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Effect of Hypoxemia on Fetal Hemoglobin Synthesis during Late Gestation

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ABSTRACT. This study was carried out to investigate the effect of fetal hypoxemia, as a result of acute intermittent maternal hypoxia, on the switchover from fetal to adult type Hb as well as Hb oxygen affinity and 2,3-diphosphoglycerate levels in the near-term fetus. These experiments were carried out using 10 fetal lambs with gestational ages ranging from 132 to 140 d. After the installation of appropriate fetal catheters, five of the ewes were exposed to an air mixture containing 10% O_2 for 90 min/d for 4 consecutive d. Blood samples were withdrawn before the beginning of the hypoxic experiments and between the 5th and 6th d after the first episode of hypoxia. These samples were for the determination of 2,3-diphosphoglycerate concentration, arterial O_2 pressure at which Hb is 50% saturated, and Hb type synthesis. Blood gases were monitored during each hypoxic episode. During the hypoxia, fetal arterial O_2 pressure decreased from 2.43 ± 0.36 kPa (18.2 ± 2.7 mm Hg) to 1.57 ± 0.17 kPa (11.8 ± 1.3 mm Hg). These values returned to their initial levels after cessation of the maternal hypoxia. Five control animals of the same gestational age were also followed. During the interval of the study, a decrease of fetal Hb synthesis was noted (71.7 ± 12.1 to 57.4 ± 10.2%, $p < 0.001$) in the control group. However, the level of fetal Hb synthesis did not significantly change in the hypoxic group (85.1 ± 11.1 *versus* 80.6 ± 18.9%). At the end of the study, the hypoxic group had higher levels of fetal Hb synthesis when compared with the control group (80.6 ± 18.9 *versus* 57.4 ± 10.2%, $p < 0.05$). The 2,3-diphosphoglycerate concentrations as well as the values of arterial O_2 pressure at which Hb was 50% saturated remained within normal limits. The data obtained in this study show that intermittent episodes of fetal hypoxemia cause an increase in the level of fetal Hb synthesis in relation to gestational age. (*Pediatr Res* 31: 483–485, 1992)

Abbreviations

HbF, fetal hemoglobin
2,3-DPG, 2,3-diphosphoglycerate
P_{50}, arterial O_2 pressure at which hemoglobin is 50% saturated

Previous studies have described an increase in HbF in newborns born to hypoxemic (1), diabetic (2), and toxemic mothers (3). In a more recent report, it was also shown that fetal hyperglycemia causing hypoxemia resulted in an increase in HbF synthesis in the fetal lamb (4). Fetal hypoxemia appears to be the stimulus for an increase in HbF described in the newborn infant; however, a direct relationship between *in utero* hypoxemia and increased HbF synthesis has not been established.

The fetal lamb is an interesting experimental model to evaluate the effects of hypoxemia on the physiologic properties of the fetal erythrocyte. During the perinatal period, the switchover from fetal to adult Hb synthesis that occurs in these animals, although more rapid, is similar to that observed in humans (5), and, as in humans, the ultimate determination of the configuration of the Hb oxygen dissociation curve is dependent upon the type of Hb in the red cell and the concentration 2,3-DPG (5). Fetal lamb erythrocytes have the capacity to rapidly increase their 2,3-DPG after birth, thus lowering their oxygen affinity (6). If the fetal red cell had a similar 2,3-DPG response to hypoxemia during *in utero* life, it would be detrimental to the fetus by hindering adequate transfer of oxygen from mother to fetus.

A study was therefore planned to determine the effect of intermittent periods of acute hypoxemia on the relative rates of fetal and adult Hb synthesis as well as 2,3-DPG concentrations and the position of the Hb oxygen dissociation curve. The experiments were planned to be carried out at a time in gestation when the rapid switchover from fetal to adult Hb synthesis occurs.

