PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 42–47 (1982)

Erythropoietin Elevation in the Chronically Hyperglycemic Fetal Lamb[1] (41394)

A. F. PHILIPPS,*[,2] J. A. WIDNESS,† J. F. GARCIA,‡ J. R. RAYE,* AND R. SCHWARTZ†

*Department of Pediatrics, University of Connecticut Health Center, Farmington, Connecticut 06032; ‡The Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720; and †Department of Pediatrics, Rhode Island Hospital and Brown University, Providence, Rhode Island 02902

Abstract. The effects of chronic fetal glucose infusion upon fetal oxygenation and endogenous erythropoietin (Ep) production were studied using the chronically catheterized fetal lamb. Fetal glucose infusion at rates between 5 and 20 mg/kg/min resulted in sustained fetal hyperglycemia. During glucose infusion (maximal glucose concentration achieved = 55.4 ± 3.7 mg/dl) fetal arterial oxygen contents fell from 5.8 ± 0.9 to 4.2 ± 1.0 ml/dl while no changes were observed in simultaneously sampled, noninfused twins. Although plasma insulin concentration rose in the infused fetuses, the elevations were inconstant and no relationship between fetal plasma insulin concentration and decrement in fetal oxygen content was evident. Fetal plasma arterial Ep concentrations in the control state were 13.2 ± 2.8 mU/ml. In infused fetuses, plasma Ep concentrations rose to 150.7 ± 35.9 mU/ml when the fetal arterial oxygen contents fell below 60% of their basal values. A reciprocal relationship was demonstrated between fetal arterial oxygen content and fetal plasma arterial Ep concentration ($P < 0.001$) in the pooled data of infused fetuses. The changes in plasma Ep concentration were noted prior to any significant fetal metabolic acidosis (as evidence of tissue hypoxia) and no changes in plasma Ep concentration were observed in simultaneously sampled noninfused twins. No relationship was apparent between fetal arterial plasma insulin and Ep concentrations. Since neither fetal anemia nor hemodilution occurred in these preparations, glucose-induced fetal hypoxemia is the likely mechanism behind elevated fetal Ep concentrations in these experiments. Similarities between this animal model and human fetuses and infants of diabetic mothers suggest that chronic *in utero* hypoxemia may be a common feature responsible for such diverse abnormalities as polycythemia, hyperbilirubinemia, and late fetal demise. The mechanism behind the glucose-induced fetal hypoxemia is not known.

Hyperinsulinism and macrosomia are features commonly found in infants of inadequately controlled or undiagnosed diabetic mothers (1–5) presumably the result of fetal hyperglycemia and excessive fetal pancreatic β cell stimulation (1–3). Other features such as congenital polycythemia (1) and late *in utero* demise (3), although prominent in infants of diabetic mothers, have etiologies not clearly linked to fetal hyperglycemia or hyperinsulinemia. Some have theorized that *in utero* hypoxemia may play a role (1–5), but this finding has not been clearly documented. Suggestive of this viewpoint has been the demonstration of elevation in erythropoietin (Ep) concentration in the cord blood of infants of diabetic mothers and insulin-infused fetal monkeys, using a sensitive Ep radioimmunoassay (6, 7). Whether elevation in fetal plasma Ep concentration is due primarily to hyperinsulinemia or hyperglycemia, however, is unclear, since both can induce fetal hypoxemia in experimental circumstances (8–10). The present study was performed, therefore, using a hyperglycemic twin fetal

[1] Supported in part by grants from the National Institute of Arthritis, Metabolism and Digestive Diseases (AM 26067), the National Institute of Child Health and Human Development (HD 11343), the National Heart, Lung and Blood Institute (HL 22469), the Division of Environmental Research for the U.S. Department of Energy, and the Rhode Island Hospital Fund.

[2] Address reprint requests to: Anthony F. Philipps, M.D., Department of Pediatrics, University of Connecticut Health Center, Farmington, Conn. 06032.

0037-9727/82/050042-06$01.00/0

lamb model (10) to test the hypothesis that elevation of fetal Ep levels is due primarily to fetal hypoxemia induced by fetal hyperglycemia.

