Proc. Natl. Acad. Sci. USA
Vol. 77, No. 5, pp. 2757–2761, May 1980
Cell Biology

Proportion of fetal hemoglobin synthesis decreases during erythroid cell maturation

(hemoglobin switching/erythroid colonies/erythropoietin/pregnancy)

D. H. K. CHUI, S. C. WONG, M. W. ENKIN, M. PATTERSON, AND R. A. IVES

Departments of Pathology and Obstetrics and Gynecology, School of Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Communicated by Elizabeth S. Russell, February 11, 1980

ABSTRACT **Peripheral blood mononuclear cells from pregnant and postpartum women were cultured *in vitro* with erythropoietin. Burst-forming unit (BFU-E)-derived erythroid colonies composed of immature erythroblasts with low hemoglobin contents were observed by day 8 of culture. By day 12 of culture, numerous BFU-E-derived erythroid colonies with high hemoglobin contents were present. The $\gamma/(\gamma + \beta)$ globin synthetic ratio was approximately 12% in the early cultures and 6% in the late cultures, indicating that the proportion of fetal hemoglobin synthesis decreases during erythroid cell maturation. These studies also reveal that the capacity for fetal hemoglobin production by peripheral blood BFU-E *in vitro* is not altered during pregnancy.**

The major hemoglobin in humans during intrauterine life is fetal hemoglobin, $\alpha_2\gamma_2$. Adult hemoglobin, $\alpha_2\beta_2$, is first detected at around the 10th week of gestation and accounts for approximately 10% of all hemoglobins present until about the 30th week of gestation, when increasing amounts of adult hemoglobin are produced (1). By the time the infant is 6 months old, most of the hemoglobin present is adult hemoglobin (2). The mechanisms for the switching of hemoglobins during fetal development are presently not clear (3). These orderly ontogenetic changes provide an excellent model for studying gene regulation and expression. In addition, elucidation of the mechanisms for hemoglobin switching can provide a potentially useful approach to the therapy of patients with genetic disorders involving the β globin chain.

Immature erythroid stem cells, burst-forming units that respond to erythropoietin (BFU-E), are present in adult human peripheral blood and can proliferate and differentiate into large colonies of erythroblasts *in vitro* (4, 5). These erythroblasts, derived from normal adult individuals, synthesize a substantial amount of fetal hemoglobin (6–8). Because HbF is increased in maternal peripheral blood during pregnancy (9–11), the present investigation was undertaken to determine if peripheral blood BFU-E in pregnant women have an increased capacity for HbF production in culture and to study the pattern of HbF synthesis during erythroid cell maturation *in vitro*. Evidence is presented that the capacity for HbF production by peripheral blood BFU-E *in vitro* is not altered during pregnancy. These studies also reveal that the proportion of HbF synthesis decreases during erythroid cell maturation. A preliminary report of these studies has been published elsewhere (12).

METHODS

Subjects. Forty-four pregnant and postpartum (3–7 months) women were studied. All women were healthy and had no known diseases or complications. Appropriate consent was obtained. Forty milliliters of venous blood was withdrawn from each person into a sterile plastic syringe containing 1000 units of heparin.

Hematological Data. Hematological data were determined by standard laboratory procedures, using a Coulter S electronic counter. Hemoglobin studies included starch gel electrophoresis (13) and determination of HbA_2 (normal range 1.8–3.3%) by microcolumn chromatography (14) and HbF (normal range 0.4–1.2%) by the Betke method (15). Iron status was evaluated by the measurement of the free erythrocyte protoporphyrin (FEP) (normal range 15–50 μg/dl of erythrocytes), using the Piomelli procedure (16).

Erythroid Cell Culture. Peripheral blood mononuclear cells were obtained by the Ficoll/Hypaque cell separation technique (4). BFU-E-derived erythroid colonies were cultured according to the method of Heath *et al.* (17) with minor alterations. Linbro microtitration multiwell plates (Flow Laboratories, McLean, VA) were sterilized by exposure to ultraviolet irradiation for 1 hr. Each plasma clot culture of 0.1 ml final volume usually contained 2.5×10^5 cells. Either 0.2 or 1.0 unit of sheep plasma erythropoietin, step 3 (specific activity, 14 units/mg of protein; Connaught Laboratory, Willowdale, ON) was added to each plasma clot at onset of culture. To induce clot formation, 0.1 unit of bovine plasma thrombin, grade 1 (Sigma) was added to each plasma clot. In each experimental point, at least 2 clots were prepared for cytological examination and 10 clots for biochemical analysis. These cultures were incubated for up to 15 days at 37°C in a humidified atmosphere of 5% CO_2 in air. The cultures were harvested and cells were stained with benzidine (18). The cells were examined with a light microscope at ×63 magnification. Erythroid colonies consisting of either 250 or more erythroblasts each or five subcolonies each of which was made up of at least 50 erythroblasts were scored as BFU-E-derived colonies.

