Fetal plasma erythropoietin in pregnancies complicated by maternal diabetes mellitus

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OBJECTIVE: Our purpose was to investigate the relationship between fetal plasma erythropoietin concentration and measures of short-term and long-term glycemic control and fetal oxygenation in pregnancies complicated by maternal diabetes mellitus.

STUDY DESIGN: A cross-sectional study was performed at The Harris Birthright Research Centre for Fetal Medicine, London. Cordocentesis was performed in 31 diabetic pregnancies for the measurement of umbilical venous blood pH, Po_2, Pco_2, lactate and glucose concentration, erythroblast count, hemoglobin, and plasma erythropoietin concentrations.

RESULTS: The mean pH was significantly lower and the Pco_2, lactate, erythropoietin, hemoglobin, and erythroblast counts were significantly higher than the appropriate normal mean for gestation. There were significant associations betwen (1) fetal erythropoietin and erythroblast count, (2) fetal erythroblast count and hemoglobin, (3) fetal hemoglobin and maternal glycosylated hemoglobin, and (4) maternal glucose and fetal glucose, pH, and lactate.

CONCLUSIONS: We postulate that maternal hyperglycemia causes fetal hyperglycemia and acidemia. Increased erythropoietin may be caused by tissue hypoxia or hyperinsulinemia. The increase in fetal hemoglobin may be the consequence of increased erythropoiesis, mediated by either erythropoietin or hyperinsulinemia. (Am J Obstet Gynecol 1993;168:88-94.)

Key words: Diabetes mellitus, cordocentesis, erythropoietin, blood gases

In pregnancies complicated by maternal diabetes mellitus the increased incidence of fetal death and intrapartum fetal distress late in gestation may have its origins in chronic or acute fetal hypoxemia.[1-4] Analysis of fetal blood obtained by cordocentesis from uncomplicated diabetic pregnancies has documented that some fetuses are indeed polycythemic and acidemic but not hypoxemic.[5] Furthermore, significant associations were found between maternal glucose concentrations and fetal blood pH and between maternal glycosylated hemoglobin percentage and fetal hematocrit. It was suggested that fetal acidemia may be a consequence of short-term maternal hyperglycemia, whereas fetal polycythemia reflects poor long-term glycemic control.[5]

Studies of cord blood obtained at delivery from pregnancies complicated by maternal diabetes mellitus have reported increased erythropoietin and hemoglobin concentrations and increased erythroblast counts.[1, 2, 6, 7] Furthermore, Widness et al.[2] demonstrated a significant association between mean maternal glycosylated hemoglobin during the last month of pregnancy and umbilical venous erythropoietin at delivery. However, fetal erythropoietin concentration can change acutely,[8, 9] and it is therefore uncertain whether increased concentrations in cord blood at delivery reflect acute or chronic fetal distress.

The aim of the current study was to investigate in uncomplicated diabetic pregnancies, by the use of samples obtained by cordocentesis, whether fetal erythropoietin is increased and its relationship to parameters of short-term or long-term maternal glycemic control or fetal oxygenation.

Patients and methods

In 31 women with established (n = 23) or gestational (n = 8) diabetes mellitus plasma erythropoietin concentration was measured in umbilical venous blood obtained by cordocentesis up to 24 hours before delivery. Before giving their written consent, all patients were counseled that the procedure was experimental and that the results would not give any direct benefit to their current pregnancy. The study was approved by our hospital ethics committee.

The patients were recruited from our combined diabetic antenatal clinic and all were insulin treated. In the

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group with established diabetes, according to the White classification,[10] eight belonged to group B, 14 to group C, and one to group D. Gestational age was established from the maternal menstrual history and was confirmed by ultrasonography (n = 26) or by an early ultrasonographic scan in those with uncertain dates (n = 5). In all cases the fetal anatomy, by both antenatal ultrasonography and postnatal examination, was normal.

In two cases elective delivery was undertaken at 36 weeks' gestation because of previously unexplained intrauterine deaths at 37 weeks. In the remaining cases the antenatal course was also uncomplicated, and according to our current policy elective delivery was undertaken at 37 to 39 weeks' gestation. Induction of labor was undertaken in 14 patients, nine of whom had vaginal deliveries; the remaining five were delivered by emergency cesarean section because of suspected cephalopelvic disproportion (n = 1) or fetal distress (n = 4). In 17 patients elective cesarean section was performed because of previous cesarean delivery (n = 13), congenital abnormality of the maternal pelvis (n = 1), fetal macrosomia (n = 1), and breech presentation (n = 2). All infants survived, and seven required admission to the special care baby unit because of isolated hypoglycemia (n = 6) or hypoglycemia and transient tachypnea of the newborn (n = 1). The mean gestational age at delivery was 38 weeks (range 36 to 39).

