

EXTENDED THERAPY WITH INTRAVENOUS ARGININE BUTYRATE IN PATIENTS WITH β -HEMOGLOBINOPATHIES

GRAHAM D. SHER, M.B., B.CH., GORDON D. GINDER, M.D., JANE LITTLE, M.D., SUYA YANG, M.SC., GEORGE J. DOVER, M.D., AND NANCY F. OLIVIERI, M.D.

Abstract Background. Enhanced production of fetal hemoglobin lessens the severity of β -thalassemia and sickle cell disease. Intravenous infusion of arginine butyrate can increase the number of reticulocytes containing fetal hemoglobin in patients with these disorders, and it has induced a substantial increase in hemoglobin in one patient with thalassemia. We therefore tested the efficacy of this agent in patients with β -hemoglobinopathies.

Methods. We treated 10 patients with severe β -thalassemia or sickle cell disease with arginine butyrate at an initial dose of 500 mg per kilogram of body weight per day (final dose, 2000 mg per kilogram per day), 6 days per week, for a mean (\pm SD) of 10 ± 1.2 weeks (range, 9 to 13). A hematologic response was defined as an increase in the hemoglobin concentration of at least 2 g per deciliter

in patients with thalassemia and as a twofold increase in fetal hemoglobin in patients with sickle cell disease.

Results. There were increases in γ -globin messenger RNA and in reticulocytes containing fetal hemoglobin, but no increases in hemoglobin, in the patients with thalassemia. A small, unsustained increase in fetal hemoglobin was observed in two patients with sickle cell disease. Drug toxicity was minimal at standard doses. One patient had a grand mal seizure after inadvertently receiving 2000 mg of arginine butyrate per kilogram over a period of six hours.

Conclusions. Ten weeks of intravenous arginine butyrate did not produce a hematologic response in 10 patients with either severe β -thalassemia or sickle cell disease. (N Engl J Med 1995;332:1606-10.)

ENHANCED production of fetal hemoglobin ($\alpha_2\gamma_2$) lessens the severity of the two major β -hemoglobinopathies, β -thalassemia and sickle cell disease. In homozygous β -thalassemia, reduced or absent production of β chains results in an excess of unpaired α -globin chains, which precipitate within the red cell, thus causing ineffective erythropoiesis and severe anemia. After the first year of life, when the switch from the production of γ chains to β chains normally occurs, most patients with homozygous β -thalassemia begin to require regular transfusions of red cells. Increased synthesis of γ chains reduces the imbalance between γ chains and β chains in thalassemia by increasing the synthesis of non- α chains. Increased synthesis of fetal hemoglobin reduces the severity of sickle cell disease through a different mechanism: it inhibits the polymerization of sickle hemoglobin and reduces sickling in vivo.¹⁻⁶ The investigational use of chemotherapeutic agents to stimulate the production of fetal hemoglobin⁷⁻²² has aroused concern about the long-term effects of these drugs in nonmalignant disorders. A group of nonchemotherapeutic compounds, the butyric acid analogues, might be used as an alternative therapy for the β -hemoglobinopathies. The rationale for their use comes from reports of a delay in the normal switch from production of γ globin to β globin in newborn infants of diabetic mothers,^{23,24} a finding subsequently attributed to elevated plasma concentrations of α -amino-

n-butyric acid.^{25,26} Studies of the effects of butyrate on fetal-globin genes in vitro²⁶⁻²⁹ and in animals^{25,30,31} were followed by a pilot trial³² in which the administration of arginine butyrate to one patient with β -thalassemia increased the total hemoglobin concentration by 6 g per deciliter, a response that stimulated much enthusiasm for butyrate in the treatment of these disorders.³³ We now report the results of a study of extended administration of arginine butyrate to patients with β -thalassemia or sickle cell disease.