Globin Synthesis. Five microliters of L-[4,5-^{3}H]leucine (specific activity 50–60 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), obtained from New England Nuclear, was added to each plasma clot on either day 6 or day 7, and either day 11 or day 12 of culture. Twenty-four hours later, 10 plasma clots thus labeled were pooled and washed twice with phosphate-buffered saline. Three milligrams of Pronase (B grade; Calbiochem) in 3 ml of medium consisting of 95% α minimal essential medium and 5% fetal calf serum (Flow Laboratories) were added to digest the plasma clots; digestion usually was complete after about 20 min at room temperature. The erythroblasts released from the lysed plasma clot cultures were washed twice with phosphate-buffered saline. One milliliter of hemolysate containing approximately 100 mg of hemoglobin, which was made up of human cord and adult hemolysates at a ratio of 2:1, was added to the erythroblasts. Erythroblasts were lysed by freezing

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: BFU-E, burst-forming units that respond to erythropoietin; CFU-E, colony-forming units that respond to erythropoietin; FEP, free erythrocyte protoporphyrin.

Table 1. Hematological data

Gestation, weeks	No. of subjects	Hb, g/dl	Hemato-crit, liters/liter	Mean corpuscular volume, fl	Mean corpuscular Hb, pg	HbF, %	HbA_2, %	FEB, μg/dl erythrocytes
9–19	7	12.1 ± 0.4	0.37 ± 0.01	92.0 ± 1.1	30.3 ± 0.5	0.8 ± 0.2	2.7 ± 0.1	27.4 ± 1.8
		(9.7 – 13.3)	(0.34 – 0.39)	(89.0 – 96.0)	(28.4 – 31.9)	(0.4 – 1.7)	(2.4 – 3.0)	(18.0 – 32.0)
21–25	7	11.6 ± 0.3	0.35 ± 0.01	92.4 ± 1.4	30.2 ± 0.4	0.6 ± 0.1	2.7 ± 0.2	37.4 ± 12.0
		(10.6 – 12.8)	(0.32 – 0.39)	(88.0 – 99.0)	(29.2 – 32.3)	(0.4 – 0.8)	(1.9 – 3.1)	(22.0 – 85.0)
31–39	7	12.1 ± 0.3	0.37 ± 0.01	90.7 ± 1.8	29.9 ± 0.9	0.8 ± 0.2	2.7 ± 0.1	34.0 ± 2.1
		(11.0 – 13.1)	(0.34 – 0.40)	(83.0 – 98.0)	(25.8 – 32.2)	(0.4 – 1.6)	(2.2 – 3.1)	(27.0 – 42.0)
Postpartum	7	12.5 ± 0.4	0.37 ± 0.01	89.5 ± 0.6	30.3 ± 0.6	0.8 ± 0.1	2.6 ± 0.1	24.8 ± 4.1
		(11.2 – 13.5)	(0.34 – 0.40)	(84.0 – 96.0)	(28.7 – 32.4)	(0.5 – 1.5)	(2.3 – 3.0)	(16.0 – 35.0)

Data are expressed as mean ± SEM. The figures in parentheses represent the range.

and thawing three times. The hemolysate thus obtained was stored frozen until further use.

When thawed, the hemolysate was passed through a Sephadex G-100 column, using 0.05 M Tris·HCl, pH 7.4, buffer for elution. The fractions containing hemoglobins were combined and globin was prepared by the acid/acetone method. Globin chains were isolated by carboxymethylcellulose/urea column chromatography. Starting buffer was 8 mM sodium phosphate, and final buffer was 36 mM sodium phosphate, both in 8 M urea and at pH 6.7, containing 150 mg of dithiothreitol (Sigma) per liter. The nonlinear phosphate gradients were made with an LKB Ultra-grad and were pumped at 24 ml per hour for 17 hr. The absorbances were determined with Gilford model 2400 spectrophotometer at 280 nm. One milliliter of each fraction was added to 10 ml of either Aquasol or Aquassure (New England Nuclear), and the samples were assayed for radioactivity in a Beckman liquid scintillation counter.