During the same period (August 1990 through December 1991) there were an additional 31 diabetic patients (White class B, n = 4; class C, n = 9; class D, n = 3; gestational diabetes, n = 15) who were delivered at 37 to 40 weeks' gestation and were not included in this study; in 14 cases the patient refused to have cordocentesis, in 15 cases the patient went into spontaneous labor before the date of planned delivery, and in two patients who had cordocentesis there was insufficient fetal plasma to perform the erythropoietin assay.

Cordocentesis was performed without fetal paralysis or maternal fasting or sedation, and all procedures were uncomplicated. Fetal blood was collected into a heparinized syringe (350 μl) for blood gas analysis (Radiometer ABL330, Copenhagen) and for measurement of whole blood glucose and lactate concentration (YSI 23A, Yellow Springs Instruments, Yellow Springs, Ohio). Fetal blood (180 μl) was also collected in 20 μl of isotonic edetic acid solution (0.5 mmol/L in 0.15 mmol/L sodium chloride), and contamination with maternal blood was excluded by the acid-elution (Kleihauer-Betke) method. The hemoglobin concentration and total nucleated blood cell counts were determined with a Coulter Stacker Automated Cytometer (Coulter Electronics, Luton, England). Blood films were stained by the May-Grünwald-Giemsa method, and the number of erythroblasts was counted.

At cordocentesis a maternal venous blood sample was taken from the antecubital fossa for the measurement of blood glucose concentration and glycosylated hemoglobin percentage (Corning scanner, Corning, Halstead, England).

The blood samples for erythropoietin were centrifuged for 10 minutes at 5000 revolutions/min, and the plasma was separated and stored at −20° C. The assays were performed in one batch by enzyme immunoassay (Clinigen, Amgen Diagnostics, Thousand Oaks, Calif.). The reference curve was standardized against the World Health Organization second international reference preparation of human urinary erythropoietin. The limit of sensitivity of the assay was 2 mU/ml (limit at 3 SD from the zero erythropoietin standard). The intrassay coefficient of variability was 4.5% for a mean erythropoietin value of 40 mU/ml (n = 10), and the upper assay limit necessitating dilution of samples was 200 mU/ml (this did not apply to any of our samples).

Statistical analysis. Because erythropoietin concentration and erythroblast count are distributed in a nonparametric manner in normal pregnancy, for both variables the logarithm of actual values was used. Two-tailed Student t test was applied to determine whether the measurements in the fetuses of diabetic mothers differed significantly from the appropritae normal mean for gestation.[11-15] Furthermore, linear regression analysis was used to determine the significance of associations beween the measured parameters. Although in normal pregnancy many of the parameters studied change with gestational age, within the narrow gestational range of the current study group none of the parameters was significantly associated with gestation.

Results

In the samples obtained by cordocentesis the mean umbilical venous blood pH was lower, and the mean Pco_2, lactate, erythropoietin, and hemoglobin concentrations and erythroblast count were higher than the appropriate normal mean for gestational age; the mean Po_2 was not significantly different (Figs. 1 through 3, Table I). The mean birth weight and the maternal glycosylated hemoglobin percentage were significantly higher than the appropriate normal mean for gestation (Table I). The mean ± SD maternal and fetal glucose concentrations were 5.38 ± 2.4 mmol/L (range 1.8 to 12.4) and 4.30 ± 1.8 mmol/L (range 1.2 to 8.8), respectively.

The interrelationships between fetal blood gases, blood glucose, lactate, hemoglobin, erythroblast count, plasma erythropoietin, and birth weight and maternal blood glucose and maternal glycosylated hemoglobin percentage are shown in Table II. There were significant associations between (1) fetal erythropoietin and erythroblast count, (2) fetal erythroblast count and fetal hemoglobin, (3) fetal hemoglobin and maternal glyco-

