The in vitro biosynthesis of acetylated human fetal hemoglobin (Hb F) was investigated in umbilical cord blood from two normal newborns and in peripheral blood from five adults with different hematologic disorders accompanied by elevated levels of fetal hemoglobin. Hemoglobin biosynthesis was measured by [3H]leucine incorporation into hemoglobin components which were separated by chromatography on Bio Rex 70 cation-exchange resin. Following incubation of the samples for times ranging from 2% to 120 min, the specific radioactivities of newly synthesized Hb F ranged from 2.1 to 3.9 times those corresponding values for the major fetal hemoglobin component (Hb F,) which is not acetylated. For both umbilical cord blood and the δβ/β+ thalassemia blood, the ratio of specific radioactivities (Hb F/Hb F,) decreased significantly during the initial 20 min of incubation. Pulse-chase experiments revealed that the newly synthesized Hb F was stable. These results indicate that in the circulating reticulocytes of the newborn and in those of adults with elevated fetal hemoglobin, the rate of biosynthesis of acetylated fetal hemoglobin is higher than that expected from the amounts of this hemoglobin existing in the blood.

Radiolabeled blood cells from an adult patient with δβ/β+ thalassemia were separated into five zones by centrifugation in a density gradient. Although each of the five zones contained similar amounts of cellular hemoglobin, about 84% of the protein [3H]-radioactivity was present in the lightest (top) zone with the remainder distributed almost equally among the other zones. The Hb F/Hb F, specific radioactivity ratio was about 1.5 in the lightest gradient zone and ranged from 11.1 to 18.4 in the four heaviest zones. These data suggest that much of the Hb F synthesis occurred in a separate population of erythroid cells heavier than, and probably more mature than, the cells responsible for most of the hemoglobin synthesis. This result may occur because of increased activity of an acetyltransferase enzyme which could serve as a marker for erythroid cell maturation.

A wide variety of proteins are acetylated at the NH2 terminus of the polypeptide chain (1-3). The acetylation event is catalyzed by an acetyltransferase enzyme using acetyl-CoA as substrate and occurs early in the life of a protein molecule, perhaps during growth of the nascent polypeptide chain (4- 8). Only a few types of hemoglobins, which have served as model macromolecules to study various aspects of protein structure and synthesis, have N-acetylated polypeptide chains (9-18). One of these is a modification of human fetal hemoglobin (Hb F) (19-22).

Hb F is composed of two α- and two γ-polypeptide chains. The acetylated form, Hb F, differs in structure from the major component Hb F, only by the presence of acetyl groups at the NH2 termini of the γ chains (19, 20). Human fetal hemoglobin differs from other acetylated proteins in that about 80-90% of the molecules remain unacetylated. We have studied the relative rates of synthesis of Hb F and Hb F, in human blood cells from a variety of sources and have examined the role of cell maturation on γ chain acetylation.

**Materials and Methods**

Blood from the umbilical cord of two normal fetuses and from five adult individuals with elevated levels of Hb F were withdrawn in EDTA and chilled to 4°C. The hematologic disorders and blood hemoglobin compositions of the five adult donors were: A. W., heterozygous pancellular hereditary persistence of fetal hemoglobin (HPFH), 30.1% F,, 3.3% F; A. S., doubly heterozygous for Hb S and heterocellular HPFH, 14.4% F,, 1.1% F; J. R., homozygous for Hb S and heterocellular HPFH, 14.4% F,, 1.1% F; A. S., doubly heterozygous for δβ and β+ thalassemia, 6-7% F, 10% F, 7% A, and 15% A.; S. O., homozygous for β+ thalassemia, Hb F, 9.2%, Hb F, 82.1%. The red cells were isolated by centrifugation and washed three times with saline solution (0.1 M NaCl, 0.005 M KCl, 0.0075 M MgCl2). Samples of the cells were incubated at 37°C with [3H]leucine (60-85 Ci/mmol) in a medium supporting protein synthesis, as described (23). Replicate reaction mixtures of the cord blood cells were incubated for various times from 2% to 120 min for one sample, and 2, 5, and 60 min for a sample from a second newborn. In a pulse-chase experiment, an aliquot of cord blood cells was incubated with [3H]leucine for 5 min at 37°C, after which the cells were washed thrice in ice-cold saline, and then subjected to incubation for 55 min at 37°C in a medium with nonradioactive leucine substituted for [3H]leucine. Blood cells from three of the adult donors were incubated for 60 min. Blood cells from one adult donor (S. O.) were incubated for 5 and 60 min and cells from another (C. G.) were incubated for 1/4 to 120 min. The reaction mixtures were chilled on ice to 4°C, and excess cold saline solution was added to each to stop the [3H]-labeling reaction.