Methods. Five pregnant ewes of timed gestation were selected between 110 and 125 days of gestation (term gestation: 147 days). Four had twins and one, a singleton fetus. Ewes were anesthetized with sodium pentobarbital and intrathecal pontocaine. After hysterotomy, catheters were placed in the distal fetal aorta (i.e., preplacental) and inferior vena cava via fetal pedal or femoral artery and pedal vein and in the maternal femoral artery for blood sampling and infusion. In twin preparations, both fetuses received venous and arterial catheters. Catheters were tunnelled subcutaneously to a pouch on the ewe's flank. Experiments were performed only after at least four postoperative days when all ewes were stable and feeding *ad libitum*. During a 2-day control period three to four arterial blood samples (2–3 ml/sample) were drawn from each fetus (including both fetuses in each twin pair) and analyzed for arterial plasma glucose, insulin, and Ep concentrations, as well as whole blood O_2 content and blood gases (pO_2, pCO_2, pH). Hematocrit and total serum solids were monitored daily during control and experimental periods to assure that infusion and sampling did not result in hemodilution or fetal anemia. Blood withdrawal for hematocrit and total serum solids was performed prior to initial blood sampling for other parameters. Maternal blood was also withdrawn daily during control and experimental periods and analyzed for plasma glucose, insulin, and blood gases. Care was taken to minimize fetal blood drawing and to withdraw equal blood volumes from fetuses in each twin pair.

At the end of the control period, the singleton fetus and one fetus in each of the twin pairs were begun on a continuous glucose infusion through the caval catheter by means of a precalibrated syringe pump. Glucose in sterile water (35 or 50%) was used for the infusate which was replaced in the syringes daily. The infusate was delivered at rates between 5 and 20 mg glucose/kg estimated fetal weight/min which required volumes of delivery between 0.5 and 2.5 ml/kg/hr. Glucose infusions were begun at rates of 5–10 mg/kg/min and increased in a stepwise fashion in increments of 2.5–5.0 mg/kg/min every 2 to 3 days. In three studies severe hypoxemia[3] or acidosis intervened and the infusion was halted for 1 to 2 days before being resumed. During infusion, serial blood samples were obtained from infused and noninfused fetuses daily to assess changes in arterial plasma glucose, insulin, and Ep concentrations, and whole blood O_2 content as well as acid base alterations. Thus, the noninfused twins served as simultaneous *in utero* controls. In these preliminary studies, parameters of erythropoietic stimulation and/or response other than hematocrit were not assessed. Catheter dislodgement and fetal demise were the major factors limiting total infusion time.

Assay of plasma glucose was performed using a glucose analyzer (Beckman Instruments). Whole blood O_2 content was measured with the Lex-O_2-Con (Lexington Instruments) after withdrawing fetal blood into NaF-treated tubes. The Lex-O_2-Con was calibrated daily, with distilled water saturated with O_2 at 0°. Insulin radioimmunoassay was performed using a double antibody separation technique (11) and ovine insulin standards (kindly supplied by Dr. M. A. Root, Eli Lilly Laboratories). Ep assay was performed using rabbit anti-Ep serum and human Ep standards (6). Purity of labeled Ep was 70,400 U/mg protein (supplied by Dr. Eugene Goldwasser, Department of Biochemistry, University of Chicago). Inter- and intraassay coefficients of variation for this assay are 8.4 and 9.7%, respectively.

Statistical significance was assessed by means of the paired Student's t test. Regression analysis utilized the least-squares

[3] In this study the term hypoxemia is used arbitrarily to designate fetal arterial O_2 content $< 50\%$ of basal and acidosis to signify a fetal arterial base deficit > 5 meq/liter. In both cases, these limits are outside 2 SDs from the mean control values obtained during these experiments.