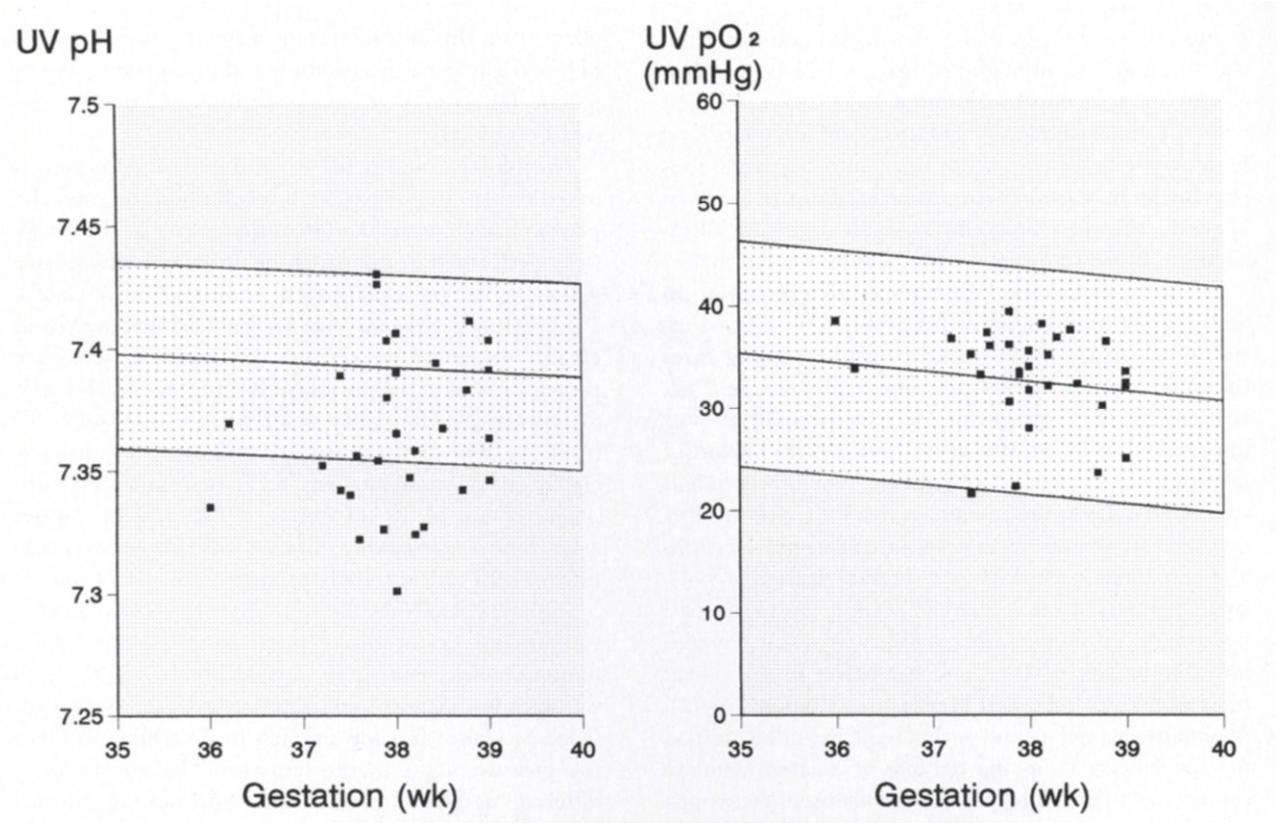

Fig. 1. Umbilical venous *(UV)* blood pH and Po_2 at cordocentesis plotted on appropriate reference range (mean, 95th and 5th percentiles) with gestation.