RESULTS

Hematological Data. The erythroid cell cultures of 8 subjects were not successful. Of the remaining 36 subjects, the data of 8 others were not included in this report because the labeling with radioactive tracer in those cultures was not carried out on days 6–7 or 7–8 and days 11–12 or 12–13 of culture. In total, experimental results of 28 women were analyzed in detail (Table 1).

The mean hemoglobin levels of all three groups of pregnant women and postpartum women were similar to each other and fell within the normal range of hemoglobin during pregnancy (19, 20). One woman had a hemoglobin level of 9.7 g/dl, but all her other hematological values were otherwise within normal limits.

The erythrocyte indices, including mean corpuscular volume in all women studied, were normal. FEP levels were normal in all subjects except for one woman whose FEP was mildly elevated at 85 μg/dl of erythrocytes. Her hemoglobin was 10.6 g/dl, mean corpuscular volume was 99 fl, and HbA_2 was 2.9%. The HbA_2 levels were also normal in all women. Taken together, these results indicate that the subjects in this study were not deficient in iron, folate, or vitamin B-12. In addition, there was no evidence that any of them had the thalassemia trait.

HbF levels were also well within the normal range in all except for five women: two (1.2%; 1.2%) at 14 weeks of pregnancy, one (1.7%) at 19 weeks, one (1.6%) at 38 weeks, and one (1.5%) at 7 months postpartum (Table 2).

Erythroid Colonies in Culture. Peripheral blood mononuclear cells obtained by the Ficoll/Hypaque cell separation technique were cultured in plasma clots with erythropoietin (1.0 unit per clot) added at onset of culture. By day 7 or 8 of culture, there were a number of partially hemoglobinized colonies, each of which consisted of a number of subcolonies and morphologically appeared to be BFU-E derived (Fig. 1 *Left*). In addition, there were some other colonies that were equally or even more cellular than the partially hemoglobinized colonies and that were composed of cells resembling immature erythroid precursors cytologically but staining only faintly or not at all with benzidine. By day 11 or 12 of culture, there was luxurious growth of many well-hemoglobinized BFU-E-derived erythroid colonies (Fig. 1 *Right*). The number of hemoglobinized BFU-E-derived erythroid colonies found in day 12

Table 2. Subjects with high and low HbF *in vivo*: Hematological data and *in vitro* culture results

Subject	Age, years	Gestation, weeks	Hb, g/dl	Mean corpuscular volume, fl	FEP, μg/dl erythrocytes	HbF, %	HbA_2, %	$\gamma/(\gamma+\beta)$ synthetic ratio, %		No. of BFU-E/10^6 cells plated	
								Early cultures	Late cultures	Early cultures	Late cultures
M.G.	26	19	12.6	95	31	1.7	2.4	23.4	10.6	36	150
S.H.	27	38	12.6	92	37	1.6	2.8	18.0*	24.9	8*	26
B.P.	31	7 months postpartum	13.5	86	—	1.5	—	16.8	6.2	21	83
K.S.	25	14	12.0	94	31	1.2	3.1	15.2	2.1†	34	88†
L.A.	27	14	11.7	96	32	1.2	2.4	11.1	1.4‡	40	88‡
J.K.	25	9	13.3	89	26	0.4	2.8	12.8	2.6‡	62	164‡
D.D.	28	19	9.7	94	26	0.4	2.5	13.5	8.1	26	124
K.H.	24	22	12.8	92	22	0.4	2.7	5.8	3.8‡	15	78‡
D.G.	34	31	12.1	90	29	0.4	2.4	21.5	7.9	14	68

Early cultures are those harvested on days 7–8. Late cultures are those harvested on days 11–12.

* Poor cultures as shown cytologically and by low [^{3}H]leucine incorporation into globin chains.

† Days 13–14 cultures.

‡ Days 12–13 cultures.