Hemolyzates were prepared from the [3H]-labeled erythroid cells by lysis of washed cells followed by centrifugation (24). To compare the synthetic patterns of the two fetal hemoglobin species, 0.50 ml (10-25 mg of hemoglobin) of each of the [3H]-labeled soluble phases was dialyzed at 4°C for 3 h against 500 ml of 0.05 M phosphate (potassium) containing 0.01 M KCN, pH 6.60, and applied to a column (1.2 x 15 cm) of Bio Rex 70 resin equilibrated with the same buffer (25). The hemoglobins were separated by development of the column with the phosphate buffer at 15 ml/h in the cold. Fractions (2 ml) of the eluate were collected, and their absorbances at 415 nm were determined.

After the Hb F component eluted, a linear salt gradient consisting of 0 or 150 ml of 0.25 M NaCl in the 0.05 M phosphate buffer (reservoir chamber) and 100 (or 150) ml of the phosphate buffer without salt (mixing chamber) was applied to elute the Hb F and other, more basic, hemoglobin components. Finally, the column was eluted with buffer containing 0.25 M NaCl to ensure complete recovery of the Hb F.
A and Hb A2. For protein radioactivity analyses, samples of the eluted 
fractons were added to 0.50 mg of carrier casein, treated with 1 N 
NaOH for 2 min to hydrolyze [3H]leucyl-tRNAs possibly present, and 
precipitated with 5% trichloroacetic acid. The [3H]-labeled precipitates 
were washed onto cellulose nitrate membrane filters for assay of 
radioactivity in a dioxane-based liquid scintillation system as de-
scribed (26).

In some experiments, selected column fractions were pooled and 
added to unlabeled carrier lysate containing 40–50 mg of hemoglobin. 
Globin was prepared from these mixtures by precipitation with acid-
ified acetone, and the α- and non-α chains were separated on a column 
(1.2 x 12 cm) of CM-cellulose in 8 M urea at pH 6.8 (27). The 
distribution of [3H]-radioactivity among the globin chains was deter-
mined by adding each eluted fraction (1 ml) to 10 ml of ACS counting 
fluid (Amersham) and counting in a liquid scintillation counter.

In one experiment, cells from C.G. were incubated with [3H]leucine 
for 60 min and washed by centrifugation with cold saline solution. A 
1.5-ml sample of the packed, [3H]-labeled cells was suspended in an 
equal volume of a 24% dextran (Sigma No. D-4133, 40,000 average 
molecular weight) solution prepared in standard medium as described 
by Abraham et al. (28). One-half of the cell suspension was pipetted 
onto each of two density gradients prepared in layers by adding 
succecssively 2.8 ml of 30%, 28%, 26%, and 24% dextran solutions to a 
14-ml cellulose nitrate centrifuge tube (Beckman No. 331101). Cells 
of various densities were separated by centrifugation for 90 min at 
27,000 rpm and 5°C in an SW 40-Ti rotor. Zones of cells located at 
the top, bottom (pellet), and the three interfaces of the four dextran 
solutions (see Fig. 1) were harvested with the aid 
of a tube slicer 
(beckman No. 303811). The separated cells were washed with the 
modified saline solution, and hemolysates of these cells and a control 
batch, labeled similarly with [3H]leucine but not fractionated in a 
dextran gradient, were prepared. Samples of the lysates were analyzed 
for absorbance at 540 nm (total hemoglobin), for total protein 3H-
radioactivity (0-