METHODS

Patients

Ten patients (mean [\pm SD] age, 15.1 ± 10.5 years; range, 2.6 to 38), were admitted to the hospital for the administration of intravenous arginine butyrate (Table 1). Five patients had sickle cell disease: four of them had sickle cell anemia and one had compound heterozygosity for hemoglobin S and β^0 -thalassemia. Of the five patients with thalassemia, four had homozygous β -thalassemia and one had compound heterozygosity for hemoglobin E and β^0 -thalassemia. Seven patients had received red-cell transfusions, which were discontinued 4 to 25 weeks before the start of the study. Mutations in the α -globin and β -globin gene clusters were determined as previously described.³⁴ The nucleotide substitution of threonine for cysteine 158 base pairs downstream from the 5' end of the G γ -globin gene was determined by analysis with the restriction enzyme *Xmn*I.³⁵ The presence of this substitution is noted as *Xmn*I+, and its absence as *Xmn*I- (Table 1).

Arginine butyrate was administered at a dose of 500 mg per kilogram of body weight during the first 24 hours of treatment. In nine patients, the dose was increased over the subsequent 48-hour period to a maximum of 2000 mg per kilogram per day. In the 10th patient the maximal dose was 1500 mg per kilogram per day because of severe nausea with any further increase in the dose. Arginine butyrate was infused through a central venous catheter 24 hours a day 5 to 6 days a week for a mean (\pm SD) of 10 ± 1.2 weeks (range, 9 to 13). In two patients (Patients 6 and 9) this regimen was followed by intermittent therapy (30 to 50 hours per week for another 16 and 5 weeks, respectively). All patients received daily folic acid.

The study was approved by the institutional review boards of the Hospital for Sick Children and Toronto Hospital, the Food and Drug Administration, and the Health Protection Branch, Ottawa,

From the Hospital for Sick Children and the University of Toronto, Toronto (G.D.S., N.F.O.); the University of Minnesota School of Medicine, Minneapolis (G.D.G., J.L., S.Y.); and Johns Hopkins University School of Medicine, Baltimore (G.J.D.). Address reprint requests to Dr. Olivieri at the Haemoglobinopathy Program, Hospital for Sick Children, Rm. 6324, 555 University Ave., Toronto, ON M5G 1X8, Canada.

Supported in part by the Connaught Transformative Research Grant Program, University of Toronto; the Medical Research Council of Canada; the Ontario Heart and Stroke Foundation; the Cooley's Anemia Foundation; and the National Institutes of Health (DK 29902). Dr. Olivieri is a Career Scientist of the Ontario Ministry of Health.

Table 1. Characteristics and Transfusion Histories of 10 Patients Receiving Arginine Butyrate.

PATIENT NO.	AGE (YR)	SEX	GENOTYPE*	TRANSFUSION HISTORY
β-Thalassemia				
1	2.6	F	Homozygous β -thalassemia (619-bp deletion) - $\alpha^{37}/\alpha\alpha$ <i>XmnI</i> -/-	No previous transfusions
2	5.0	F	Homozygous β^+ -thalassemia (IVS-1#6/IVS-1#110) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Irregular transfusions; last transfusion 5 wk before study entry
3	5.8	F	Homozygous β^0 -thalassemia (41/42[-CTTT]/A \rightarrow T at codon 17) - $\alpha^{37}/\alpha\alpha$ <i>XmnI</i> -/-	Transfusion-dependent; last transfusion 4 wk before study entry
4	16.1	F	Homozygous β^+ -thalassemia (IVS-1#6/IVS-1#110) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Infrequent transfusions; last transfusion >1 yr before study entry
5	38.4	M	β^0 -thalassemia/hemoglobin E (41/42[-CTTT]/G \rightarrow A at codon 28) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> +/-	No previous transfusions
Sickle cell disease				
6	8.8	F	Hemoglobin SS - $\alpha^{37}/\alpha\alpha$ <i>XmnI</i> -/-	No previous transfusions
7	16.9	M	Hemoglobin S/ β^0 -thalassemia (loss of CT at codon 5) $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> not determined	Regular transfusions; last transfusion 3 mo before study entry
8	17.5	F	Hemoglobin SS $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> not determined	Frequent, irregular transfusions; last transfusion >6 mo before study entry
9	18.8	M	Hemoglobin SS $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Red-cell exchange 4 wk before study entry
10	21.3	F	Hemoglobin SS $\alpha\alpha/\alpha\alpha$ <i>XmnI</i> -/-	Infrequent transfusions; last transfusion >1 yr before study entry

*The abbreviation bp denotes base pair, *XmnI*+ the presence of a C \rightarrow T substitution 158 base pairs downstream from the 5' end of the G γ -globin gene, and *XmnI*- the absence of this mutation.