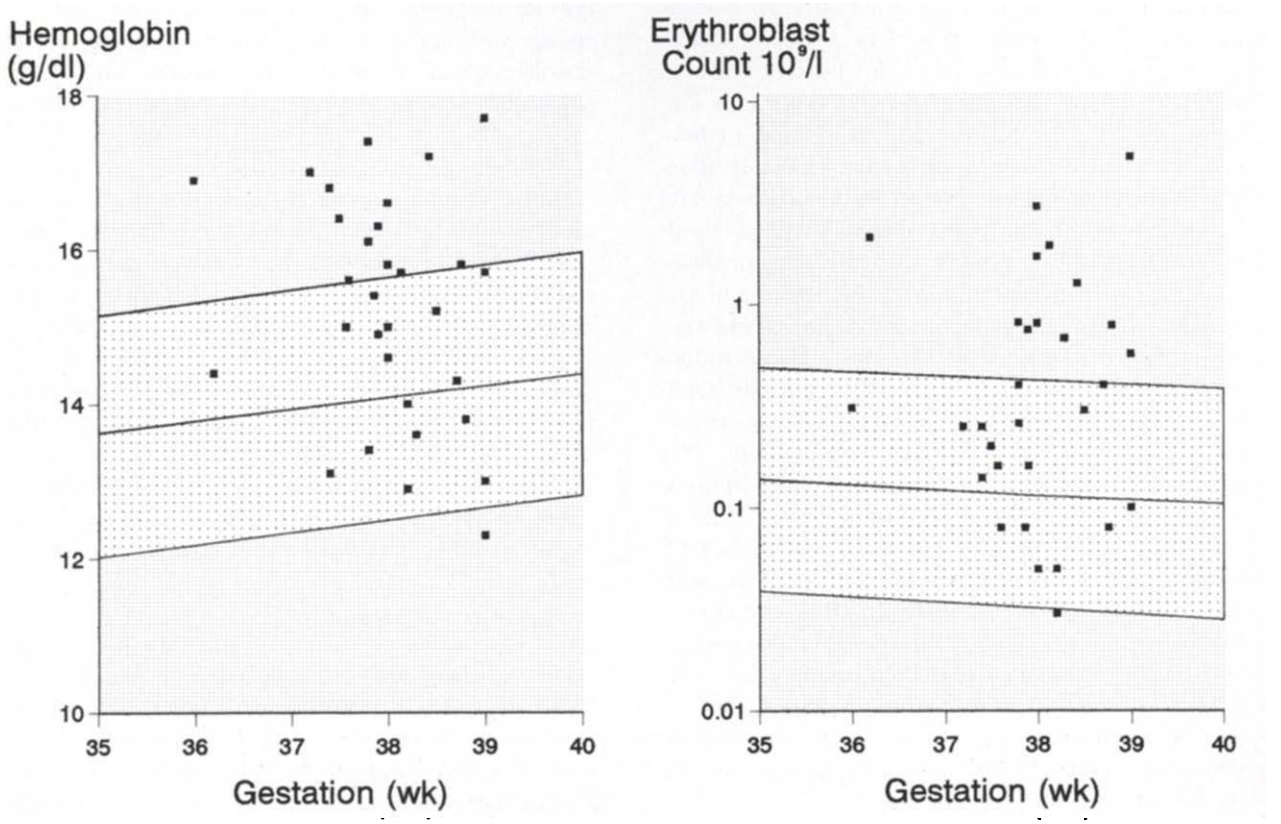

Fig. 2. Fetal blood hemoglobin and erythroblast count at cordocentesis plotted on appropriate reference range (mean, 95th and 5th percentiles) with gestation.