Table 3. *In vitro* cultures

Gestation, weeks	No. of subjects	Culture	Globin synthesis, cpm × 10^{-3}/10^6 cells plated			Synthetic ratio, %		No. of BFU-E/ 10^6 cells plated
			α	β	γ	$\gamma/(\gamma+\beta)$	$(\gamma+\beta)/\alpha$	
9–19	7	Early	16.4 ± 6.6	13.8 ± 6.4	1.8 ± 0.7	12.3 ± 2.1	0.90 ± 0.04	34.4 ± 7.7
		Late	46.9 ± 11.1	39.4 ± 8.6	2.7 ± 1.3	5.4 ± 1.2	0.91 ± 0.03	101.8 ± 19.0
21–25	7	Early	23.9 ± 8.6	20.2 ± 7.8	1.7 ± 0.4	10.9 ± 2.1	0.88 ± 0.03	31.9 ± 7.3
		Late	60.8 ± 9.6	54.2 ± 8.8	2.3 ± 0.6	5.2 ± 1.1	0.93 ± 0.01	99.5 ± 9.0
31–39	7	Early	12.3 ± 3.4	9.4 ± 3.0	1.3 ± 0.2	15.6 ± 2.5	0.83 ± 0.04	19.3 ± 5.8
		Late	67.5 ± 14.5	60.1 ± 13.8	3.7 ± 1.2	9.1 ± 3.4	0.92 ± 0.03	81.8 ± 18.9
Postpartum	7	Early	16.2 ± 4.2	12.9 ± 3.0	1.4 ± 0.4	8.7 ± 2.1	0.92 ± 0.03	30.1 ± 6.3
		Late	86.4 ± 18.9	78.5 ± 17.9	3.9 ± 1.1	4.8 ± 0.8	0.96 ± 0.02	68.5 ± 7.5

Data are expressed as mean ± SEM. Early cultures were labeled with [^{3}H]leucine on days 7–8, except for two cultures that were labeled on days 6–7. Late cultures were labeled on days 11–12 or 12–13.

cultures was 2- to 4-fold of that found in day 8 cultures (Table 3). In general, colonies in late cultures were larger and much better hemoglobinized than those in early cultures.

Globin Synthesis *in Vitro*. Preliminary experiments showed that there was no detectable globin synthesis in cultures to which exogenous erythropoietin had not been added. Moreover, the results of globin synthetic studies were similar whether or not the plasma clots were dissolved with Pronase at harvest, or whether or not the hemolysates were passed through Sephadex G-100 columns. For all subsequent studies, the plasma clots were dissolved by treatment with Pronase and the hemolysates were partially purified by Sephadex G-100 columns.

In all cultures with erythropoietin added there were substantial amounts of both HbA and HbF being produced as evidenced by the synthesis of α, β, and γ globin chains (Table 3 and Fig. 2). The $(\gamma+\beta)/\alpha$ globin synthetic ratio was close to unity in all cultures examined (Table 3). The total γ globin synthetic rate in the late cultures was 2- to 3-fold higher than that in the early cultures (Table 3). However, the degree of augmentation of β and α globin synthesis in the late cultures compared to early cultures was even higher—i.e., 3- to 6-fold increase (Table 3). Thus, the proportion of HbF produced as measured by γ chain synthesis was less in late cultures compared to early cultures (Table 3 and Fig. 3), and the difference was statistically highly significant ($P < 0.01$). This observation is also consistent with the results of those studies involving the other eight women whose erythroid cell cultures were successful but whose data were not tabulated in this report due to different dates of radioactive tracer labeling. Similar results were obtained in erythroid cell cultures derived from peripheral blood mononuclear cells from two normal male subjects (data not shown).

The proportion of γ globin synthesis in erythroid cultures was not correlated with the amount of total globin synthesis in these cultures. Moreover, there was no direct relationship between the proportion of γ chain synthesis *in vitro* and the gestational stage under study (Fig. 4). Similar analysis also failed to detect a significant correlation between the proportion of γ chain synthesis *in vitro* compared to the total hemoglobin or HbF levels in the peripheral blood of pregnant or postpartum women *in vivo*. Of those subjects with the highest and lowest HbF levels in peripheral blood *in vivo*, there was no obvious correlation with the proportion of HbF synthesized in early and late erythroid cultures or the plating efficiency of BFU-E-derived erythroid colonies (Table 2).

Effects of Two Doses of Erythropoietin in Cultures. In cultures of peripheral blood mononuclear cells obtained from three pregnant and four postpartum women, parallel experiments were done with either 1.0 or 0.2 unit of erythropoietin added to each plasma clot culture. The data are summarized in Table 4.