Ontario. Each patient or a parent gave informed consent before the study.

A clinical response was defined as an increase in the hemoglobin concentration of at least 2 g per deciliter in patients with thalassemia and as a twofold increase in the percentage of fetal hemoglobin in patients with sickle cell disease. These were considered the primary and only clinically important end points of the study. Blood counts were monitored twice weekly. Fetal hemoglobin was measured by alkali denaturation and by densitometry when the concentration exceeded 15 percent of the total hemoglobin concentration. The absolute fetal hemoglobin concentration was calculated as the product of the percentage of fetal hemoglobin and the total hemoglobin concentration.

Secondary end points of the study included biologic markers anticipated to change during butyrate therapy, including the concentration of messenger RNA (mRNA) of γ and β globin, ratios of globin-chain synthesis, and the number of reticulocytes containing fetal hemoglobin (F reticulocytes). The S1 nuclease protection assay was used to measure mRNA of γ and β globin in peripheral blood.²⁵ Total cellular RNA isolated by extraction with guanidium isothiocyanate was analyzed by hybridization to probes specific for human γ and β globin that were end-labeled with [γ^{32} P]ATP. All samples, run in parallel with a human cell line expressing γ globin (K562), were standardized by simultaneous hybridization to a β -actin probe. After digestion, protection products were subjected to electrophoresis in a

denaturing gel (6 M urea and 6 percent polyacrylamide), which was then dried and autoradiographed. We determined the ratio of γ -globin mRNA to γ -globin + β -globin mRNA before and during therapy. The value during therapy is expressed as a multiple of the pre-treatment ratio.

The ratios of globin-chain synthesis³² and the proportions of F reticulocytes³⁶ were determined by assay as previously described. All systems were reviewed and a physical examination was conducted daily, and chemical profiles were obtained twice weekly in all patients.

RESULTS

Primary End Points

Overall, the response to butyrate therapy was disappointing. In no patient with thalassemia were significant changes in total hemoglobin concentration, fetal hemoglobin concentration, or the imbalance between α -globin and non- α -globin chains observed. One patient with thalassemia had an apparent increase in fetal hemoglobin (Patient 3 in Table 2), but increases in the percentage of hemoglobin and the absolute fetal-hemoglobin concentration were consistent with a return of a β^0 -thalassemia phenotype that had been suppressed by regular transfusions. In one patient red-cell survival, studied with ⁵¹Cr-tagged autologous red cells before and after butyrate therapy, was unchanged from base line (half-life, 250 hours vs. 248 hours).

The increases in fetal hemoglobin in patients with sickle cell disease were minor. Two patients had transient, moderate increases in fetal hemoglobin during butyrate therapy. In the first patient (Patient 7 in Table 2), fetal hemoglobin increased from 4.7 percent to 11.5 percent of the total hemoglobin concentration; the absolute fetal-hemoglobin concentration increased by 0.18 g per deciliter. In Patient 10, fetal hemoglobin increased from 6.9 to 17.3 percent, and the absolute fetal-hemoglobin concentration increased by 0.5 g per deciliter. An increase in fetal hemoglobin from 0 to 4.2 percent in Patient 9, in whom values of approximately 5 percent had been previously documented, was consistent with a return to a phenotype suppressed by transfusions. No significant increase in mean hemoglobin was observed in patients with sickle cell disease.