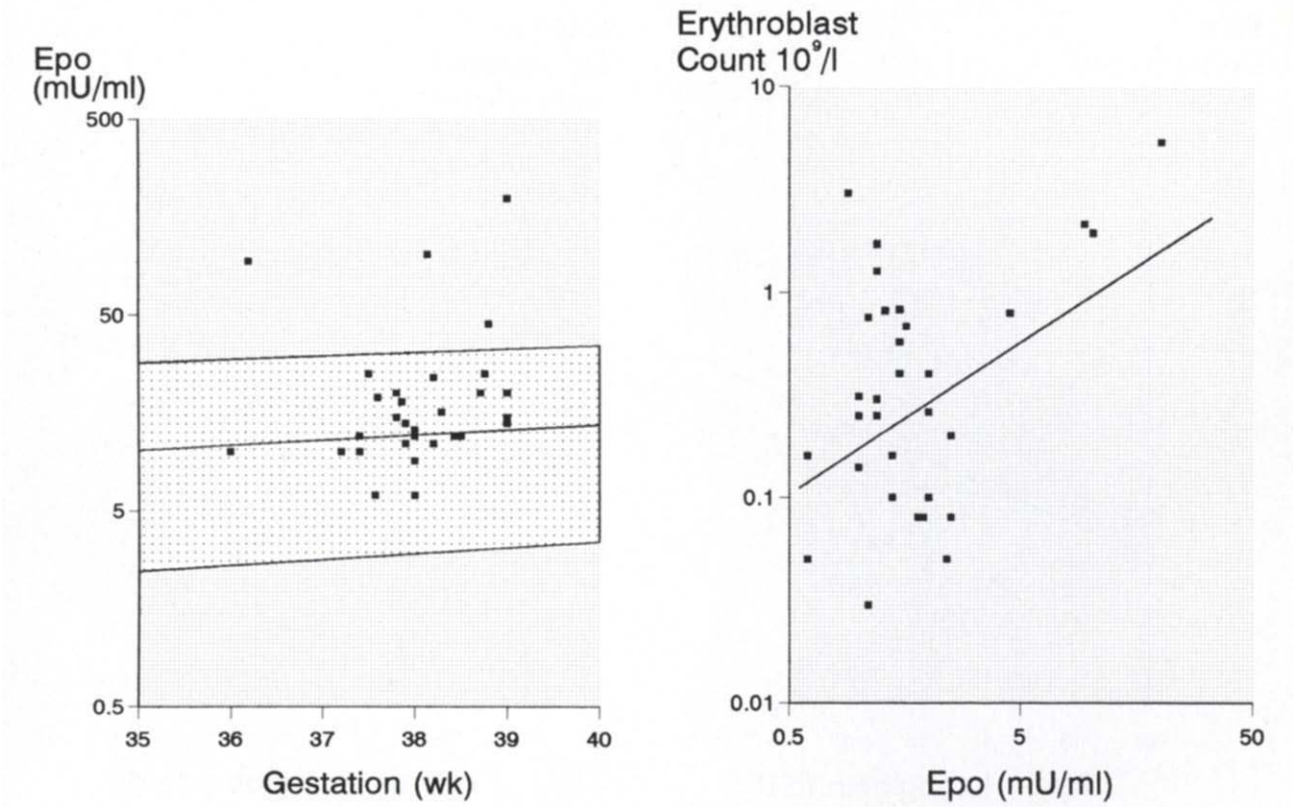

Fig. 3. Umbilical venous plasma erythropoietin concentration *(Epo)* at cordocentesis plotted on appropriate reference range (mean, 95th and 5th percentiles) with gestation and relationship between fetal erythropoietin and erythroblast count ($r = 0.452$, $p < 0.05$).

Table I. Comparison of mean values in 31 pregnancies complicated by maternal diabetes mellitus, with appropriate normal mean

	Diabetics		*Normal mean*	*Mean difference*	*Student t test*	*Significance*
	Mean	*Range*				
Blood pH	7.366	7.301-7.430	7.392	−0.026	−4.44	$p < 0.001$
Blood Po_2 (mm Hg)	32.95	21.6-39.3	32.53	0.42	0.50	NS
Blood Pco_2 (mm Hg)	40.63	33.2-47.0	37.05	3.58	6.20	$p < 0.0001$
Blood lactate (mmol/L)	0.99	0.50-1.60	0.76	0.23	5.88	$p < 0.0001$
Log_{10} plasma erythropoietin (mU/ml)	1.245	0.778-2.301	1.068	0.177	2.92	$p < 0.01$
Hemoglobin (gm/dl)	15.22	12.30-17.70	14.05	1.17	4.44	$p < 0.0001$
Log_{10} erythroblast count (10^9/L)	−0.479	−1.523-0.725	−0.943	0.464	4.53	$p < 0.0001$
Birth weight (kg)	3.59	2.65-5.32	3.199	0.391	4.32	$p < 0.001$
Maternal glycosylated hemoglobin (%)	8.63	5.50-11.90	6.9	1.730	6.98	$p < 0.0001$

NS, Not significant.

sylated hemoglobin percentage (Figs. 3 and 4), and (4) maternal glucose and fetal glucose, pH, and lactate (Table II).

Comment

The findings of this study, that in some pregnancies complicated by maternal diabetes mellitus fetal plasma erythropoietin and hemoglobin concentrations and erythroblast count are increased, are compatible with the data of previous studies that examined cord blood obtained at delivery.[1, 2, 6, 7] It is tempting to suggest that maternal hyperglycemia leads to fetal tissue hypoxia, which in turn causes release of erythropoietin with consequent stimulation of erythropoiesis and compensatory polycythemia (Fig. 5). However, in our study the increase in fetal erythropoietin was not related to measurable parameters of either short-term or long-term maternal glycemic control or fetal oxygenation.