 RESULTS

Umbilical cord blood cells (6.5% reticulocytes) were incubated with [3H]leucine for various times to study simultaneously 
the syntheses of the minor acetylated fetal hemoglobin F1 and the major unacetylated fetal hemoglobin F0. The [3H]- 
labeled cells were lysed, and the hemoglobin of each lysate 
were separated by chromatography on a column of Bio Rex 
70, a cation exchange resin. Fig. 2 shows the elution pattern 
obtained when cells were labeled for 60 min. The fraction of 
the total fetal hemoglobin in acetylated form (F1) was about 
10%, a value similar to that reported previously for cord 
erythrocytes (21, 22). During the incubation, [3H]leucine was 
incorporated into Hb F0, Fα, and A. The specific radioactivity 
(counts per min per mg of protein) of Hb F1 was about 2½ 
times that of Hb Fα.

To further identify the nature of the Hb F1 and Hb Fα 

radioactivities, samples of these hemoglobin subunits were eluted from the Bio Rex column, were converted to globin, and the [3H]-labeled 
α and γ (or γ α) subunit chains were separated on columns of 
CM-cellulose in 8 M urea. As shown in Fig. 3A, virtually all of the radioactivity incorporated into Hb F1 was recovered in 
radioactive peaks that precisely co-chromatographed with 
acetylated γ chains and α chains. The γα/α radioactivity ratio 
was 2.14. In like manner, nearly all of the radioactivity from the isolated Hb F0 peak was recovered as nonacetylated γ 
chains and α chains with a γα/α radioactivity ratio of 1.68 (Fig. 
3B). These results show that the higher specific radioactivity of 
Hb F1 was reflected in the relative γα and γ α chain specific 
radioactivities of the two hemoglobins and did not occur 
because of a difference in the specific radioactivities of the α 
chain subunits. In another experiment, separation of the globin 
chains of total hemolysates isolated from cord blood cells 
incubated with [3H]leucine for 2, 5, or 60 min showed that the 
(γα + γ + β)/α radioactivity ratios were 1.12, 1.00, and 1.12, 
respectively. The relatively higher incorporation of γα and γ 
chain radioactivity into the chromatographically purified Hb 
F1 and Hb Fα, components together with a balanced α and non-
α subunit synthesis in the total lysate infer the presence of a 
pool of free α chain intermediates in the assembly of the

FIG. 1. Schematic illustration of the dextran gradient used 
to separate blood cells of various densities and definition of 
the separated zones of cells after centrifugation (see "Materi-
als and Methods").

FIG. 2. Elution pattern after chromatography of a hemoly-
sate from [3H]-labeled cord blood cells on a column of Bio Rex 
70. A sample of cord blood cells was incubated with [3H]leucine 
at 37°C for 60 min (see "Materials and Methods"). The [3H]- 
labeled soluble phase was isolated and 0.50 ml was dialyzed for 3 h against 
500 ml of 0.05 M potassium phosphate containing 0.01 M KCN, pH 
6.20, and applied to a column (1.2 x 15 cm) of Bio Rex 70 equilibrated 
at 4°C with the same buffer. Hemoglobin F0, Fα, and A were separated 
by column development, which included the application of a linear 
NaCl gradient (200 ml total volume) between fractions 48 and 145 
(see "Materials and Methods"). The absorbance at 415 nm (-----) and 
protein [3H]-radioactivity (•—••—••) of each eluted fraction were deter-
mined; 100% of the hemoglobin (24.7 mg) and 84% of the protein [3H]- 
radioactivity (8.02 x 106 cpm) applied to the column were recovered 
in the fractions. Similar elution patterns were obtained for other Bio 
Rex 70 chromatograms reported in this paper. In peaks of Hb F1, 
there was excellent (95%) agreement between specific activities of 
adjacent fractions. In Hb Fα, both the absorbance and radioactivity 
peaks were symmetrical but the radioactivity lagged slightly behind 
absorbance. Peak homogeneity was further documented by the fact 
that subsequent analysis of labeled globin subunits on CM-cellulose 
chromatography in 8 M urea revealed symmetrical peaks with minimal 
extaneous radioactivity.