Secondary End Points

Changes in the ratio of γ -globin mRNA to γ -globin + β -globin mRNA of 1.8-fold and 3.1-fold were observed in both patients whose fetal hemoglobin increased dur-

Table 2. Biologic Markers of Efficacy Measured before and during Treatment with Arginine Butyrate in Five Patients with β -Thalassemia and Five Patients with Sickle Cell Disease.*

PATIENT NO.	γ -GLOBIN: γ -GLOBIN + β -GLOBIN mRNA [†]		α -GLOBIN CHAINS: NON- α -GLOBIN CHAINS		F RETICULOCYTES			FETAL HEMOGLOBIN			TOTAL HEMOGLOBIN		
	PEAK	MEAN	PRE	MEAN	PRE	PEAK	MEAN	PRE	PEAK	MEAN	PRE	PEAK	MEAN
					percent			percent			g/dl		
β-Thalassemia													
1	1.00	0.96	4.83	4.13	67	76.0	74.3	98.0	98.0	98.0	8.6	8.2	7.6
2	1.16	0.93	2.85	2.87	56	69.1	58.5	18.1	48.4	31.6	9.3	7.0	5.8
3	1.06	0.99	4.24	4.33	0	98.6	93.3	2.2	15.1	13.1	9.0	6.7	5.3
4	1.14	1.05	2.68	2.76	65.0	78.0	67.4	58.4	60.5	57.0	8.3	9.3	8.4
5	1.53	1.15	3.87 \ddagger	2.53	24.2	65.4	55.5	49.6	55.0	46.8	7.8	7.3	6.7
Mean \pm SD	1.18 \pm 0.21	1.02 \pm 0.09	3.69 \pm 0.92	3.32 \pm 0.84	42.3 \pm 29.4	77.4 \pm 12.9	69.8 \pm 15.1	45.3 \pm 37.3	55.4 \pm 29.6	49.4 \pm 31.9	8.6 \pm 0.6	7.7 \pm 1.1	6.8 \pm 1.3
Sickle cell disease													
6	1.28	1.28	ND	0.99	2.0	24.7	17.6	6.8	7.5	7.2	8.8	8.8	8.6
7	1.84	1.45	2.44	2.71	21.0	28.7	26.1	4.7	11.5	7.9	6.0	8.0	6.1
8	0.79	0.58	0.90	1.22	19.3	20.7	18.8	6.9	7.3	6.6	8.0	9.1	7.4
9	1.02	0.91	1.16	1.25	ND	13.0	10.3	0	4.2	4.2	10.3	11.7	10.2
10	3.11	1.80	1.23	1.27	17.0	34.0	30.4	6.9	17.3	11.7	9.9	11.8	10.6
Mean \pm SD	1.61 \pm 0.93	1.20 \pm 0.47	1.43 \pm 0.67	1.49 \pm 0.69	10.3 \pm 10.2	24.2 \pm 8.0	20.6 \pm 7.8	5.1 \pm 3.0	9.6 \pm 5.0	7.5 \pm 2.7	8.6 \pm 1.7	9.9 \pm 1.8	8.6 \pm 1.9

*Pre refers to data obtained before therapy with arginine butyrate, peak to the single highest value measured during therapy, and mean to the mean of all measurements obtained during therapy. ND denotes assay not done.

[†]We determined the ratio of γ -globin mRNA to γ -globin + β -globin mRNA before and during therapy. The value during therapy is expressed as a multiple of the pretreatment ratio. Base-line determinations were assigned a value of 1.00; see the Methods section for further explanation.

[‡]The determination of the ratio in this base-line sample may be inaccurate because of evidence of sample degradation. No other measurements were obtained before therapy with arginine butyrate was begun.

ing the study. In two other patients (Patients 5 and 6) very small (1.5-fold and 1.3-fold, respectively) increases were also observed, but without increases in fetal hemoglobin.

