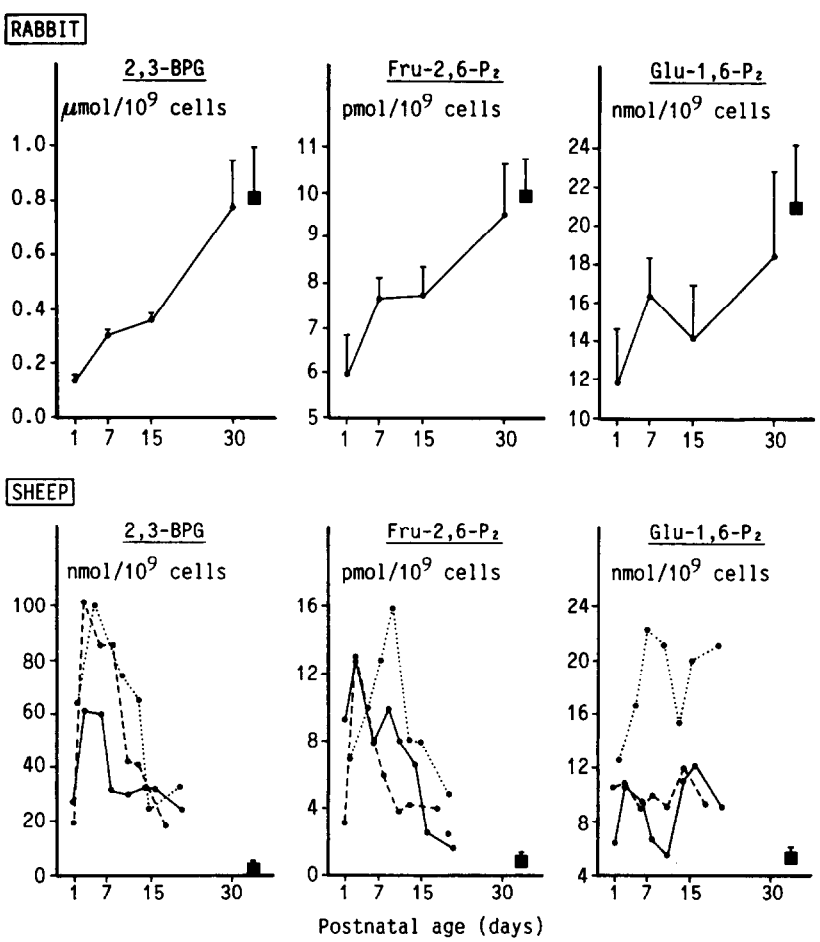

Fig.1. Levels of bisphorphorylated metabolites in rabbit and sheep erythrocytes during postnatal development. Values corresponding to sheep erythrocytes refer to three different animals. (■) Adult values.

has been found to present a transitory rise after birth. This reaches a maximum value after 4–5 days and then decreases to the very low adult levels. The concentration of Fru-2,6-P_2 also presents a postnatal increase and a subsequent fall, although neither the rise nor the decrease are as great as those of 2,3-BPG. In contrast to 2,3-BPG and Fru-2,6-P_2 levels, the concentration of aldohexoses 1,6-bisphosphate does not increase after birth, but for at least 20 days the postnatal values are higher than in adult erythrocytes.

The data of figs 2 and 3 show that in both species, rabbit and sheep, the changes in Fru-2,6-P_2 and aldohexose 1,6-bisphosphate concentrations in red blood cells during postnatal development correlate with the changes that present the levels of the enzymatic activities involved in their synthesis: PFK-2 and Glu-1,6-P_2 synthase. It has been reported that in lamb the concentration of plasma glucose more than doubles in the first 2 days and that the first four glycolytic intermediates are significantly increased [9]. The rise in glucose 6-P and fructose 6-P levels could contribute to the neonatal high concentrations of hexoses 1,6-bisphosphate and Fru-2,6-P_2 in lamb red blood cells reported herein.