TABLE I. CHANGES IN FETAL ARTERIAL GLUCOSE AND OXYGEN IN GLUCOSE-INFUSED AND NONINFUSED TWIN FETAL LAMBS

Animal No.	Length of infusion (days)	Plasma glucose (mg/dl)		Whole blood oxygen content (ml/dl)	
		Control period	Experimental period	Control period	Experimental period
1	11	27.8/29.0[a]	61.0/23.4	6.3/5.6	3.6/5.5
2	4	18.8/18.2	62.0/16.1	5.4/5.3	4.4/6.7
3[b]	7	16.8	60.6	5.8	3.4
4	3	14.0/15.0	44.0/14.5	2.9/1.6	1.7/2.1
5	9	16.5/16.0	49.4/13.8	10.4/9.2	7.8/9.1
Mean ±1 SEM					
Infused		18.8 ± 2.4	55.4 ± 3.7*	6.2 ± 1.2	4.2 ± 1.0*
Noninfused		19.6 ± 3.2	17.0 ± 2.2	5.4 ± 1.6	5.9 ± 1.5
Pooled		19.1 ± 5.5	—	5.8 ± 0.9	—

[a] Infused fetus/noninfused fetus.
[b] Singleton lamb fetus.
* Significantly ($P < 0.01$) different from control.

method. Results are expressed as mean ± SEM unless stated otherwise.

Results. Glucose infusion in the five preparations was maintained for an average of 7 days (range 3–11 days). Pooled control fetal plasma glucose concentration was 19.1 ± 5.5 mg/dl and rose to a mean level of 55.4 ± 3.7 mg/dl ($P < 0.001$) during infusion (Table I). Noninfused control fetuses had plasma glucose concentrations (17.0 ± 2.2 mg/dl) not significantly different from their values in the control period. Maternal glucose concentrations were not significantly altered during fetal glucose infusions. Fetal plasma insulin concentration rose from a mean control value of 5.6 ± 1.1 μU/ml to a mean peak concentration of 21.8 ± 3.2 μU/ml ($P < 0.01$) during infusion in the infused fetuses. However, the insulin elevations were inconstant. During periods of significant fetal hypoxemia (which developed in four of the five infused fetuses), mean plasma insulin concentration (11.5 ± 2.2 μU/ml) was not significantly different from the mean control value. An example is depicted in Fig. 1A. No changes in plasma insulin concentrations were noted in the noninfused fetuses or ewes during the infusion period.

Control fetal hematocrit was 40 ± 2%. No differences were noted between infused and noninfused twins during the control period and neither group's hematocrit was altered during the experimental period. A similar lack of change was noted in fetal total serum solids (mean control value 4.7 ± 0.2 g/dl.

Control fetal arterial O_2 content for all fetuses was 5.8 ± 0.9 ml/dl (Table I). During glucose infusion fetal O_2 content decreased in the infused hyperglycemic fetuses to mean levels of 4.2 ± 1.0 ml/dl, which were significantly lower than their mean control values ($P < 0.01$). Mean values in the simultaneously sampled noninfused twins (5.9 ± 1.5 ml/dl), however, did not differ from control values. Metabolic acidosis developed in three of the infused twins (base deficit = 10.7 ± 1.8 meq/liter) when the fetal arterial oxygen content had fallen to 20.0 ± 3.8% of basal levels.

Preinfusion plasma Ep concentrations from the pooled results from all fetuses ($n = 9$) were 13.2 ± 2.8 mU/ml. Plasma Ep levels in noninfused fetuses during the experimental period remained low, 6.5 ± 1.3 mU/ml (not significantly different from pooled control values). Although biological variability in arterial O_2 content was present in the noninfused fetuses, no relationship between plasma Ep concentration and blood O_2 content was observed. In contrast, plasma Ep concentration rose in infused fetuses during glucose infusion, but