With 0.2 unit of erythropoietin added to each plasma clot, the early cultures had fewer hemoglobinized erythroid colonies and the globin synthetic rate was 1/4th that of the culture with 1.0 unit of erythropoietin added. In six sets of these cultures, the $\gamma/(\gamma+\beta)$ synthetic ratio was higher in those cultures with 0.2 unit of erythropoietin added compared to those with 1.0 unit of erythropoietin added. In one other set of cultures, no γ chain

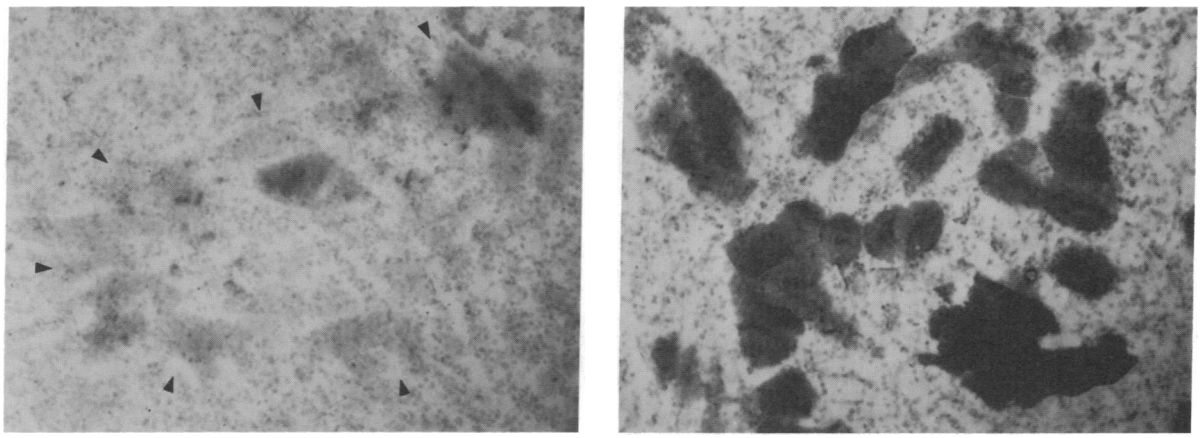

FIG. 1. Photomicrographs of erythroid cell cultures derived from peripheral blood mononuclear cells obtained from a pregnant woman at 21 weeks of gestation. (Original magnification, ×125.) About 2.2 × 10^5 cells were present in a plasma clot 0.1 ml in total volume. Erythropoietin (1.0 unit) was added at onset of culture. (*Left*) The culture was harvested for cytological examination on day 7. Note that there were a number of subcolonies (arrows) with various degrees of hemoglobinization as visualized by the benzidine staining. (*Right*) The culture was harvested on day 11. Note that the subcolonies were bigger and much better hemoglobinized.

Table 4. Comparison between cultures with 1.0 and 0.2 unit of erythropoietin added

Culture	No. of subjects	Erythropoietin, units	Globin synthesis, cpm × 10^{-3}/10^6 cells plated			Synthetic ratio, %		No. of BFU-E/ 10^6 cells plated
			α	β	γ	$\gamma/(\gamma+\beta)$	$(\gamma+\beta)/\alpha$	
Early	7	1.0	19.8 ± 6.8	16.9 ± 6.2	2.0 ± 0.6	10.4 ± 1.6	0.95 ± 0.03	35.4 ± 7.6
	7	0.2	4.7 ± 1.1	3.7 ± 1.0	0.6 ± 0.2	13.6 ± 3.9	0.90 ± 0.06	21.1 ± 3.4
Late	7	1.0	57.1 ± 15.4	50.5 ± 12.8	2.9 ± 1.1	5.2 ± 0.8	0.96 ± 0.02	83.9 ± 14.8
	7	0.2	45.2 ± 4.6	40.4 ± 4.0	2.4 ± 0.4	5.7 ± 1.1	0.95 ± 0.02	89.9 ± 24.5

Data are expressed as mean ± SEM.

synthesis was detected in the culture with less erythropoietin added, while the $\gamma/(\gamma+\beta)$ synthetic ratio was 4.8% in the culture with more erythropoietin added.

After 11–13 days in culture, there was virtually no difference between cultures with high or low doses of erythropoietin added in terms of the number of BFU-E-derived colonies formed, total globin production, and proportion of γ globin chain synthesis. Regardless whether 0.2 or 1.0 unit of erythropoietin was added to each plasma clot culture, the $\gamma/(\gamma+\beta)$ synthetic ratio was higher in the early cultures than in the late cultures.