The progressive rise in the concentration of 2,3-BPG in rabbit erythrocytes after birth seems to be mainly due to the increase in the phosphofructokinase/pyruvate kinase activity ratio (from 0.03 in the newborn to 1.1 in the adult), already reported by others [17,19], which would rise 1,3-bisphosphoglycerate levels [20], However, the increase in the 2,3-BPG synthase/2,3-BPG-

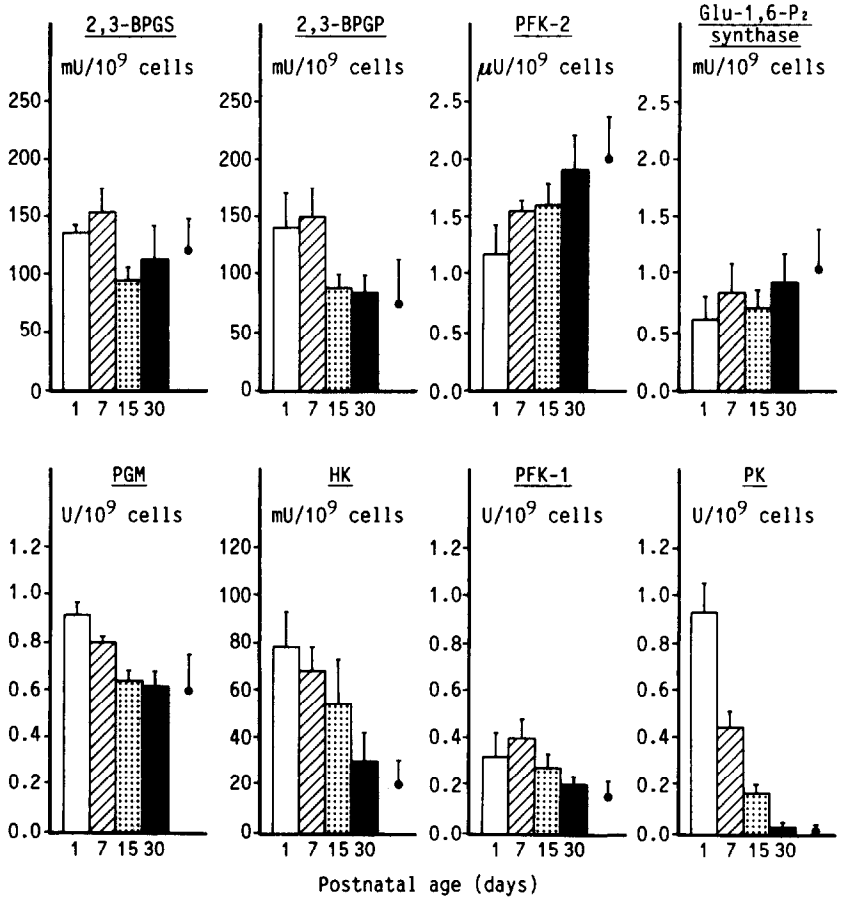

Fig.2. Levels of enzymatic activities in rabbit erythrocytes during postnatal development Values are means ± SD of four animals. (●) Adult values.