MATERIALS AND METHODS

Studies were performed on 10 time-mated pregnant ewes. They were prepared between 126 and 135 d of gestation as previously described (4). Two polyethylene catheters were introduced in the fetal neck, one in the carotid artery and the other in the jugular vein. The catheters were passed through an s.c. canal and exteriorized on the maternal flank. During the first 48 h after surgery, antibiotics (500 mg ampicillin i.v. and 500 mg streptomycin intramuscularly) were administered prophylactically. Five animal preparations were used for the hypoxia experiment, and five were monitored in ambient air and served as controls (These animals were to be used for other experiments only after the control period was complete.). The arterial fetal blood gas and pH values were measured with a blood-gas analyzer, model ABL30 (Radiometer, Copenhagen, Denmark). The P_{50} was determined by tonometry, and the 2,3-DPG levels were determined by the enzymatic method as described previously (7).

Experimental protocol. On the 5th postoperative day, fetal blood samples were drawn for the determination of the blood gases and the P_{50}, as well as for 2,3-DPG concentrations and Hb type synthesis. If the blood gases were within normal range (PO_2 = 2.4 ± 0.5 kPa, pH = 7.36 ± 0.05, PCO_2 = 5.0 ± 0.7 kPa), fetal hypoxemia was then created in the study group by having the ewe breathe an air mixture containing 10% O_2 and 3% CO_2. The procedure lasted 90 min, and arterial blood was sampled at 15-min intervals to monitor the blood gases. At the end of the hypoxia, the animal was allowed to recover for 30 min, after

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which the blood gases were again controlled. This procedure was repeated once a day for 4 consecutive d. Between 5 and 6 d after the first period of hypoxia, the final state was established by a second determination of the Hb type synthesis, 2,3-DPG levels, and P_{50} values. The total amount of blood withdrawn from these late-gestation fetuses for each of the experiments was 10 mL. This was the quantity required to determine the initial values of Hb synthesis, P_{50}, and 2,3-DPG at the basal state as well as to measure all the blood gases obtained during the 4 d of the study. The second analysis of Hb synthesis, P_{50}, and 2,3-DPG was carried out at the end of the experiment.

Hb synthesis. The Hb of the sheep used in this investigation were either AB or BB type. The red cells obtained were incubated in an amino acid mixture containing ^{14}C-leucine and then subjected to column chromatography on carboxymethyl cellulose, a method similar to those described previously (4). The carboxymethyl cellulose provided the separation of the individual globin chains. Finally, the radioactivity incorporated into each of the globin chains was determined by liquid scintillation counting. The percentage of radioactive HbF to total radioactive Hb was based on the ratio of $\gamma/\gamma + \beta^{(A+B)}$. Pre-$\gamma$ and pre-α radioactivities were included in the respective γ-chain and α-chain totals.

Data were expressed as mean ± SD and analyzed by the *t* test for paired and unpaired groups. In accordance with the rules of our research center, these animal experiments were performed with the highest standards of humane care.

RESULTS

The baseline blood gases and the hematocrits are shown in Table 1. These values were all within the physiologic limits, and there were no significant differences between the two groups. During the interval of the maternal hypoxia, the fetal arterial O_2 pressure decreased from their baseline values of 2.43 ± 0.36 (kPa (18.2 ± 2.7 mm Hg) to 1.57 ± 0.17 kPa (11.8 ± 1.3 mm Hg) and returned to baseline values after the recovery period. The pH values of 7.360 ± 0.044 at baseline were 7.331 ± 0.069 at the end of the experiment. The globin-chain synthesis profiles of the basal and final states after carboxymethyl cellulose separation are shown in Figure 1. The blood samples were from a fetal lamb that was homozygous for β-B adult Hb. The percentage of γ-chain synthesis to the total non-α-chain synthesis was 80.1% at the basal state (135 d of gestation) and 83.1% at the end of the intermittent hypoxic experiments (140 d of gestation).

