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Postnatal Changes in Oxygen-Hemoglobin Affinity and Erythrocyte 2,3-Diphosphoglycerate in Piglets

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Extract

Serial changes in hemoglobin, hematocrit, erythrocyte (RBC) 2,3-diphosphoglycerate (2,3-DPG), and whole blood oxygen affinity were studied in piglets. In the newborn piglet, the P_{50} averaged 19.0 mm Hg at birth and the erythrocyte 2,3-DPG was 1,670 mμmol/ml RBC's. Within 48 hr of birth, these values had increased to 27.4 mm Hg and 6,600 mμmol/ml RBC's, respectively. Normal adult values were reached by 1 month of age. The P_{50} bore a direct relationship to the erythrocyte 2,3-DPG concentration.

Speculation

The mechanism triggering the rapid rise in erythrocyte 2,3-DPG and the abrupt increase in P_{50} in the newborn piglet remains unexplained. The rise in 2,3-DPG may be a result of alterations in plasma pH, a sudden rise in plasma inorganic phosphate, or the presence of a metabolizable substrate such as dihydroxyacetone that was not present in the fetal environment. It remains to be determined whether the 2,3-DPG effect on P_{50} is a result of direct interaction with hemoglobin or a consequence of alterations in intracellular pH.

The goat was the first species in which differences between the oxygen affinity of maternal and fetal blood was observed (12). With the exception of the cat (16), in all species studied to date (3), the blood of the fetus has been found to have a higher affinity for oxygen than that of the mother. In man, the most mammalian species (goat, sheep, rabbit, lion, and dog), the postnatal decrease of oxygen affinity of the whole blood to that of the normal adult occurs during the first six months of life (2, 8, 9, 10, 11, 16, 17, 18, 19).

Factors causing the postnatal decrease in blood oxygen affinity include the replacement of fetal by adult types of hemoglobin, alterations in red cell hydrogen ion concentration, and changes in the concentration of red cell organic phosphates such as 2,3-diphosphoglycerate (4, 5, 8).

The present study was conducted in an attempt to characterize the nature and time of the change in the whole blood oxygen affinity of piglets.

MATERIALS AND METHODS

Fifty-nine piglets, weighing 750 to 1350 grams at birth, from seven different litters were studied. In most instances, the animals were studied at birth, at 12 and 48 hours of life, 4, 10, and 17 days of age, and then weekly until two and one-half months of age. In addition, randomly selected pigs were studied at 3, 5, and 12 months of age.

Six to ten milliliters of blood were obtained from each piglet by either cardiac or jugular vein puncture. The samples were collected in heparin and kept chilled in wet ice until the time of analysis.

In all samples, measurements of hemoglobin, hematocrit, percent fetal hemoglobin (21), red cell 2,3-diphosphoglycerate (2,3-DPG) concentration, and determination of the hemoglobin-oxygen equilibrium curve were performed.

Red cell 2,3-DPG was measured by a modification of the Krimsky (15) method previously described (8).

The oxygen-hemoglobin equilibrium curves were obtained by directly measuring the oxygen tension, pH, and oxygen saturation following equilibration of whole blood (at a constant carbon dioxide tension) at varying oxygen contents. Four to seven points were obtained for each curve with oxygen saturation ranging from 30 to 70 percent at a constant temperature of 37° C. The P_{50}, which is the partial pressure of oxygen for 50% oxyhemoglobin saturation, was then obtained from the regression line drawn through the data points of the steep part of the curve (8). All P_{50}s were corrected to a pH of 7.40 using the Bohr factor of -0.485 (20) and temperature 39.6° Centigrade (normal pig temperature). Oxygen capacity in milliliters of oxygen per 100 ml of blood was calculated by multiplication of the hemoglobin concentration in grams per 100 ml by 1.39 (23).

Samples of blood from each litter, as well as blood from the sow, were employed for hemoglobin electrophoresis on starch gel, pH 8.6 (13).

RESULTS

The data obtained from the studies of 59 piglets is presented in Table I and Figure 1. At term birth, the mean P_{50} was 19.0 + 1.3 mm Hg and the red cell 2,3-DPG averaged 1670 + 610 muMoles/ml RBC's. During the first 48 hours of life, both the P_{50} and the red cell 2,3-DPG rose rapidly and, by the end of the second day of life, averaged 27.4 mm Hg and 6600 muMoles/ml RBC's respectively.

The P_{50} and the red cell 2,3-DPG concentration then gradually increased and reached normal adult values by the third to fourth week of life. Between the fourth and tenth week of life, both the P_{50} and the red cell 2,3-DPG exceeded the values observed in the mature pig.

A highly significant direct relationship (r = 0.94) was observed between the P_{50} value and the red cell 2,3-DPG concentration (Figure 2).

At no time was fetal hemoglobin demonstrable by either alkali denaturation or hemoglobin electrophoresis.

DISCUSSION

In the piglet, unlike the human infant, it would appear that the decrease in hemoglobin-oxygen affinity that occurs in the postnatal period is entirely mediated via changes in red cell 2,3-DPG concentration. In the human, both red cell 2,3-DPG concentration as well as the amount of fetal hemoglobin serve to determine the position of the curve (8). Extensive studies employing blood from both adult and fetal pigs employing techniques that included electrophoresis on starch block and cellulose acetate, chromatography on DEAE-sephadex, denaturation by acid, alkali and heat, spectral analysis failed to reveal any significant differences (14). In addition, chromatography, electrophoresis, and finger-printing of separated alpha and non-alpha chains of fetal pig hemoglobin failed to reveal the presence of any fetal type hemoglobin during any stage of development (22). These studies and the observed rapid changes in P_{50} reported in this study make it highly unlikely that changes in hemoglobin type are responsible for the alterations in red cell oxygen affinity that occur so promptly in the newborn period in the piglet.

