vided the blood meals for the Fort Washington mosquitoes rule out the possibility that this chicken served as a virus source but similarly document the failure of infected mosquitoes to transmit virus by this feeding. The chicken used for feeding the Fort Mifflin mosquitoes died during exposure to the insects. The fact that both of the 1977 isolates came from mosquitoes which had been held in the insectary for at least 15 days may be significant.

There are two possible explanations for the presence of virus in these mosquitoes. One is that they became infected through transovarial transmission, even though work by Chamberlain *et al.* (*3*) fails to support this theory; the other is that these females took viremic blood meals prior to hibernation. Since the feasibility of the latter occurring has been demonstrated (*8*), we consider this to be the most likely explanation. In spite of several demonstrations of transovarial transmission of arboviruses by mosquitoes, all have involved bunyaviruses of the California encephalitis group and mosquitoes which overwinter in the egg stage; research by Tesh and Gubler (*14*) suggests that the phenomenon may be restricted to this combination. Our data support an overwintering mechanism in which female *C. pipiens* that are diapause-conditioned (that is, exposed to short day lengths and cool temperatures) take a viremic blood meal, overwinter, and emerge infected. Whether or not such overwintering mosquitoes can introduce SLE virus to the summer cycle upon emergence remains to be demonstrated.

CHARLES L. BAILEY
BRUCE F. ELDRIDGE
DAVID E. HAYES
DOUGLAS M. WATTS
RALPH F. TAMMARIELLO
JOEL M. DALRYMPLE

Departments of Entomology and Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20012

References and Notes

1. W. C. Reeves, *Prog. Med. Virol.* **17**, 193 (1974).
2. R. E. Bellamy, W. C. Reeves, R. P. Scrivani, *Am. J. Hyg.* **67**, 90 (1958).
3. R. W. Chamberlain, W. D. Sudia, R. H. Gogel, *ibid.* **80**, 254 (1964).
4. W. C. Reeves, R. E. Bellamy, R. P. Scrivani, *ibid.* **67**, 78 (1958).
5. J. S. Blackmore and J. F. Winn, *Proc. Soc. Exp. Biol. Med.* **91**, 146 (1956).
6. L. H. Wang, *J. Korean Med. Assoc.* **14**, 871 (1971).
7. M. E. Slaff and W. J. Crans, *Mosq. News* **37** (No. 1), 11 (1977).
8. B. F. Eldridge, *Science* **151**, 826 (1966).
9. L. C. Lamotte, Jr., *Mosq. News* **23** (No. 4), 330 (1963).
10. H. A. McCordock, M. G. Smith, E. Moore, *Proc. Soc. Exp. Biol. Med.* **37**, 288 (1937).
11. J. Mallack, K. W. Ludlam, R. M. Altman, *Proc. N.J. Mosq. Exterm. Assoc.* **1977**, 98 (1977).
12. Verification of our identification of these strains as SLE virus and antigenic differences by neutralization was performed by C. Calisher, Vector-Borne Disease Division, Center for Disease Control, Fort Collins, Colo.
13. P. A. Jumars, F. J. Murphey, R. W. Lake, *Proc. N.J. Mosq. Exterm. Assoc.* **1969**, 219 (1969).
14. R. B. Tesh and D. J. Gubler, *Am. J. Trop. Med. Hyg.* **24**, 876 (1975).
15. D. Chiewsilp and J. M. McCown, *Appl. Microbiol.* **24**, 288 (1972).
16. J. F. Kent and E. H. Fife, Jr., *Am. J. Trop. Med. Hyg.* **12**, 103 (1963).
17. In conducting the research described in this report, we adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

12 December 1977

Erythroid Progenitors Circulating in the Blood of Adult Individuals Produce Fetal Hemoglobin in Culture

Abstract. *Erythroid colonies, raised from erythroid stem cells circulating in the peripheral blood of normal adult individuals, synthesize considerable amounts of fetal hemoglobin. In cultures from persons with sickling disorders, amounts of hemoglobin F that are known to inhibit sickling in vivo are produced. The results provide evidence that primitive erythroid progenitors are able to express the hemoglobin F production program and that cultures of mononuclear cells of the adult blood can be used to investigate the mechanisms involved in regulation of γ-globin gene switching.*

We report that the erythroid progenitor cells found in the circulation of the normal adult individuals produce, in vitro, erythroid colonies that synthesize considerable amounts of fetal hemoglobin. These circulating erythroid progenitors are considered to be relatively primitive committed erythroid stem cells with proliferative potentials of the erythroid stem-cell class operationally defined as burst-forming unit erythroid (BFUe) (*1*). They are capable of producing clus-