The changes in γ-chain synthesis as a percentage of non-α-chain synthesis in both groups of animals at the start and the end of the experimental period are shown in Figure 2. The data from both groups are superimposed on the range of normal sheep fetuses (5). Four of the five hypoxic fetuses did not show a decrease in HbF synthesis. Table 2 summarizes the changes in Hb type synthesis obtained in this study. There was no significant difference in the amounts of HbF synthesis between the controls and the hypoxemic fetuses at the basal state. There was a decrease in the HbF synthesis in the control group by the end of the experiment (71.7 ± 12.1 to 57.4 ± 10.2%, $p < 0.001$). There was no significant difference in HbF synthesis between the basal and final states in the hypoxic group (85.1 ± 11.1 *versus* 80.6 ± 18.9%), but there was a significant difference between the control group and the hypoxemic group at the end of the experiments

Table 1. *Baseline blood gases and hematocrit*

	Controls (group II) ($n = 5$)	Hypoxia (group I) ($n = 5$)
Hematocrit (%)*	38.40 ± 4.16	36.10 ± 2.66
Pa_{O_2}† (kPa)	2.55 ± 0.28	2.43 ± 0.36
P_{CO_2} (kPa)	4.95 ± 0.25	5.24 ± 0.16
pH	7.347 ± 0.022	7.360 ± 0.044

* SI unit conversion × 0.01.

† Pa_{O_2}, arterial O_2 pressure.

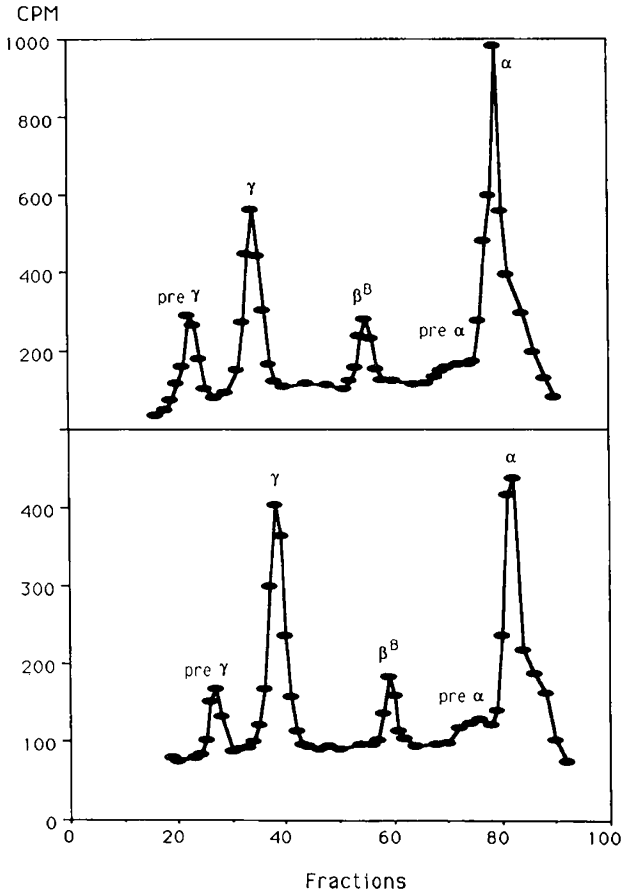

Fig. 1. Carboxylmethyl cellulose chromatography of ^{14}C-leucine-labeled globins obtained from a hypoxemic fetal lamb. The HbF synthesis was 80.1% before the hypoxemic experiments (*top*) and 83.1% at the end of the study (*bottom*).

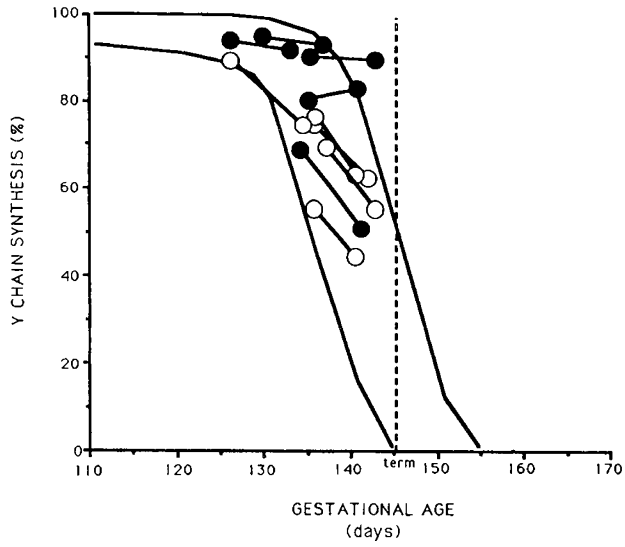

Fig. 2. HbF synthesis as a percentage of total Hb synthesis in two groups of fetal lambs. ●, the hypoxemic group; O, the control group. The area between the two lines is the normal range of HbF synthesis from reported data (5).