DISCUSSION

Immature erythroid stem cells, BFU-E, are present in peripheral blood of normal adults (4, 5) and pregnant women. These precursor cells can be induced by erythropoietin to proliferate and differentiate into large erythroid colonies capable of synthesizing a considerable amount of HbF (6–8). The more mature erythroid progenitor cells, colony-forming units that respond to erythropoietin (CFU-E), are present in bone marrow, but not in normal adult peripheral blood (4, 5, 21). Operationally, CFU-E from adult individuals can be distinguished from BFU-E by the much lower amount of erythropoietin required for growth *in vitro*, the shorter time necessary for colony formation, the much smaller size and compact appearance of the colonies, and their inability to synthesize HbF *in vitro* (7, 8, 22). In the present study, no CFU-E-derived colonies could be identified with confidence in cultures of peripheral blood mononuclear cells from pregnant women.

Under the present experimental conditions, the erythroid colonies observed on days 6–8 of culture, composed of immature and poorly hemoglobinized erythroblasts, are clearly BFU-E derived as demonstrated by their cellularity, morphological appearance, and capacity to synthesize a high proportion of HbF. By day 12 of culture, numerous BFU-E-derived large erythroid colonies made up of mature and well-hemoglobinized erythroblasts are present, but proportionally less HbF is synthesized in these colonies. This phenomenon is not significantly altered whether 0.2 or 1.0 unit of erythropoietin is present in each culture. These studies are highly suggestive that the proportion of HbF synthesis varies during human erythroid cell maturation and that proportionally less HbF is produced in the

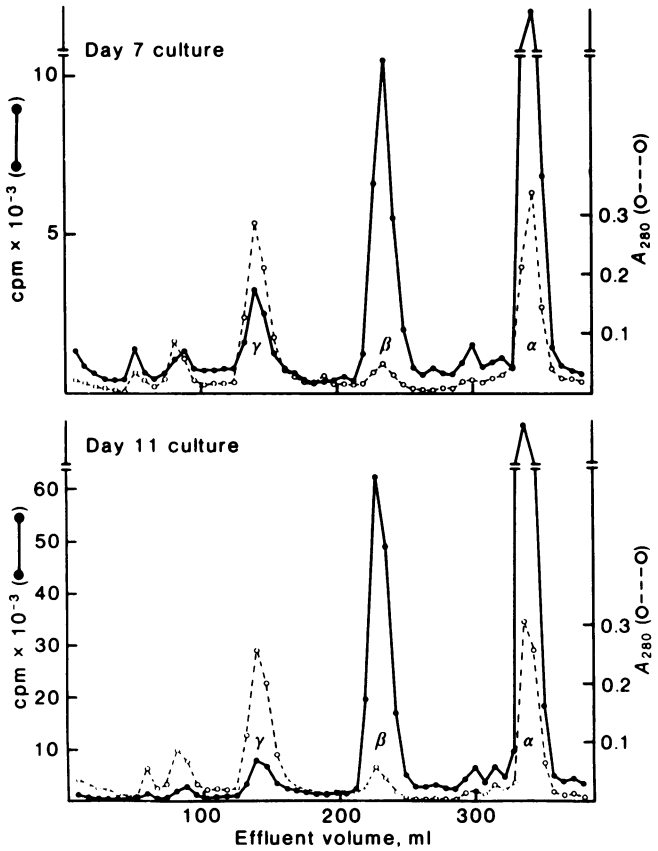

FIG. 2. Globin chain synthesis in erythroid cell cultures labeled with [^{3}H]leucine on days 7–8 of culture (*Upper*) and on days 11–12 of culture (*Lower*). The donor was a pregnant woman, age 26, at 19 weeks of gestation. At the onset of culture, 1.0 unit of erythropoietin was added to each plasma clot culture (0.1 ml final volume). $\gamma/(\gamma+\beta)$ synthetic ratio was 23.4% in the early cultures and 10.6% in the late cultures.