FIG. 3. A, Separation of radioactively labeled fetal hemoglo-
bin subunits from [3H]-labeled cord blood cells on a column of 
Bio Rex 70 chromatograms reported in this paper. In peaks of Hb F1, 
there was excellent (95%) agreement between specific activities of 
adjacent fractions. In Hb Fα, both the absorbance and radioactivity 
peaks were symmetrical but the radioactivity lagged slightly behind 
absorbance. Peak homogeneity was further documented by the fact 
that subsequent analysis of labeled globin subunits on CM-cellulose 
chromatography in 8 M urea revealed symmetrical peaks with minimal 
extraordinary radioactivity.

...
were incubated with $[^3H]leucine$ for 60 min. When the hemoglobins of each $[^3H]leucine$-incorporating donor, which was unassociated with significant absorbance eluted immediately prior to Hb Fr, were chromatographed on a column of Bio Rex 70 (see "Materials and Methods" and Fig. 2). The specific radioactivities (cpm/mg protein) of Hb F1 and Hb Fr were calculated from the total protein radioactivities and total absorbances of each eluted hemoglo-

**TABLE I**

**Biosynthesis of Hb F1 and Hb F2 in adult red cells**

Replicate samples of peripheral blood cells were incubated with $[^3H]leucine$ for various times and the hemoglobins of each $[^3H]leucine$-labeled lysate were separated by chromatography on a column of Bio Rex 70 (see "Materials and Methods" and Fig. 2). The specific radioactivities (cpm/mg protein) of Hb F1 and Hb Fr were calculated from the total protein radioactivities and total absorbances of each eluted hemoglobin.

<table>
<thead>
<tr>
<th>Donor type</th>
<th>Time of $[^3H]$-labeling</th>
<th>mg Hb F1/mg Hb Fr</th>
<th>cpm/mg Hb F1</th>
<th>cpm/mg Hb Fr</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, PanHPFH(A. W.)</td>
<td>60.0</td>
<td>0.11</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>AS, HetHPFH(S., R.)</td>
<td>60.0</td>
<td>0.09</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>SS, HetHPFH(S. J., R.)</td>
<td>60.0</td>
<td>0.13</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>$^\delta^6/\beta$-thal.(C. G.)</td>
<td>60.0</td>
<td>0.14</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>$\beta$-thal/$\beta$-thal.(S. O.)</td>
<td>5.0</td>
<td>0.11</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>$\beta$-thal/$\beta$-thal.(S. O.)</td>
<td>60.0</td>
<td>0.12</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

*PanHPFH, pancellular hereditary persistence of fetal hemoglobin; HetHPFH, heterocellular HPFH; $\beta$-thal, $\beta$-thalassemia.*

hemoglobin tetramers. Indeed, the protein radioactivity which eluted immediately prior to Hb Fr during Bio Rex chromatography and which was unassociated with significant absorbance (Fig. 2) consisted entirely of $[^3H]leucine$-labelled $\alpha$ chains (data not shown).

Blood cells from adults with a variety of disorders resulting in elevated levels of Hb F (see "Materials and Methods") also were incubated with $[^3H]leucine$ for 60 min. When the hemoglobins of these cells were analyzed on columns of Bio Rex 70, the elution patterns were similar to that of Fig. 2. Table I shows that the ratio of pre-existing hemoglobins F1 to F2 in these adult cells ranged from 0.09 to 0.14. These values are similar to those reported by others (22) for people with hereditary persistence of fetal hemoglobin or non-$\alpha$ thalassemia. Hb F1 had a substantially higher specific radioactivity than Hb Fr in the $[^3H]leucine$-labeled erythroid cells of each of the adults (Table I). Moreover, the specific radioactivity of Hb F1 was consistently higher than that of Hb Fr in both cord blood cells and cells from the adult donor (C. G.) with $\delta^6/\delta^6$ thalassemia throughout a 2-h period of $[^3H]leucine$ incorporation (Table II). These results suggest that there was an enhanced relative synthesis of pre-existing fetal hemoglobin in these cells.