The percentage of F reticulocytes, determined by an assay that identifies any reticulocyte containing fetal hemoglobin in concentrations exceeding 2 to 3 pg per cell,³⁶ increased in two patients with thalassemia: from 0 to 98.6 percent in Patient 3, as a result of the increase in fetal-hemoglobin synthesis previously suppressed by transfusions, and from 24.2 to 65.4 percent in Patient 5 in the absence of an increase in the percentage of fetal hemoglobin. The percentage of F reticulocytes also increased in two patients with sickle cell disease: from 2.0 to 24.7 percent without an increase in fetal hemoglobin in Patient 6, and from 17.0 to 34.0 percent in parallel with the moderate increase in fetal hemoglobin observed in Patient 10.

Consistent increases in the mean red-cell volume and mean corpuscular hemoglobin concentration were observed in two patients, in parallel with an increase in F reticulocytes (Patient 5) and with increases in both F reticulocytes and fetal hemoglobin (Patient 10). In no patient were consistent decreases in plasma free hemoglobin, serum lactate dehydrogenase, or bilirubin observed — a finding consistent with the lack of improvement in erythropoiesis or hemolytic anemia.

Toxicity

Hypokalemia requiring oral potassium supplementation occurred in eight patients; nausea requiring parenteral antiemetics or anorexia was noted in nine patients. In one patient daily infusion of arginine butyrate

at a dose of 1500 mg per kilogram produced constant nausea; increases in the dose to a maximum of 2000 mg per kilogram per day resulted in intractable vomiting. Mean blood urea nitrogen concentrations increased from 9.6 ± 3.7 to 29.2 ± 10.9 mg per deciliter ($P \leq 0.005$) during therapy, returning to normal within 24 hours after treatment with butyrate was stopped. Serum creatinine levels remained unchanged.

Because of a labeling error during the production of the study drug, one patient received a dose of 2000 mg of arginine butyrate per kilogram over a period of six hours, after which she suffered a grand mal seizure. The results of all metabolic investigations, computed tomography, and electroencephalography were normal. The patient recovered without sequelae and completed the study after a two-week butyrate-free interval.

DISCUSSION

An increased synthesis of fetal hemoglobin lessens the severity of β -thalassemia and sickle cell disease. In patients with β^0 -thalassemia, the absolute lack of the synthesis of β -globin chains upsets the normal balance between α -globin and β -globin chains in erythrocytes. Augmenting the production of the γ chains of fetal hemoglobin reduces this imbalance, thus improving erythropoiesis and ameliorating anemia.³⁷ In patients who are homozygous for hemoglobin S, coinheritance of a determinant for high expression of fetal hemoglobin results in a relatively benign form of sickle cell disease.^{4,5,35,38,39} Moreover, any increment of fetal hemoglobin reduces mortality in sickle cell disease.⁴⁰

Several cell-cycle-specific agents, including azaciti-

dine, cytarabine, vinblastine, and hydroxyurea, stimulate the synthesis of γ -globin and fetal hemoglobin.⁷⁻²² Because the toxicity of these drugs poses at least a theoretical long-term risk to patients with nonmalignant disorders,⁴¹ noncytotoxic agents that augment fetal-hemoglobin production are of great interest. A pilot trial³² showed that short-term infusions of arginine butyrate increased the number of F reticulocytes and the synthesis of γ -globin mRNA in a small number of patients with β -hemoglobinopathies. In this same study, an increase in total hemoglobin was observed in one patient with thalassemia. The present study aimed to determine the efficacy of extended administration of arginine butyrate in a larger group of patients, in whom hematologic response was defined as a clinically important increase in total hemoglobin or the percentage of fetal hemoglobin.

In contrast to the pilot study, this study found that extended administration of arginine butyrate did not increase total hemoglobin in patients with thalassemia, nor did it cause sustained increases in fetal hemoglobin in patients with sickle cell disease. The moderate increases in γ -globin chains in a few patients were not associated with sustained hematologic responses over a 10-week period. These findings seem inconsistent with the proposed effect of butyrate — augmentation of the expression of the γ -globin gene.²⁷⁻³¹ However, there is evidence that butyric acid may increase the expression of α globin, although not to the same extent as that of γ globin.⁴² Increases in the expression of both α -globin genes and non- α -globin genes (including γ globin) would not fully reduce the imbalance between α -globin chains and non- α -globin chains, which is the cause of the ineffective erythropoiesis in severe thalassemia.