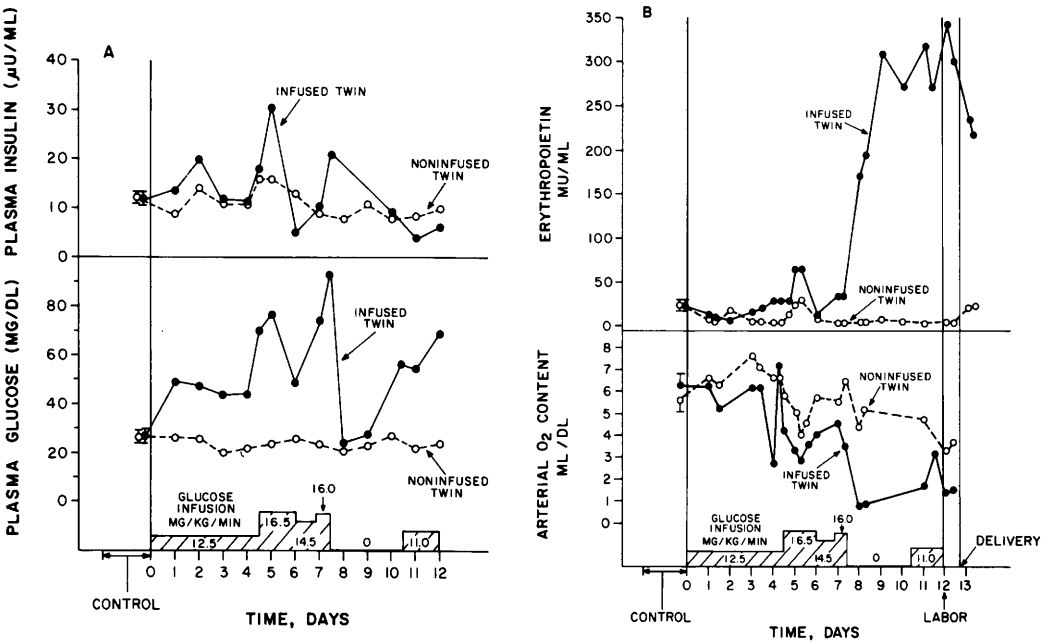

FIG. 1. Results of glucose infusion into one of twin fetal lambs (animal No. 1). (A) Changes in plasma arterial glucose and insulin (mean control concentrations are plotted ±SEM). (B) Changes in fetal arterial oxygen content and plasma erythropoietin concentration (mean control concentrations are plotted ±SEM).

only during significant fetal hypoxemia (<50% basal O_2 content). Figure 1B depicts such changes in the same twin preparation as shown in Fig. 1A.

When data from all five preparations were pooled, a reciprocal relationship was evident between fetal plasma Ep concentration and arterial O_2 content in infused fetuses ($P < 0.001$). Although individual responses were variable, a fall in arterial blood O_2 content to less than 60% of basal values (2 SD below the mean) resulted in a plasma Ep concentration of 150.7 ± 35.9 mU/ml ($P < 0.01$). In the only glucose-infused fetus that did not respond with a rise in plasma Ep (Fig. 2), arterial O_2 content did not fall below 50% of basal and only one point was below 60%. The elevations in plasma Ep concentration observed in these preparations were not related to changes in fetal insulin concentration and occurred before development of metabolic acidosis.

Discussion. In previous work (10), the relationship between experimentally induced fetal hyperglycemia and altered fetal oxygenation was assessed since several investigators had reported the development of fetal acidosis and *in utero* demise during chronic fetal glucose administration in lamb models. When fetal arterial oxygen content rather than p_aO_2 was used as an index of subtle changes in tissue oxygenation (14), chronic glucose infusion resulted in arterial hypoxemia in the fetal lamb (10). The decrement in arterial oxygen content was related both to the rate of glucose infusion and to the degree of hyperglycemia produced. Although metabolic acidosis (as evidence for tissue hypoxia) was noted during severe hypoxemia (see Methods), lesser decrements in arterial O_2 content were not associated with abnormalities in acid–base status. Although fetal lactate concentrations were not measured in these preparations, it is assumed that significant fetal metabolic acidosis was due to elevation of fetal blood lactate concentration induced by tissue hypoxia. Fetal lactate concentration in normoxemic, hyperglycemic fetal sheep is statistically similar to values obtained in the basal state (10). The mechanism(s) involved in the induction of the

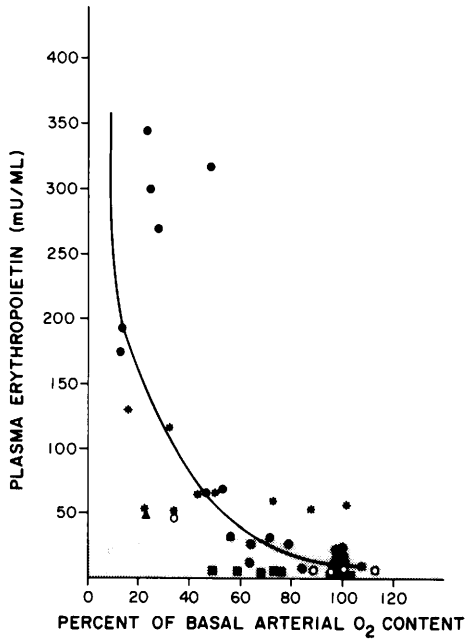

FIG. 2. Correlation between fetal arterial oxygen content in infused fetuses (expressed as percentage of basal oxygen content) and fetal arterial plasma erythropoietin concentration ($y = 3218.2/x - 8.1$, $r = 0.62$, $P < 0.001$). Shaded zone represents 2 SD above mean control plasma erythropoietin concentration.