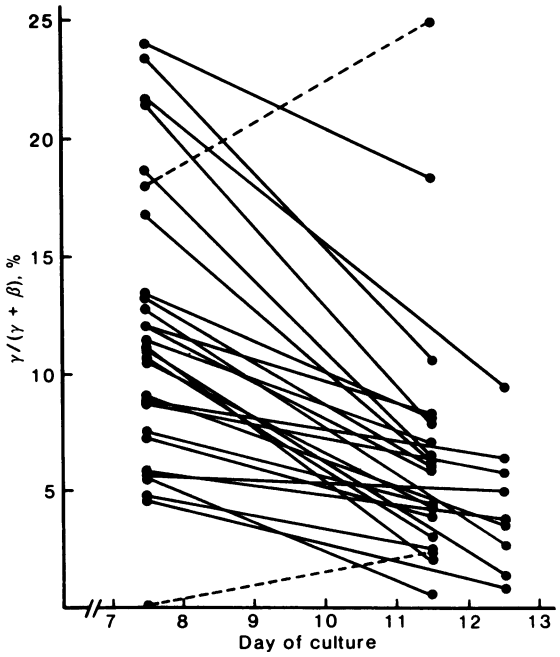

FIG. 3. The $\gamma/(\gamma+\beta)$ synthetic ratio in erythroid cell cultures from each individual subject was plotted against the time of labeling with [^{3}H]leucine. Note that in 26 out of 28 instances the ratio is lower in the late cultures than in the early cultures. The two exceptions were: one with poor early culture as shown cytologically and by low [^{3}H]-leucine incorporation into globin chains, and the other with little γ chain synthesis in both early and late cultures.

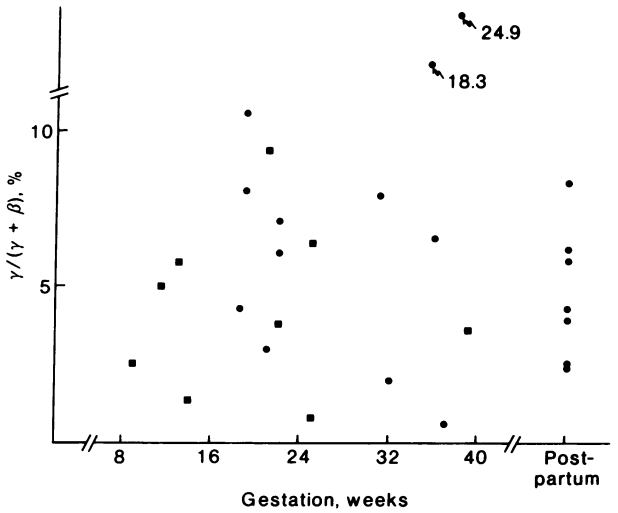

FIG. 4. Synthetic ratios of $\gamma/(\gamma + \beta)$ in late cultures plotted against the time of gestation of the subjects studied. The cultures were labeled with [^{3}H]leucine on either days 11–12 (●) or days 12–13 (■).

more mature erythroblasts. During mouse fetal erythropoiesis, it has been well documented that the proportions of embryonic and adult hemoglobin synthesis also vary during erythroid cell maturation (23–25).

An alternative interpretation of these experimental data is that different classes of BFU-E differentiating at different rates may account for the varying proportion of HbF synthesized in early and late cultures. At least two classes of BFU-E have been reported to be present in adult bone marrow (21). However, peripheral blood BFU-E appears to represent a relatively homogenous population of immature erythroid stem cells (4, 5). Moreover, the degrees of hemoglobinization and the cytological appearance of the erythroblasts present in early and late cultures are consistent with these erythroid cells being progeny of a relatively homogeneous class of immature BFU-E differentiating and maturing as a cohort.

Pregnancy is the only physiological condition in adults in which the amount of HbF in peripheral blood is elevated (26). The increase of HbF in pregnant women is the result of alterations in maternal erythropoiesis and not due to transplacental bleeding from the fetus (9–11). In the present study, culturing peripheral blood BFU-E *in vitro*, HbF synthesis is increased to as high as 25% of the total hemoglobin produced in erythroid cell cultures, although the degree of augmentation varies considerably. There is no significant difference between the HbF-producing capabilities *in vitro* of peripheral blood BFU-E obtained from pregnant or postpartum women. Whether or not bone marrow BFU-E in pregnant women are different in their capability to produce HbF *in vitro* compared to nonpregnant subjects has not been investigated. In other conditions in which the HbF in blood is increased, such as sickle cell anemia, both peripheral blood and bone marrow BFU-E produce significantly increased amounts of HbF *in vitro* (7, 8, 27).

During pregnancy, the total erythrocyte mass is increased due to increased maternal erythropoietic activity, probably via the erythropoietin pathway (20). It is therefore possible that the slight elevation of HbF in peripheral blood of pregnant women is the reflection of stimulated erythropoiesis and release of erythroid cells at earlier maturational stages (28). Similar mechanisms may be in operation to account for the increased level of HbF observed in a number of pathological conditions such as leukemia and aplastic anemia.