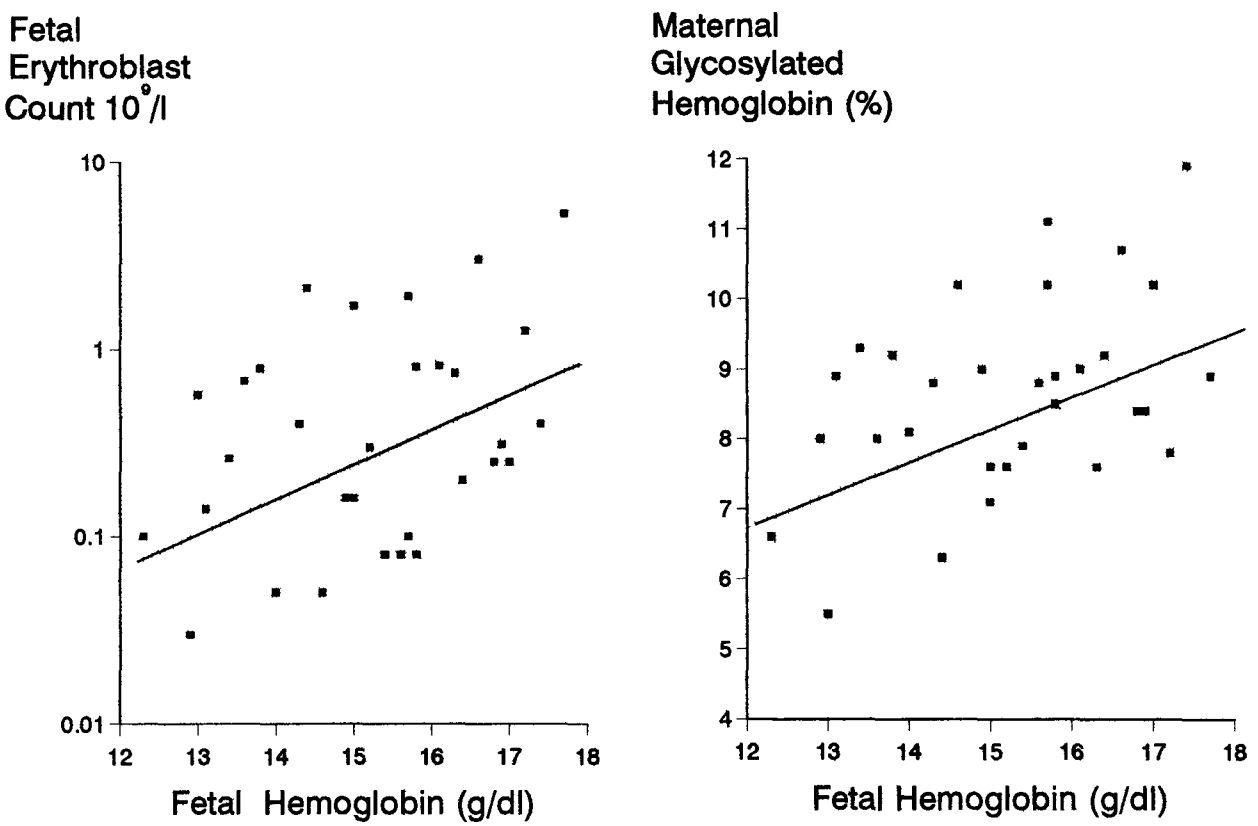

Fig. 4. Relationship between fetal hemoglobin concentration and erythroblast count (*left:* $r = 0.376$, $p < 0.05$) and maternal glycosylated hemoglobin percentage (*right:* $r = 0.452$, $p < 0.05$).

Table II. Interrelationships between umbilical venous blood pH, Po_2, Pco_2, lactate, glucose, hemoglobin, $\log_{10}$ erythroblast count, $\log_{10}$ erythropoietin, maternal glucose, maternal glycosylated hemoglobin percent, and birth weight

	Pco_2	*Lactate*	*Po_2*	*Umbilical glucose*	*Hemoglobin*	*Log_{10} erythroblast count*	*Log_{10} erythropoietin*	*Maternal glucose*	*Maternal glycosylated hemoglobin percent*	*Birth weight*
pH	−0.559*	−0.568†	0.344	−0.450‡	0.007	0.174	−0.078	0.510*	0.109	0.016
Pco_2		0.213	−0.686†	0.004	−0.123	−0.144	0.096	0.085	−0.090	−0.017
Lactate			−0.184	0.326	0.039	0.202	0.236	0.397‡	−0.194	−0.055
Po_2				0.117	0.295	0.211	0.034	0.066	0.113	0.227
Umbilical glucose					0.136	0.126	−0.149	0.954§	−0.078	0.072
Hemoglobin						0.376‡	0.071	0.050	0.452‡	0.249
Log_{10} erythroblast count							0.452‡	0.099	−0.027	0.154
Log_{10} erythropoietin								−0.124	−0.041	−0.109
Maternal glucose									−0.098	0.080
Maternal glycosylated hemoglobin percent										0.113

*$p < 0.01$.
†$p < 0.001$.
‡$p < 0.05$.
§$p < 0.0001$.

In normal fetal life the number of circulating erythroblasts decreases with gestation, reflecting the normal switch from hepatic to medullary erythropoiesis.[12] In hepatic erythropoiesis erythroblasts enter the peripheral circulation freely, whereas with marrow erythropoiesis the nucleated erythroid precursors are confined to the parenchyma in which hematopoiesis takes place.[16] In maternal diabetes mellitus the increase in

fetal erythroblast count suggests that the fetal polycythemia is at least partly a consequence of recruitment of hepatic erthropoiesis. Although there was a significant association between erythropoietin and erythroblast count, the former explained only 20% of the variance in the latter, suggesting the presence of additional erythrogenic stimulants.