hypoxemia are not known but may involve alteration of the oxyhemoglobin dissociation curve or stimulation of fetal or uteroplacental O_2 consumption. In addition, several investigators have demonstrated that fetal insulin infusion can increase fetal O_2 consumption and induce arterial hypoxemia (8, 9). Endogenous hyperinsulinemia was not noted uniformly in the present studies, but may have contributed to the fall in O_2 content. The reasons behind the relative inconstancy in glucose-induced elevation of fetal insulin concentration are unclear but may be related to the duration of hyperglycemia as well as the resulting arterial hypoxemia.

Erythropoietin, although well known for its effects upon stimulation of proliferation of erythroid precursors in the adult (15), also exerts a major influence upon the control of erythropoiesis in the fetus and infant (16). In the adult, Ep secretion occurs primarily in the kidney (15, 17), while *in utero* the fetal liver appears to be the major synthetic site (18). Both *in* and *ex utero,* the primary factor responsible for changes in Ep secretion is the relatively complex relationship between tissue O_2 consumption and O_2 delivery (17, 19). Using an Ep bioassay as well as radioimmunoassay, several authors (16, 18, 19) have demonstrated elevations in plasma Ep concentrations in response to hypoxia or anemia in the fetus and neonate. In one study (7), significant Ep elevations in cord plasma also were noted in both infants of diabetic mothers and monkey fetuses chronically infused with insulin. The authors speculated that Ep elevation might be the result of either hyperinsulinemia or chronic *in utero* hypoxemia. Although certain hormones such as thyroxine, growth hormone, and catecholamines can stimulate Ep production (20) no evidence is currently available regarding a possible stimulatory effect of insulin per se upon Ep secretion.

The present experiments indicate that endogenous fetal Ep secretion is attuned to subtle alterations in fetal oxygenation and is stimulated when fetal arterial O_2 content falls below 60% of basal levels. This value corresponds with approximately 2 m*M* O_2 content in the fetal lamb, the same content below which fetal organ blood flow alterations occur as a compensatory mechanism in fetal hypoxic states (21). Prolonged Ep stimulation in the hypoxemic fetal lamb would, presumably, have resulted in an elevation in fetal red blood cell mass and, therefore, in fetal O_2 carrying capacity. In addition to the elevations of fetal Ep with arterial hypoxemia, a blunting of fetal glucose-induced insulin response was also demonstrated, an effect well documented in the hypoxic adult (22). Thus, the common mechanism(s) underlying elevations in plasma Ep seen in the glucose-infused fetal lamb and insulin-infused fetal monkey preparations may have been *in utero* hypoxemia induced by increased substrate and oxygen consumption, rather than direct stimulation by insulin.

Some infants of diabetic mothers have findings of venous polycythemia (1), accelerated carbon monoxide and bilirubin production (23), and increased hepatic hematopoietic elements (5), all suggestive of

excessive stimulation of erythropoiesis *in utero*. Data from the chronically hyperglycemic fetal lambs are interpreted to indicate that these abnormal findings in the human may represent an hematopoietic response to endogenous fetal Ep secretion. Chronic *in utero* hypoxemia resulting from excessive maternal–fetal glucose transfer is suggested as the major stimulus for the rise in fetal plasma Ep concentration and, thus, for a compensatory rise in fetal red blood cell mass.

The authors wish to acknowledge the expert secretarial assistance of Mary Ann Peifer.