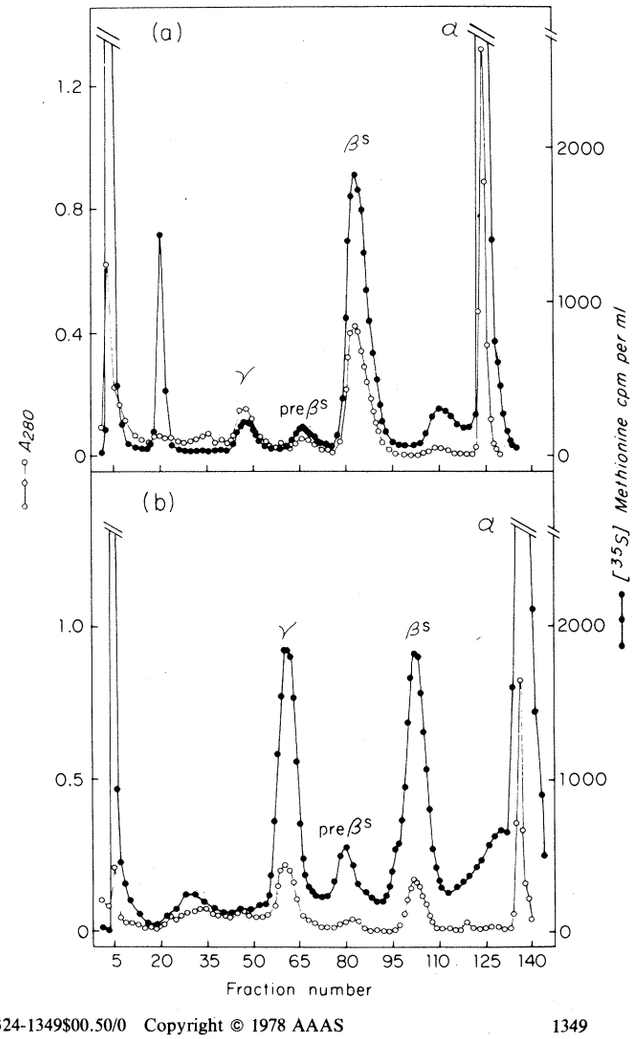

Fig. 1. Carboxymethylcellulose chromatography of globin chains from the hemoglobins produced by the cells of a subject with sickle cell/β-thalassemia. (a) Globin chain synthesis in blood reticulocytes. (b) Globin chains synthesized in blood BFUe cultures. There is a striking increase of γ chain radioactivity in (b).

0036-8075/78/0324-1349$00.50/0

Table 1. Globin-chain synthesis in plasma-clot cultures derived from circulating BFUe's.

Subject number	Hemoglobin genotype	γ chain synthesis in vivo (%)*	Plating efficiency†	Globin synthesis in culture (10^3 count/min)‡			γ chain (%)*
				α	β	γ	
183	A/A	<1	88	122.5	92.9	18.3	16.5
186	A/A	<1	256	210.7	163.0	33.8	17.1
188	A/A	<1	108	44.0	36.4	7.8	17.6
194	A/A	<1	144	17.6	18.6	2.8	13.0
198	A/A	<1	146	26.7	28.0	3.1	9.9
126	S/S	1.7	276	14.8	9.8	4.1	29.5
173	S/β-thal	4.6	270	158.8	74.1	38.7	34.3
193	S/S	1.9	322	61.7	88.0	32.3	26.8

*As percentage of non-α chain. †Colonies per 10^5 inoculated cells (9). ‡Total [^{35}S]methionine counts were adjusted for the presence of two methionyl residues in each α and γ chain and one each in β chain.

ters of subcolonies or erythroid bursts in vitro, each of which originates from a single BFUe (2). The ability of these cells to direct fetal hemoglobin production was detected by quantifying the radioactive γ chains of fetal hemoglobin (Hb F: $\alpha_2\gamma_2$) and β chains of adult hemoglobin (Hb A: $\alpha_2\beta_2$) that were synthesized in culture.

Erythroid cultures were initiated with the mononuclear cells separated with Ficoll-Hypaque from blood samples (100 ml each). After incubation in tissue-culture medium for 1 hour at 37°C, the nonadherent cells were collected, adjusted to a concentration of 10^6 nucleated cells per milliliter, mixed with the medium (3) containing 2.0 I.U. (international units) of erythropoietin per milliliter (4), and plated in tissue culture dishes. Cell growth was monitored by daily observations of developing erythroid clones under the inverted microscope. On days 11 or 12, 30 to 50 μCi of [^{35}S]methionine were added to each dish, and, 24 hours later, erythroid colonies were collected and lysed (5). Carrier [^{3}H]methionine-labeled hemoglobins F and A were added, and the fresh lysates were applied to Sephadex G-100 columns equilibrated and subsequently developed with 0.05*M* tris-HCl buffer, *p*H 7.4. The globin chains of the hemoglobins purified by gel filtration were separated on carboxymethylcellulose columns, and the relative proportions of γ, β, and α chains eluted from the columns were calculated after their ^{35}S radioactivities were measured and corrected for methionine content and contamination (6).

The proportion of [^{35}S]methionine incorporated into the globin chains produced by the circulating reticulocytes and the erythroid clones raised from circulating BFUe's is shown in Fig. 1 and Table 1. No synthesis of γ chain was detected in reticulocytes from five normal subjects after short-term incubation with [^{35}S]methionine, indicating that only traces of Hb F were synthesized in vivo; the BFUe cultures produced an average of 14.8 percent γ chains. Although the biosynthetically determined levels of Hb F constituted less than 2 percent of the hemoglobin synthesized in reticulocytes from the two Hb S homozygotes and only 4.6 percent of the hemoglobin synthesized in reticulocytes from a subject heterozygous for Hb S and a β-thalassemia gene (sickle cell/β-thalassemia). an average of 30.2 percent Hb F was produced in BFUe cultures, an amount known to protect the sickle cell patients from the pathophysiological consequences of sickling in vivo (7).