**TABLE II**

**Biosynthesis of Hb F1 and Hb F2 in red cells**

Incubations and analyses were performed as in Table I. In two experiments, cells were $[^3H]leucine$-labeled for 5 min, washed, and subsequently incubated (chased) for 55 min in nonradioactive medium.

<table>
<thead>
<tr>
<th>Donor type</th>
<th>Time of $[^3H]$-labeling</th>
<th>Hb F1</th>
<th>Hb Fr</th>
<th>cpm/mg Hb F1</th>
<th>cpm/mg Hb Fr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn No. 1</td>
<td>2.5</td>
<td>16,260</td>
<td>4,360</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Hb F1 = 7.9%</td>
<td>5</td>
<td>44,800</td>
<td>12,750</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Hb Fr = 77.1%</td>
<td>20</td>
<td>109,200</td>
<td>50,720</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Hb F1 = 10%</td>
<td>60</td>
<td>456,000</td>
<td>179,500</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Hb Fr = 60%</td>
<td>120</td>
<td>334,000</td>
<td>159,800</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Newborn No. 2</td>
<td>5</td>
<td>11,400</td>
<td>3,590</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Hb F1 = 5.8%</td>
<td>12</td>
<td>51,590</td>
<td>23,040</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Hb Fr = 64.9%</td>
<td>5</td>
<td>47,090</td>
<td>21,600</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Hb A = 14.0%</td>
<td>60</td>
<td>395,300</td>
<td>186,800</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

Adult

| Hb F1 | 1.25 | 11,770 | 2,240 | 3.6 |
| Hb Fr | 5    | 42,560 | 17,080 | 2.5 |
| Hb A = 7% | 50   | 142,700 | 81,800 | 1.7 |
| 60 | 442,100 | 215,200 | 2.1 |
| 5 pulse | 56 chase | 79,910 | 31,080 | 2.6 |

*Thal, thalassemia.*

When cord or adult blood cells were pulse-labeled with $[^3H]leucine$ for 5 min and incubated subsequently (chased) in nonradioactive medium for an additional 55 min, the specific radioactivity of Hb Fr remained at about 2½ times that of Hb F1 (Table II). In the adult sample, the radioactivities of both Hb F1 and Hb Fr increased during the chase incubation, probably because the excess newly synthesized $\alpha$ chains of this thalassemic individual were converted into the tetramers. These results indicate that newly synthesized Hb Fr was stable for at least several hours after synthesis.

However, there may be some Hb F1, synthesized in the erythropoietic cells of the donor, which is catabolized slowly during the life of the circulating erythrocyte to provide the relative levels of Hb F1 and Hb Fr observed in the blood. If so, the intracellular level of Hb F1 should decrease selectively as
Synthesis of Hb F;

Relative amounts of hemoglobin components and specific radioactivities of Hb F1 and Hb F, in erythroid cells of increasing density

Blood cells were incubated with [3H]leucine for 60 min and separated into zones of various densities by centrifugation into a dextran gradient (see "Materials and Methods" and Fig. 1). The proportion of nucleated red cells (RBC) in each zone was determined from smears. A sample (about 15 mg of hemoglobin) of the cellular lysate from each zone was chromatographed on a Bio Rex 70 column, and the distribution of the total eluted absorbance among the hemoglobin components was determined. "Pre-F1" designates the absorbance representing the minor components which eluted before Hb F1 (see Fig. 2 and Ref. 22). The specific radioactivities of the Hb F1 and Hb F, separated on each Bio Rex column also were determined, as described in the legend of Table I. Data were obtained in a similar manner from a sample of cells (control) which were incubated with [3H]leucine but not separated in a dextran gradient.