Although previous reports have considered the increased erythropoietin concentration to be synonymous with fetal hypoxemia, in our study the fetal blood Po_2 was below the 5th percentile for gestation in only one case. Nevertheless, some of the fetuses were acidemic, and there was a significant association between maternal blood glucose concentration and fetal blood glucose, pH, and lactate. These findings are compatible with the previous suggestion that in maternal diabetes mellitus fetal acidemia is the consequence of anaerobic metabolism and that fetal blood pH is an index of short-term maternal hyperglycemia.[5] The lack of a significant association between fetal erythropoietin and pH suggests that in diabetes mellitus umbilical venous blood pH may be a poor indicator of tissue oxygenation. Alternatively, acidemia reflects tissue hypoxia, but blood pH is liable to acute fluctuations; the lack of a significant association between blood pH and plasma erythropoietin concentration is because the latter increases after medium-term (4 to 5 hours) tissue hypoxia.[8, 9] Similarly, the relationship between maternal glycosylated hemoglobin percentage and fetal hemoglobin concentration but the lack of significant association between fetal erythropoietin and the increased hemoglobin is presumably because the latter is an index of long-term maternal hyperglycemia and fetal hypoxia. In contrast to maternal diabetes mellitus, in fetal growth retardation caused by uteroplacental insufficiency there are high correlations between pH and erythropoietin, presumably because tissue hypoxia is the consequence of chronic impairment of placental perfusion and not of short-term fluctuations in the level of maternal hyperglycemia.[17]

An alternative explanation for increased fetal erythropoietin and hemoglobin concentrations, but no significant association between them, is that in maternal diabetes mellitus there is a direct and variable effect of fetal insulin on both erythropoietin production and erythropoiesis. In vitro studies have shown that insulin has erythrogenic properties, and both animal and human studies have demonstrated that erythropoietin is increased in association with hyperinsulinemia in the absence of hypoxemia.[1, 2, 18, 19]

We postulate that in pregnancies complicated by diabetes mellitus maternal hyperglycemia results in fetal hyperglycemia and acidemia. Furthermore, the increase in fetal erythropoietin is likely to be a consequence of tissue hypoxia. The increase in fetal hemoglobin is presumably caused by increased erythropoiesis, mediated by either erythropoietin or hyperinsulinemia (Fig. 5). Decreased fetal blood pH may be considered a short-term measure of tissue hypoxia, whereas increased erythropoietin and hemoglobin concentrations are medium and long-term measures, respectively.

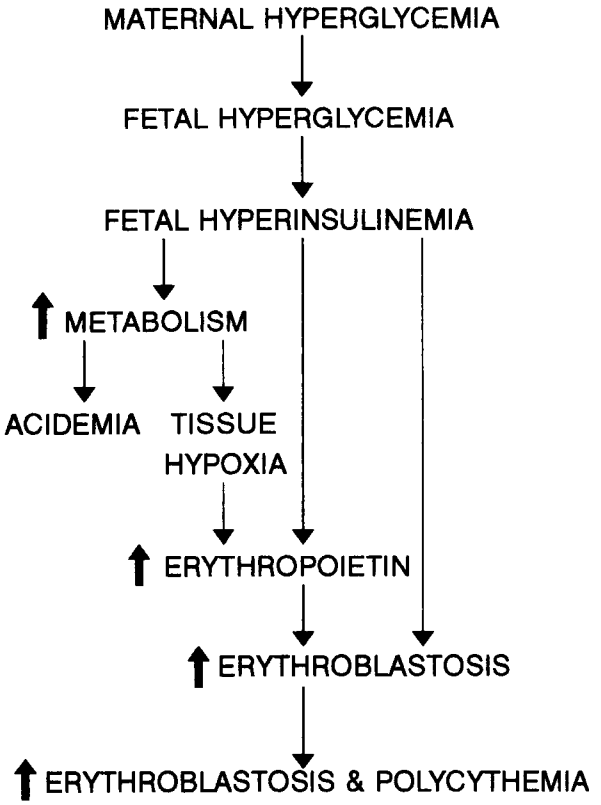

Fig. 5. Suggested mechanism for fetal erythroblastosis and polycythemia in pregnancies complicated by maternal diabetes mellitus.