One finding from the pilot study was confirmed in the present study: increases in F reticulocytes were observed in approximately half the patients. However, in two patients with thalassemia the increases were consistent simply with a return of the phenotype suppressed by regular transfusions, whereas the greatest increase in a patient with sickle cell disease was not associated with any hematologic response during six months of therapy. The importance of a butyrate-induced increase in F reticulocytes is unclear. This assay identifies any reticulocyte containing fetal hemoglobin in concentrations exceeding 2 to 3 pg per cell³⁶; it is therefore possible that small increases in γ -globin mRNA may be insufficient to cause measurable increases in fetal hemoglobin.

Our findings, disappointing in view of an earlier pilot study, should stimulate an investigation of the possible influences of genotype; the usefulness of other short-chain fatty acids,^{43,44} acylators, and butyrate analogues⁴⁵⁻⁴⁷; and the therapeutic role of these compounds in combination with other agents that augment fetal hemoglobin in patients with β -hemoglobinopathies.

We are indebted to Dr. Tim Ley for his gift of the globin gene probes; to Dr. Anne Collins, Dr. Barbara Entsuah, Dr. Janet MacKinnon, Ms. Lebe Chang, Ms. Abby Mays, Ms. Mary Saukas, and Ms. Trish Griffin for help and support; to the staff of the Clinical Investigation Unit of Toronto Hospital and the Haematology/Oncology Ward nurses of the Hospital for Sick Children; to Dr. John Wayne for analysis of the α -globin and β -globin gene clusters; and to Dr. Susan Perrine for arranging the initial supply of arginine butyrate.