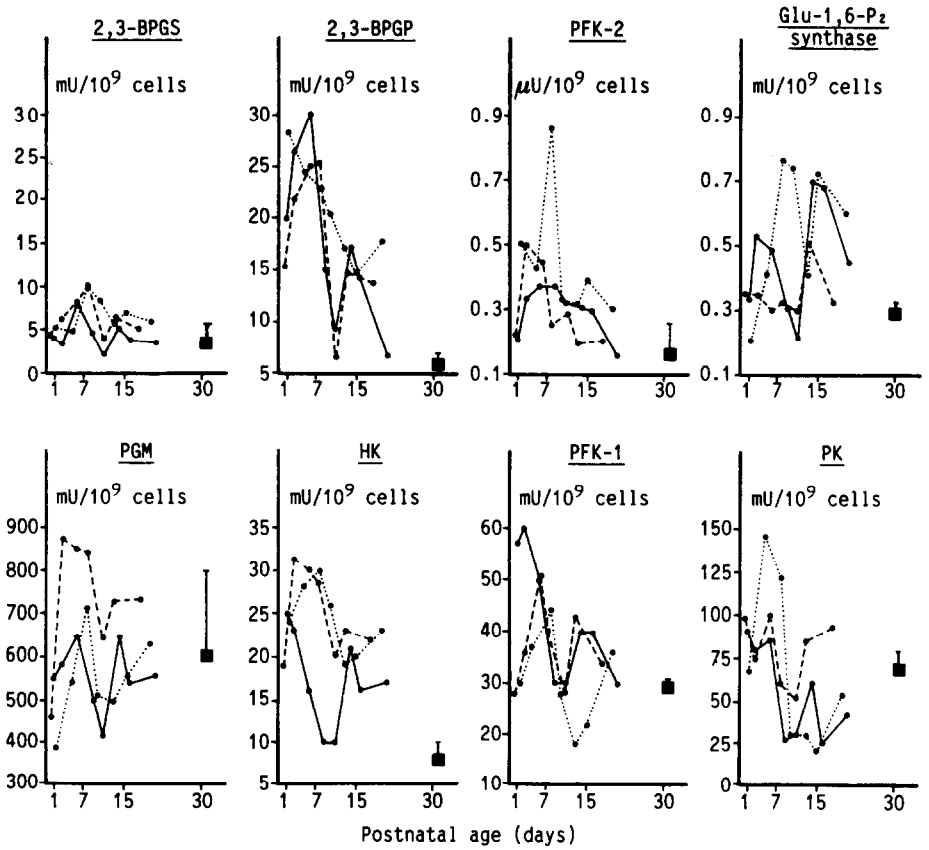

Fig.3. Levels of enzymatic activities in sheep erythrocytes during postnatal development. Each line corresponds to a different animal. (■) Adult values.

phosphatase activity ratio (from 0.9 in the newborn to the 1.6 in the adult), now reported, can also be involved. It is known that in the erythrocytes with high 2,3-BPG content, 2,3-BPG metabolism is controlled by two enzymes: the bifunctional 2,3-BPG synthase/phosphatase (EC 2.7.5.4/EC 3.1.3.13) and the phosphoglycerate mutase (EC 2.7.5.3) which in addition to the mutase activity also possesses some 2,3-BPG synthase and 2,3-BPG phosphatase activities [21]. The decrease of phosphoglycerate mutase (fig.2) could be responsible for the postnatal decrease of the total 2,3-BPG-phosphatase activity produced in rabbit red blood cells during postnatal life.

In contrast with the rabbit, the transitory rise in the concentration of 2,3-BPG in lamb erythrocytes after birth cannot be explained by the change in either the 2,3-BPG synthase/2,3-BPG phosphatase or the phosphofructokinase/pyruvate kinase activity ratios (fig.3). It could result from several fractors described by others [11,18,22]: the increase in plasma glucose, the activation of phosphofructokinase by a transitory rise in blood pH and the activation of glyceraldehyde 3-P dehydrogenase by an increase in plasma inorganic phosphate.

It is concluded that in mammalian erythrocytes after birth, in addition to the changes in the overall 2,3-BPG concentration produced as adaptation to the postnatal respiratory requirements, changes in the overall concentrations of Fru-2,6-P_2 and Glu-1,6-P_2 take place which could contribute to the change in red blood cell glycolysis produced during postnatal development [8]. The neonatal changes in red cell metabolism could be explained by the switch from fetal-type to adult-type red cells produced after birth, although additional factors cannot be excluded [22]. In the rabbit, as in the rat

[23], change over in red cell populations from larger to smaller cells is suggested by the decrease of the mean cell volume during development (96 ± 1.4 at birth, 78.3 ± 2.5 at 7 days and 73.7 ± 2.0 at 30 days). In contrast, in the sheep the mean cell volume remains essentially constant (data not shown, and [22]).

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