<table>
<thead>
<tr>
<th>Gradient zone</th>
<th>Nucleated RBC %</th>
<th>Fraction of total A450</th>
<th>Hb F1 %</th>
<th>Hb F, %</th>
<th>Hb A %</th>
<th>Hb F1 cpm/mg</th>
<th>Hb F, cpm/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Light</td>
<td>0.83</td>
<td>1.98</td>
<td>7.8</td>
<td>74.3</td>
<td>8.92</td>
<td>1,345,000</td>
<td>909,700</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
<td>2.45</td>
<td>9.3</td>
<td>71.7</td>
<td>8.07</td>
<td>274,800</td>
<td>24,830</td>
</tr>
<tr>
<td>3</td>
<td>0.14</td>
<td>3.23</td>
<td>9.9</td>
<td>70.5</td>
<td>6.75</td>
<td>240,400</td>
<td>13,040</td>
</tr>
<tr>
<td>4</td>
<td>0.03</td>
<td>4.54</td>
<td>10.5</td>
<td>68.6</td>
<td>6.00</td>
<td>218,000</td>
<td>14,900</td>
</tr>
<tr>
<td>5 Heavy</td>
<td>0.00</td>
<td>4.11</td>
<td>10.2</td>
<td>67.7</td>
<td>7.13</td>
<td>238,000</td>
<td>14,340</td>
</tr>
<tr>
<td>Control</td>
<td>0.44</td>
<td>3.40</td>
<td>10.1</td>
<td>73.0</td>
<td></td>
<td>442,100</td>
<td>215,200</td>
</tr>
</tbody>
</table>

![Fig. 4. Distribution of the total hemoglobin and protein 3H-radioactivity among cells of various densities after separation in a dextran gradient.](source)

The total amounts of Hb F1 and Hb F, 3H-radioactivity in the cells from each of the dextran gradient zones of the experiment described in Table III were determined from the Bio Rex chromatography patterns of the lysates. The total protein 3H-radioactivity of each lysate was determined from an appropriate counting sample. The results for each hemoglobin (or total lysate) is expressed as a fraction of the total radioactivity recovered from the five gradient zones. These total protein 3H-radioactivities were: Hb F1, 2.82 × 10^4 cpm; Hb F, 9.30 × 10^3 cpm; and total lysate, 37.38 × 10^4 cpm.

Table IV

Distribution of total Hb F1 and Hb F, synthesis among erythroid cells of increasing density

<table>
<thead>
<tr>
<th>Protein 1H cpm</th>
<th>Hb F1</th>
<th>Hb F,</th>
<th>Total lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradient zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Light</td>
<td>47.6</td>
<td>92.4</td>
<td>84.7</td>
</tr>
<tr>
<td>2</td>
<td>13.0</td>
<td>2.7</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>14.4</td>
<td>1.7</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>11.3</td>
<td>1.5</td>
<td>3.6</td>
</tr>
<tr>
<td>5 Heavy</td>
<td>13.6</td>
<td>1.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Control</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The red cell ages in vivo. To investigate this possibility, red cells from C. G. labeled with [3H]leucine for 60 min were centrifuged into a dextran gradient to separate the cells into five zones of various densities (see "Materials and Methods" and Fig. 1). Other investigators have shown that, when blood cells from normal individuals are separated after centrifugation into similar densities, the denser fractions are enriched in older erythrocytes while the lighter fractions contain younger cells (30, 31). Fig. 4 shows that, after centrifugation of the thalassemic donor’s cells into the dextran gradient, the total cellular hemoglobin was divided about equally among the five zones of separated cells. A sample of the hemolysate from the cells of each zone was analyzed on a Bio Rex 70 column. Table III shows that the amounts of the acetylated Hb F1 and of the minor hemoglobins which eluted before Hb F1 (pre-F1, see Ref. 22) increased, while that of Hb A decreased, with increasing density of the separated cells. Several years ago, Gabuzda et al. (32) showed that cells of the same thalassemic patient (C. G.) that were relatively rich in Hb A had a shorter survival in vivo than those containing predominantly Hb F. Thus, the pattern of distribution of the hemoglobin components among the dextran gradient zones was consistent with the hypothesis that red cell aging in vivo parallels increased cell density. The failure to find a decrease in Hb F1 concentration with increasing cell age (Table III) suggests that the elevated Hb F1/Hb F, specific radioactivity ratios of [3H]leucine-labeled erythroid cells cannot be explained by a slow turnover of some of the newly synthesized Hb F1 during the life span of the circulating red cell.