REFERENCES

1. Weatherall DJ, Clegg JB. The thalassemia syndromes. 3rd ed. Oxford, England: Blackwell Scientific, 1981:148-319.
2. Noguchi CT, Rodgers GP, Serjeant G, Schechter AN. Levels of fetal hemoglobin necessary for the treatment of sickle cell disease. *N Engl J Med* 1988; 318:96-9.
3. Goldberg MA, Husson MA, Bunn HF. Participation of hemoglobins A and F in polymerization of sickle hemoglobin. *J Biol Chem* 1977;252:3414-21.
4. Perrine RP, Brown MJ, Clegg JB, Weatherall DJ, May A. Benign sickle-cell anaemia. *Lancet* 1972;2:1163-7.
5. Wood WG, Pembrey ME, Serjeant GR, Perrine RP, Weatherall DJ. Hb F synthesis in sickle cell anaemia: a comparison of Saudi Arab cases with those of African origin. *Br J Haematol* 1980;45:431-45.
6. Brittenham GM, Schechter AN, Noguchi CT. Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes. *Blood* 1985;65:183-9.
7. DeSimone J, Heller P, Hall L, Zwiers D. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. *Proc Natl Acad Sci U S A* 1982;79: 4428-31.
8. Ley TJ, DeSimone J, Noguchi CT, et al. 5-Azacytidine increases γ -globin synthesis and reduces the proportion of dense cells in patients with sickle cell anemia. *Blood* 1983;62:370-80.
9. Charache S, Dover G, Smith K, Talbot CC Jr, Moyer M, Boyer S. Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the $\gamma\delta\beta$ -globin gene complex. *Proc Natl Acad Sci U S A* 1983;80: 4842-6.
10. Dover GJ, Charache S, Boyer SH, Vogelsang G, Moyer M. 5-Azacytidine increases HbF production and reduces anemia in sickle cell disease: dose-response analysis of subcutaneous and oral dosage regimens. *Blood* 1985; 66:527-32.
11. Papayannopoulou T, Torrealba de Ron A, Veith R, Knitter G, Stamatoyannopoulos G. Arabinosylcytosine induces fetal hemoglobin in baboons by perturbing erythroid cell differentiation kinetics. *Science* 1984;224:617-9.
12. Veith R, Galanello R, Papayannopoulou T, Stamatoyannopoulos G. Stimulation of F-cell production in patients with sickle-cell anemia treated with cytarabine or hydroxyurea. *N Engl J Med* 1985;313:1571-5.
13. Letvin NL, Linch DC, Beardsley GP, McIntyre KW, Nathan DG. Augmentation of fetal-hemoglobin production in anemic monkeys by hydroxyurea. *N Engl J Med* 1984;310:869-73.
14. Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. *J Clin Invest* 1984;74:652-6.
15. Dover GJ, Humphries RK, Moore JG, et al. Hydroxyurea induction of hemoglobin F production in sickle cell disease: relationship between cytotoxicity and F cell production. *Blood* 1986;67:735-8.
16. Charache S, Dover GJ, Moyer MA, Moore JW. Hydroxyurea-induced augmentation of fetal hemoglobin production in patients with sickle cell anemia. *Blood* 1987;69:109-16.
17. Rodgers GP, Dover GJ, Noguchi CT, Schechter AN, Nienhuis AW. Hematologic responses of patients with sickle cell disease to treatment with hydroxyurea. *N Engl J Med* 1990;322:1037-45.
18. Constantoulakis P, Mangahas JL, Papayannopoulou T, Enver T, Constantini F, Stamatoyannopoulos G. Locus control region-A gamma transgenic mice: a new model for studying the induction of fetal hemoglobin in the adult. *Blood* 1991;77:1326-33.
19. Dover GJ, Charache S. Hydroxyurea induction of fetal hemoglobin synthesis in sickle-cell disease. *Semin Oncol* 1992;19:Suppl 9:61-6.
20. Rodgers GP. Spectrum of fetal hemoglobin responses in sickle cell patients treated with hydroxyurea: the National Institutes of Health experience. *Semin Oncol* 1992;19:Suppl:67-73.
21. Charache S, Dover GJ, Moore RD, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. *Blood* 1992;79:2555-65.
22. Nathan DG. Pharmacologic manipulation of fetal hemoglobin in the hemoglobinopathies. *Ann N Y Acad Sci* 1990;612:179-83.