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REFERENCES

1. Widness JA, Susa JB, Garcia JF, et al. Increased erythropoiesis and elevated erythropoietin in infants of diabetic mothers and in hyperinsulinemic rhesus fetuses. J Clin Invest 1981;67:637-42.
2. Widness JA, Teramo KA, Clemons GK, et al. Direct relationship of antepartum glucose control and fetal erythropoietin in human type I (insulin-dependent) diabetic pregnancy. Diabetologia 1990;33:378-83.
3. Madsen H. Fetal oxygenation in diabetic pregnancy: with special reference to maternal blood oxygen affinity and its effectors. Dan Med Bull 1986;33:64-74.
4. Mimouni F, Miodovnik M, Siddiqi TA, Khoury J, Tsang RC. Perinatal asphyxia in infants of insulin-dependent diabetic mothers. J Pediatr 1988;113:345-53.
5. Salvesen DR, Brudenell MJ, Nicolaides KH. Fetal polycythemia and thrombocytopenia in pregnancies complicated by maternal diabetes mellitus. Am J Obstet Gynecol 1992;166:1287-92.
6. Kaiser IH, Goodlin RC. Alterations in pH, gases and haemoglobin in blood and electrolytes in plasma of fetuses of diabetic mothers. Pediatrics 1985;22:1097-109.
7. Shannon K, Davis J, Kitzmiller JL, Fulcher SA, Koenig

HM. Erythropoiesis in infants of diabetic mothers. Pediatr Res 1986;20:161-5.
8. Widness JA, Teramo KA, Clemons GK, et al. Temporal response of immunoreactive erythropoietin to acute hypoxemia in fetal sheep. Pediatr Res 1986;20;15-9.
9. Widness JA, Clemons GK, Garcia JF, Oh W, Schwartz R. Increased immunoreactive erythropoietin in cord serum after labor. Am J Obstet Gynecol 1984;148:194-7.
10. White P. Classification of obstetric diabetes. Am J Obstet Gynecol 1978;130:228-30.
11. Ireland R, Abbas A, Thilaganathan B, et al. Fetal and maternal erythropoietin levels in normal pregnancy. Fetal Diagn Ther 1992;7:1-11.
12. Nicolaides KH, Thilaganathen B, Mibashan RS. Cordocentesis in the investigation of fetal erythropoiesis. Am J Obstet Gynecol 1989;161:1197-200.
13. Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH and lactate in appropriate and small for gestational age fetuses. Am J Obstet Gynecol 1989;161:996-1001.
14. Yudkin PL, Aboualfa M, Eyre JA, Redman CWG, Wilkinson AR. New birth weight and head circumference centiles for gestational ages 24-42 weeks. Early Hum Dev 1987; 15:45-52.
15. Worth R, Potter JM, Drury J, Fraser RB, Cullen DR. Glycosylated haemoglobin in normal pregnancy: a longitudinal study with two independent methods. Diabetologia 1985;28:76-9.
16. Factors thought to contribute to the regulation of egress of cells from marrow. In: Silber R, LoBue J, Gordon AS, eds. The year of hematology. New York: Plenum, 1978:243.
17. Snijders RJM, Abbas A, Melby O, Ireland RM, Nicolaides KH. Fetal plasma erythropoietin concentration in severe growth retardation. Am J Obstet Gynecol 1993;168:615-9.
18. Golde DW, Bersche N, Cline MJ. Hormonal effects on erythroid stem cells. In: Stamatoyannopoulos G, Niehuis AW, eds. Cellular and molecular regulation of haemoglobin switching. New York: Grune & Stratton, 1979:305-21.
19. Perrine SP, Greene MP, Lee PDK, Cohen RA, Faller DV. Insulin stimulates cord blood erythroid progenitor growth: evidence for an aetiological role in neonatal polycythaemia. Br J Haematol 1986;64:503-11.

Placental interleukin-6 production is enhanced in intrauterine infection but not in labor

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OBJECTIVE: Because interleukin-6 is an important mediator in the host defense mechanism against infection and tissue damage, we studied the capacity of placentas with or without either labor or chorioamnionitis in the third trimester to produce interleukin-6.

STUDY DESIGN: The placental blocks were cultured, and their interleukin-6 titers were measured by a bioassay.

RESULTS: Placentas with labor produced a similar amount of interleukin-6 to placentas without labor. In contrast, placentas with chorioamnionitis produced much more interleukin-6 than the placentas with or without labor ($p < 0.0001$).

CONCLUSION: Placental interleukin-6 is thus surmised to participate in potentiation of the placental and fetomaternal defense mechanisms together with placental interleukin-1 during chorioamnionitis. (Am J Obstet Gynecol 1993;168:94-7.)

Key words: Placenta, trophoblast, interleukin-6, interleukin-1, and chorioamnionitis

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Interleukin-6 exhibits multiple biologic activities and is produced by various cell types, including monocytes-macrophages, endothelial cells, fibroblasts, endometrial stromal cells,[1] and trophoblasts.[2] Interleukin-6 elicits various changes in the biochemical, physiologic, and immunologic status of the host. Especially in the host response to infection and tissue damage, interleukin-6 induces the "acute phase" protein responses and mediates changes in the plasma protein response, thereby

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