A probable explanation for the enhanced relative synthesis of acetylated fetal hemoglobin in the donor’s peripheral blood erythrocyte cells was obtained when the pattern of distribution of the newly synthesized hemoglobins among the dextran gradient zones was examined. Fig. 4 shows that 84% of the total protein 3H-radioactivity was in the lightest zone of separated cells, while the remaining 16% was divided about equally among the four densest zones. The finding that most of the hemoglobin synthesis and most of the nucleated red cells (Table III) were in the top zone was consistent with the hypothesis that the younger erythroid cells were located selectively in the lighter zones. Although only a small fraction of the total hemoglobin production occurred in the more dense cells, Table III shows that there was a 10-fold increase of the Hb F1/Hb F, specific radioactivity ratio in the four densest zones compared to the lightest zone of cells. These results suggest that much of the Hb F1 synthesis occurred in a separate population of cells heavier than the cells responsible for most of the hemoglobin synthesis. The distribution of total Hb F1 synthesis among the cells from the various dextran gradient zones was determined. Table IV shows that at least 50% of the total Hb F1 synthesis occurred in cells heavier than those responsible for almost all of the Hb F, synthesis. Per-
haps an even higher fraction of Hb F₁ synthesis in the denser cells was not observed because of incomplete separation of the cells which synthesized Hb F₁ from the other erythroid cells.

**DISCUSSION**

The enhanced synthesis of acetylated Hb F₁ relative to Hb F₀ appears to be a general phenomenon occurring in a variety of blood specimens containing increased levels of fetal hemoglobin. The consistency of this observation makes it unlikely that it is an artifact of in vitro incubation or imbalance of chain synthesis. The increased specific radioactivity of Hb F₁ is unlikely to be due either to conversion of Hb F₁ to Hb F₀, or to preferential catabolism of newly synthesized Hb F₁. Not all of the newly synthesized Hb F₁ can be an intermediate in the biosynthesis of Hb F₀; at least some of the synthesized Hb F₁ molecules must be stable because 10–15% of the total fetal hemoglobin molecules in the peripheral blood remain acetylated. Although there was a decrease in the F₁/F₀ specific radioactivity ratio as the labeling of cord blood cells with [³H]leucine progressed (see Table II), the finding that this ratio was still significantly higher than unity at the longer incubation times is consistent with the stability of Hb F₁. Furthermore, pulse-chase experiments also indicate that newly synthesized Hb F₁ is stable.

Our finding that much of the Hb F₁ synthesis occurred in cells which could be isolated on a density gradient provides a more plausible explanation of the increased specific activity of Hb F₁ compared to Hb F₀. The acetylated and unacetylated fetal tetramers may be synthesized in different subsets of erythropoietic cells or at different stages in maturation of a uniform erythroid cell line. An example of the former possibility would exist if the acetyltransferase enzyme were active in only a few clones of erythroid cells; the latter possibility would exist if most of the Hb F₁ synthesis occurred early in erythroid cell development, perhaps in the nucleated red cells, while much of the Hb F₀ synthesis occurred at a later stage, perhaps in reticulocytes. Accordingly, the decline in the ratio of specific radioactivities of Hb F₁/Hb F₀ during 2-h incubations (Table II) may reflect the enhanced preferential synthesis of Hb F₁ in late reticulocytes which steadily lose their biosynthetic capability during the 2-h incubation. Either explanation is consistent with the incomplete acetylation of human fetal hemoglobin, a circumstance unique to this protein.

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