23. Bard H, Prossman J. Relative rates of fetal hemoglobin and adult hemoglobin synthesis in cord blood of infants of insulin-dependent diabetic mothers. *Pediatrics* 1985;75:1143-7.
24. Perrine SP, Greene MF, Faller DV. Delay in the fetal globin switch in infants of diabetic mothers. *N Engl J Med* 1985;312:334-8.
25. Ginder GD, Whitters MJ, Pohlman JK. Activation of a chicken embryonic globin gene in adult erythroid cells by 5-azacytidine and sodium butyrate. *Proc Natl Acad Sci U S A* 1984;81:3954-8.
26. Perrine SP, Miller BA, Greene MF, et al. Butyric acid analogues augment γ globin gene expression in neonatal erythroid progenitors. *Biochem Biophys Res Commun* 1987;148:694-700.
27. Glauber JG, Wandersee NJ, Little JA, Ginder DG. 5'-Flanking sequences mediate butyrate stimulation of embryonic globin gene expression in adult erythroid cells. *Mol Cell Biol* 1991;11:4690-7.
28. Perrine SP, Miller BA, Faller DV, et al. Sodium butyrate enhances fetal globin gene expression in erythroid progenitors of patients with Hb SS and beta thalassemia. *Blood* 1989;74:454-9.
29. Fibach E, Prasanna P, Rodgers GP, Samid D. Enhanced fetal hemoglobin production by phenylacetate and 4-phenylbutyrate in erythroid precursors derived from normal donors and patients with sickle cell anemia and beta-thalassemia. *Blood* 1993;82:2203-9.
30. Perrine SP, Rudolph A, Faller DV, et al. Butyrate infusions in the ovine fetus delay the biologic clock for globin gene switching. *Proc Natl Acad Sci U S A* 1988;85:8540-2.
31. Constantoulakis P, Knitter G, Stamatoyannopoulos G. Butyrate stimulates HbF in adult baboons. *Prog Clin Biol Res* 1989;316B:351-61.
32. Perrine SP, Ginder GD, Faller DV, et al. A short-term trial of butyrate to stimulate fetal-globin-gene expression in the β -globin disorders. *N Engl J Med* 1993;328:81-6.
33. Desforges JF. My life at the *Journal*, 1961-1993. *N Engl J Med* 1993;329:1038-9.
34. Waye JS, Cai S-P, Eng B, et al. High hemoglobin A₂ β^0 -thalassemia due to a 532-basepair deletion of the 5' β -globin gene region. *Blood* 1991;77:1100-3.
35. Miller BA, Olivieri N, Salameh M, et al. Molecular analysis of the high-hemoglobin-F phenotype in Saudi Arabian sickle cell anemia. *N Engl J Med* 1987;316:244-50.
36. Dover GJ, Boyer SH, Bell WR. Microscopic method for assaying F cell production: illustrative changes during infancy and in aplastic anemia. *Blood* 1978;52:664-72.
37. Cao A, Galanello R, Rosatelli MC. Genotype-phenotype correlations in β -thalassemias. *Blood Rev* 1994;8:1-12.
38. Pembrey ME, Wood WG, Weatherall DJ, Perrine RP. Fetal haemoglobin production and the sickle gene in the oases of eastern Saudi Arabia. *Br J Haematol* 1978;40:415-29.
39. Miller BA, Salameh M, Ahmed M, et al. High fetal hemoglobin production in sickle cell anemia in the eastern province of Saudi Arabia is genetically determined. *Blood* 1986;67:1404-10.
40. Platt OS, Thorington BD, Brambilla DJ, et al. Pain in sickle cell disease: rates and risk factors. *N Engl J Med* 1991;325:11-6.
41. Vichinsky EP, Lubin BH. A cautionary note regarding hydroxyurea in sickle cell disease. *Blood* 1994;83:1124-8.
42. Pace B, Li Q, Peterson K, Stamatoyannopoulos G. α -Amino butyric acid cannot reactivate the silenced γ gene of the β locus YAC transgenic mouse. *Blood* 1994;84:4344-53.
43. Little JA, Tuchman M, Ginder GD. Elevated fetal hemoglobin levels in propionic acidemia. *Clin Res* 1994;42:238A. abstract.
44. Little JA, Dempsey NJ, Tuchman M, Ginder GD. Metabolic persistence of fetal hemoglobin. *Blood* 1995;85:1712-8.
45. Dover GJ, Brusilow S, Samid D. Increased fetal hemoglobin in patients receiving sodium 4-phenylbutyrate. *N Engl J Med* 1992;327:569-70.
46. Dover GJ, Brusilow S, Charache S. Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. *Blood* 1994;84:339-43.
47. Collins AF, Pearson HA, Giardina P, McDonagh KT, Brusilow SW, Dover GJ. Oral sodium phenylbutyrate therapy in homozygous β thalassemia: a clinical trial. *Blood* 1995;85:43-9.

IMAGES IN CLINICAL MEDICINE

Images in Clinical Medicine, a weekly *Journal* feature, presents clinically important visual images, emphasizing those a doctor might encounter in an average day at the office, the emergency department, or the hospital. If you have an original unpublished, high-quality color or black-and-white photograph representing such a typical image that you would like considered for publication, send it with a descriptive legend to Kim Eagle, M.D., University of Michigan Medical Center, Division of Cardiology, 3910 Taubman Center, Box 0366, 1500 East Medical Center Drive, Ann Arbor, MI 48109. For details about the size and labeling of the photographs, the requirements for the legend, and authorship, please contact Dr. Eagle at 313-936-5275 (phone) or 313-936-5256 (fax), or the *New England Journal of Medicine* at images@edit.nejm.org (e-mail).