The mechanisms involved in the maintenance of Hb F during intrauterine life and in the transition to adult hemoglobin formation after birth remain unknown. Previous observations (2, 8) of Hb F synthesis in cultures of bone marrow cells, have suggested (i) that Hb F synthesis in culture is clonal, indicating that differences in commitment to Hb F production exist among erythroid progenitors, and (ii) that cells mainly responsible for the formation of clones displaying Hb F synthesis have the erythropoietin dependence and growth characteristics of the primitive progenitors defined as BFUe's. These observations led us to propose that expression of the γ gene is dependent on erythroid stem-cell differentiation and is a characteristic of less mature erythroid stem cells (2). Our data provide further support to our hypothesis, for they show a consistent activation of Hb F formation in erythroid cultures that are of pure BFUe origin. Expression of the Hb F production program thus appears to be a property of cells derived from primitive erythroid progenitors; this property apparently is lost when the erythroid progenitors of the adult individual further differentiate in vivo.

The delineation of the mechanism of Hb F regulation has been hampered by the fact that Hb F appears in only a few large animals during a restricted stage of development. Our findings suggest that the cellular and molecular mechanisms that regulate the expression of hemoglobin genes might be investigated in vitro with the use of easily accessible cells, that is, the erythroid cell progenitors circulating in human peripheral blood.

TH. PAPAYANNOPOULOU
B. NAKAMOTO
J. BUCKLEY, S. KURACHI
P. E. NUTE, G. STAMATOYANNOPOULOS
Divisions of Hematology and *Medical Genetics, Department of Medicine* and *Department of Anthropology, University of Washington, Seattle 98195*

References and Notes

1. H. Hara and M. Ogawa, *Exp. Hematol. (Copenhagen)* **5**, 161 (1977); B. J. Clarke and D. Housman, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1105 (1977).
2. Th. Papayannopoulou, M. Brice, G. Stamatoyannopoulos, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2923 (1977).
3. Plasma-clot cultures were set up as originally described by Tepperman *et al.* [A. D. Tepperman, J. E. Curtis, E. A. McCulloch, *Blood* **44**, 659 (1974)], with minor modifications. Cultures were established in 35-mm Falcon tissue culture dishes at a concentration of 10^6 nucleated cells per milliliter, in the presence of 1 percent beef embryo extract, 1 percent bovine serum albumin, $10^{-4}M$ β-mercaptoethanol, anemic plasma sheep erythropoietin, and 30 percent fetal calf or adult human (AB) serum. Clotting was achieved by the addition of 10 percent citrated bovine plasma.
4. Anemic sheep plasma erythropoietin step III preparation (specific activity 4.8 I.U. per milligram of protein; Connaught Research Laboratories, Toronto, Canada).
5. At the end of the labeling period, [^{35}S]methionine cultures were washed twice with phosphate-buffered saline, and the plasma clots were dissolved by treatment with 1 ml of fresh solution of 0.1 percent Pronase (Calbiochem grade B) in alpha medium containing 5 percent fetal calf serum, for 10 to 20 minutes at room temperature. By this treatment, colonies located at the center of the dish were freed and collected by micromanipulation under the inverted microscope. For complete collection of peripherally located colonies, repetition of the treatment was required.
6. The purity of the ^{35}S-labeled material in the γ peak was checked by high-voltage paper electrophoresis of a tryptic digest at 2000 V for 120 minutes [D. J. Nathan, H. F. Lodish, Y. W. Kan, D. Housman, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2514 (1971)], with the use of a pyridine–acetic acid buffer (10 percent aqueous pyridine, by volume, adjusted to *p*H 6.58 with glacial acetic acid). Under these conditions, peptide β_5 migrates about 10 cm toward the anode, γ_{15} about 18 cm toward the cathode, and γ_5 remains near the origin. From the amounts of radioactivity incorporated into peptides β_5 and γ_{15}, the proportions of γ and pre-β chains in the γ chain peak of the carboxymethylcellulose column were calculated. Pure γ chain counts appear in Table 1.
7. Persons who are heterozygous either for Hb S and a gene for hereditary persistence of fetal hemoglobin or for Hb S and a gene for Hb F thalassemia produce from 15 to 30 percent Hb F in vivo; the Hb F is distributed equally among erythrocytes in the former or unequally in the latter. These patients have minimal manifestations of intravascular sickling [see D. J. Weatherall and J. B. Clegg, *The Thalassemia Syndromes* (Blackwell, Oxford, 1972)].
8. Th. Papayannopoulou *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2033 (1976).
9. Isolated colonies and closely associated aggregates were counted as single colonies, with no attempt to assign them to individual BFUe's.
10. Supported by NIH grants HL 20899, GM 15253, and RR 00166.

14 November 1977; revised 13 January 1978