(57.4 ± 10.2 *versus* 80.6 ± 18.9%, $p < 0.05$). In the two groups, the 2,3-DPG concentrations and P_{50} did not differ significantly from the onset to the end of the experiments, and all of the values obtained remained within the normal range determined in a previous study (7). The values obtained at baseline and at the end of the experiments were 2.2 ± 0.4 and 4.2 ± 1.8 μmol/

Table 2. *Comparison of two groups of experiments*

	Controls (group II) ($n = 5$)		Hypoxia (group I) ($n = 5$)	
	Basal	End of experiment	Basal	End of experiment
Gestational age (d)	134.2 ± 4.7	140.0 ± 3.4	132.0 ± 3.9	138.2 ± 3.9
HbF synthesis (%)	71.7 ± 12.1	57.4 ± 10.2*	85.1 ± 11.1	80.6 ± 18.9†
2,3-DPG (μmol/g Hb)	2.2 ± 0.4	2.1 ± 0.1	2.8 ± 0.4	4.2 ± 1.8
P_{50} (kPa)	2.47 ± 0.17	2.45 ± 0.16	2.43 ± 0.23	2.48 ± 0.05

* $p < 0.001$ compared with basal level in control group.
† $p < 0.05$ compared with controls at end of experiment.

g Hb, respectively, for 2,3-DPG and 2.45 ± 0.24 kPa (18.4 ± 1.8 mm Hg) and 2.43 ± 0.23 kPa (18.2 ± 1.7 mm Hg), respectively, for P_{50}.

DISCUSSION

The purpose of this study was to determine if increased HbF synthesis could be stimulated by hypoxemia alone in a stable fetal maternal preparation. The duration of each hypoxic exposure was limited to maintain a stable preparation, yet was long enough for a hypoxemic effect on the erythropoietic system. This hypothesis was based on the time interval required for an increase in serum erythropoietin in both human and animal studies, which showed that after 1.5 h of hypoxemia in fetal sheep serum erythropoietin levels would be significantly increased (8, 9). Increased plasma erythropoietin has been shown to be associated with hypoxemia in fetal sheep (10), and others (11) have demonstrated, by administrating erythropoietin to baboons, that there exists a direct relationship between increases in erythropoietin and an increase in the production of HbF.

An interval of 5 to 6 d was considered adequate to determine if fetal hypoxemia would have an effect on HbF synthesis because results obtained using primates, cell cycle-specific drugs, acute bleeding, or recombinant erythropoietin showed that there was an increase in HbF production within 3–4 d after treatment that peaked at 5–6 d post-treatment (12). There was no significant change in 2,3-DPG or P_{50} during the study. This lack of an effect of hypoxemia on 2,3-DPG synthesis in the fetal lamb is in agreement with the data described by others (13).

Normally, during the switchover from fetal to adult Hb synthesis, the proportion of fetal to adult Hb being produced is dependent upon gestational age. Hypoxemia could possibly have a direct effect on γ-globin gene expression similar to that described in a study by Perrine *et al.* (14), in which butyrate infusions in the ovine fetus delayed the fetal to adult globin-chain switchover. However, the increased HbF during the switchover period could also be explained by using the concept suggested by Stamatoyannopoulos *et al.* (15), which proposes a model relating the pattern of Hb synthesis to the maturation of the erythroid precursors. Conditions such as hypoxemia result in an increase in erythropoietin that causes a recruitment of immature precursor cells. These immature precursor cells are forced to enter terminal differentiation rather than maturation pathways. The result is that a number of reticulocytes appear in the circulation, producing more HbF than is expected for the developmental age of the fetus.

This study demonstrates that fetal hypoxemia uncomplicated by other factors can be the cause of levels of HbF synthesis that are greater than can be expected for the period of gestation. Increased production of HbF during the perinatal period would be indicative of fetal hypoxemia. This finding could have important clinical significance.

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