We thank M. Snyder, who recruited many of the subjects for this study, M. Lawry, who designed the nonlinear gradient for globin chain separation, and T. W. Brotherton, who modified the method for harvesting erythroblasts in plasma clot cultures. We are grateful to those women who participated in this study by donating their blood. This investigation was supported by a grant (MT-5004) from the Medical Research Council of Canada.

1. Wood, W. G. (1976) *Br. Med. Bull.* **32**, 282–287.
2. Bard, H. (1975) *J. Clin. Invest.* **55**, 395–398.
3. Nienhuis, A. W. & Stamatoyannopoulos, G. (1978) *Cell* **15**, 307–315.
4. Clarke, B. J. & Housman, D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1105–1109.
5. Ogawa, M., Grush, O. C., O'Dell, R. F., Hara, H. & MacEachern, M. D. (1977) *Blood* **50**, 1081–1092.
6. Papayannopoulou, T., Nakamoto, B., Buckley, J., Kurachi, S., Nute, P. E. & Stamatoyannopoulos, G. (1978) *Science* **199**, 1349–1350.
7. Kidoguchi, K., Ogawa, M., Karam, J. D. & Martin, A. G. (1978) *Blood* **52**, 1115–1124.
8. Clarke, B. J., Nathan, D. G., Alter, B. P., Forget, B. G., Hillman, D. & Housman, D. (1979) *Blood* **54**, 805–817.
9. Pembrey, M. E., Weatherall, D. J. & Clegg, J. B. (1973) *Lancet* **i**, 1350–1355.
10. Boyer, S. H., Belding, T. K., Margolet, L., Noyes, A. N., Burke, P. J. & Bell, W. R. (1975) *Johns Hopkins Med. J.* **137**, 105–115.
11. Popat, N., Wood, W. G., Weatherall, D. & Turnbull, A. C. (1977) *Lancet* **ii**, 377–379.
12. Chui, D. H. K., Wong, S. C., Enkin, M. W., Patterson, M. & Ives, R. A. (1978) *Blood* **52**, Suppl. 1, 110 (abstr.).
13. Efremov, G. D., Huisman, T. H. J., Smith, L. L., Wilson, J. B., Kitchens, J. L., Wrightstone, R. N. & Adams, H. R. (1969) *J. Biol. Chem.* **244**, 6105–6116.
14. Efremov, G. D., Huisman, T. H. J., Bowman, K., Wrightstone, R. N. & Schroeder, W. A. (1974) *J. Lab. Clin. Med.* **83**, 657–664.
15. Betke, K., Warte, H. R. & Schlicht, L. (1959) *Nature (London)* **184**, 1877–1878.
16. Piomelli, S. (1973) *J. Lab. Clin. Med.* **81**, 932–940.
17. Heath, D. S., Axelrad, A. A., McLeod, D. L. & Shreeve, M. M. (1976) *Blood* **47**, 777–792.
18. Chui, D. H. K., Liao, S.-K. & Walker, K. (1978) *Blood* **51**, 539–547.
19. Lund, C. J. & Sisson, T. R. C. (1958) *Am. J. Obstet. Gynecol.* **76**, 1013–1024.
20. Mansac, B. & Jepson, J. (1969) *Can. Med. Assoc. J.* **100**, 687–691.
21. Eaves, C. J. & Eaves, A. C. (1978) *Blood* **52**, 1196–1210.
22. Papayannopoulou, T., Brice, M. & Stamatoyannopoulos, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2923–2927.
23. Craig, M. L. & Southard, J. L. (1967) *Dev. Biol.* **16**, 331–340.
24. Fantoni, A., de la Chapelle, A. & Marks, P. A. (1969) *J. Biol. Chem.* **244**, 675–681.
25. Chui, D. H. K., Brotherton, T. W. & Gauldie, J. (1979) in *Cellular and Molecular Regulation of Hemoglobin Switching*, eds. Stamatoyannopoulos, G. & Nienhuis, A. W. (Grune & Stratton, New York), pp. 213–225.
26. Weatherall, D. J., Pembrey, M. E. & Pritchard, J. (1974) *Clinics in Hematol.* **3**, 467–508.
27. Papayannopoulou, T., Nute, P. E., Kurachi, S. & Stamatoyannopoulos, G. (1978) *Blood* **51**, 671–679.
28. Stohlman, F., Jr., Ebbe, S., Morse, B., Howard, D. & Donovan, J. (1968) *Ann. N.Y. Acad. Sci.* **149**, 156–172.