In the piglet and the human infant, the affinity of hemoglobin for oxygen eventually reaches values that are even lower than that observed in the normal adult. In the pig, the normal adult values are achieved by 5 months of age, while in the human these values may not be reached until pubescence (7).

Because of this apparent pure 2,3-DPG effect on modulating hemoglobin's affinity for oxygen, the piglet appears to be an ideal model for the study of the relationships between red cell 2,3-DPG, hemoglobin-oxygen affinity, and oxygen transport and delivery.

SUMMARY

Sequential measurements of red cell 2,3-diphosphoglycerate (2,3-DPG) and whole blood oxygen affinity were performed in piglets from birth. These studies demonstrate a precise correlation between the 2,3-DPG content of the erythrocytes and the position of the oxygen-hemoglobin equilibrium curve and the absence of a fetal hemoglobin.

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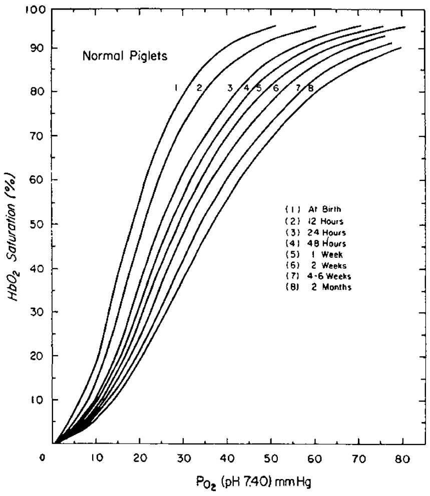

Figure 1. Oxygen-hemoglobin equilibrium curves of blood from piglets at different postnatal ages; each curve represents the mean value of the piglets studied in each age group.

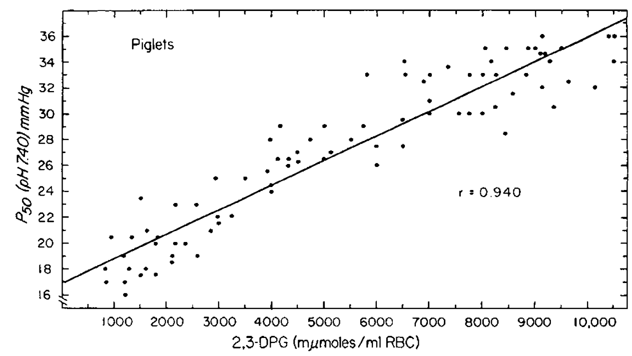

Figure 2. The P_{50} and total red cell 2,3-diphosphoglycerate for all piglets at different postnatal ages.

TABLE I

POSTNATAL OXYGEN TRANSPORT OF 59 PIGLETS

Number of Piglets	Age of Piglets	Total Hb gm/100 ml of Blood	O_2 capacity ml/100 ml of Blood	Hct in (%)	P_{50} at 7.40 (mmHg)	2,3-DPG mμMoles/ml RBC's
10	Preterm	10.2 ±1.5	14.1 ±1.9	31.3 ±6.5	19.7 ±1.5	1450 ±580
15	Birth	10.5 ±1.6	14.6 ±2.0	35.1 ±5.2	19.0 ±1.3	1670 ±610
13	12 hrs	10.9 ±1.2	14.8 ±1.8	36.6 ±5.3	20.8 ±1.1	2100 ±610
13	24 hrs	9.8 ±1.0	13.6 ±0.8	33.1 ±5.1	25.8 ±0.6	4800 ±700
19	48 hrs	9.1 ±1.3	12.6 ±1.2	30.3 ±5.7	27.4 ±1.9	6600 ±700
12	4 days	8.7 ±0.8	12.0 ±0.6	29.3 ±2.6	28.9 ±1.6	6000 ±600
11	10 days	9.3 ±0.6	13.0 ±1.0	31.3 ±2.5	29.5 ±1.0	7100 ±540
10	17 days	10.3 ±0.7	14.3 ±0.8	34.4 ±2.9	31.3 ±1.1	7330 ±400
10	23 days	11.3 ±0.6	15.7 ±0.9	37.8 ±2.4	33.0 ±0.7	9800 ±650
10	31 days	10.8 ±0.6	15.0 ±0.7	36.0 ±2.3	33.0 ±0.6	10380 ±590
10	38 days	11.1 ±0.4	15.5 ±0.5	37.2 ±0.2	33.5 ±0.4	9850 ±400
12	60 days	10.5 ±0.5	14.6 ±0.6	36.5 ±1.3	35.0 ±0.2	12400 ±680
10	75 days	10.8 ±0.7	14.7 ±0.8	36.5 ±1.6	35.5 ±0.4	12800 ±560
12	3 months	10.0 ±0.9	13.9 ±0.7	36.0 ±2.1	33.5 ±0.6	10500 ±800
6	5 months	11.4 ±0.8	15.9 ±0.8	37.7 ±1.3	33.0 ±1.0	10650 ±650

All values are given as mean ± one standard deviation (SD).