1. Cornblath M, Schwartz R. Disorders of Carbohydrate Metabolism. Philadelphia, Saunders, p115, 1976.
2. Pedersen J, Bojson-Moller B, Poulsen H. Blood sugar in newborn infants of diabetic mothers. Acta Endocrinol **15**:33–52, 1954.
3. Gellis SS, Hsia DY. The infant of the diabetic mother. Amer J Dis Child **97**:1–41, 1959.
4. Sosenko IR, Kitzmiller JL, Loo SW, Blix P, Rubenstein AH, Gabbay KH. The infant of the diabetic mother. Correlation of increased cord C-peptide levels with macrosomia and hypoglycemia. N Engl J Med **301**:859–862, 1979.
5. Naeye RL. Infants of diabetic mothers. Pediatrics **35**:980–988, 1965.
6. Garcia JF, Sherwood J, Goldwasser E. Radioimmunoassay of erythropoietin. Blood Cells **5**:405–419, 1979.
7. Widness JA, Susa JB, Garcia JF, Singer DB, Sehgal P, Oh W, Schwartz R, Schwartz HC. Increased erythropoiesis and elevated erythropoietin in infants born to diabetic mothers and in hyperinsulinemic Rhesus fetuses. J Clin Invest **67**:637–642, 1981.
8. Milley JR, Rosenberg AA, Philipps AF, Molteni RA, Jones MD Jr, Simmons MA. Circulatory effects of hyperinsulinemia in the near term ovine fetus. Pediat Res **15**:672, 1981.
9. Carson BS, Philipps AF, Simmons MA, Battaglia FC, Meschia G. Effects of a sustained insulin infusion upon glucose uptake and oxygenation of the ovine fetus. Pediat Res **14**:147–152, 1980.
10. Philipps AF, Dubin JW, Matty PJ, Raye JR. Hypoxemia and hyperinsulinemia in the chronically hyperglycemic fetal lamb. Pediat Res, in press.
11. Morgan CR, Lazarow A. Immunoassay of insulin: Two antibody system. Diabetes **12**:115–126, 1963.
12. Bassett JM, Madill P. Influence of prolonged glucose infusions on plasma insulin and growth hormone concentrations of foetal lambs. J Endocrinol **62**:299–309, 1974.
13. Robillard J, Sessions C, Kennedy RL, Smith FG Jr. Metabolic effects of constant hypertonic glucose infusion in well oxygenated fetuses. Amer J Obstet Gyn **130**:199–203, 1978.
14. Meschia G. Supply of oxygen to the fetus. J Reprod Med **23**:160–165, 1979.
15. Jacobsen LO, Goldwasser E, Fried W, Plzak L. Role of the kidney in erythropoiesis. Nature (London) **179**:633–634, 1957.
16. Zanjani ED, Mann LI, Burlington LH, Gordon AS, Wasserman LR. Evidence for a physiologic role of erythropoietin in fetal erythropoiesis. Blood **44**:285–290, 1974.
17. Erslev AJ, Kansu E, Caro J. The biogenesis and metabolism of erythropoietin. In: Golde DW, Cline MJ, Metcalf D, Fox CF, eds. Hematopoietic Cell Differentiation. New York, Academic Press, pl, 1978.
18. Zanjani ED, Ascensao JL, McGlave PB, Banisadre M, Ash RC. Studies on the liver to kidney switch of erythropoietin production. J Clin Invest **67**:1183–1188, 1981.
19. Stockman JA, Garcia JR, Oski FA. The anemia of prematurity: Factors governing the erythropoietin response. N Engl J Med **296**:647–650, 1977.
20. Adamson JW, Popvic WJ, Brown JE. Hormonal influences on erythroid colony growth in culture. In: Murphy MJ, ed. In Vitro Aspects of Erythropoiesis. New York, Springer-Verlag, p95, 1978.
21. Sheldon RE, Peeters LLH, Jones MD Jr, Makowski EL, Meschia G. Redistribution of cardiac output and oxygen delivery in the hypoxemic fetal lamb. Amer J Obstet Gynecol **135**:1071–1078, 1979.
22. Baum D, Griepp R, Porte D Jr. Glucose-induced insulin release during acute and chronic hypoxia. Amer J Physiol **237**:E45–E50, 1979.
23. Stevenson DK, Bartoletti AL, Ostrander CR, Johnson JD. Pulmonary excretion of carbon monoxide in the human infant as an index of bilirubin production. II. Infants of diabetic mothers. J Pediat **94**:956–958, 1979.

Received October 12, 1981. P.S.E.B.M. 1982, Vol. 170.